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American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

AJEONP

American Journal of Essential Oils and Natural Products

ISSN: 2321 9114
AJEONP 2016; 4(1): 32-35

Received: 17-11-2015
Accepted: 18-12-2015

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Essential oil composition and antiradical activity of two *Artemisia* species endemic to the island of Crete (Southern Greece)

Emad Shehata, Sofia Loupassaki, Dimitris P Makris

Abstract
Artemisia arborescens and *Artemisia inculta* Delile, two species endemic to the island of Crete (southern Greece) were examined with regard to their essential oil composition. The main constituents of *A. arborescens* essential oil were found to be camphor (30.51%), *trans*-thujone (18.42%), and chamazulene (15.72%), while those of *A. inculta* were 1,8-cineole (28.10%), *cis*-thujone (17.48%), *trans*-sabinol (12.49%), and *trans*-thujone (11.97%). The determination of the antiradical activity of the essential oils showed that the potency of *A. arborescens* is 40 times higher compared with that of *A. inculta*, a finding attributed to the high amount of chamazulene in *A. arborescens*.

Keywords: Antiradical activity; *Artemisia;* essential oil.

1. Introduction
Almost 50% of all licensed drugs that were registered worldwide in the 25-year period prior to 2007 were natural products or their synthetic derivatives [1]. The Asteraceae family embraces more than 20,000 species and approximately 500 species from the genus *Artemisia* grow mainly in Asia, Europe and North America, some of which are economically important [2]. The essential oils produced from *Artemisia* species contain a large spectrum of secondary metabolites, including terpenoids [3], and several of them are frequently used for the treatment of diseases such as hepatitis, cancer, malaria, inflammation, and infections by fungi, bacteria and viruses [4].
The chemical composition of essential oils from several *Artemisia* species from around the world has been extensively studied and many investigations have shown that *Artemisia* species display significant intraspecific variations in the terpene constituents of their essential oils [5]. This is because the quality and yield of essential oils is influenced by the harvesting season, the soil type, the choice and stage of drying conditions, the geographic location, chemotype or subspecies, choice of plant part or genotype, or extraction method. On such a ground, this investigation was performed with the view to investigating the analytical essential oil profile from two scarcely studied *Artemisia* species, namely *A. inculta* Delile and *A. arborescens*, endemic to the island of Crete (southern Greece). Furthermore, the oils obtained were also tested for antiradical activity, to obtain evidence regarding their antioxidant potency.

2. Materials and methods
2.1 Chemicals and reagents
2,2-diphenyl-1-picrylhydrazyl(DPPH) was from Sigma-Aldrich (Steinheim, Germany). Sodium sulphate (anhydrous) was from Fisher (New Jersey, U.S.A.).

2.2 Plant material
The plant material used in this study consisted of the aerial parts of *Artemisia inculta* and *Artemisia arborescens*, which were provided by the Mediterranean Plant Conservation Unit (M.A.I.Ch., Chania, Crete), where voucher specimens were deposited (*A. inculta*: 9493 MAIC; *A. arborescens*: 9504 MAIC).

2.3 Sample preparation
After collection and deposition, the aerial parts of *A. arborescens* and *A. inculta* were left to dry at room temperature for 15 days. Then 50 grams of dried aerial parts were distilled in a Clevenger-type apparatus for 4 h. The essential oil was dried over anhydrous sodium sulphate and stored at 4 °C.

2.4 Gas chromatography-mass spectrometry (GC/MS)

The analysis of the essential oil was performed using a GC-MS QP 2010 Shimadzu system equipped with a ZB-5 MS, Zebron, Phenomenex capillary column (0.25 µm film thickness × 30 m × 0.25 mm) and a QP 2010 mass selective detector. Helium was the carrier gas, at a flow rate of 1.03 mL min^{-1}. Column temperature was initially kept at 60 °C, and then gradually increased to 240 °C at a 3 °C min^{-1}, held for 5 min. Mass range was recorded from *m*/*z* 35 to 400. Injection port temperature was at 230 °C. The ionization mode used was electronic impact at 70 eV. For mass spectrometer, the ion source temperature and the interface temperatures were set at 200 °C and 245 °C, respectively. Diluted samples of 1.0 µL (depending on the concentration of the sample) were injected manually in the split-less mode. The components were identified based on the comparison of their relative retention times and mass spectra with those of standards, Wiley and NIST library data of the GC/MS system, and literature data [6].

2.5 Determination of the antiradical activity (A_{AR})

A well-established protocol was employed [7]. Briefly, 0.025 mL sample was mixed with 0.975 mL DPPH solution (100 µM in methanol) and the absorbance at 515 nm was read immediately after mixing ($A_{515(i)}$) and after exactly 30 min ($A_{515(f)}$). The A_{AR} was determined using the following equation:

$$A_{AR}(\mu mol\ DPPH\ g^{-1}) = \frac{C_{DPPH}}{C_{extr}} \times \left(1 - \frac{A_{515(f)}}{A_{515(i)}}\right)$$

Where C_{DPPH} is the initial molar concentration of DPPH (µmol mL^{-1}) and C_{extr} is the concentration of the extract, expressed as mg mL^{-1}. Results were expressed as µmol DPPH per g of extract.

2.6 Statistics

All treatments were carried out in duplicate. All determinations were carried out at least in triplicate and values were averaged. Statistics was performed with Microsoft™ Excel 2010 and SigmaPlot™ 12.0.

3. Results and discussion

3.1 Essential oil composition

Distillation of the aerial part of *A. arborescens* gave a blue essential oil in good yield (1.2%). The identified compounds, along with their % contribution are analytically listed in Table 1. The main constituents of *A. arborescens* essential oil were found to be camphor (30.51%), *trans*-thujone (18.42%), chamazulene (15.72%), isobornyl acetate (7.98%), terpinen-4-ol (3.86%), camphene (2.96%), β-eudesmol (2.71%), α-pinene (2.46%), caryophyllene oxide (1.70%), linalool (1.50%), 1,8-cineole (1.41%), β-myrcene (1.21%), γ-terpinene (1.20%), and borneol (1.08%) (Fig. 1, left chromatogram).

Distillation of the aerial part of *A. inculta* gave essential oil in a very good yield (2.7%). The analysis of the oil revealed the presence of thirty seven compounds. The essential oil chromatogram is shown in Fig. 1 (lower chromatogram) and the identified compounds, along with their % contribution are listed in Table 2.The main constituents of *A. inculta* essential oil were found to be 1,8-cineole (28.10%), *cis*-thujone (17.48%), *trans*-sabinol (12.49%), *trans*-thujone (11.97%), camphor (11.76%), borneol (4.46%), camphene (3.53%), 4-terpineol (1.70%), artemisia alcohol (1.47%), and myrtenol (1.31%).

With respect to *A. inculta* essential oil composition (syn. *Artemisia herba-alba*), there have been some investigations providing informative data. Monoterpene hydrocarbons and oxygenated monoterpenes, such as camphor, 1,8-cineole, *p*-cymene and davanone were shown to be the most abundant components [8, 9]. The intraspecific chemical variability of essential oils (50 samples) isolated from the aerial parts of *A. inculta* growing wildly also demonstrated that the main compounds were β-thujone and α-thujone, followed by 1,8-cineole, camphor, chrysanthenone, *trans*-sabinyl acetate, *trans*-pinocarveol and borneol. These data were further corroborated by a study on the chemical composition of essential oil of *A. herba-alba* from west Azerbaijan (Iran), where β-thujone (35.66%), camphor (34.94%), 1,8-cineole (7.42%) and α-thujone (4.12%) were found to be the major constituents of the oil[10]. The examination of the chemical composition of *A. inculta* growing in south Jordan identified fifty-eight components accounting for 98.8% of the oil, with oxygenated monoterpenes accounting for about 75% of the total oil content. Major identified compounds were *cis*-chrysanthenol (13.83%), 1,8-cineole (12.84%), *cis*-limonene (12.57%), α-terpineol (6.97%), and γ-muurolene (4.50%) [11].

Regarding *A. arborescens*, it has been reported that the composition of the essential oils from specimens growing on the Isle of La Maddalena (Sardinia, Italy), belonged to the β-thujone/chamazulene chemotype, notably with the highest amount of chamazulene (ca. 52%) ever detected up to now in the *Artemisia* genus and, in general, in essential oils[5]. Other studies [12, 13] showed that in *A. arborescens* leaves collected in the countryside around Usellus (Sardinia, Italy), during full blossom, the most abundant components were camphor, β-thujone, and chamazulene. Furthermore, it has been reported [14] that the chemical composition of the *Artemisia arborescens* L. essential oil growing in Lebanon was composed of forty three compounds, representing 95.33% of the oil sample. The major component was β-thujone (68.5%), followed by chamazulene (12.3%), and lesser amounts of terpinen-4-ol (1.8%), myrcene (1.3%), α-thujone (1.2%), linalool (1%), *cis*-thuyanol (1%), carvacrol (0.9%), β-cubebene (0.8%) and camphor (0.8%). Similar results were drawn from other investigations [15] that studied the chemical composition of fresh plant samples of *A. arborescens* growing in Sicily (Italy). Forty-three compounds, accounting for more than 92% of the oil, were identified; β-thujone (20.5–55.9%), chamazulene (15.2–49.4%), camphor (1.3–10.7%) and germacrene D (2.3–3.4%) were the principal compounds. Chemical composition was found to be influenced by phenological stage, with an increase in the monoterpene fraction at flowering stage; both in flowering and vegetative stages, the main compounds were always the oxygenated monoterpene β-thujone and the sesquiterpene hydrocarbon chamazulene.

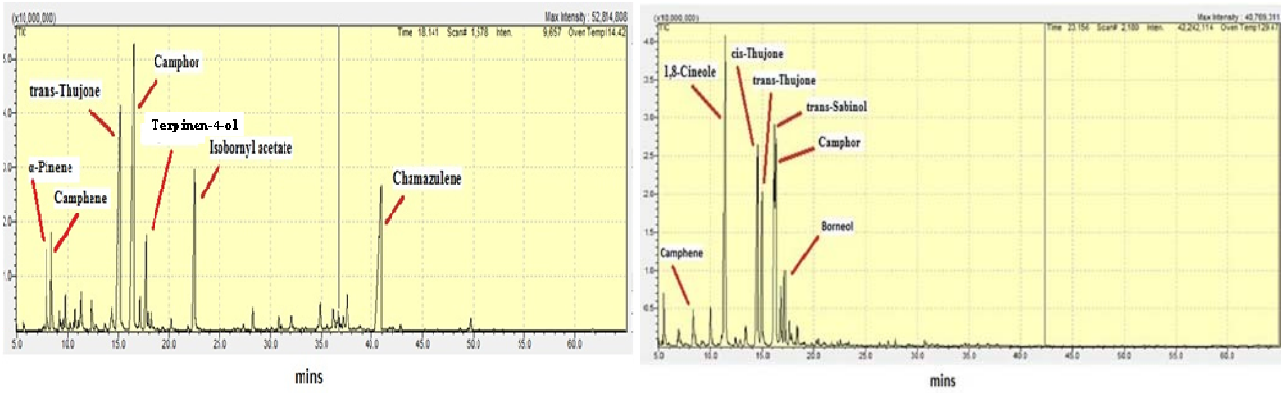

Fig 1: GC/MS chromatograms illustrating the major constituents identified in *A. arborescens* (left chromatogram) and *A. inculta* (right chromatogram) essential oils.

Table 1: Analytical composition of the essential oil obtained from *A. arborescens*

No	Compound	Rt (min)	Kovats Index	Area (%)
1	α-Thujene	7.7	930	0.16
2	α-Pinene	7.9	938	2.46
3	Camphene	8.4	954	2.96
4	Sabinene	9.2	977	0.50
5	β-Pinene	9.3	982	0.25
6	β-Myrcene	9.7	994	1.21
7	α-Phellandrene	10.3	1009	0.27
8	α-Terpinene	10.8	1020	0.83
9	*p*-Cymene	11.1	1028	0.33
10	Limonene	11.2	1032	0.36
11	1,8-Cineole	11.3	1035	1.41
12	γ-Terpinene	12.4	1062	1.20
13	*cis*-Sabinene	12.8	1071	0.32
14	α-Terpinolene	13.6	1092	0.31
15	Linalool	14.4	1110	1.50
16	*trans*-Thujone	15.2	1128	18.42
17	Camphor	16.6	1160	30.51
18	Pinocarvone	17.0	1169	0.13
19	Borneol	17.2	1173	1.08
20	Terpinen-4-ol	17.7	1186	3.86
21	α-Terpineol	18.2	1197	0.55
22	*trans*-Piperitol	18.4	1201	0.15
23	*cis*-Carveol	19.4	1223	0.15
24	Isobornyl acetate	22.5	1293	7.98
25	Carvacrol	23.0	1306	0.05
26	α-Terpinyl acetate	25.1	1353	0.04
27	Eugenol	25.4	1362	0.04
28	α-Copaene	26.3	1382	0.10
29	β-Bourbonene	26.7	1391	0.16
30	β-Elemene	27.0	1397	0.06
31	*cis*-Jasmone	27.2	1402	0.06
32	Methyl Eugenol	27.4	1407	0.23
33	*trans*-Caryophyllene	28.2	1428	0.95
34	α-Humulene	29.6	1462	0.14
35	Germacrene (D)	30.8	1489	0.56
36	β-Selinene	31.0	1495	0.24
37	Elemol	33.5	1556	0.25
38	Nerolidol (E)	34.0	1569	0.09
39	Caryophyllene oxide	34.9	1593	1.70
40	β-Eudesmol	37.5	1663	2.71
41	Chamazulene	40.9	1756	15.72

Table 2: Analytical composition of the essential oil obtained from *A. inculta* Delile.

No	Compound	Rt (min)	Kovats Index	Area (%)
1	Tricyclene	7.4	922	0.01
2	Artemisia triene	7.7	933	0.05
3	α-Pinene	7.8	936	0.09
4	Camphene	8.2	948	3.53
5	Sabinene	9.1	973	0.04
6	β-Pinene	9.2	976	0.30
7	Dehydro-1,8-Cineole	9.7	994	0.09
8	δ-2-Carene	10.7	1020	0.17
9	*p*-Cymene	11.1	1028	0.87
10	1,8-Cineole	11.4	1037	28.10
11	γ-Terpinene	12.4	1061	0.54
12	Artemisia alcohol	13.4	1087	1.47
13	Terpinolene	13.7	1093	0.16
14	*cis*-Thujone	14.6	1114	17.48
15	*trans*-Thujone	15.0	1124	11.97
16	*trans*-Sabinol	16.2	1150	12.49
17	Camphor	16.3	1154	11.76
18	Pinocarvone	16.9	1167	0.46
19	Borneol	17.2	1173	4.46
20	4-Terpinen-4ol	17.6	1183	1.70
21	α-Terpineol	18.2	1196	0.30
22	Myrtenol	18.4	1201	1.31
23	Verbenone	19.0	1214	0.06
24	Isobornyl formate	19.8	1232	0.23
25	Cumin aldehyde	20.3	1244	0.11
26	Carvone	20.5	1248	0.43
27	*cis*-Verbenylacetate	21.7	1276	0.18
28	Isobornyl acetate	22.3	1289	0.23
29	*p*-Cymen-7-ol	22.6	1295	0.38
30	Carvacrol	23.0	1305	0.06
31	*cis*-Jasmone	27.2	1402	0.11
32	*trans*-Caryophyllene	28.2	1427	0.37
33	Germacrene D	30.8	1489	0.11
34	Bicyclogermacrene	31.4	1504	0.08
35	δ-Cadinene	32.4	1530	0.16
36	Spathulenol	34.6	1586	0.08
37	(Z)-α-Santalol acetate	41.3	1767	0.05

3.2 Antiradical activity (A_{AR})

The determination of A_{AR} is a credible criterion regarding the antioxidant activity, providing informative data with respect to the ability of an extract to trap radicals [16]. The determination of A_{AR} of the essential oils obtained suggested that there is a very high difference in the antioxidant potency (Fig. 2), with that of *A. arborescens* being 40 times higher than that of *A.*

inculta. Apparently this striking difference would be attributed to the different composition of the two oils, and most probably could be correlated with major substances occurring in *A. arborescens,* such as chamazulene.

The structure of chamazulene, the major constituent of *A. arborescens* essential oil, is a conjugated system that permits the molecule to behave as classic lipophilic antioxidant, through various resonance forms. Thus it is likely that the strong A_{AR} exerted by *A. arborescens* essential oil is due to high amounts of chamazulene. However, the proportionality between certain compounds and the antioxidant activity is not a general rule, as suggested by previous studies [17, 18]. It should also be emphasised that the expression of antioxidant effects of a mixture should be carefully interpreted, due to phenomena of synergism and antagonism amongst the various constituents [19].

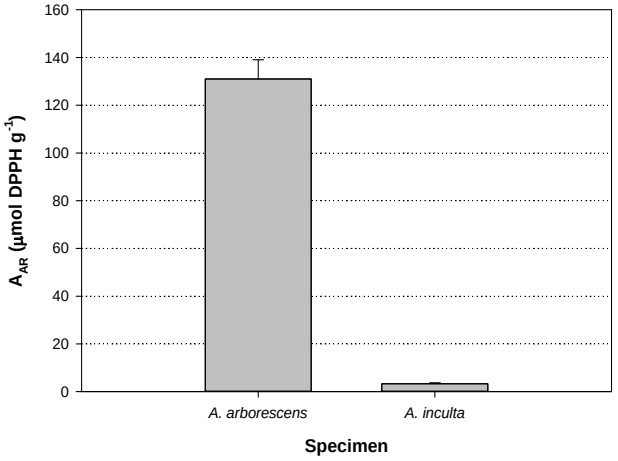

Fig 2: Graph illustrating the A_{AR} of the essential oils obtained from *A. arborescens* and *A. inculta* Delile. Values represent means of tiplicate determination (±standard deviation).

4. Conclusions

The study presented herein is the first report concerning the composition of the essential oils obtained from two *Artemisia* species, native to the island of Crete. The composition of the essentials oils examined displayed significant differences and while *A. arborescens* essential oil was dominated by camphor, *trans*-thujone and chamazulene, the essential oil of *A.inculta* contained mainly 1,8-cineole, *cis*-thujone, *trans*-sabinol and *trans*-thujone. The ability of the *A. arborescens* to scavenge free radicals was 40 times higher than that of *A. inculta*, which was ascribed to the abundance of chamazulene in the *A. arborescens* essential oil.

References

1. Kennedy DO, Wightman EL. Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of human brain function. Advances in Nutrition: An International Review Journal. 2011; 2(1):32-50.
2. Giang PM, Binh NT, Matsunami K, Son PT. Three new eudesmanes from Artemisia japonica. Natural Product Research. 2014; 28(9):631-5.
3. Brown GD. The biosynthesis of artemisinin (Qinghaosu) and the phytochemistry of Artemisia annua L. (Qinghao). Molecules. 2010; 15(11):7603-98.
4. Piao GC, Li YX, Yuan HD, Jin GZ. Cytotoxic fraction from Artemisia sacrorum Ledeb. Against three human cancer cell lines and separation and identification of its compounds. Natural Product Research 2012; 26(16):1483-91.
5. Abad MJ, Bedoya LM, Apaza L, Bermejo P. The Artemisia L. genus: a review of bioactive essential oils. Molecules 2012; 17(3):2542-66.
6. Adams R. Identification of essential oil components by gas chromatography. Mass Spectrometry. Allured Publishing Corp., IL, U.S.A., 1995, 237-43.
7. Shehata E, Grigorakis S, Loupassaki S, Makris DP. Extraction optimisation using water/glycerol for the efficient recovery of polyphenolic antioxidants from two Artemisia species. Separation and Purification Technology 2015; 149:462-9.
8. Lupidi G, Bramucci M, Quassinti L, Fornari E, Avenali L, Khalife H *et al.* Antiproliferative activities of *A*rtemisia herba-albaethanolic extract in human colon cancer cell line (HCT116). Alternative Medicine Studies 2011; 1(1):14.
9. Mohamed A, El-Sayed MA, Hegazy ME, Helaly SE, Esmail AM, Mohamed NS. Chemical constituents and biological activities of Artemisia herba-alba. Records of Natural Products. 2010; 4(1):1-25.
10. Sharifian I, Aghaei M, Alizadeh M. Chemical composition of essential oil of Artemisia herba-alba from west Azerbaijan, Iran. e-Journal of Environmental, Agricultural and Food Chemistry. 2011; 10(6):2413-2416.
11. Al-Shuneigat J, Sarayreh SA, Al-Qudah M, Al-Tarawneh I, Al–Saraireh Y, Al-Qtaitat A. GC-MS Analysis and antibacterial activity of the essential oil isolated from wild Artemisia herba-alba grown in south Jordan. British Journal of Medicine & Medical Research. 2015; 5(3):297-302.
12. Lai F, Loy G, Manconi M, Manca ML, Fadda AM. Artemisia arborescens L essential oil loaded beads: preparation and characterization. AAPS Pharm Sci Tech 2007; 8(3):E126-E32.
13. Sinico C, De Logu A, Lai F, Valenti D, Manconi M, Loy G *et al*. Liposomal incorporation of Artemisia arborescens L. essential oil and in vitro antiviral activity. European Journal of Pharmaceutics and Biopharmaceutics. 2005; 59(1):161-8.
14. El Beyrouthy M, Arnold-Apostolides N, Labaki M, Cazier F, Najm S, AbouKais A. Chemical composition of the essential oil of the Artemisia arborescens L. growing wild in Lebanon. Lebanese Science Journal. 2011; 12(1):71-78.
15. Militello M, Carrubba A, Blázquez MA. Artemisia arborescens L.: essential oil composition and effects of plant growth stage in some genotypes from Sicily. Journal of Essential Oil Research. 2012; 24(3):229-35.
16. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry. 2005; 53(6):1841-56.
17. Karvela E, Makris DP, Kalogeropoulos N, Karathanos VT, Kefalas P. Factorial design optimisation of grape (Vitisvinifera) seed polyphenol extraction. European Food Research and Technology. 2009; 229(5):731-42.
18. Khiari Z, Makris DP, Kefalas P. An investigation on the recovery of antioxidant phenolics from onion solid wastes employing water/ethanol-based solvent systems. Food and Bioprocess Technology. 2009; 2(4):337-43.
19. Karvela E, Makris DP, Karathanos VT. Implementation of response surface methodology to assess the antiradical behaviour in mixtures of ascorbic acid and α-tocopherol with grape (Vitisvinifera) stem extracts. Food Chemistry. 2012; 132(1):351-9.