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Volatile specialised metabolites of *Achillea cappadocica* Hausskn. & Bornm.

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Short communication

Volatile specialised metabolites of *Achillea cappadocica* Hausskn. & Bornm.

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Running title: VOLATILE METABOLITES OF *ACHILLEA CAPPADOCICA*

Abstract – Phytochemical analyses of the aerial parts of *Achillea cappadocica* (Asteraceae), an endemic species of Turkish flora, resulted in the identification of 36, 34 and, 31 volatile specialised metabolites (VSM) for three wild populations. Oxygenated monoterpenes, such as eucalyptol, camphor, and γ-terpineol, were detected as the main volatiles depending on the populations. This is the first report documenting the content and composition of VSM of this endemic species. Data such as this is important for identifying heterogeneous germplasms and underutilized wild species.

Keywords: *Achillea cappadocica*, chemodiversity, wild populations, volatile specialised metabolites

Introduction

Achillea is a widespread genus of the Asteraceae family and includes 110-140 species growing naturally in various climatic zones of the world, especially in Southeastern Europe, Southwestern Asia, and North America (Ehrendorfer and Guo 2006). *Achillea* species have been used for many centuries worldwide as healing agents to treat several health problems such as painful periods, kidney disorders, anemia, epilepsy, gastrointestinal ailments, urinary tract infections, etc. (Mohammadhosseini et al. 2017). Previous reports have highlighted *Achillea* spp. as a rich source of various bioactive secondary metabolites, namely volatiles such as monoterpenes, diterpenes, and sesquiterpenes, as well as different kinds of phenolics such as flavonoids, caffeoylquinic acids, and their derivatives (Farhadi et al. 2020). Türkiye is the main intersection point and the largest distribution area in the world for the *Achillea* genus with the presence of 60 flora species, 33 of which are reported to be endemic (Semiz et al. 2022).

Achillea cappadocica Hausskn. & Bornm. is an endemic perennial herb of Turkish flora. This species, locally known as "buyucan", has been used in Turkish folk medicine as an anti-edema, emmenagogue, and gastric agent for centuries (Altundag and Ozturk 2011). A review of literature revealed only two previous reports on the chemistry and extract bioactivity of this endemic species. First, Ertaş et al. (2014) described the fatty acid composition and identified oleic, palmitic, and

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linoleic acids as its main components. The authors also reported that its alcoholic extracts showed moderate anticholinesterase and antimicrobial activities. More recently, Raudone et al. (2022) evaluated the comprehensive distribution patterns of phenolic compounds in its different plant parts and assessed their antioxidant activity. According to the results, considerable amounts of hydroxycinnamic acids, flavanols, and flavones were identified and quantified in its methanolic extract, and a remarkable antiradical capacity was observed. However, to the best of our knowledge, there is no published report on the volatile chemistry of *A. cappadocica*. Thus, in this study, we present the first report documenting the content and composition of volatile secondary metabolites (VSM) of this endemic species.

Material and methods

A brief description of the plant material

The stems of *Achillea cappadocica* Hausskn. & Bornm. are erect, 25-50 cm, longitudinally striated to rounded-angular, frequently with an upturned pubescent. The upper leaves are green, sparsely pilose, 1-3 pinnate and heteromorphic while the basal leaves have short petioles, are oblong-linear, 7-10 x 1.5-2 cm, 3-pinnatipartite. Capitula are 50-150 or more, corymbs 4.5-12 cm wide, the peduncle 4-8 mm. The ligulate flowers are 4-7, golden yellow, 1.5 mm; the tubular flowers 20-40 (Davis 1988) (Fig. 1). Its habitats are dry slopes, steppes and crop fields at high elevations (1200-2100 m).

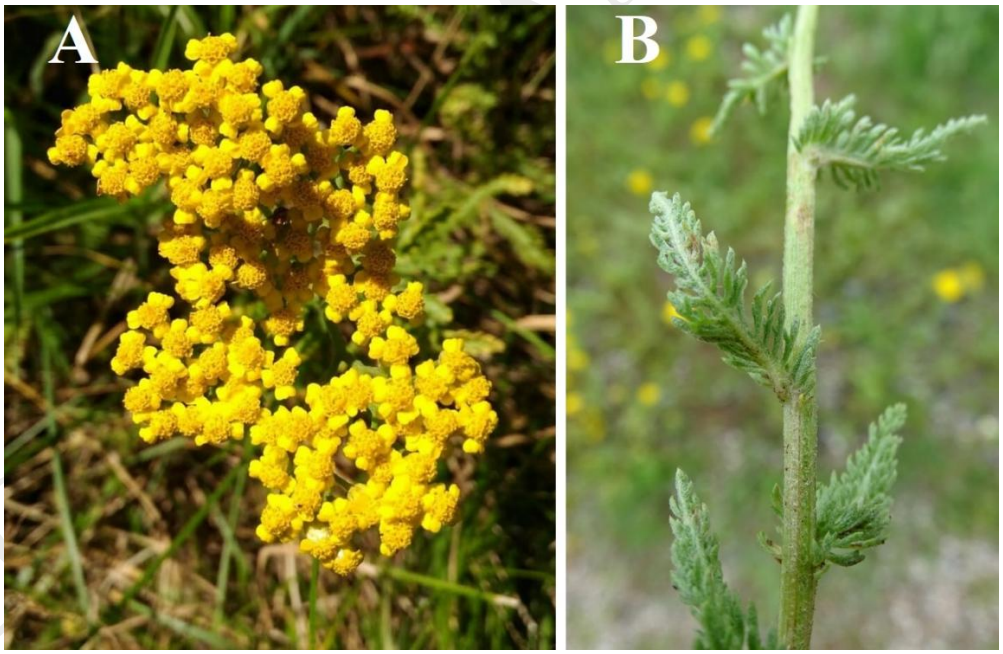

Fig. 1. Phenotype of flowers (A) and leaves (B) of *Achillea cappadocica,* an endemic species of Turkish flora.

Sampling and identification procedures

The upper 2/3 of the flowering part, including fully opened flowers, leaves, and stems, of 30 individuals were gathered and bulked from three wild populations located in the Central and Southern parts of Türkiye from July 25 to July 30, 2023 (37°20′ N Lat., 34°48′ E Long., and 1186

m elevation for population 1; 38°29′ N Lat., 34°57′ E Long., and 1311 m elevation for population 2; 38°48′ N Lat., 34°56′ E Long., and 1213 m elevation for population 3). After drying them at room temperature (20 ± 2 °C), three homogenous replicates of the bulked plant material for each population were analyzed for volatile components by solid-phase microextraction (SPME) supported gas chromatography-mass spectrometry (GC-MS) analyses as detailed in our previous work (Cirak and Seyis 2025). Botanical identification of taxa was performed by Dr. Samim Kayikci, Mustafa Kemal University, Faculty of Arts and Sciences, Department of Biology, Türkiye. Voucher specimens were deposited in the herbarium of Ondokuz Mayis University, Vocational High School of Bafra (BMYO # 212, 213, and 214 for the populations 1, 2, and 3, respectively).

Sample preparation and analysis of the volatile specialised metabolite composition

Sample preparation and analysis of the volatile specialised metabolite composition were performed according to Aykutlu et al. (2025). The instrumental system used for the analysis comprised a Shimadzu GC-2010 Plus gas chromatograph, a QP2020 mass spectrometer, and a multifunctional autosampler (AOC-5000 Plus) equipped with a solid-phase microextraction (SPME) module and a split/splitless injection inlet.

The SPME extraction was performed using a polydimethylsiloxane (PDMS) fibre (1 cm × 100 μm thickness) purchased from Sigma-Aldrich (Supelco, Bellefonte, USA). The fibre was preconditioned for 5 minutes at 250 °C prior to analysis and reconditioned for 10 minutes at 250 °C after each run.

For each sample, 1.0 g of plant material was placed in a 20 mL SPME glass vial, followed by the addition of 100 μL of hexane. The same procedure was applied to all samples, which were sealed with silicone/PTFE septa. Volatile compounds were extracted under optimised headspace SPME (HS-SPME) conditions, consisting of a 5-min equilibration period and a 15-min extraction period at 100 °C with an agitation speed of 500 rpm. Analytes were desorbed for 1 minute in the GC injection port at 250 °C using a straight Ultra Inert SPME liner operating in split mode. Each sample was analyzed in triplicate.

All analyses were conducted using a Shimadzu GC-2010 Plus gas chromatograph coupled with a QP2020 mass selective detector. Separation was carried out using a Rtx-5MS low-bleed capillary column (30 m × 0.25 mm × 0.25 μm; Restek, USA). Helium served as the carrier gas at a constant pressure of 80 kPa. The oven temperature program was optimised as follows: an initial temperature of 40°C (held for 2 min), increased to 250 °C at a rate of 4°C/min, and held at 250 °C for 3 min, resulting in a total run time of 55 min. The interface and ion source temperatures were set at 250 °C and 200 °C, respectively. Electron ionization (EI) was conducted at 70 eV with an m/z scan range of 40–500 and a scan speed of 1666 u/sec.

Data acquisition and processing were carried out using GCMS solution software (Shimadzu, Japan). Compound identification was performed by comparing mass spectra to the Wiley FFNSC 3rd Edition Library (Mass Spectra of Flavours and Fragrances of Natural and Synthetic Compounds) and by matching calculated retention indices (RIs) with those listed in the FFNSC 3rd Edition Library, the NIST Chemistry WebBook (SRD 69), and the PubChem database.

Data analysis

Data for VSM contents of the bulked plant material for each population were subjected to ANOVA and significant differences among mean values were tested with the least significant difference test ($P < 0.01$) by using XLSTAT 2020 statistical software. The standard deviations of

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the mean values of quantified volatiles for each population were also calculated. Mean values of the chemical contents were normalised using $x' = \sqrt{x} + 1$ transformation before conducting ANOVA, when necessary, because some chemicals were not detected in several cases.

Results and discussion

The results of phytochemical analyses revealed qualitatively and quantitatively different VSM profiles for the investigated populations, which were mostly dominated by oxygenated monoterpenes. A total of 36, 34, and 31 components were quantified for populations 1, 2, and 3 respectively, which represent 100% of VSM content (Tab. 1). Among the quantified volatiles, eucalyptol (1,8-cineole) (40.40%), camphor (10.10%), and α-terpinyl acetate (13.91%) were recognised as the main ingredients for population 1, followed by heptylidene acetone (6.64%) and isoborneol (5.19%).

Tab. 1. The volatile composition and percentage of each component in three wild populations of the Turkish flora *Achillea cappadocica*. RI - Kovats Retention Index. Values are a mean of three replicates ± standard deviation and the values that are followed by different small letters in each row are significantly different ($P < 0.01$) according to the least significant difference test.

No.	Compounds	RI	Populations		
			1	2	3
	Monoterpene Hydrocarbons				
1	α-Pinene	931	1.02±0.01^{a}	0.44±0.002^{b}	-
2	Camphene	945	0.57±0.003^{b}	0.97±0.0017^{a}	-
3	β-Pinene	975	0.76±0.005	-	-
4	α-Phellandrene	1005	-	-	0.25±0.002
5	α-Terpinene	1016	-	0.94±0.0015^{a}	0.99±0.006^{a}
6	Limonene	1026	-	0.32±0.0042	-
7	γ-Terpinene	1059	0.49±0.0036	-	-
8	Terpinolene	1085	0.34±0.01	-	-
9	*p*-Cymene	1090	4.23±0.002^{b}	6.71±0.02^{a}	3.92±0.0012^{b}
Total			**7.41**	**9.28**	**5.16**
	Oxygenated monoterpenes				
10	1,4-Cineole	1017	0.78±0.0038^{c}	1.14±0.005^{b}	8.37±0.005^{a}
11	Eucalyptol (1,8 cineole)	1029	40.40±0.07^{a}	4.760±0.0013^{b}	4.70±0.036^{b}
12	Trans sabinene hydrate	1097	0.69±0.0074	-	-
13	2-Isopropyl-5-methyl-hex-2-enal	1104	1.07±0.006^{c}	1.75±0.002^{b}	3.91±0.0053^{a}
14	β-Thujone	1105	1.01±0.0001	-	-
15	Rose oxide	1112	-	-	0.44±0.002
16	Trans pinocarveol	1139	-	-	0.39±0.01
17	Trans-*p*-menth-2-en-1-ol	1140	0.80±0.02^{a}	0.36±0.01^{c}	0.53±0.05^{b}
18	Camphor	1142	10.10±0.015^{b}	25.84±0.002^{a}	2.49±0.0095^{c}
19	Pinocarvone	1163	0.48±0.01^{a}	-	0.29±0.0076^{b}
20	Verbenol	1164	-	0.33±0.003	-
21	Isoborneol	1166	5.19±0.005^{a}	0.49±0.0006^{b}	0.34±0.009^{b}
22	Terpinen-4-ol	1178	1.18±0.001	-	-

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Tab. 1 continued

23	Cymen-8-ol	1182	0.27±0.004	-	-
24	*p*-Cymen-8-ol	1185	0.28±0.003	-	-
25	α-Terpineol	1190	2.55±0.0755[a]	0.30±0.0064[b]	-
26	Myrtenal	1197	0.40±0.05	-	-
27	γ-Terpineol	1200	-	28.52±0.001[b]	39.21±0.05[a]
28	Safranal	1201	0.68±0.05	-	-
29	Verbenone	1224	-	-	0.50±0.012
30	Pulegone	1240	0.42±0.0036	-	-
31	Carvone	1246	-	0.32±0.005	-
32	Phellandral	1262	0.57±0.0153[a]	0.52±0.002[a]	-
33	α-Terpinyl formate	1301	0.42±0.005[b]	0.89±0.0029[a]	-
Total			**67.19**	**65.22**	**61.17**
	Sesquiterpene hydrocarbons				
34	α-Cedrene	1418	-	0.28±0.01	-
35	Germacrene D	1479	-	-	0.34±0.0055
Total			**-**	**0.28**	**0.34**
	Oxygenated sesquiterpenes				
36	Dehydro-sesquicineole	1466	-	-	1.93±0.01
37	Nerolidol	1564	0.29±0.015	-	-
38	Caryophyllene oxide	1587	0.58±0.005[b]	1.47±0.01[a]	0.24±0.0055[c]
39	Viridiflorol	1614	-	2.27±0.005	-
40	β-Eudesmol	1657	0.91±0.0029[a]	0.87±0.007[a]	-
41	α-Bisabolol	1688	0.29±0.02	-	-
42	α-Bisabolone oxide A	1686	-	0.25±0.01	-
Total			**2.07**	**4.86**	**2.17**
	Alkanes, alkenes, alkynes, arenes				
43	Docosane	2200	-	-	0.26±0.01
Total			-	-	**0.26**
	Alcohols, ketones, aldehydes, furans				
44	Yomogi alcohol	1001	-	12.47±0.005[b]	17.75±0.0087[a]
45	Allyl caproate	1066	-	0.30±0.0153[b]	1.20±0.02[a]
46	Ethyl butyl ketone	1078	-	-	0.98±0.01
47	3,4-Dimethyl-1,2-cyclopentanedione	1122	-	0.85±0.005[b]	1.26±0.0087[a]
48	Heptylidene acetone	1259	6.64±0.0808[a]	0.26±0.0046[c]	1.87±0.01[b]
49	Diamyl ketone	1286	-	-	0.23±0.0076
50	Cumin alcohol	1292	0.24±0.001	-	-
51	Eugenol	1360	0.39±0.0049	-	-
52	Trans carvyl propionate	1469	-	0.33±0.0046	-
53	α-Terpinyl isobutyrate	1480	-	0.27±0.0066[b]	0.60±0.02[a]
54	Tridec-2(E)-enal	1573	-	0.91±0.004	-
55	Tetradecanal	1614	-	-	0.46±0.0058
56	Myristic alcohol	1663	0.39±0.0053	-	-
57	Phytone	1844	0.32±0.015[a]	-	0.32±0.01[a]
58	Civetone	2015	-		1.68±0.01
Total			**7.98**	**15.39**	**26.35**
	Ethers, carboxylic acids, esters, others				
59	Isobornyl acetate	1288	1.08±0.038[a]	0.53±0.02[b]	-
60	Methyl 2-nonynoate	1300	0.27±0.021	-	-
61	α-Terpinyl acetate	1342	13.91±0.0076	-	-

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Tab. 1 continued

62	Bornyl isovalerate	1513	-	0.98±0.007[a]	3.56±0.002[b]
63	Neryl isovalerate	1582	-	2.49±0.01	-
64	Methyl jasmonate	1652	-	-	0.26±0.01
65	Pentadecanolide	1827	-	0.87±0.03[a]	0.67±0.0117[b]
Total			**15.26**	**4.87**	**4.49**
Number of detected compounds			**36**	**34**	**31**
General total			**99.91**	**99.90**	**99.94**

Contrary to population 1, populations 2 and 3 had somewhat similar VSM profiles and both were characterised by the dominance of γ-terpineol (28.52 and 39.21% for populations 2 and 3, respectively) and yomogi alcohol (12.47 and 17.75% for populations 2 and 3, respectively), which were not detected in population 1. It is interesting to note that α-terpinyl acetate, one of the main volatiles of population 1, was also absent in populations 2 and 3, indicating the presence of different kinds of chemotypes among wild populations of this endemic species. Camphor and eucalyptol (1,8-cineole) were previously designated as the main volatile constituents of many *Achillea* species of Turkish origin (Turkmenoğlu et al. 2015, Başer 2016, Yener et al. 2020), as can also be observed in the present study. Along with these chemicals, several others, such as borneol, isoborneol, α- and β-pinenes, α- and β-thujones, santolina alcohol, germacrene D, chamazulene, and spathulenol, were reported as the major VSMs of *Achillea* species from different localities in the world (Nemeth 2005, Radulovic et al. 2007, Mohammadhosseini et al. 2017). Many of these components were also detected in our GC-MS analyses.

The apparent difference between the VSM profiles of the tested populations may be due to environmental factors such as elevation, temperature, UV radiation, precipitation, etc., which vary evidently with the plant's environment. Thus, Rahimmalek et al. (2009) indicated the soil type and climatic conditions of the plant's growing habitats as the possible reason for the remarkable intraspecific and interspecific chemical polymorphism they observed among 19 accessions of the six Iranian species of *Achillea* namely, *A. millefolium* L., *A. filipendulina* Lam., *A. tenuifolia* Lam., *A. santolina* L., *A. biebersteinii* Afan., and *A. eriophora* DC. Elevation, for example, was reported to significantly change the VSM profile of 16 *A. millefolium* populations of Italian origin and a rising altitudinal gradient resulted in the occurrence of 4*β*-hydroxygermacra-1(10),5-diene, germacrene D, *β*-eudesmol, caryophyllene and camphor chemotypes for the most well-known member of the *Achillea* genus (Pecetti et al. 2012). Chrysanthenone, trans-carveol, linalool, neoiso-dihy drocarveol acetate, and camphor were detected as the main volatile components depending on changing habitats among 20 Iranian populations of *Achillea wilhelmsii* K. Koch and the tested populations fell into five chemotypes based on their VSM profiles (Saeidi et al. 2018). Similarly, VSM profiles and the main VSM ingredients were significantly different among five populations of Turkish *A. biebersteinii* (Tabanca et al. 2011). Artemisia ketone, α-thujone, β-thujone, 1,8-cineole were the major VSMs of Iranian *A. nobilis* L. and their contents fluctuated significantly among the 22 field-grown accessions from different geographic localities (Azimi et al. 2016). Moreover, geographic isolation from other germplasms triggered the formation of local ecotypes, and over time, chemically distinct gene pools emerged (Pecetti et al. 2012). Thus, the evident chemo-diversity among the tested populations of *A. cappadocica* in the present study may also be due to the possible genetic differences of plant samples.

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Conclusion

In this study, we report the content and composition of volatile secondary metabolites of three wild populations of *A. cappadocica* for the first time. The current research provides new insights into the chemical diversity of this endemic species, and data such as this is therefore important for identifying heterogeneous germplasms and underutilized wild species for medicinal and/or commercial purposes.

Author contribution statement

Cuneyt Cirak: Writing - review & editing, Visualisation, Software, Resources, Methodology, Investigation, Conceptualisation. Fatih Seyis: Visualisation, Formal analysis, Data curation.

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