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Composition and Antioxidant Activity of the Essential Oil from *Achillea moschata* Wulfen Growing in Valchiavenna and Valmalenco (Italian Central Alps)

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Abstract

Achillea moschata Wulfen (Asteraceae) is an endemic species growing on the Alps and traditionally used in several medicinal remedies. For its aromatic properties, it is also collected for food purposes. Chemical content and biological activity of *A. moschata* oil are not extensively studied. We investigated whether the different lithological and geomorphological features of two alpine areas including Vallone dello Scerscen and Valle dei Ratti, located in the Sondrio Province (Northern Italy), can influence the essential oil (EO) composition and the related antioxidant activity of the respective *A. moschata* populations. The essential oils obtained by steam distillation from the dried aerial parts were investigated by GC/FID and GC/MS. Several compounds were identified belonging to different chemical classes, including monoterpenes and sesquiterpenes as the most abundant constituents, together with ketones, alcohols, phenols, acids and esters. A variation in the quantitative composition of several constituents was recorded in the two oils. The main constituents were camphor (23.4-34.2%), 1,8-cineole (9.8-16.4%), *trans*-tujone (3.6-13.4%), sesquicineole (3.5-5.8%), borneol (1.1-4.8%), β-caryophyllene (2.7-4.4%) and myrcene (1.3-4.2%). Their antioxidant activity was measured *in vitro* by the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS·+) and 2,2-diphenyl-picryl hydrazyl (DPPH·) stable radical assays. The essential oils showed similar and significant scavenger effects, possibly due to the synergistic action of all components including compounds present in small quantities.

Keywords: Alpine area, Asteraceae, Endemic species, Free radicals, Officinal plants, Terpenes, Yarrow.

Introduction

Achillea is a genus of perennial flowering plants, commonly known as yarrows, belonging to the family Asteraceae. They are typical of the temperate regions of the northern hemisphere, native to Europe, North America and some areas of Asia. Among the 23 Italian species, 20 grow on the Alps, up to 3400 meters above sea level (Pignatti, 1982). Some of them, including *A. moschata* Wulfen, are traditionally used by the local population for the appreciated medicinal properties. The aromatic traits of *A. moschata*

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are also exploited in various food preparations such as aperitifs, grappa, herbal teas, liqueurs, main dishes, desserts (Grabherr, 2009; Fasoli et al., 2012; Vitalini et al., 2013; Abbet et al., 2014; Vitalini et al., 2015). It is an endemic herbaceous species, subject in the past to intensive collection, now strictly controlled by regional laws. Until recently, in fact, the plant was widely marketed and its sale constituted an additional source of income for the families of the mountain pastures. Despite of its tradition use, probably due to the limited distribution area, previous studies on *A. moschata* are few and aimed to investigate mainly the seed characteristics (Stöcklin and Bäumler, 1996; Schwienbacher and Erschbamer, 2002; Schwienbacher et al., 2010; Aiello et al., 2017), karyological data (Maffei et al., 1989; Baltisberger and Widmer, 2016), and taxonomy (Valant-Vetscher and Wollenweber, 2001; Galasso et al., 2018). The chemical content and biological activity of *A. moschata* have only been partially studied by our research team and few others (Maffei et al., 1989; Duke, 1992; Vitalini et al., 2016; Argentieri et al., 2020). In this case, we decided to check whether the different lithological and geomorphological features of two alpine areas such as Vallone dello Scerscen and Valle dei Ratti, located in the Sondrio Province (Northern Italy), can influence the essential oil (EO) composition and the related antioxidant activity of the respective *A. moschata* populations. The first one, inserted in a serpentinite valley, is a basin of alluvial debris, characterized by the presence of hydrothermal caves, while the second one (a large granite valley) belongs to the peculiar oligocene intrusion of the Masino - Bergell pluto.

Material and methods

Plant material

The aerial parts of *A. moschata* were harvested at flowering stage (July 2013) in two different areas of the Rhaetian Alps, namely Valle dei Ratti (Valchiavena, 2300 m a.s.l.) (VC) e Vallone dello Scerscen (Valmalenco, 2450 m a.s.l.) (VM). Both areas are characterized by a cool-continental climate (Df), with average temperature of the coldest month < −3 °C, average of the warmest month between 10 °C and 15 °C and annual average between 3 °C and 6 °C (Martinelli and Matzarakis, 2017). *A. moschata* prefers slightly calcareous soils and sunny sites. It withstands temperatures below −20 °C.

Voucher specimen plants were identified according to Flora d'Italia (Pignatti 1982), exsiccated and deposited at the Department of Agricultural and Environmental Sciences of the Milan State University (Italy).

Essential oil extraction

The EOs were extracted from 25 g of each *A. moschata* air-dried sample by steam distillation for 1 h in a Clevenger-type apparatus. The distillate was saturated with NaCl, extracted with freshly distilled Et_2O (3 x 100 mL), dried over anhydrous Na_2SO_4 and concentrated with a rotary evaporator at 30 °C. The oils were diluted with Et_2O and then used for gas chromatographic analyses. All the chemicals were from Sigma Aldrich.

Essential oil characterization

- ***Gas Chromatography-Flame Ionization Detector (GC/FID)***

GC/FID analyses were carried out using a Perkin Elmer model 8500 GC equipped with a 30 m x 0.32 mm i.d. Elite-5MS capillary column (0.32 µm film thickness). Samples (0.5 µL) were injected in the "split" mode (1:30) with a column temperature programme of 40 °C for 5 min, then increased to 260 °C at 4 °C/min and finally held at this temperature for 10 min. The injector and the detector were set at 250 °C and 300 °C, respectively. The carrier gas was He (99.9999% purity) with a head pressure of 12.0 psi.

- ***Identification and quantitation of the essential oil constituents***

The identification of the EO components

was performed by their Retention Indices (marked as RI in Table 1) and their mass spectra, by comparison with a NIST database mass spectral library as well as with literature data (Adams, 1998; Iriti et al., 2014). Authentic reference compounds purchased from Sigma-Aldrich were also used. Retention indices were calculated using a n-alkane series (C_6–C_{32}) under the same GC conditions as for the samples. The relative amounts of individual components of the EOs were expressed as percent peak area relative to total peak area from the GC/FID analyses of the whole extracts.

Table 1. Percentage composition of the essential oil from two different populations of *Achillea moschata* collected in Valchiavenna (VC) and Valmalenco (VM), Italy.

#	Compound	RI[a]	RI[b]	Relative amount (%)	
				VC	VM
1	santolina triene	902	906	0.05	0.68
2	tricyclene	916	921	Tr	0.10
3	artemisia triene	923	923	0.06	1.07
4	α-thujene	928	924	0.32	1.09
5	camphene	943	946	0.66	1.79
6	sabinene	968	969	0.53	3.08
7	β-pinene	971	974	0.27	0.70
8	1,8-dehydrocineole	986	988	0.01	0.02
9	myrcene	992	988	1.34	4.16
10	α-terpinene	1013	1013	0.08	0.11
11	p-cymene	1021	1020	1.20	1.02
12	1,8-cineole	1028	1026	9.78	16.43
13	γ-terpinene	1055	1054	1.04	0.66
14	*cis*-sabinene hydrate	1067	1065	1.92	2.22
15	trans-sabinene hydrate	1097	1098	0.63	0.51
16	*cis*-thujone	1102	1101	1.64	2.34
17	isovaleric acid 2-methylbutyl ester	1109	1103	0.17	tr
18	*trans*-thujone	1114	1112	3.61	13.39
19	chrysantenone	1117	1124	0.06	1.68
20	camphor	1142	1141	34.22	23.45
21	pinocarvone	1157	1160	0.32	0.06
22	borneol	1167	1165	4.80	1.05
23	terpinen-4-ol	1176	1177	0.83	1.15
24	α-terpineol	1191	1186	1.56	2.42
25	*trans*-piperitol	1205	1207	0.01	0.83
26	bornylacetate	1280	1287	2.01	1.10
27	thymol	1289	1289	0.17	0.16
28	carvacrol	1297	1298	0.02	0.03
29	β-caryophyllene	1412	1419	4.43	2.75
30	sesquicineole	1508	1515	5.82	3.48
31	sesquiterpene hydrocarbon $C_{15}H_{24}$ MW=204	1558	-	2.28	1.87
32	oxygenated sesquiterpene $C_{15}H_{22}O$ MW=218	1565	-	0.36	0.05
33	oxygenated sesquiterpene $C_{15}H_{26}O$ MW=222	1569	-	1.25	0.02
34	caryophyllene oxide	1573	1582	1.23	0.88
35	oxygenated sesquiterpene $C_{15}H_{24}O$ MW=220	1607	-	0.63	0.50
36	oxygenated sesquiterpene $C_{15}H_{26}O$ MW=222	1625	-	0.60	0.42
37	oxygenated sesquiterpene $C_{15}H_{24}O$ MW=220	1629	-	0.04	0.13
38	oxygenated sesquiterpene $C_{15}H_{26}O$ MW=222	1646	-	0.06	0.01
39	unidentified $C_{15}H_{26}O_2$ MW=238	1679		3.41	0.63
40	unidentified $C_{15}H_{26}O_2$ MW=38	1743		2.81	3.15
	Total monoterpenes			67.12	81.32
	Total sesquiterpenes			16.70	10.10
	Others			6.40	3.78
	Total			90.23	95.21

RI[a]: Retention Indices from literature data (Adams 2007). RI[b]: Retention Indices calculated by GC/MS using *n*-alkane series (from C_6 to C_{32}) under the same analytical conditions as for the samples.

Antiradical activity

The 2,2-diphenyl-picryl hydrazyl (DPPH·) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS·+) radical-scavenging tests was performed according to Iriti et al. (2014). All the chemicals were from Sigma Aldrich.

Results

Characterization of the essential oils

The air-dried aerial parts from the two populations of *A. moschata* subjected to steam distillation produced pale-blue yellow oils in the yield of 0.72% (VC) and 0.75% (VM) (w/w), respectively. In both EOs, analyzed by GC/FID and GC/MS, forty components were identified, corresponding to 90.23% of VC total content oil and to 95.21% of VM. The obtained results are shown in Table 1, where the compounds are listed in elution order from the DB5 capillary column. They constituted a complex mixture with oxygenated monoterpenes, monoterpene hydrocarbons and oxygenated sesquiterpenes occurring in higher proportions.

VC and VM EOs were similar in terms of the two main components. Both consisted mainly of camphor (34.22% and 23.45%, respectively) and 1,8-cineole (9.78% and 16.43%), as shown, for example, in the chromatogram relating to the VC population, where the corresponding peaks are evident (12 and 20, respectively) (Fig. 1). A third abundant compound in the VM EO was *trans*-thujone (13.39%). The main monoterpene hydrocarbon was myrcene (1.34% and 4.16%, respectively) (peak 9, Fig. 1) Among the oxygenated sesquiterpenes, the total percentages were similar in VC and VM populations (5.62% and 5.49%, respectively) and the main compound was always sesquicineole (5.82% and 3.48%, respectively) (peak 30, Fig.1).

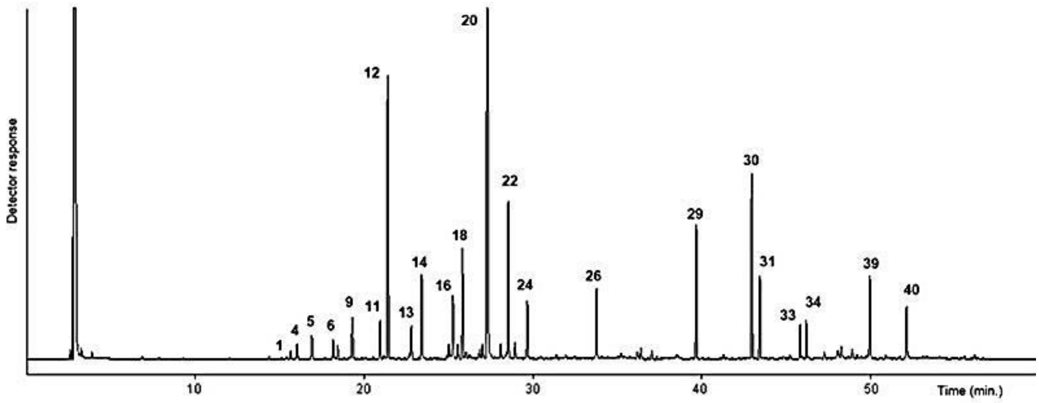

Fig. 1. Gas chromatogram of the essential oil from *Achillea moschata* collected in Valchiavenna (VC), Italy.
(1) santolina triene; (4) α-thujene; (5) camphene; (6) sabinene; (9) myrcene; (11) p-cymene; (12) 1.8-cineole; (13) γ-terpinene; (14) *cis*-sabinene-hydrate; (16) *cis*-tujone; (18) *trans*-tujone; (20) camphor; (22) borneol; (24) α-terpineol; (26) bornylacetate; (29) β-caryophyllene; (30) sesquicineole; (31) sesquiterpene hydrocarbon $C_{15}H_{24}$ MW=204; (33) oxygenated sesquiterpene $C_{15}H_{26}O$ MW=222; (34) caryopyhllene oxide; (39) and (40) unidentified $C_{15}H_{26}O_2$ MW=238.

Antioxidant activity

The biological activity of *A. moschata* EOs from different valleys of Rhaetian Alps was investigated in terms of antioxidant power towards DPPH· and ABTS·+ stable radicals. VC and VM oils proved similar radical-scavenging activity showing a good agreement between the two tests. In both cases, the essential oils were able to arrest ABTS·+ cation radical leading to a decrease in its absorbance corresponding to significant values of activity (Table 2). Similarly, VC and VM oils exhibited appreciable antioxidant potential against DPPH· with IC_{50} values around 5.00 x 10^{-5} M (Table 2).

Table 2. *In vitro* antiradical capacity of *A. moschata* essential oils measured by the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS·+) radical cation and 2,2-diphenyl-picryl hydrazyl (DPPH·) free radical assays.

Essential oil	ABTS (μmol eq Trolox/mL)	DPPH (IC_{50}) (M)
Valchiavenna (VC)	6.70 ± 0.11[a]	4.98×10^{-5}
Valmalenco (VM)	6.51 ± 0.10	5.63×10^{-5}
Quercetin	n.a.	4.92×10^{-6}

[a] Results are mean ± standard deviation of three independent analyses.

Discussion

The obtained results supplemented the characterization of *A. moschata* EO given in Maffei and co-workers (1989) and Vitalini et al. (2016). In all cases, most of the EO components were monoterpenes ranging from 67.12% to 81.80%, followed by sesquiterpenes (between 7.75% and 18.82%) and others (up to 6.40%). However, while VC and VM EOs were characterized by the presence of camphor as the more abundant compound, the main component in the *A. moschata* EO from Valle d'Ayas was terpineol-4-ol (22.32%) (Maffei et al., 1989). It was detected in very low quantities in VC (0.83%) and VM (1.15%) EOs, as well as in our previous sample (VF, 1.26%) (Vitalini et al., 2016). Likewise, bornylacetate was lower (2.01% and 1.10% in VC and VM, respectively) than that found by Maffei et al. (1989) equal to 7.94%, comparable, instead, to the percentage value in VF (6.21%) (Vitalini et al., 2016). Both β-phellandrene and the β-bisabolene, among the major compounds showed by Maffei et al. (1989), were absent in VC, VM and VF EOs (Vitalini et al., 2016), while similar percentage values were noticed for 1,8-cineole (9.78% vs 7.58%). The latter monoterpene, together with camphor, is often reported as one of the main constituents of EOs from *Achillea* species (Dokhani et al., 2005; Rahimmalek et al., 2009; Farhadi et al., 2020). It has been proved that a wide range of factors has significant impacts on the profile of secondary metabolites in plants. Different place of origin, soil composition, meteorological conditions, altitude, temperature, UV radiations, nutritional status as well as plant age, plant part, microbial attack, and competition can influence the EO composition. The presence or absence of some compounds and their concentrations may be the result of the plants' active response to the environmental conditions, including biotic and abiotic stresses (Gudaityte and Venskutonis, 2007; Teixeira Duarte and Rai, 2016). Rahimmalek et al. (2009) confirmed the importance of geo-ecological factors in EO production of some *Achillea* species showing the relationship among the identified chemotypes, soil type and climate, as well as their interactions. For example, they proposed a possible link between a high level of camphor in the EO and a soil rich in organic matter in the surface horizon and characterized by the $CaCO_3$ accumulation. As far for the antioxidant potential, equally to other EOs characterized by camphor and 1,8-cineole as main components, VC and VM samples showed good antioxidant activity (Kordali et al., 2005; Rašković et al., 2014; Wu et al., 2019). Nevertheless, these two pure compounds tested against DPPH were not able to be effective scavengers (Kordali et al., 2005). According to previous reports, the noticed activity might be affected by the presence of minor phenolic compounds and their synergistic interaction (Sotelo-Mendez et al., 2017).

Conclusion

The analysis of the EOs of the two populations of *A. moschata* showed that their composition was equivalent from a qualitative point of view but differed quantitatively. However, their action in inhibiting the radicals was comparable. Therefore, *A. moschata*, regardless of the origin area, appears to be a natural source of antioxidants that can be exploited in food preparations in order to prevent oxidative damage and consequent spoilage, as well as for its taste properties claimed by traditional knowledge.

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Conflict of interest
No potential conflict of interest was reported by the authors.

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