



Article Chemical Composition and Antioxidant Activity of Artemisia argyi Essential Oil and Hydrolate

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Abstract: *Artemisia argyi*, Chinese mugwort, is a plant widely used in China for various purposes from traditional medicine to food. The plant is less known in Europe. From plants grown in Austria, essential oils and their respective hydrolates were obtained, and their compositions were studied. Oxidized monoterpenes 1,8-cineole (32–42%), camphor (12–14%), and borneol (10–12%) were the main components present in both the essential oils and hydrolates. The essential oils also contained 6.6–10.5% monoterpene hydrocarbons such as β -caroyphyllene, camphene, and p-cymene. The hydrolate volatile fractions were devoid of hydrocarbons because of the low solubility of these compounds in water. Neointermedeol (selin-11-en-4- α -ol), a rather rare compound, and caryophyllene oxide were the major oxidized sesquiterpenes in the essential oils and were also present in low levels in the hydrolate volatiles. Furthermore, small amounts of eugenol were in the hydrolate volatiles. The essential oils and hydrolates showed some antioxidant activities in the DPPH and FRAP assays. Essential oils diluted 1:100 corresponded to gallic acid equivalents of 212–274 µg/mL in the FRAP assay and 26.1–30.7 µg/mL in the DPPH assay, while the ranges of activity for the hydrolates corresponded to gallic acid equivalents of 109–597 and 10.5–31.7 µg/mL for FRAP and DPPH assays, respectively.

Keywords: Chinese mugwort; essential oil composition; hydrolate volatiles; 1,8-cineole; camphor

1. Introduction

Artemisia argyi H. Lév. & Vaniot (Asteraceae, Sect. Artemisia), also known as silvery wormwood or Chinese mugwort, is an aromatic herbaceous perennial up to 150 cm tall and a plant native to eastern Asia. Midstem leaves are 1–2 pinnatipartite and cleft, the uppermost leaves and leaflike bracts 3-partite, or in entirety. The blades are abaxially densely gray arachnoid-tomentose and adaxially incanous pubescent. The capitules are in narrow panicles [1]. The plant is widely used in its original form, and applications range from traditional medicines to food purposes. The phytochemistry and bioactivities of *A. argyi* have been recently reviewed [2]. Biologically active ingredients include essential oils, flavonoids, organic acids, sesquiterpenes, and triterpenes. In addition to antioxidant, immunomodulatory, as well as anti-inflammatory activities, anticancer and neuroprotective activities have also been reported. Therefore, *A. argyi* can be considered as a dietary plant with multiple health benefits [2]. Sometimes, it is called the longevity grass in eastern countries for its warm effect to cure many cold diseases [3].

According to *The Chinese Pharmacopoeia*, *A. argyi* has the effects of warming the meridian, stopping bleeding, dispersing cold, and relieving pain [4]. Additionally, in China, *A. argyi* is used for moxibustion, where a cone of dried leaf powder is placed on the skin and burned to stimulate blood flow at specific points [5].

In China, *A. argyi* is a widely eaten traditional food and is used to make Chinese dishes [4]. The essential oil inhibiting melanogenesis could be useful in acting as a natural



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). antioxidant in skin care products [6]. Some flavones isolated from *A. argyi* presented antitumor activity, inhibiting the proliferation of a couple of cell lines [7]. The essential oil obtained by hydrodistillation [8] or by simultaneous distillation–extraction [9] showed good antimicrobial activity against various microbes.

Although *A. argyi* plants are already available in some specialized nurseries in Europe, the quality of these materials has not yet been documented. The present work reports the composition and antioxidant activity of the essential oils and the respective hydrolates from *A. argyi* grown in central Europe.

2. Materials and Methods

2.1. Plant Material

Plant material was obtained from Rühlemann's mail order nursery (Germany) and then cultivated for several years in a private garden in Upper Austria, Austria. The plant was identified according to the Flora of China, and voucher specimens (WU 0125375– WU 0125384) were deposited in the Herbarium of the University of Vienna, Austria (WU Generale, https://www.jacq.org/#database, accessed on 27 August 2023).

The first harvest was conducted in autumn 2021 on flowering plants. In spring 2022, from the regrowth in a vegetative development stage, a harvest was conducted again. The plants were dried in the shade in ambient air.

2.2. Hydrodistillation and Hydrolates

The bulk of the material was distilled in a (semi)professional device with the plant material from autumn 2021 to give essential oils from leaves (EO1) and leaves with stems (EO2) as well as the respective hydrolates (H1, H2). Further plant material was collected from the vegetative regrowth in spring 2022. Additionally, laboratory-scale hydrodistillations using a Clevenger-type apparatus were performed. In these cases, about 12 g of leaves or 25 g of cut stems were distilled with 400 mL water for two hours. In the Clevenger apparatus, the water trapped in the reflux tube was taken as the hydrolate. With this method, leaf (EO3) and stem (EO4) oils from autumn plants and leaf (EO5) and stem (EO6) oils from the spring plants were obtained as well as the respective hydrolates (H3-H6). The amount of essential oil from the leaves was measured in the capillary of the device, and the oil was collected and stored at 4 °C until further analysis. For the stems low in essential oil, 1 mL hexane was added into the distillation unit to obtain the oils.

2.3. GC and GC/MS

An Agilent Technologies 7890A gas chromatograph equipped with a 5975 C quadrupole mass selective detector (MSD) and a CTC-PAL autosampler (Agilent Technologies, Santa Clara, CA, USA), as described in Chizzola et al., 2018, was used with slight modifications. The separation was performed in a 30 m × 0.25 mm fused silica column coated with 0.25 μ m HP5-MS. The temperature program of the oven started with isothermal at 50 °C for 1 min, then increased to 220 °C at a rate of 5 °C/min, and finally increased further to 280 °C at a rate of 15 °C/min. The injector temperature was set to 250 °C and the split was at 20:1. The injection volume was 1 μ L. The total ion current (*m*/*z* 40 to 400) from the MSD was used to identify the compounds according to their mass spectra in comparison to the spectral libraries Wiley 275 and NIST20 and their retention indices relative to the n-alkanes [10]. For GC-FID analysis, an Agilent Technologies 8690N gas chromatograph was operated using the same type of column and temperature program as indicated above.

2.4. SPME-GC/MS

Headspace solid-phase microextraction (HS-SPME) is a solvent-free method with simple sample preparation for the analysis of volatile compounds. This technique was applied to the distilled oils, the hydrolates, and the air-dried plant material from the spring harvest. A 10 mL sample vial was charged with 10 μ L of cyclododecanone as the internal standard (0.5 mg/mL in methanol), 10 μ L of a 1:100 dilution of the leaf essential

oil, and 10 μ L stem essential oil in hexane, or 100–300 μ L hydrolate or 400 mg dried cut leaf or stem pieces. The vial was tightly closed with a septum and further processed in the CTC-PAL autosampler with the mounting for SPME fibers. The SPME fiber (PDMS-DVB, polydimethylsiloxanedivinylbenzene, Supelco, Bellefonte, PA, USA) was exposed for 30 min at 50 °C while stirring the headspace sample. Afterwards, the fiber was introduced into the injection port of the GC system and desorbed for 5 min at 250 °C at a split ratio of 10:1. The conditions for GC/MS were the same as indicated above.

2.5. Antioxidant Activity

The measurements of polyphenols and antioxidative activity rely on colorimetric reactions and were adapted for measurement using an iMark microplate reader (BioRad, Hercules, CA, USA). On a microplate, 4 replicates of each sample or standard were carried out as described by Chizzola et al. [11].

2.6. Total Phenolics

Phenolic compounds react in alkaline conditions with the Folin–Ciocalteu reagent to give a blue color. In the wells of the microplate, a 10 μ L portion of essential oil or hydrolate was added to 100 μ L distilled water, which was followed by the addition of 5 μ L Folin–Ciocalteu reagent, 10 μ L Na₂CO₃ (35% in distilled water.) and again 125 μ L of distilled water. After storing for 1 h in the dark, the absorbance was measured at 750 nm. To calibrate the color formation, increasing concentrations (ranging from 1.5 to 6.0 μ g of catechin in 110 μ L methanol) were taken instead of the sample and the initial water volume. The essential oils and hydrolates were diluted 1:20 with ethanol before the assay. The total phenolics value was presented as milligrams catechin equivalent per g of dried sample (mg CE/g DW).

2.7. DPPH (2,2-diphenyl-1-picrylhydrazyl)-Assay

The antioxidant activity of the essential oils and the hydrolates was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl radical). In this assay, the pink color of DPPH is decolorized by the reaction with antioxidant compounds. In the microplate wells, to an increasing portion of the sample (5–25 μ L) made up to 100 μ L with methanol, an aliquot of 100 μ L of the DPPH reagent (0.015% in methanol) was added. It was kept in the dark for 30 min. Increasing volumes (0–8 μ L) of trolox (0.62 mg/mL in ethanol) made up to 100 μ L with methanol instead of the samples were used to construct a calibration curve. A preparation consisting of 50 μ L trolox, 50 μ L distilled water and 100 μ L of the DPPH reagent, wherein the DPPH was completely decolorized was taken as blank and subtracted from all measurements. The decoloration was measured at 490 nm. The essential oils and hydrolates used in the assay were diluted 1:10 with ethanol. The resulting DPPH radical scavenging activity was presented as milligrams trolox equivalent per g of dried sample (mg Trolox/g DW).

2.8. Ferric Reducing Antioxidant Power (FRAP) Assay

The principle relies on the ability of antioxidants to reduce ferric (Fe+++) ions. The resulting ferrous ions (Fe++) form a deep blue complex with TPTZ (2,4,6-tripyridyl-s-triazine) [12]. The reaction was carried out in the microplate wells by mixing 10 μ L sample, 15 μ L methanol and 180 μ L working reagent. After 5 min, the color formation was measured at 595 nm. The working reagent consisted of 25 mL acetate buffer (300 mmol/L) pH 3.6, 2.5 mL of 10 mmol/L TPTZ in 40 mmol/L HCl and 2.5 mL FeCl₃ solution (20 mmol/L). A calibration curve was constructed using increasing amounts of FeSO₄7H₂O, from 1.25 to 6.25 μ g/well, instead of the samples. The sample dilution before the assay was 1:20 with ethanol. The reducing activity was presented as nmol ferrous ions per g of dried sample (nMol Fe/g DW).

3. Results

3.1. Essential Oils

The essential oil yield of the leaves harvested in 2021 and analyzed in spring 2022 was 1.3%, while the leaves from the regrowth in 2022 gave 1.9% (v/w) essential oil. Stems had at both occasions less than 0.02% essential oil. The composition of the essential oils is presented in Table 1. Leaf essential oil contained over all oxidized monoterpenes (75–79%): 1,8-cineole (32–42%), camphor (12–14%), borneol (10–12%), α -terpineol (3–4%), terpinen-4-ol (2–4%) and *trans*-carveol (2–3%). Selin-11-en-4- α -ol and caryophyllene oxide were the most prominent oxidized sesquiterpenes. Stem essential oils were lower in oxidized monoterpenes (7–10% 1,8-cineol, 3–5% camphor, 3–5% borneol) but higher in oxidized sesquiterpenes (13–23% caryophyllene oxide, 10–12% selin-11-en-4- α -ol) than leaf essential oils. Eight monoterpene hydrocarbons, amongst them camphene and *p*-cymene, made up together up to 10% of the leaf or stem oil, each of them accounting for less than 2%. Several sesquiterpene hydrocarbons were present (6.6–10.7%); amongst them, β -caryophyllene ranged from 1% to 8% in the essential oils. All essential oils contained also less than 1% eugenol.

Table 1.	Composition	of the essential	oils	(%).
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RI	Compound	EO1	EO2	EO3	EO4	EO5	EO6
	Monoterpene Hydrocarbons						
939	Camphene	2.9	3.0	1.5	0.6	2.9	1.4
968	Sabinene	1.1	1.5	0.4	0.2	1.1	0.4
971	β-Pinene	0.6	0.7	0.3	0.8	0.6	1.7
1016	α-Terpinene	0.4	0.4	0.6	0.4	0.8	0.6
1024	p-Cymene	2.1	2.4	1.8	1.5	2.0	2.1
1028	Limonene	0.9	1.4	1.1	0.9	1.6	1.5
1058	γ-Terpinene	0.7	0.7	1.3	1.9	1.5	2.4
1088	Terpinolene	0.2	0.2	0.3	0.4	0.3	0.4
	Sum	8.8	10.4	7.4	6.6	10.7	10.5
	Oxidized Monoterpenes						
989	2,3-Dehydro-1,8-cineole	0.1	0.1	0.2	0.3	0.2	0.8
1030	1,8-Cineol	41.6	39.0	31.7	6.8	37.7	10.0
1066	cis-Sabinene hydrate	2.8	3.0	1.2	0.1	2.1	0.4
1097	trans-Sabinene hydrate	2.0	1.9	1.1	0.1	1.4	0.3
1105	Filifolone			0.1	0.2		
1121	cis-p-Menth-2-en-1-ol	0.4	0.3	0.5	0.2	0.4	0.4
1124	Chrysanthenone	0.7	0.7	0.6	0.1	0.7	0.2
1138	trans-p-Mentha-2,8-dien-1-ol	0.2	0.1	0.3	0.1		
1140	trans-p-menth-2-en-1-ol	0.2	0.2	0.2			
1143	Camphor	13.5	11.7	13.9	3.4	14.0	5.2
1145	p-Mentha-1,5-dien-8-ol						0.4
1156	Isoborneol	0.2	0.2	0.3	0.1	0.2	0.2
1165	Borneol	9.1	8.1	11.7	3.7	9.7	5.3
1177	Terpinen-4-ol	1.7	1.6	4.0	1.4	2.6	1.6
1188	Dihydrocarveol					0.1	
1191	α-Terpineol	2.9	3.1	4.3	0.9	4.0	1.2
1195	cis-Piperitol					0.1	
1211	Verbenone			0.2	0.1	0.2	
1219	trans-Carveol	1.9	2.1	2.8	0.6	2.1	0.9
1227	cis-p-Mentha-1(7),8-dien-2-ol					0.1	0.2
1231	cis-Carveol	0.2	0.2	0.3	0.0	0.3	
1238	Ascaridol					0.3	0.3
1244	Carvone	0.2	0.2	0.3	0.1	0.2	
1274	Perillaldehyde	0.1	0.2	0.2	0.2	0.2	0.2
1285	Bornyl acetate	0.6	0.7	0.7	0.8	0.6	1.0
1291	p-Mentha-1(7),8(10)-dien-9-ol					0.2	0.1
1304	Carvacrol					0.3	
1338	trans-Carvyl-acetate	0.2	0.3	0.2	0.4	0.2	0.4
	Sum	78.5	73.7	74.6	19.7	77.7	29.0
	Sesquiterpene Hydrocarbons						

RI	Compound	EO1	EO2	EO3	EO4	EO5	EO6
1375	α-Copaene	0.1	0.1		0.2		0.3
1419	β-Caryophyllene	4.7	4.6	1.0	2.8	1.4	8.1
1452	α-Humulene	0.4	0.5	0.1	1.4	0.2	8.5
1457	E-β-Farnesene						0.2
1480	Germacrene D	1.7	1.8	0.1	0.2	0.3	1.4
1484	γ-Himachalene		0.2		0.5	0.1	1.4
1495	β-Selinene	0.2	0.3	0.2	0.4	0.2	0.4
1500	Bicyclogermacrene	0.5	0.6				
	Sum	7.7	8.1	1.3	5.4	2.2	20.3
	Oxidized Sesquiterpenes						
1564	E-Nerolidol		0.2	0.1	2.0	0.2	1.6
1577	Spathulenol	0.3	0.4	0.9	2.1	0.5	2.1
1582	Caryophyllene oxide	0.3	0.5	2.3	22.8	0.7	13.2
1593	Salvial-4(14)-en-1-one				0.2		0.3
1609	Humulene epoxide II			0.2	1.4		0.7
1630	Isospathulenol				1.6		
1637	Caryophylla-4(12),8(13)-dien-5β-ol		0.1	0.5	6.1	0.2	1.5
1655	Selin-11-en-4-α-ol	1.5	2.8	7.1	12.3	4.1	9.5
1677	Oxidized sesquiterpene *			0.3	4.3		
1692	Amorpha-4,9-dien-2-ol			0.4	5.6	0.1	3.0
	sum	2.0	4.0	11.9	58.4	5.7	32.0
	Other						
977	1-Octen-3-ol	0.7	0.8	0.8	0.1	0.9	0.2
1363	Eugenol	0.6	0.8	1.0	0.2	0.9	0.2

Table 1. Cont.

* Mass spectrum: 159 (100), 109 (90), 91 (81), 105 (76), 79 (67), 131 (57), 117 (56), 77 (49), 121 (46), 220 (11, M+).

3.2. Hydrolates

The hydrolate volatiles (Table 2) comprised more than 90% oxidized monoterpenes, mainly 1,8-cineole (33.6–50.4%), camphor (17.2–23.1%), borneol (8.7–13.5%), terpinen-4-ol (5.1–7.0%), α -terpineol (3.5–6.8%) and *trans*-carveol (3.7–6.5%). Overall, the hydrolates from leaves and stems distillation had comparable composition. The hydrolate volatiles had a higher eugenol percentage (up to 4.8%) than the essential oils but a lower selin-11-en-4- α -ol percentage (up to 1.9%). Caryophyllene oxide and spathulenol were very low in the hydrolate volatiles. Additionally, in contrast to the distilled oils, no monoterpene or sesquiterpene hydrocarbons were found in the hydrolates. A further comparison of the hydrolates with the respective essential oils is given in Figure 1 where the ratio of the percentages in both sample types is presented for selected compounds. Giving a ratio clearly below one, caryophyllene oxide, spathulenol, *trans*-sabinene hydrate and selin-11-en-4- α -ol were present mainly in the essential oil, while compounds having a ratio around one (cis-p-menth-2-en-1-ol, 1,8-cineol, borneol and camphor) occurred equally in hydrolates and essential oils. Finally, a ratio greater than one indicates compounds found preferentially in the hydrolates.

3.3. Volatiles Analyzed by SPME

Furthermore, the composition of volatile fractions has been examined using SPME as a solvent-free technique. By this way, the volatiles in the headspace of the sample were first adsorbed to a fiber and then directly desorbed in the GC/MS system. This technique was also used to obtain the volatile fingerprints from the dried plant, the distilled oils and the respective hydrolates as presented in Table 3. The main compounds as in the distillates and extracted hydrolates could be also found with this technique but in different ratios. 1,8-Cineole values and ranges were 25.8% in the dried plant, 11.1–15.9% in the leaf essential oils and 12.4–29.1% in the hydrolates. SPME from the leaf essential oils gave 10.9–22.4% borneol and 9.3–13.0% camphor; in the leaf hydrolate volatiles, these two compounds accounted for 11.2–17.7% and 12.0–15.4%, respectively. β -Caryophyllene levels were around 9% in the dried plant, up to 12.4% in essential oils and absent in hydrolates. Reaching 13.6%, selin-11-

en-4- α -ol was highest in the spring leaf essential oil (EO5). Eugenol was remarkably high (18.4%) in the SPME volatiles of the leaf hydrolate from the spring harvest (H5).

RI	Compound	H1	H2	H3	H4	H5	H6
NI	•		112	115	117	115	110
	Oxidized Monoterpenes						
989	2,3-Dehydro-1,8-cineole					0.4	0.2
1030	1,8-Cineol	41.5	42.7	50.4	49.0	33.6	39.8
1066	cis-Sabinene hydrate			0.1		0.4	0.2
1097	trans-Sabinene hydrate			0.2	0.1	0.4	0.1
1105	Filifolone	0.2	0.3		0.1		
1121	cis-p-Menth-2-en-1-ol	0.5	0.1	0.5	0.5	0.6	0.7
1124	Chrysanthenone					0.1	
1138	trans-p-Mentha-2,8-dien-1-ol					0.1	0.2
1140	trans-p-Menth-2-en-1-ol	0.1	0.1	0.2	0.0	0.3	0.5
1141	<i>cis-p-</i> Mentha-2,8-dien-1-ol	0.4	0.1	0.3	0.5		
1143	Camphor	17.2	15.5	16.8	20.0	19.3	23.1
1156	Isoborneol	0.1	0.1	0.0	0.2		0.2
1165	Borneol	12.3	11.6	8.7	10.4	11.5	13.5
1177	Terpinen-4-ol	7.0	7.0	5.1	6.7	5.4	6.9
1186	<i>p</i> -Cymen-8-ol	0.2	0.3	0.2	0.0	0.2	0.1
1188	Dihydrocarveol	0.2	0.1	0.1	0.2		0.2
1191	α-Terpineol	5.4	5.8	4.8	3.5	6.8	4.1
1191	Myrtenol	0.2	0.1		0.2		
1195	cis-Piperitol	0.1	0.1	0.1	0.1	0.1	0.2
1209	trans-3(10)-Caren-2-ol	0.1		< 0.05	0.1	< 0.05	0.1
1211	Verbenone	0.5	0.4	0.4	0.1	0.9	0.2
1219	trans-Carveol	4.2	4.4	4.4	2.4	6.5	3.7
1227	cis-p-Mentha-1(7),8-dien-2-ol	0.2	0.2	0.2	0.2	0.2	0.3
1231	cis-Carveol	0.4	0.7	0.4	0.2	0.7	0.3
1244	Carvone	0.3	0.4	0.3	0.3	0.3	0.4
1274	Perillaldehyd	0.2	0.3	0.2	0.2	0.4	0.2
1294	<i>p</i> -Mentha-1(7),8(10)-dien-9-ol	0.4	0.4	0.2	0.1	0.6	0.2
1302	Perillyl alcohol	0.7	0.7	0.5	0.2	0.8	0.2
1304	Carvacrol	0.1	0.1			1.4	0.1
	Sum	92.7	91.5	94.0	95.3	91.3	95.6
	Oxidized Sesquiterpenes		,	,		,	,
1577	Spathulenol	0.3		0.1	0.3		
1582	Caryophyllene oxide	0.1		0.1			
1637	Caryophylla-4(12),8(13)-dien-5β-ol	0.1	0.1	0.1	0.1		
1655	Selin-11-en-4- α -ol	1.9	1.4	0.8	0.6	0.6	0.5
	sum	2.4	1.5	1.0	1.0	0.6	0.5
	Other		1.0	1.0	1.0	0.0	0.0
977	1-Octen-3-ol	0.6	0.8	0.8	0.8	0.9	0.9
1363	Eugenol	2.8	3.9	2.6	0.7	4.8	0.9

Table 2. Composition of the hydrolate volatile fractions (%).

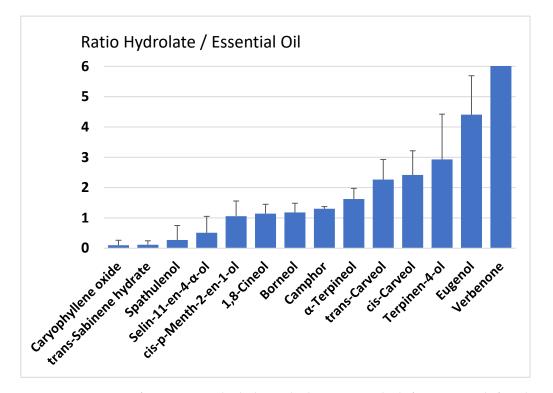


Figure 1. Mean ratio of percentage in hydrolate volatiles to essential oils for compounds found in both sample types from leaves (n = 4, error bar: standard deviation).

Table 3. SPME fingerprints of essential oil, hydrolate and dried plant of A. argyi.

RI	Compound	EO1	EO2	EO3	EO4	EO5	EO6	Dried Leaves	Dried Stems
1030	1,8-Cineole	12.9	11.1	11.4		15.9	2.5	25.8	25.8
1143	Camphor	9.3	10.4	13.0		12.7	2.9	12.1	14.2
1165	Borneol	16.0	15.5	22.4		10.9	4.1	7.8	7.1
1177	Terpinen-4-ol	2.3	2.8	6.7		3.1	1.3	1.2	0.6
1191	α -Terpineol	7.1	7.0	9.1		5.7	1.3	4.6	1.9
1219	trans-Carveol	5.3	4.4	5.2		3.6	1.1	2.9	1.9
1363	Eugenol	2.6	2.1	2.0		2.8	0.2	2.2	0.1
1419	β-Caryophyllene	12.4	12.2	3.2		4.2	9.7	8.7	9.5
1457	E - β -Farnesene					0.3	9.7	0.7	10.4
1480	Germacrene D	7.2	6.3	0.3		1.3	2.5	4.0	1.5
1582	Caryophyllene oxide	1.2	0.9	3.9		2.7	14.4	1.3	4.2
1655	Selin-11-en-4-α-ol	1.2	1.1	2.7		13.6	9.7	1.2	0.9
	Sum	77.3	73.8	79.8		76.9	59.4	72.6	77.9
		H1	H2	H3	H4	H5	H6		
1030	1,8-Cineol	26.7	26.4	12.6	24.0	12.4	29.1		
1143	Camphor	13.9	15.4	14.8	20.4	12.0	22.5		
1165	Borneol	14.2	15.5	17.7	17.1	11.2	18.0		
1177	Terpinen-4-ol	9.1	9.1	7.1	11.5	4.3	6.7		
1191	α -Terpineol	7.4	7.2	10.5	7.4	8.0	5.6		
1219	trans-Carveol	5.2	5.2	11.1	5.2	10.3	6.4		
1363	Eugenol	5.7	4.2	5.2	1.4	18.4	3.0		
1582	Caryophyllene oxide	0.1	0.8	0.5	0.6	0.0	0.6		
1655	Selin-11-en-4-α-ol	5.0	5.2	7.7	2.5	1.3	2.3		
	Sum	87.3	89.1	87.1	90.1	77.9	94.2		

3.4. Antioxidant Activities

Finally, antioxidant activity has been tested in essential oils and hydrolates (Table 4). The essential oils from leaves or leaves + stems (EO1, 2, 5) were diluted 1:100 prior to analysis. Essential oil EO6 from the stems was directly taken up in hexane in the distillation apparatus, so the true dilution could not be measured. The results are expressed in gallic acid equivalents on the basis of this dilution. Comparing the gallic acid equivalents obtained in the three distinct assays (total phenolics, FRAP and DPPH) shows that they respond differently to the active compounds in the samples. In the FRAP and the DPPH assay, the hydrolates from spring leaves (H5) appeared more active than those from autumn leaves (H1, 2). Carvacrol and eugenol, both known for a high antioxidant potency [13], are present in this sample (H5) and might contribute to this higher activity. The activities of the stem essential oil and hydrolate (EO6, H6) were low because of their low essential oil content.

	TP μg/mL Gallic	Acid Equivalents	
EO 1	95.9	H1	107.8
EO 2	132.5	H 2	114.7
EO 5	98.6	H 5	100.6
EO 6	20.7	H 6	16.8
	FRAP µg/mL Galli	c Acid Equivalents	
EO 1	211.8	H1	108.7
EO 2	312.3	H 2	113.7
EO 5	274.0	H 5	597.3
EO 6	8.5	H 6	48.6
	DPPH µg/mL Galli	c Acid Equivalents	
EO 1	30.0	H1	10.5
EO 2	30.7	H 2	18.6
EO 5	26.1	H 5	31.7
EO 6	3.0	H 6	9.2

Table 4. Total phenolics and antioxidant activity in the essential oils and hydrolates from A. argyi.

EO 1, EO 2 and EO 5: diluted 1:100, EO 6: in 1 mL hexane, distilled from 25 g dried plant.

4. Discussion

The present research found 1.3 to 1.9% essential oil in the leaves, which is higher than the contents being between 0.2 and 0.7% for aerial parts as reported by various authors [6,8,9,14–18]. Most authors report 1,8-cineole as the major compound, and camphor, borneol, terpinen-4-ol and α -terpineol were also present in most samples. However, essential oils with more than 30% 1,8-cineole, 12% camphor and 10% borneol as in the present study were reported occasionally only: for instance, from the provinces Hebei and Jainxi [19,20]. Other authors found lower levels of these three compounds. Intermedeol (=Selin-11-en-4- α -ol) is a compound present consistently, usually accounting for 5–12% of the oil. Some *A. argyi* essential oils contained artemisia ketone and artemisia alcohol [16,21] or up to 16% α -thujone [8,20,22]. Further compounds reported include β -caryophyllene and caryophyllene oxide; their percentage reached up to 15% each [22].

The main components 1,8-cineole, camphor and borneol of the present essential oils are widespread in essential oils. For instance, they can be found in in rosemary, lavender, sage or eucalyptus [23]. Also, terpinene-4-ol, α -terpineol, β -caryophyllene and caryophyllene oxide occur often in essential oils. 1,8-Cineol shows a wide range of pharmacological activities; it has therapeutic effects on many respiratory diseases and proved to be a potential anticancer agent against various cancer types both in vitro and in vivo [24]. An *A. argyi* essential oil which had similar high contents of 1,8-cineole, camphor and borneol than in the actual findings showed considerable anti-inflammatory activity, which was caused by a down-regulation of the JAK/STATs signaling pathway [20]. In human gingival

fibroblasts, an anti-inflammatory response has been measured with the application of a sage infusion and/or their volatile compounds 1,8-cineole, camphor, borneol and thujone [25].

Data about the composition of hydrolate volatiles from *A. argyi* are not available in the literature. The present essential oils contained around 85% oxidized compounds; most of them could be found also in the respective hydrolates, while hydrocarbons were absent. Hydrolates or hydrosols are generally poor in hydrocarbons because of the low solubility of this compound class in water. Similarly, an essential oil from *Artemisia annua* had 38.6% monoterpene and sesquiterpene hydrocarbons, while the respective hydrolate was devoid of hydrocarbons [26]. In the same way, essential oils from *Pinus cembra* comprised approximately 95% mono- and sesquiterpene hydrocarbons and 5% their oxidized derivatives, while in the corresponding hydrolates volatiles, the ratio of hydrocarbons to oxidized compounds was around 5:95 reversed [27]. Rosemary essential oils having the pinenes, 1,8-cineole, camphor, borneol, and bornyl acetate as the main compounds were related to hydrolates with 1,8-cineole, camphor, borneol, terpinene-4-ol and α -terpineol in similar ratios as in the present *A. argyi* hydrolate volatiles [28].

The antioxidant activity of *A. argyi* reported in the literature refers to extracts with various solvents and demonstrated considerable activity due to the presence of organic acids, phenolic compounds and flavonoids [29]. The present essential oils and hydrolates gave some antioxidant activities. An essential oil having 1,8-cineole as the main compound (23.7%) significantly scavenged 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) ABTS radicals [6]. Similarly, various essential oils from rosemary cultivars cultivated in Italy containing 1,8-cineole, camphor, and borneol as major compounds displayed a good antioxidant activity in the FRAP and DPPH assays [30]. However, there is also a notice that 1,8-cineole was not active in the DPPH assay [31].

5. Conclusions

Essential oils and hydrolates rich in 1,8-cineole, camphor, and borneol from the aromatic *Artemisia argyi* grown in central Europe can be obtained in different seasons. These volatile fractions containing mostly oxidized (mono)terpenes and showing some antioxidant activity might be of interest for various applications.

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