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Essential Oil Composition and Antioxidant Activity of Endemic *Achillea lingulata* Waldst. & Kit. Compared to Common *A. millefolium* L.

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Abstract: In this study, the chemical composition and antioxidant activity of the hydrodistilled essential oil of *Achillea lingulata*, an endemic species of the Euro-Mediterranean region, originating from Bosnia and Herzegovina, was investigated for the first time. For comparison, an analysis of the essential oil of the widely distributed *Achillea millefolium*, which grows together in the same habitat, was made. Ninety-six components were identified in *A. lingulata* and *A. millefolium* oils comprising 97.8% and 85.8%, of the total oil, respectively. The oil of *A. lingulata* was characterized by a high content of oxygenated monoterpenes (76.8%). The main compounds were borneol (30.1%), *trans*-verbenol (15.5%), 2-tridecanone (12.2%), fragranol (8.3%), and myrtenol (7.9%). In contrast, essential oil of *A. millefolium* had oxygenated sesquiterpenes (60.8%) as the most abundant compounds, with elemol (32.9%) as the main constituent. In addition, γ-eudesmol (12.9%), caryophyllene oxide (7.7%), *trans*-caryophyllene (5.7%) and γ-muurolene (4.7%) were present in a significant percentage in *A. millefolium* oil. Antioxidant activity was tested by three methods, ABTS, DPPH and FRAP, and the obtained results showed low activity of both investigated oils.

Keywords: *Achillea lingulata*; *Achillea millefolium*; GC-MS; essential oil; terpenes; antioxidant activity.

1. Introduction

Plant chemistry represents the basis for the use of herbs for various therapeutic purposes. A good knowledge of the chemical composition of plants enables a better understanding of their possible medicinal effects. The pharmacological effects of plants depend on their phytochemical constituents. Secondary plant metabolites have played an important role in traditional medicine and folk use. Today,

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they represent the main compounds for the development of new drugs for the treatment of various diseases [1].

Family Asteraceae (Compositae) contains about 10% of all flowering plants with over 1700 genera and 25000 species [2]. The genus *Achillea* L. (tribe *Anthemideae*) includes 110-140 species, suffruticose to herbaceous perennials, mainly native to SW Asia and SE Europe, and widespread in Euroasia and North America. Its representatives display significant ecological amplitude from the coastal to the alpine (nival) region and from arid to waterlogged habitats [3, 4].

Achillea species have a long history of use in traditional medicine due to their many health benefits. The main therapeutic applications are anti-inflammatory, wound healing, spasmolytic, choleretic abdominal pain, stomachache, symptomatic relief of colds, ulcer, and diarrhea [5, 6].

Phytochemical studies of the genus *Achillea* have shown that many chemical constituents of this genus are bioactive. Literature data indicate the presence of flavonoids, terpenoids, lignans, amino acid derivates, fatty acids and alkamides [7, 8].

Essential oils, as a part of the plant immune system, have numbers of important impacts in plant metabolism, their protection from predators and pests, in attracting pollinators and supporting seed dispersal by animals, the interactions of the plants with their environment etc. In addition, these oils are a powerful tool for a better understanding of plants phylogeny [9,10].

Achillea lingulata Waldst. & Kit. (*Achillea* sect. *Anthemoideae* s.l.) [4] an endemic species of the Euro-Mediterranean region is distributed throughout the SE parts of Europe, mainly the Balkan Peninsula, Carpathians and Belarus [11]. Populations of *A. lingulata* inhabit high mountain regions: pastures, grassy places and rarely rocky ground [12].

Achillea millefolium L. (*Achillea* sect. *A. millefolium* agg.) [4] is a common widespread plant native to North and Central America, Asia and Europe [11].

There are numerous scientific studies on the biological activity of essential oils and extracts of *Achillea* species such as antioxidant, anti-inflammatory, antibacterial, antifungal and herbicidal activity [5-7, 13-16]. Antioxidants play an important role in protecting against free radicals that cause oxidative stress in the body. Some natural plant products have the ability to neutralize free radicals and because of that more and more research is being conducted today leading to the discovery of new bioactive compounds. Methods for determining antioxidant activity are based on different mechanisms that determine the ability of individual compounds to act in different ways.

There is no data on the determination of the volatile constituents of the *A. lingulata* of Bosnian origin. According to literature data, the chemical composition of essential oil of *A. lingulata* was investigated by several authors for samples from Serbia [17-22], showing that these samples belong to the borneol chemotype. Some other authors have reported the presence of lignans [23, 24], fatty acids [25] and phenolic compounds [26, 27].

The composition of the essential oil of the commercially available sample *A. millefolium* from Bosnia and Herzegovina was determined by Vidic [15]. Also, this and closely related species growing in other regions of the world, has been the subject of numerous studies for years, mainly due to their medicinal properties. According to different authors, camphor, borneol, 1,8-cineole, α- and *β*-pinene, caryophyllene oxide, α-bisabolol, nerolidol α- and *β*-thujone, ascaridole, chamazulene, piperitone, and artemisia ketone have been found as the main components in the essential oil of *A. millefolium* [15, 28-35].

Volatile compounds can be a useful tool for chemotaxonomic classification that allows *Achillea* species to be divided into several groups. For species from the Balkan Peninsula, the most common group is with "1,8-cineole-camphor-borneol" chemotype [33]. *Achillea lingulata* can be observed separately because compounds in its essential oil are not significantly shared with other species. Research on the essential oil from *A. millefolium* has shown that ten different compounds can define the chemotype of this species [32].

The aim of this study was to investigate the volatile constituents of essential oils and antioxidant activity of *A. lingulata*, an endemic species of the Euro-Mediterranean region, and to compare its data, using the same analysis, with the well-known medicinal plant *A. millefolium* growing wild together in the same habitat and under the same environmental conditions.

These are the first data on the chemical composition of essential oil of *Achillea lingulata* from Bosnia and Herzegovina and its antioxidant activity.

2. Materials and Methods

2.1. Plant Material

Plant material of *Achillea lingulata* and *Achillea millefolium* were collected in full blooming stage, on mountain Jahorina (Rajska vrata, cca 1600 m of altitude), Bosnia and Herzegovina, in July 2020. The vouchers are stored in the Herbarium of National Museum of Bosnia and Herzegovina, Herbarium code SARA (*A. lingulata* SARA - 52474, *A. millefolium* SARA - 52475). The samples were air-dried in room with ventilation at ambient temperature and stored in paper bags in a dry place, until use.

2.2. Essential Oil Isolation

The essential oils were isolated by hydrodistillation from aerial parts of the plant for 3h. The oil was extracted with dichloromethane and dried over anhydrous sodium sulfate. Dichloromethane was completely removed and extracted essential oil was stored at 4°C in the dark, until analysis. All of the applied chemicals were of pro analysis purity and were purchased from Sigma Aldrich.

2.3. GC-MS Analysis

Determination of volatile compounds from the aerial parts of the plants was analyzed by GC–MS Agilent Technologies Inc. GC7890A; MS 5975C with autosampler 7983. The GC conditions were: fused silica HP-5 column, carrier gas He (1.0 mL/min), temperature was programmed from 60°C to 240°C with a temperature increase of 3°C/min; the injection port temperature was 250°C; detector temperature was 280°C. Ionization of the sample components was performed in the EI mode (70 eV).

A mixture of *n*-alkanes (C8-C40) was injected under the above conditions to calculate the retention indices using the generalized equation [36] Retention indices of *n*-alkanes were used for recalculating retention indices of volatile constituents.

The identification of volatile constituents was performed by comparing their retention indices and MS spectra with those presented in the databases available in the licensed MassFinder 4 software (EssentialOil 4a and Adams2205 [37] databases). Oil samples were dissolved in *n*-hexane prior to GC-MS analysis.

2.4. Antioxidant Capacity Assays

2.4.1. ABTS Capacity

The antioxidant activity of analyzed essential oils was determined by ABTS radical cation decolorization assay [38]. For this assay ABTS radical cation was prepared by mixing equal volumes of 7 mM ABTS solution and 2.45 mM solution of potassium persulfate, allowing the mixture to stand in the dark at temperature of 4°C for 12–16 h before use. To determine the antioxidant activity of the oils, $ABTS^{\cdot+}$ solution was diluted with ethanol to achieve an absorbance of 0.70–0.90 at 734 nm.

After adding 100 μL of the sample solution in dimethyl sulfoxide to 1 mL of $ABTS^{\cdot+}$ solution, mixture was left in the dark for 10 minutes and absorbance was measured spectrophotometrically at 743 nm. All measurements were performed in triplicate. The percentage inhibition of the $ABTS^{\cdot+}$ radical by the oils was calculated according to the formula:

$$\%AA = (A_0\text{-}A_t)/A_0 \times 100$$

A_0 – absorbance of ABTS t = 0 min, A_t – absorbance of ABTS with antioxidant t = 10 min

The calibration curve was performed using Trolox as a standard, and the results were expressed as Trolox equivalent per gram of oil (μM (TE)/g).

2.4.2. DPPH Capacity

The antioxidant activity of the oils was measured in terms of hydrogen donating radical scavenging ability, using the stable radical (2,2-diphenyl-1-picrylhydrazyl), DPPH [39]. Solution of DPPH radical was prepared dissolving DPPH in ethanol, and diluting to achieve absorbance of 0.70–

0.90, at 517 nm. After addition of an aliquot (100 μL) of diluted essential oil to 1 mL of DPPH solution, decrease of absorbance was measured at 517 nm after 30 minutes. Ethanol was used as a blank. All measurements were performed in triplicate.

The percentage of antioxidant activity for scavenging DPPH radical by essential oils was calculated according to the formula:

$$\%AA = (A_0\text{-}A_t)/A_0 \times 100$$

(1) A_0–absorbance of DPPH t = 0 min, A_t-absorbance of DPPH with antioxidant t = 30 min

The calibration curve was performed using Trolox as a standard and the results were expressed as Trolox equivalent per gram of oil (μM (TE)/g).

2.4.3. Ferric-Reducing Antioxidant Power Assay (FRAP)

The FRAP (Ferric-Reducing Antioxidant Power Assay) assay is carried out under acidic conditions (pH 3.6) to maintain iron solubility and more importantly, to stimulate electron transfer. The FRAP assay was performed according to the method described by Benzie and Strain [40] with minor modifications. The FRAP reagent was prepared fresh just before use by mixing: 300 mmol/L acetate buffer, 10 mmol/L TPTZ/EtOH solution, and 20 mmol/L ferric chloride in this order at a volume ratio of 10:1:1 and then heated to 37°C before use. An aliquot of 0.1 mL of sample was allowed to react with 3.0 mL of the FRAP working solution and 0.3 mL of water. All readings were then taken at 593 nm against a blank reagent sample at the end of 6 min. The results are expressed as L(+)-ascorbic acid equivalent per gram of oil, (mg AAE)/g).

3. Results and Discussion

3.1. Essential Oil Composition

The hydrodistillated oils of *A. lingulata* and *A. millefolium* were analysed by GC-MS technique. Despite the fact that both species grow in the same habitat under the same environmental conditions, significant differences in the content and composition of essential oils were observed. Both oils were light yellow color with a pleasant smell. The yield of hydrodistilled essential oil from the aerial parts of *A. lingulata* was low 0.01%, while for *A. millefolium* it was 0.1%. In general, *A. lingulata* was not rich in essential oil content, and its content varied from 0.05-0.22 % [17-22], depending on the parts of the plant that were used to obtain the oil.

The chemical composition of the essential oils for both species is presented in Table 1. Forty-two constituents were identified in *A. lingulata* oil, of which twenty-nine comprise 97.8% of the total oil. In addition, 13 components were identified, but their content was lower than 0.1% and they were marked as trace (t). The oxygenated monoterpenes were dominant compounds (76.8%), with a very low content of monoterpene hydrocarbons (1.2%).

The obtained data showed that the essential oil of *A. lingulata* belongs to the borneol chemotype, with a borneol content of 30.1%. Most samples from Serbia also had borneol as the main compound, (20.3%) [17], (29.9%) [18], (23 - 40.7%) [20], (30.7%) [21], only Boskovic [19] reported τ-cadinol (22.48%) as the main compound. The essential oil from underground parts (root) had neryl tiglate (16.2%), as the main component [22].

The second most abundant compound is *trans*-verbenol (15.5%), also oxygenated monoterpene. To the best of our knowledge this is the first report on the *trans*-verbenol content in the oil of the *A. lingulata*. As the main compound, in others *Achillea* species, verbenol (27.98%) was found in *A. lycaonica*, an endemic species from Turkey [16]. Also, fragranol was detected for the first time in a significant amount (8.3%) in the analyzed essential oil.

Components that do not belong to terpenoids are marked as others (OT) comprising (14.1%), including 2-tridecanone (12.2%) and alkanes. The presence of a high content of verbenol, fragranol and 2-tridecanone makes the chemical profile of *A. lingulata* from Bosnia and Herzegovina specific.

Table 1. Chemical composition of essential oil of *A. lingulata* and *A. millefolium*

Compounds	**RI[a,b]**	**RI**	**Relative content (%)**		**IM[c]**
			A. lingulata	***A. millefolium***	
Thuja-2,4 (10)-diene	953[a]	946	0.8	-	RI[a], MS
m-Cymene	1013[b]	1002	0.2	-	RI[b], MS
p-Cymene	1020 [a]	1020	0.2	-	RI[a], MS
1,8-Cineol	1026 [a]	1026	0.6	0.4	RI[a], MS
cis-Sabinene hydrate	1065 [a]	1062	0.3	t	RI[a], MS
trans-Sabinene hydrate	1098 [a]	1095	0.4	t	RI[a], MS
dehydro Sabina ketone	1117 [a]	1117	0.4	-	RI[a], MS
trans-Pinocarveol	1135 [a]	1135	3.3	0.1	RI[a], MS
trans-Sabinol	1137 [a]	1137	t	-	RI[a], MS
Camphor	1141 [a]	1140	-	0.5	RI[a], MS
trans-Verbenol	1140[b]	1141	**15.5**	-	RI[b], MS
Sabina ketone	1154 [a]	1154	0.4	-	RI[a], MS
Pinocarvone	1160 [a]	1159	1.6	0.1	RI[a], MS
Borneol	1165 [a]	1162	**30.1**	2.0	RI[a], MS
Lavandulol	1165 [a]	1164	-	1.0	RI[a], MS
Terpinen-4-ol	1174 [a]	1174	2.6	0.2	RI[a], MS
p-Cymene-8-ol	1179 [a]	1182	0.3	-	RI[a], MS
α-Terpineol	1186 [a]	1188	0.1	1.0	RI[a], MS
Myrtenol	1194 [a]	1193	**7.9**	0.2	RI[a], MS
α-Camphonelol	1190[b]	1200	-	t	RI[b], MS
Verbenone	1204 [a]	1206	1.3	-	RI[a], MS
Fragranol	1214 [a]	1212	**8.3**	-	RI[a], MS
trans-Carveol	1215 [a]	1216	t	0.1	RI[a], MS
m-Cumenol	1224 [a]	1226	t	-	RI[a], MS
Cumin aldehyde	1238 [a]	1237	0.2	-	RI[a], MS
Bornyl acetate	1284 [a]	1284	2.2	0.2	RI[a], MS
p-Cymen-7-ol	1289 [a]	1288	t	-	RI[a], MS
Chrysanthenone epoxide	1290[b]	1316	t	-	RI[b], MS
p-vinyl-Guaiacol	1309 [a]	1327	-	0.2	RI[a], MS
trans-Carvyl acetate	1339 [a]	1337	-	t	RI[a], MS
Fragranyl acetate	1331[b]	1343	1.3	-	RI[b], MS
Eugenol	1356 [a]	1356	t	0.2	RI[a], MS
Anethol epoxide	1347[b]	1363	-	0.2	RI[b], MS
α-Copaene	1374 [a]	1373	-	t	RI[a], MS
β-Bourbonene	1387 [a]	1382	-	0.2	RI[a], MS
β-Cubebene	1387 [a]	1388	-	t	RI[a], MS
β-Elemene	1389 [a]	1390	-	0.1	RI[a], MS
(Z)-Jasmone	1392 [a]	1397	t	t	RI[a], MS
a-Chamipinene	1396 [a]	1400	-	t	RI[a], MS
trans-Caryophyllene	1417 [a]	1417	-	**5.7**	RI[a], MS
β-Copaene	1430 [a]	1426	-	0.1	RI[a], MS
β-Gurjunene	1431 [a]	1433	-	t	RI[a], MS
Aromadendrene	1439 [a]	1441	-	t	RI[a], MS
α-Humulene	1452 [a]	1450	-	1.0	RI[a], MS
allo-Aromadendrene	1458 [a]	1457	-	0.1	RI[a], MS
cis-Cadina-1(6)-4-diene	1461 [a]	1460	-	t	RI[a], MS
Dauca-5,8-diene	1471 [a]	1474	-	0.3	RI[a], MS
Dodecanol	1469 [a]	1475	0.5	-	RI[a], MS

Table 1 continued..

γ-Muurolene	1478 [a]	1478	-	**4.7**	RI[a], MS
ar -Curcumene	1479 [a]	1481	-	0.7	RI[a], MS
Germacrene D	1484 [a]	1489	-	t	RI[a], MS
α-Zingiberene	1493 [a]	1494	-	0.9	RI[a], MS
Viridiflorene	1496 [a]	1501	-	t	RI[a], MS
2-Tridecanone	1495 [a]	1504	**12.2**	-	RI[a], MS
trans-*β*-Guaiane	1502 [a]	1505	-	t	RI[a], MS
β-Bisabolene	1505 [a]	1507	-	t	RI[a], MS
γ-Cadinene	1513 [a]	1511	-	0.4	RI[a], MS
(E)-*γ*-Bisabolene	1521[b]	1519	-	0.6	RI[b], MS
δ-Cadinene	1522 [a]	1522	-	1.1	RI[a], MS
(E)-iso-*γ*-Bisabolene	1528 [a]	1529	-	t	RI[a], MS
α-Cadinene	1537 [a]	1535	-	0.2	RI[a], MS
Elemol	1548 [a]	1551	-	**32.9**	RI[a], MS
Salviadienol	1545[b]	1554	-	t	RI[b], MS
(E)-Nerolidol	1561 [a]	1564	-	0.6	RI[a], MS
Spathulenol	1577 [a]	1576	3.3	t	RI[a], MS
Caryophyllene oxide	1582 [a]	1579	t	**7.7**	RI[a], MS
Lemnalol	1579[b]	1583	t	-	RI[b], MS
Ledol	1602 [a]	1600	t	-	RI[a], MS
Eremoligenol	1629 [a]	1616	-	t	RI[a], MS
γ-Eudesmol	1630 [a]	1631	-	**12.9**	RI[a], MS
Caryophylla-3(15),7(14)-dien-6-ol	1635[b]	1635	-	1.1	RI[b], MS
Hinesol	1640 [a]	1637	-	t	RI[a], MS
β-Eudesmol	1649 [a]	1646	1.5	-	RI[a], MS
α-Cadinol	1652 [a]	1650	t	-	RI[a], MS
α-Eudesmol	1652 [a]	1652	-	t	RI[a], MS
Germacra-4 (15),5,10(14)-trien-1-*α*-ol	1685 [a]	1684	0.9	1.8	RI[a], MS
Shyobunol	1688 [a]	1688	-	1.1	RI[a], MS
Eudesm-7(11)-en-4-ol	1700 [a]	1702	-	0.2	RI[a], MS
Chamazulene	1730 [a]	1725	-	0.8	RI[a], MS
6S,7R-Bisabolone	1748 [a]	1744	-	0.6	RI[a], MS
β-Costol	1766 [a]	1762	-	0.6	RI[a], MS
α-Costol	1773 [a]	1768	-	0.6	RI[a], MS
Cryptomeridiol	1813 [a]	1807	-	0.5	RI[a], MS
Hexahydrofarnesyl acetone	1817[b]	1845	-	0.2	RI[b], MS
Cembrene	1937 [a]	1937	-	t	RI[a], MS
trans-2,6-Dimethyl-10(*p*-tolyl)-undeca-2,6-diene	1945[b]	1951	-	0.7	RI[b], MS
cis-*γ*-Curcumyl-2-methylbutyrate	2011[b]	2008	-	0.2	RI[b], MS
Octadecanol	2077 [a]	2085	t	-	RI[a], MS
Docosane	2200 [a]	2200	t	-	RI[a], MS
Tricosane	2300 [a]	2300	0.5	0.4	RI[a], MS
Tetracosane	2400 [a]	2400	0.1	t	RI[a], MS
Pentacosane	2500 [a]	2499	0.8	0.3	RI[a], MS
Heptacosane	2700 [a]	2700	-	0.1	RI[a], MS
Nonacosane	2900 [a]	2900	-	0.1	RI[a], MS

Table 1 continued..

Monoterpene hydrocarbons	1.2	-
Oxygenated monoterpenes	**76.8**	6.4
Sesquiterpenes	-	16.0
Oxygenated sesquiterpenes	5.7	**60.6**
Diterpenes	-	0.7
Oxygenated diterpenes	-	0.2
Other	14.1	1.9
TOTAL	**97.8**	**85.8**

t-trace (< 0.1), RI[a,b] - retention index from literature, [a] – Adams 2205 database [37], [b] - EssentialOil 4a database, RI- Retention indices calculated from retention times in relation to those of a series of *n*-alkanes C8-C40 on a 30m DB-5 capillary column, MS- mass spectra, IM[c] – Identification method: MS, based on comparison with Adams 2205 [37] and EssentialOil 4a databases

Sesquiterpene hydrocarbons were not identified, while oxygenated sesquiterpenes accounted for 5.7%, and spathulenol (3.3%) were the most abundant representatives.

In general, *A. millefolium* oil was characterized by a high percentage of oxygenated sesquiterpenes. Besides elemol (32.9%) as the main compound, other compounds with a high amount were γ-eudesmol (12.9%) and carophyllene oxide (7.7%). Monoterpene hydrocarbons were not detected, while oxygenated monoterpenes comprised 6.7% of the total oil. Two diterpenes and one oxygenated diterpene were also identified in *A. millefolium* oil.

The only data on the chemical composition of essential oil of *A. millefolium* from Bosnia and Herzegovina [15] are those for a commercially available sample from an herbal store. This sample was characterized by a high content of oxygenated monoterpenes (80.4%), with camphor (19.2%) and borneol (15.1%) as the main constituents. Oil of *A. millefolium* shows differences in chemical composition depending on origin, phenologic phase, and the part of the plant [28-30, 41, 42].

According to Nemeth [32], more than 10 components were found to be the main constituents of *A. millefolium* essential oils, which making it difficult to define a characteristic chemotype. Our results confirmed the complexity of the chemical composition of essential oil of *A. millefolium* which is evidenced by the new chemotype for *A. millefolium* from Bosnia and Herzegovina.

It is known that the content of some components in essential oils is highly dependent on environmental factors, such as altitude. The content of *α*- and *β*-thujone, which occurs in *A. millefolium*, decreases and eventually disappears with increasing elevation, [41], while the content of some other components e.g., *trans*-caryophyllene and γ–muurolene remains unchanged. The results of our analyzes for *A. millefolium* oil are in accordance with these statements, since for samples collected at about 1700 m above sea level, none of the thujones was detected in the oil, while the presence of *trans*-caryophyllene 5.7% and γ -muurolene 4.7% was recorded. These data confirm that the composition of the essential oil, as part of the plant's immune system, depends on environmental conditions.

In conclusion, the essential oil composition of two *Achillea* species that grow in the same habitat and are collected at the same time differs significantly. Quantitative and qualitative differences in the profile of essential oils of the two *Achillea* species are notable.

The main component of *A. millefolium* was sesquiterpene elemol (32.9%) which was not detected in essential oil of *A. lingulata*, while the main component of *A. lingulata* essential oil, borneol (30.1%), was identified in a low amount of 2.0% in *A. millefolium* oil. Components, other than borneol, whose content in *A. lingulata* essential oil is greater than 1%, in essential oil of *A. millefolium* oil occur in traces or in a percentage not exceeding 0.2% and *vice versa*.

Oxygenated terpenes are dominant in both oils, but oxygenated monoterpenes represent more than three quarters of the total oil (76.8%) in *A. lingulata*, while oxygenated sesquiterpenes are the most abundant in essential oil of *A. millefolium* (60.6%) (Figure 1).

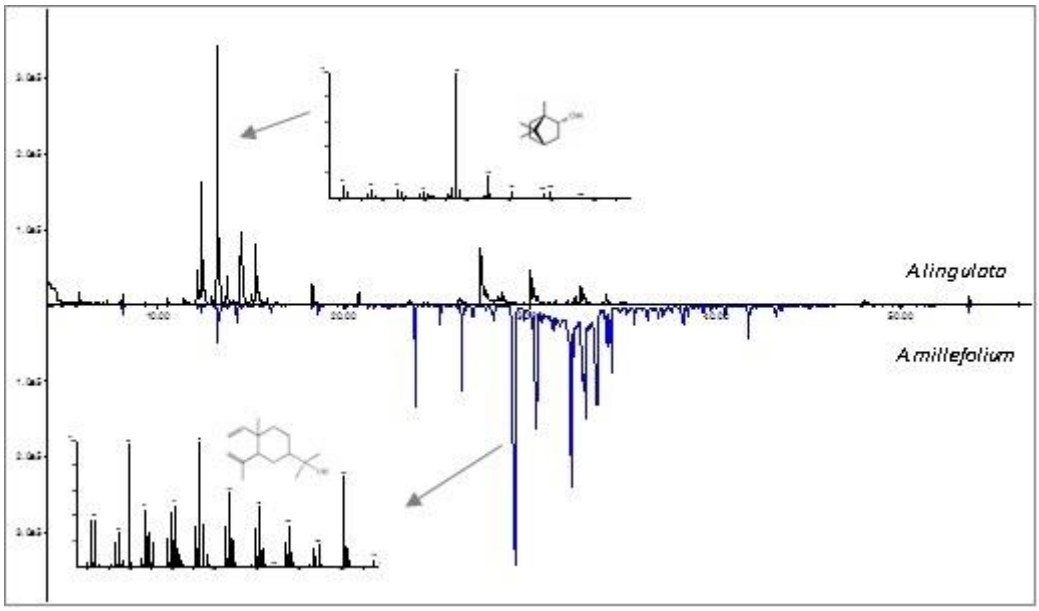

Figure 1. Gas chromatograms of *A. lingulata* and *A. millefolium*

3.2. Antioxidant Activity

The antioxidant activity of isolated essential oils was determined using three different antioxidant methods, ABTS, DPPH and FRAP. The chemical profile of the oils of two *Achillea* species showed significant differences, but although the oil composition was different, both oils showed low antioxidant activity. The results were expressed as Trolox equivalents (Table 2) for ABTS and DPPH method and as L(+)-ascorbic acid equivalent for FRAP method.

Table 2. Antioxidant activity of essential oil of *A. lingulata* and *A. millefolium*

Sample	ABTS	DPPH	FRAP
	µM (TE)/g	µM (TE)/g	mg (AAE)/g
A. lingulata	17.86±0.76	0.0158±0.0006	12.88±0.24
A. millefolium	71.21±0.33	0.0099±0.0001	32.03±0.91

(µM (TE)/g) - Trolox equivalent per gram of oil, (mg (AAE)/g) - L(+)-ascorbic acid equivalent per gram of oil

These are the first results of the antioxidant activity of essential oil for *A. lingulata*. The only data on the antioxidant activity of *A. lingulata* are related to extracts obtained with different solvents and the results showed that extracts had a good antioxidant activity depending on the used solvent [27].

For ABTS method, *A. lingulata* oil had 17.86 µM (TE)/g and the ability to reduce the stabile DPPH radical was 0.0158 µM (TE)/g. The ability to reduce stabile DPPH radical was also weak for *A. millefolium* oil and was 0.0099 µM (TE)/g, but for the ABTS method this oil had almost four times higher activity than *A. lingulata* oil and amounted 71.21 µM (TE)/g. The results obtained by the FRAP method also showed low antioxidant activity for both analyzed essential oils. *Achillea millefolium* oil had a better antioxidant activity 32.03 mg (AAE)/g, than *A. lingulata* 12.88 mg (AAE)/g.

According to literature data [15], the antioxidant activity of oil obtained from a commercial sample of *A. millefolium* from Bosnia and Herzegovina, was low for DPPH assay (IC_{50}=32.75 g/mL) while the ability to reduce ABTS radical was higher (IC_{50}, 0.34 mg/mL). Low antioxidant activity for the DPPH method was also reported by Kazemi [43] (IC_{50}=22.11 mg/mL), although some samples of essential oil of *A. millefolium* showed high activity (IC_{50}=1.56 µg/mL) [44] (15.12 µg/mL) [45].

Our results showed significant differences in the chemical composition of the oils of two *Achillea* species growing wild together. In conclusion, the chemical composition of *A. lingulata* essential oil was different from other previous analyzes of this oil. To the best of our knowledge this is the first time that

trans-verbenol, fragranol and 2-tridecanone have been detected in *A. lingulata*. The sample of *A. millefolium* differed from other published data, as elemol chemotype oil.

It is well known that antioxidant activity depends on the chemical composition where all constituents are in a synergistic or antagonistic relation. Good antioxidant activity of the essential oil is related to the content of the phenolic type terpenes such as thymol, carvacrol, carnosol, carnosolic acid, which are not found in the analyzed oils. Both oils showed low antioxidant activity by DPPH, ABTS and FRAP methods, so regardless of the differences in chemical composition, we can conclude that the components presented in the oil of the two investigated species are not good antioxidants and they have no synergistic effect.

The significance of this research is in the presentation of the content and composition of the essential oil of endemic *Achillea lingulata* from Bosnia and Herzegovina for the first time and its antioxidant activity, as well as the detection of a new chemotype of the well-known *A. millefolium*.

These results confirm again that the chemical profile of the essential oil is variable with respect to environmental factors. This variability forms the backbone of the problem in chemotaxonomic studies.

On the other hand, differences found in the composition of essential oils between two *Achillea* species growing in the same habitats indicate their genetic determinism.

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