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The chemical composition, antimicrobial and antiradical properties of the essential oil of *Achillea grandifolia* aerial parts from Serbia

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ABSTRACT:
Aromatic plants and essential oils have many applications in medicine, pharmaceuticals, cosmetics, and the food industry. The essential oil of the flowering aerial parts of *Achillea grandifolia*, obtained by hydrodistillation, was analyzed for its constituents and investigated for antimicrobial and radical scavenging activity.
The essential oil was characterized by a high amount of oxygenated monoterpenes (72.7%) with 1,8-cineole (29.2%) and camphor (23.4%) being the most abundant. Sesquiterpenes were present in smaller quantities (4.8%). Antimicrobial activity was tested against eight ATCC bacterial strains and two ATCC strains of *Candida albicans*. The essential oil exhibited highly pronounced antimicrobial activity against *Micrococcus luteus* with a MIC value of 3.50 μg/mL, as well as significant antimicrobial activity (<100 μg/mL) against *Staphylococcus aureus*, *S. epidermidis* and *Bacillus subtilis*. Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* were resistant. *Achillea grandifolia* essential oil exhibited concentration-dependent antiradical activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical with an SC_{50} value of 5.4 mg/mL. The TLC-DPPH assay revealed two main light yellow spots indicating components with anti-DPPH activity, which after isolation were identified as 1,8-cineole and camphor.

Keywords:
Achillea grandifolia, essential oil, 1,8-cineole, camphor, antimicrobial activity, antiradical activity

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INTRODUCTION

Essential oils, as complex mixtures of natural compounds, possess various biological activities including antimicrobial, antioxidant, anti-inflammatory, analgesic, immunomodulatory, antithrombotic and insecticidal activity. The interest in aromatic plants and essential oils has grown substantially in the previous decades due to their widespread use in medicine, pharmacy, agriculture, cosmetics and the food industry (Carson & Hammer 2011).

In recent years, many studies have focused on research into essential oils and their components as potential antimicrobial and antioxidant agents (Yang *et al.* 2018; Valdivieso-Ugarte *et al.* 2019).

The genus *Achillea* L., one of the most important genera of the family Asteraceae, includes 110-140 species widely distributed in Europe, Asia, and North Africa, with centers of diversity in Southeast Europe and Southwest Asia (Baltisberger & Widmer 2016). The taxa vary broadly in morphology, life cycle, and ecology. The genus exhibits a complex phyletic structure due to excessive hybridization and polyploidy (Guo *et al.* 2004; Nedelcheva 2008).

Achillea species are important and frequently used medicinal plants. Among them, the species within the *Mille-*

folium section are of the greatest significance. Due to their antiinflammatory, antispasmodic, stomachic and choleretic properties, *Achillea* species are traditionally used for the treatment of spasmodic gastrointestinal complaints, hepatobiliary disorders, minor spasms associated with menstrual periods as well as for temporary loss of appetite and wound healing (Saeidnia *et al.* 2011; Mohammadhosseini *et al.* 2017; Aminkhani *et al.* 2019, 2020).

Considering their vast distribution and various traditionally known applications, the *Achillea* species have attracted great interest among scientists regarding their chemical constituents, pharmacological activities and therapeutic applications (Saeidnia *et al.* 2011; Mohammadhosseini *et al.* 2017). The majority of investigated *Achillea* essential oils exhibit substantial antimicrobial and antioxidant effects (Mohammadhosseini *et al.* 2017).

Achillea grandifolia Friv. is one of the 19 *Achillea* species recorded in the flora of Serbia (Gajić 1975). This perennial is a relict and Balkan endemic species which grows in rocky and shaded places on limestone or siliceous bedrocks. It is also distributed in Albania, North Macedonia, Greece, Bulgaria and western Anatolia (Richardson 1976). This species is phylogenetically isolated within the *Millefolium* section (Nedelcheva 2008).

Research has shown that an infusion prepared from the flowerheads of *A. grandifolia*, originating from Turkey, exhibited antioxidant activity, which was correlated with the total phenol and flavonoid contents (Konyalioglu & Karamenderes 2004, 2005). Various phenolic compounds were identified in *A. grandifolia* flowers, leaves and steams (luteolin-7-O-glucoside, rutin, luteolin, quercetin and chlorogenic acid) (Özek 2018). The methanol extract of *A. grandifolia* was also tested for antibacterial and antioxidant properties (Stanković *et al.* 2016a).

The essential oil of *A. grandifolia* has gained the attention of numerous researchers based on its chemical composition (Hanlidou *et al.* 1992; Radulović *et al.* 2010; Küçükbay & Çetin 2012; Stanković *et al.* 2016b; Özek 2018), antimicrobial (Stanković *et al.* 2016b), antioxidant (Stanković *et al.* 2016b; Özek 2018), as well as acetylcholinesterase and α-amylase inhibitory activities (Özek 2018).

Bearing in mind that the use and research of essential oils as natural antioxidants and antimicrobial agents is a field of growing interest, as well as the chemical diversity of the essential oil of *A. grandifolia* from different localities, the aim of this study was to investigate the chemical composition, antimicrobial and antiradical properties of the essential oil of the flowering aerial parts of *A. grandifolia* from Serbia.

MATERIALS AND METHODS

Plant material and the isolation of the essential oil. The aerial parts of *A. grandifolia* were collected in May 2018, in southeastern Serbia (Sićevo Gorge, N 43.316623°, E 22.175822°), during the period of full flowering. A voucher specimen is deposited at the Herbarium of the Natural History Museum, Belgrade (BEO, K05012018/16).

The essential oil was isolated from the air-dried plant material. The flowering aerial parts were cut and subjected to hydrodistillation for 2.5 h, using a Clevenger-type apparatus, according to the European Pharmacopoeia (Ph. Eur. 2017). The hydrodistillation was performed three times. The essential oil was dried over anhydrous sodium sulfate and kept at 4°C until analysis.

GC-FID/MS analysis. Gas chromatographic analysis (GC-FID/MS) was carried out using an Agilent 6890N gas chromatograph equipped with a flame-ionization detector (FID) combined with an Agilent 5975C MS detector. The chromatographic separation was performed on an HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The carrier gas was He (1.0 mL/min), the temperature of the injector was 200°C and the oven temperature programmed from 60°C to 280°C at a rate of 3°C/min. The injected volume was 1 μL (1% solution of oil in absolute ethanol), the split ratio 10:1. FID and the MSD transfer line temperatures were set at 300 and 250°C, respectively. The EI mass spectra (70 eV) were obtained over the *m/z* range of 35-550.

The identification of the compounds was based on the comparison of their retention indices (RI), their retention times and mass spectra with those from the NIST/NBS and Wiley libraries and the literature (Adams 2007). Additionally, the identification of the compounds was confirmed by co-injection of available authentic standards. The linear RIs were determined in relation to a homologous series of *n*-alkanes (C_8-C_{40}) under the same operating conditions (Adams 2007). The relative percentages of the compounds were calculated based on the peak areas from the FID data.

Antimicrobial activity. The antimicrobial activity was tested against the laboratory control strains from the American Type Culture Collection (ATCC): Gram (+) bacteria *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 6633), *Micrococcus luteus* (ATCC 9341), *Enterococcus faecalis* (ATCC 29212), Gram (–) bacteria *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* NCIMB 9111, *Pseudomonas aeruginosa* ATCC 27853 and two strains of yeast *Candida albicans* (ATCC 10231 and ATCC 24433). The microorganisms were provided by the Institute for Immunology and Virology, Torlak, Belgrade.

For the determination of the minimal inhibitory concentration (MIC) a broth microdilution assay was used (CLSI 2014). The test strains were suspended in the medium (Müller-Hinton broth for bacterial strains and Sabouraud dextrose broth for *C. albicans*) to give a final density of 5×10^5 cfu/mL. Twofold serial dilutions of essential oil in dimethylsulfoxide were prepared in 96-well microtiter plates to obtain final concentrations of the essential oil in

the range of 3.5-1925 μg/mL. Microbial growth was determined after incubation for 24 h at 37°C for the bacteria and 48 h at 26°C for the yeast. All determinations were performed in triplicate. The MICs of standard antibiotics, ampicillin and nystatin were determined in parallel experiments and positive controls of growth were included.

Antiradical activity. The radical scavenging ability was determined by the DPPH assay (Cuendet *et al.* 1997). Different aliquots of the essential oil were mixed with 0.4 mL of 0.5 mM DPPH in absolute ethanol and adjusted up to 2 mL. The mixtures were shaken vigorously and left in the dark for 30 min. Absorbance was measured at 517 nm using ethanol as a blank. One milliliter of 0.5 mM DPPH diluted in 4 mL of absolute ethanol was used as a control. The DPPH radical scavenging activity was calculated using the equation: S(%)=100×(A0-As)/A0, where A0 is the absorbance of the control (containing all the reagents except the tested sample), and As is the absorbance of the tested sample. The SC_{50} value represented the concentration of the essential oil which caused the scavenging of 50% of the DPPH radicals. The activity was compared with some well-known antioxidant compounds (ascorbic acid, rutin and quercetin).

TLC-DPPH test. The thin layer chromatography (TLC)-DPPH assay was employed to determine the active compounds. Fifty microliters of essential oil diluted in absolute ethanol (1:5) were applied to the TLC plate (Silica gel 60 F254, Merck) and developed in toluene-ethyl acetate (93:7) as the mobile phase. One part of the plate was sprayed with vanillin-sulphuric acid reagent and another part with 0.2% DPPH reagent in absolute ethanol. The plates were left in the dark at room temperature and observed after 30 min, 1 and 2 hours. The yellow spots formed from the bleaching of DPPH were considered as positive DPPH radical scavengers (Cuendet *et al.* 1997).

Preparative TLC. After identification of the active zones on the TLC-DPPH plate, in order to isolate the active compounds, preparative TLC analysis was performed. One hundred microliters of essential oil diluted in absolute ethanol (1:5) were applied to two TLC plates (Silica gel 60 F254, Merck). After development in toluene-ethyl acetate (93:7), one part of both plates was sprayed with 0.2% DPPH solution (in absolute ethanol), while the rest of the plates were used to scratch off the corresponding active zones, which were extracted with absolute ethanol. The isolated compounds were identified by GC-FID/MS analysis.

RESULTS

Chemical composition of the essential oil. The average essential oil yield, determined from three hydrodistillations, was 0.35% (w/w). The isolated oil was yellow, with

Table 1. The composition of the essential oil of *Achillea grandifolia*

Compound	RI_{exp} [a]	RI_{lit} [b]	% [c]
α-Pinene	936	932	1.0
Camphene	950	946	2.4
Sabinene	973	969	2.2
β-Pinene	977	974	0.9
dehydro-1,8-Cineole	991	988	0.6
p-Mentha-1(7),8-diene	1002	1003	1.4
α-Terpinene	1015	1014	4.1
p-Cymene	1023	1020	0.5
1,8-Cineole	1028	1026	29.2
γ-Terpinene	1057	1054	0.8
cis-Sabinene hydrate	1067	1065	1.4
Terpinolene	1087	1086	0.3
trans-Sabinene hydrate	1101	1098	1.7
Isopentyl-2-methyl butanoate	1102	1100	0.3
cis-Thujone	1102	1101	tr[d]
Isopentyl isovalerate	1103	1102	0.6
trans-Thujone	1114	1112	tr
cis-*p*-Menth-2-en-1-ol	1121	1118	0.6
Chrysanthenone	1126	1124	0.6
cis-*p*-Mentha-2,8-dien-1-ol	1135	1133	0.6
Camphor	1143	1141	23.4
Pinocarvone	1162	1160	0.6
Borneol	1166	1165	4.6
Terpinen-4-ol	1175	1174	2.0
α-Terpineol	1187	1186	3.6
Myrtenol	1197	1194	0.7
Myrtenal	1197	1195	0.3
Bornyl acetate	1290	1287	1.7
Lavandulyl acetate	1291	1288	0.6
p-Cymen-7-ol	1292	1289	0.5
Eugenol	1357	1356	tr
β-Bourbonene	1388	1387	tr
(*E*)-Jasmone	1392	1390	1.2
(*E*)-Caryophyllene	1419	1417	tr
α-Humulene	1453	1452	tr
(*E*)-β-Farnesene	1455	1454	0.3
ar-Curcumene	1481	1479	tr
Germacrene D	1482	1484	1.3
(*E*)-β-Ionone	1489	1487	tr
Bicyclogermacrene	1502	1500	0.1
(*E*,*E*)-α-Farnesene	1507	1505	0.8
β-Sesquiphellandrene	1522	1521	0.3
(*E*)-Nerolidol	1563	1561	0.6
Spathulenol	1579	1577	0.9
Caryophyllene oxide	1583	1582	0.5
Caryophylla-4(12),8(13)-dien-5β-ol	1642	1639	tr
β-Eudesmol	1651	1649	tr
Identified			93.2
Grouped components			
Monoterpene hydrocarbons			13.6
Oxygenated monoterpenes			72.7
Sesquiterpene hydrocarbons			2.8
Oxygenated sesquiterpenes			2.0
Others			2.1

[a] The retention indices relative to C_8-C_{40} *n*-alkanes experimentally determined on the HP-5MS column; [b] The retention indices obtained from the literature (Adams 2007); [c] The relative area percentage-values are the mean of the three analyses; [d] trace (< 0.1%)

Table 2. The antimicrobial activity of the investigated *Achillea grandifolia* essential oil and standard antibiotics

Microorganism	*A. grandifolia* essential oil	Antibiotic	
		Ampicilin	Nystatin
	MIC[a] (mg/mL)		
Staphylococcus aureus ATCC 25923	64.17	0.5	n.t.[b]
Staphylococcus epidermidis ATCC 12228	64.17	0.25	n.t.
Bacillus subtilis ATCC 6633	96.25	0.8	n.t.
Micrococcus luteus ATCC 9341	3.50	2.8	n.t.
Enterococcus faecalis ATCC 29212	770.00	0.5	n.t.
Escherichia coli ATCC 25922	> 1540.00	2.0	n.t.
Klebsiella pneumoniae NCIMB 9111	224.58	2.0	n.t.
Pseudomonas aeruginosa ATCC 27853	> 1925.00	3.0	n.t.
Candida albicans ATCC 10231	160.42	n.t.	3.8
Candida albicans ATCC 24433	525.00	n.t.	4.2

[a] Minimal inhibitory concentration (μg/mL); [b] not tested

Table 3. Classes of terpenes and the most abundant components of the investigated and previously analyzed *A. grandifolia* essential oils

Plant part	Origin	Monoterpenes (%)		Sesquiterpenes (%)	Main components	Reference
		Oxygenated	Hydrocarbons			
Flowering aerial parts	NW Greece, Vikos Gorge	68.0	14.76	2.31	camphor (25.6%) 1,8-cineole (12.8%) *α*-thujone (11.9%) *β*-thujone (9.2%) *p*-cymene (6.7%)	Hanlidou *et al.* 1992
Flowering aerial parts	E Serbia, Jerma Gorge (Dimitrovgrad vicinity)	65.9	0.3	14.0	camphor (15.6%) ascaridole (15.5%) *α*-thujone (7.5%) (*Z*)-jasmone (6.4%) borneol (5.2%)	Radulović *et al.* 2010
Leaves and flowers	Turkey, Izmir	57.5	5.4	6.4	piperitone (34.0%) carvacrol (7.0%) *p*-cymene (5.0%)	Küçükbay & Çetin 2012
	Turkey, Aydin	64.9	10.6	3.0	1,8-cineole (32.0%) piperitone (18.7%) *p*-cymene (10.0%)	
Flowering aerial parts	E Serbia, Jerma Gorge (Trnski Odorovci)	89.15	7.19	-	camphor (45.4%), 1,8-cineole (16.4%) *α*-thujone (15.1%) borneol (8.13%)	Stanković *et al.* 2016b
Flowering aerial parts	Turkey, Antalya province	76.2	20.6	1.1	α-terpinyl acetate (54.1%) *p*-cymene (17.7%) *cis*-piperitone-oxide (7.5%)	Özek 2018
Flowering aerial parts	E Serbia, Sićevo Gorge	72.7	13.6	4.8	1,8-cineole (29.2%) camphor (23.4%) borneol (4.6%) *α*-terpinene (4.1%)	This research

an aromatic, and pleasant smell. The chemical composition of the oil is summarized in Table 1. GC-FID/MS analysis resulted in the identification of 47 components which accounted for 93.2% of the total oil.

The essential oil obtained from the flowering aerial parts of *A. grandifolia* was characterized by a high amount of oxygenated monoterpenes (72.7%), with 1,8-cineole (29.2%) and camphor (23.4%) being the most abundant components. Among the remaining compounds, which were present in less than 5%, borneol (4.6%) and α-terpinene (4.1%) were in higher amounts. Sesquiterpenes were present in much smaller quantities (4.8%).

Antimicrobial activity. The antimicrobial activity of the *A. grandifolia* essential oil was evaluated against 10 microorganisms, including five Gram-positive bacteria, three Gram-negative bacteria, and two strains of yeast *Candida albicans*. The results of the antimicrobial activity, ex-

pressed as minimal inhibitory concentrations (MIC), are presented in Table 2.

The essential oil exhibited a very pronounced antimicrobial activity against *Micrococcus luteus* with a MIC value of 3.50 μg/mL. The tested oil also showed significant antimicrobial activity (MICs<100 μg/mL) (Cos *et al.* 2006) against *Staphylococcus aureus* (MIC 64.17 μg/mL), *S. epidermidis* (MIC 64.17 μg/mL) and *Bacillus subtilis* (MIC 96.25 μg/mL), as well as moderate activity against *Candida albicans* ATCC 10231 (MIC 160.42 μg/mL). The Gram-negative bacteria *Klebsiella pneumoniae* was less sensitive with a MIC of 224.54 μg/mL, whereas *Escherichia coli* and *Pseudomonas aeruginosa* were resistant, which is in line with the accepted opinion that essential oils are generally more active against Gram-positive than against Gram-negative bacteria (Kozics *et al.* 2019).

Antiradical activity. In the DPPH test, the essential oil of *A. grandifolia* exhibited concentration-dependent antiradical activity, with a SC_{50} value of 5.4 mg/mL. Ascorbic acid and flavonoids rutin and quercetin showed substantial antiradical activities, with SC_{50} values of 4.09, 5.75 and 2.75 μg/mL, respectively. The antiradical capacity of the investigated essential oil was lower than that of standard antioxidants, but was much more pronounced compared to previous research on the anti-DPPH activity of *A. grandifolia* essential oils from the Jerma Gorge (IC_{50}=33.6 mg/mL) (Stanković *et al.* 2016b) and Antalya province (10 mg/mL) (Özek 2018).

The TLC-DPPH test revealed four DPPH radical neutralizing zones, which were extracted and identified by GC-FID/MS analysis. The zones corresponding to 1,8-cineole (Rf=0.5) and camphor (Rf=0.59) were the most active; they appeared shortly after spraying with DPPH reagent. Two more zones (Rf=0.44 and Rf=0.9), which were revealed during the following 2 hours, corresponded to components with lower anti-DPPH activity: α-terpinene and α-pinene, respectively.

DISCUSSION

A large number of *Achillea* species have been investigated in terms of the content, composition and pharmacological activities of their essential oils. Studies on the composition of essential oils of different *Achillea* species have shown that their main ingredients are primarily oxygenated monoterpenes, with camphor, 1,8-cineole, sabinene, borneol, thujones, linalool and α-terpineol as the most dominant components. The sesquiterpenoid fraction is usually less prominent, mainly represented by germacrene D, β-caryophyllene, cadinol derivatives and α-bisabolol (Kindlovits & Németh 2012; Mohammadhosseini *et al.* 2017).

The results obtained in our study are partly in line with previous investigations of *A. grandifolia* essential oils. Our sample was rich in oxygenated monoterpenes (72.7%) as were the previously analyzed samples (Table 3). In addition, similarities were also observed in terms of the dominant components. 1,8-cineole and camphor were also the most abundant compounds in previously analysed *A. grandifolia* essential oils from Vikos (Hanlidou *et al.* 1992) and the Jerma Gorge (Stanković *et al.* 2016b), but in contrast to our sample where 1,8-cineole prevailed, camphor was more dominant in the mentioned oils. Analysis of the essential oil from the leaves and flowers of *A. grandifolia* originating from the Aydin province revealed that the major compounds were 1,8-cineole (32.0%), piperitone (18.7%) and *p*-cymene (10.0%) (Küçükbay & Çetin 2012), while in our sample piperitone was not detected and *p*-cymene was present in low quantities (0.5%). In another study of *A. grandifolia* essential oil, also originating from the Jerma Gorge, camphor (15.6%) was one of the most dominant components similar to our sample, but the other abundant components, i.e. ascaridole (15.5%), α-thujone (7.5%) and (*Z*)-jasmone (6.4%) were quite different. This sample also contained a considerable amount of sesquiterpenes (14.0%) (Radulović *et al.* 2010).

On the other hand, two essential oil samples of *A. grandifolia* from Turkey showed quite different chemical compositions, one (from Antalya) (Özek 2018) with α-terpinyl acetate (54.1%), *p*-cymene (17.7%) and *cis*-piperitone-oxide (7.5%) and another (from Izmir) (Küçükbay & Çetin 2012) with piperitone (34.0%) and carvacrol (7.0%) as the main constituents (Table 3).

It should be noted that some previously analysed *A. grandifolia* essential oils contain substantial amounts of certain compounds that may pose risks to human health such as thujones (8.9-22.1%) (Hanlidou *et al.* 1992; Radulović *et al.* 2010; Stanković *et al.* 2016b), as well as ascaridole (15.5%) (Radulović *et al.* 2010). In our sample, thujones were present in traces, whereas ascaridole was not detected.

The qualitative and quantitative differences in the chemical composition of *A. grandifolia* essential oils suggest that environmental factors and probably different periods of plant material collection strongly influence its chemical composition.

In a previous investigation of antimicrobial activity, the essential oil of *A. grandifolia* demonstrated inhibitory and bactericidal effects against various microbial strains isolated from human material, although in much a higher range of MICs from 5.77-46.15 mg/mL (Stanković *et al.* 2016b) than in this study.

The demonstrated antimicrobial activity of the investigated *A. grandifolia* essential oil might be related to the high content of 1,8-cineole and camphor, which constitute more than 50% of the investigated essential oil. Both compounds have known antimicrobial properties (Hammer & Carson 2011; Bilia *et al.* 2014). In addition, the synergistic activity of 1,8-cineole and camphor against some bacteria have already been observed (Viljoen *et al.* 2003; Koroch *et al.* 2007). The components present

in lower amounts such as borneol (Santoyo *et al*. 2005), α-terpinene, α-terpineol and terpinen-4-ol (Koroch *et al*. 2007), could contribute to the antimicrobial activity since their inhibitory effects on several microorganisms have been previously reported. Also, these minor components could be included in some type of synergism with other active compounds (Marino *et al*. 2001).

Literature data indicate different antiradical capacities of DPPH radical neutralizing components revealed in the TLC-DPPH assay (Kim *et al*. 2004; Koroch *et al*. 2007; Wang *et al*. 2019). It was previously reported that 1,8-cineole, as a constituent of *Myrtus communis* essential oil, shows considerable DPPH scavenging activity in the TLC-DPPH assay (Mimica-Dukić *et al*. 2010). Both 1,8-cineole and camphor were detected and isolated from *Curcuma wenyujin* essential oil, as components with moderate and weak antiradical activity, respectively (Wang *et al*. 2020). In addition, essential oils of *Achillea millefolium* subsp. *millefolium* (Candan *et al*. 2003) and *A. vermicularis* (Polatoğlu *et al*. 2013), which share a similar chemical pattern as the investigated *A. grandifolia* essential oil (1,8-cineole and camphor as the main constituents), also exhibited significant anti-DPPH activity. Our results point out that the observed antioxidative activity of *A. grandifolia* essential oil can be attributed to its main compounds, 1,8-cineole and camphor. Still, the fact already observed by some researchers that minor compounds and synergistic effects may significantly contribute to the expressed activity, should not be neglected (Candan *et al*. 2003; Koroch *et al*. 2007).

CONCLUSION

The chemical analysis of the essential oil from the flowering aerial parts of *Achillea grandifolia* showed that 1,8-cineole and camphor were the most abundant compounds. Although some previously analyzed *A. grandifolia* essential oils contain significant amounts of certain components of concern, in the investigated sample thujones were present in traces, whereas ascaridole was not detected.

The essential oil exhibited very strong antimicrobial activity against *Micrococcus luteus*, as well as significant antimicrobial activity against Gram-positive bacteria *Staphylococcus aureus*, *S. epidermidis*, and *Bacillus subtilis*, whereas Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* were resistant.

In the DPPH assay, the essential oil exhibited concentration-dependent antiradical activity, which can be attributed to its main components, 1,8-cineole, and camphor.

The essential oil of the flowering aerial parts of *A. grandifolia* originating from Sićevo Gorge from Serbia, with its advantageous chemical profile and observed antimicrobial and radical scavenging activities, is a promising candidate for further research in order to define its potential use in the pharmacy, food, and cosmetic industries.

Since *A. grandifolia* is an endemic plant, for further research and potential application it is necessary to consider the use of cultivated plants in order to obtain a larger amount of plant material and to protect natural habitats.

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REZIME

Botanica SERBICA

Hemijski sastav, antimikrobna i antiradikalska svojstva etarskog ulja nadzemnih delova *Achillea grandifolia* iz Srbije

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Aromatične biljke i etarska ulja nalaze primenu u medicini, farmaciji, kozmetičkoj, parfimerijskoj i prehrambenoj industriji. Etarsko ulje iz nadzemnih delova u cvetu *Achillea grandifolia* izolovano je destilacijom vodenom parom i ispitivano u pogledu hemijskog sastava, antimikrobne i antiradikalske aktivnosti. Etarsko ulje se odlikovalo velikom količinom oksidovanih monoterpena (72,7%) sa 1,8-cineolom (29,2%) i kamforom (23,4%) kao dominantnim jedinjenjima. Seskviterpeni su bili prisutni u manjoj količini (4,8%). Antimikrobna aktivnost je ispitivana prema osam ATCC sojeva bakterija i dva ATCC soja *Candida albicans*. Etarsko ulje je pokazalo veoma izraženu antimikrobnu aktivnost prema *Micrococcus luteus* sa MIC vrednošću od 3,50 μg/mL, kao i značajnu aktivnost prema *Staphylococcus aureus*, *S. epidermidis* i *Bacillus subtilis*. Gram-negativne bakterije *Escherichia coli* i *Pseudomonas aeruginosa* bile su rezistentne. Etarsko ulje je ispoljilo dozno-zavisnu antiradikalsku aktivnost prema DPPH radikalu sa SC_{50} vrednošću od 5,4 mg/mL. U TLC-DPPH testu uočene su dve glavne žute zone koje odgovaraju anti-DPPH aktivnim jedinjenjima, a koja su nakon izolovanja identifikovana kao 1,8-cineol i kamfor.

Ključne reči: *Achillea grandifolia*, etarsko ulje, 1,8-cineol, kamfor, antimikrobna aktivnost, antiradikalska aktivnost