BIOLOGICA NYSSANA
13 (2) December 2022: 179-189
DOI: 10.5281/zenodo.7437345

The essential oil composition of different parts of *Artemisia absinthium* and its antibacterial activity against phytopathogenic bacteria

Original Article

Maja Radulović
Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia
maja.radulovic@bio.bg.ac.rs (corresponding author)

Milan Gavrilović
Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia

Nemanja Rajčević
Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia

Tamara Janakiev
Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia

Ivica Dimkić
Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia

Pedja Janaćković
Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia

Abstract:

The composition of essential oil (EO) from different aerial parts of *Artemisia absinthium* L. (Asteraceae), from Serbia, was analyzed. Shoots without leaves and inflorescences (SWLI), leaves (L), and inflorescences (I) were subjected to hydrodistillation using the Clevenger-type apparatus. Analysis of EO was done by gas chromatography with MS of flame ionization detection (GC/MS and GC/FID). Microdilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Organoleptic characteristics and quantitative and qualitative differences were documented between examined EOs. In total, 17 compounds were identified in SWLI, 11 in L and 18 in I. The principal constituent in all EOs was *trans*-thujone (SWLI - 23.56%, L - 60.41%, and I - 46.16%). The most susceptible strains were *Xanthomonas campestris* pv. *campestris, Erwinia amylovora* and *Rathayibacter tritici*. The strongest activity was shown for SWLI EO against *E. amylovora* (0.03 mg/mL). The same MIC values were observed for L EO against *E. amylovora* and *R. tritici* (0.09 mg/mL). The equal activity for all EOs tested was detected against *X. campestris* (0.13 mg/mL).

Key words:
wormwood, Asteraceae, hydrodistillation, volatile terpenes, antibacterial activity

Apstrakt:

Sastav etarskog ulja iz različitih delova vrste *Artemisia absinthium* i njegova antibakterijska aktivnost protiv fitopatogenih bakterija

U ovom radu je analiziran sastav etarskog ulja (EU) iz različitih nadzemnih delova vrste *Artemisia absinthium* L. (Asteraceae) iz Srbije. Izdanci bez listova i cvasti (IBLC), listovi (L) i cvasti (C) podvrgnuti su hidrodestilaciji pomoću Klevendžerove aparature. Analiza EU je izvršena upotrebom GH/MS (gasne hromatografije i masene spektrometrije). Mikrodilucionom metodom određena je minimalna inhibitorna koncentracija (MIK) i minimalna baktericidna koncentracija (MBK). Utvrđeni su prinos (%, w/w), organoleptičke karakteristike (boja i miris), kao i kvantitativne i kvalitativne razlike između ispitivanih etarskih ulja. Ukupno je identifikovano 17 komponenti u EU IBLC, 11 u EU L i 18 u EU I. Dominantno jedinjenje u svim etarskim uljima je *trans*-tujon (IBLC - 23,56%, L - 60,41%, i C - 46,16%). Najosjetljiviji sojevi bili su *Xanthomonas campestris* pv. *campestris*, *Erwinia amylovora* i *Rathayibacter tritici*. Najveću aktivnost pokazalo je EU IBLC protiv *E. amylovora* (0,03 mg/mL). Iste MIK vrednosti dobijene su za EU L protiv *E. amylovora* i *R. tritici* (0,09 mg/mL). Sva testirana etarska ulja pokazala su istu aktivnost protiv *X. campestris* (0,13 mg/mL).

Ključne reči:
pelin, Asteraceae, hidrodestilacija, isparljivi terpeni, antibakterijska aktivnost

Received: October 04, 2022
Revised: December 01, 2022
Accepted: December 02, 2022

Introduction

Artemisia absinthium L. (Anthemideae, Asteraceae), commonly known as wormwood, is a perennial semi-shrubby plant up to 120 cm high (Gajić, 1975; Bora & Sharma, 2011) which grows in dry habitats in Eurasia, North and South America (Deans & Kennedy, 2001). This herb is the main ingredient of one of the most popular alcoholic beverages – absinthe (Padosch et al., 2006). Wormwood is known for its medical properties both in traditional medicine and in modern pharmacology (Nguyen et al., 2018;

Ahamad, 2019). As a remedy, wormwood is used for stomachache, kidney and gallbladder diseases, insomnia, and for the treatment of many other diseases and disorders (Judzentiene et al., 2012). In Serbia, a few ethnobotanical studies showed that it has been traditionally used as a diuretic and against respiratory, urinary, and digestive system disorders (Jarić et al., 2015; Živković et al., 2020).

The most significant active constituents of the wormwood are volatile compounds and bitter substances which have attracted the interest of many researchers and producers throughout the world. Numerous studies have shown that wormwood displays significant intraspecific variation in the terpene constituents of the EO (Basta et al. 2007; Mohammadi et al., 2015). Many previous researchers have examined EOs from herba of *A. absinthium* and have shown that the main component was bicyclic monoterpene – thujone (Juteau et al., 2003; Blagojević et al., 2006). Both isomeric forms, *cis*- and *trans*-thujone were present, but the EO was characterized by a higher percentage of *trans*-thujone (Benkhaled et al., 2020). Besides thujone, which is convulsant and has toxic activity (Olsen, 2000), some other natural products were found in the oil: (*Z*)-epoxyocimene, chrysanthenyl acetate (Juteau et al., 2003; Nguyen et al., 2017), caryophyllene oxide, *p*-cymene, 1,8-cineole (Basta et al., 2007), sabinene, sabinyl acetate, α-phellandrene (Mihajilov-Krstev et al., 2014), camphor, and chamazulene (Benkhaled et al., 2020).

The chemical composition of wormwood EO varies significantly not only depending on the individual genetic variability, but also according to phenological stage and the plant part used (Nguyen & Németh 2016; Nguyen et al., 2018). However, data regarding EO composition of separated plant parts are scarce. There are few papers dealing with variability in volatile composition regarding plant organ in wormwood, mainly aerial parts and roots (Blagojević et al., 2006), or leaves and flowers (Judzentiene & Budiene, 2010; Riahi et al., 2013), while shoots without leaves and inflorescences have not been analyzed to date.

The wormwood EO has a broad spectrum of biological properties: antioxidant, antimicrobial (Mihajilov-Krstev et al., 2014; Riahi et al., 2015), antiparasitic (Yildiz et al., 2011), cytotoxic activity (Taherkhani, 2014), and insecticidal and repellent effect (Mihajilov-Krstev et al., 2014). Many previous studies investigated the antibacterial activity of wormwood EO, but they have been mainly focused on human pathogens (Blagojević et al., 2006; Mihajilov-Krstev et al., 2014), while only a few studies were focused on phytopathogens (Kordali et al., 2005). Unfortunately, crop loss represents a problem due to plant diseases caused by insects and plant pathogens. Since widely used synthetic chemicals in plant disease control are associated with undesirable effects and some toxic residues in the products (Isman, 2000), there has been a growing interest in research concerning alternative pesticides and active antimicrobial compounds, including the plant extracts and EOs.

To date, there are no data regarding the antimicrobial activity of separated parts of wormwood against phytopathogens, but there are only few studies against human pathogens (Joshi, 2013; Vieira et al., 2016). Investigation of the EO composition of different plant parts as well as their antimicrobial activity deserves special attention, bearing in mind that a specific chemical composition could significantly affect biological activities. Therefore, the objectives of the present work were to (1) isolate and analyze the composition of EO of separated parts of *A. absinthium*; (2) investigate their antibacterial properties against selected phytopathogenic bacterial strains; and (3) highlight their potential future importance in chemophenetics and agriculture.

Materials and Methods

Plant material

Plant material of *Artemisia absinthium* was collected during the flowering period in village Reka, near Kladovo (Eastern Serbia, 44°29'35''N, 22°24'55'' E) in August 2021. The plants were identified using appropriate professional literature (Gajić, 1975). A voucher specimen was deposited at the Herbarium of the University of Belgrade – Faculty of Biology, Institute of Botany and Botanical Garden „Jevremovac" (BEOU 17804). The collected material was dried at room temperature and then divided into three groups: (1) shoots without leaves and inflorescences (SWLI), (2) leaves (L), and (3) inflorescences (I).

Isolation of essential oil

Separated plant material (SWLI – 172 g, L – 72 g and I – 124 g) was chopped and subjected to hydrodistillation using a Clevenger type apparatus for 3 h, according to the procedure described in Ph. Eur. 6. (European Directorate for the Quality of Medicines, 2007). The obtained EOs were stored at 4 °C before the analysis by gas chromatography with MS of flame ionization detection (GC/MS and GC/FID).

GC-FID and GC/MS analysis

The GC-FID and GC/MS analyses were carried out with an Agilent 7890 A apparatus equipped with

a 5975 C mass-selective detector (MSD), a flame ionization detector (FID), and an HP-5 MSI fused-silica cap (column length 30 m, diameter 0.25 mm, film thickness 0.25 mm). The oven temperature was programmed linearly, rising from 60 °C to 240 °C at 3 °C/min; the injector temperature was 220 °C; the detector temperature was 300 °C, and the transfer-line temperature was 240 °C. The carrier gas was He (flow rate: 1.0 mL/min at 210 °C, constant pressure mode) at an injection volume of 1 μL and a split ratio of 10:1. Electron impact mass spectra (EI-MS; 70 eV) were acquired over the *m/z* range 40–550. Library search and mass spectral deconvolution and extraction were performed using the NIST AMDIS (automated mass spectral deconvolution and identification system) software, version 2.64.113.71, with the retention index (RI) calibration data analysis parameters set to the strong level and a 10% penalty for compounds without a RI. The RIs were experimentally determined using the standard method involving retention times (Rt) of *n*-alkanes, which were injected after the essential oil under the same chromatographic conditions. The search was performed against our home-made library, containing 4972 spectra. The relative contents of identified compounds were computed from the GC peak areas.

Tested bacterial strains and growth conditions

Antibacterial activity was tested using one Gram-positive (*Rathayibacter tritici*), and five Gram-negative phytopathogenic bacterial strains previously identified: *Agrobacterium tumefaciens*, *Erwinia amylovora*, *Pantoea alli*, *Pseudomonas oryzihabitans*, *Xanthomonas campestris* pv. *campestris*. The bacterial strains were cultured in LB medium (composition g/L: tryptone 10, yeast extract 5, NaCl 5) for 24 h at 30 °C. Suspensions were prepared in phosphate saline buffer (1×PBS, Sigma Aldrich, USA) in the final concentration of 10^6 CFU/mL.

MIC assay

Microdillution method (MIC assay) described in a previous study (Ristivojević et al., 2016) was used to determine the minimum inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of the *A. absinthium* essential oils. Two-fold serial dilutions with LB medium in 96-well microtiter plates were performed. Except for the sterility control, each well was inoculated with 20 μL of bacterial suspensions (1×10^6 CFU/mL), reaching a final volume of 200 μL. Besides a negative control, a sterility control, and control for the solvent (MeOH), the antibiotics gentamicin and kanamycin were tested as positive controls in the final concentration of 0.025 mg/mL. The final concentration of methanol (MeOH) as a solvent was 10%. All dilutions were done in duplicate, and the results were expressed in mg/mL. After reaching the final volume, 22 μL of resazurin as an indicator was added, and the 96-well microtiter plates were incubated for 24 h at 30 °C. According to the resazurin reaction, the lowest concentration, which showed no change in color was defined as the MIC. The lowest concentration that after sub-culturing did not show bacterial growth overnight was defined as the MBC value.

Results and discussion

Organoleptic characteristics, yield and composition of investigated EOs

The yield and organoleptic characteristics of the EOs of separated parts of *A. absinthium* are given in **Tab. 1**. The lowest yield (w/w) was recorded for SWLI EO (0.05%), while the highest was recorded for L EO (0.29%). This may be in relation with the abundance of glandular trichomes and the presence of secretory canals in the plant parts. Although secretory canals are found only in the stem, while glandular trichomes were found both on the stem and leaves of *A. absinthium* (Janaćković et al., 2019), it can be considered that certain mass of leaves, used for distillation, contain more glandular trichomes which contribute to higher EO yield. Riahi et al. (2013) recorded variation between yields of the flowers and leaves EOs. The highest yield was recorded for flowers EO (2.98%) whereas leaves EO exhibited a lower yield (1.87%) (Riahi et al. 2013). On the contrary, we have shown that leaves produced more EO than inflorescences (**Tab. 1**).

It was noted that the color of obtained EOs had changed over time. The L and I EO samples were light orange at the beginning of distillation but became dark brown at the end of distillation. The SWLI EO was orange-brown at the beginning and during distillation, but then changed color and became brown at the end of distillation. After 24 h in the refrigerator, the color of this oil was greenish, and after a few days, it was light yellow (**Tab. 1**). Wormwood EOs, obtained from aerial parts of the plant, has a dark green to orange or dark brown color (sensitive to ultraviolet/visible light) (Judzentiene, 2016). Dark blue EO was reported by Riahi et al. (2013) and Msaada et al. (2015). On the other hand, according to Pino et al. (1997), the EO obtained from the dried leaves and flowering tops of wormwood varied from dark green to brown or dark brownish green. Different colors of EO can be a consequence of the presence/absence of different constituents.

Besides the color, the smell of EOs was also specific. All of the obtained EOs had a specific and

Table 1. Yield and organoleptic characteristics of EOs of separated parts of *A. absinthium*

Sample	Dry plant material (g)	Obtained oil (g)	Yield (%, w/w)	Smell	Color			
					start of distillation	end of distillation	after 24 h	after few days
SWLI[1]	172	0.09	0.05	strong; unpleasant; like tobacco	orange-brown	dark brown	green	light yellow
L	72	0.21	0.29	strong; very unpleasant; bitter; typically wormwood	light orange	dark brown	dark brown	dark brown
I	124	0.26	0.21	less unpleasant; bitter	light orange	dark brown	dark brown	dark brown

[1]**SWLI** - shoots without leaves and inflorescences; **L** - leaves; **I** - inflorescences

unsavory odor. SWLI EO had an odor very similar to the smell of tobacco. L EO showed the strongest and most unpleasant fragrance in comparison with the other two samples. This smell was described as „typically wormwood", which reminds of bitter constituents. I EO was also recognized as fragrance with bitter characteristics. Because of high concentrations of volatile terpenes, especially in leaves and inflorescences, the EO of this species has strong aromatic smell (Nguyen & Németh, 2016).

Phytochemical analyses of investigated EOs showed some qualitative and quantitative differences. The conducted GC-FID and GC/MS analyses detected and identified a total of 27 compounds (17 in the SWLI EO, 11 in L EO, and 18 in I EO). All compounds are listed in **Tab. 2**. More compounds were identified in I EO, than in L EO, which is in agreement with the investigation done by Riahi et al. (2013). The dominant group of compounds in the L EO and I EO were oxygenated monoterpenes (81.80% and 62.63%, respectively). On the other hand, SWLI EO was not dominated by terpenes (other compounds including fatty acid derivates, esters, were found in higher abundance, 62.15%). This finding is important as there are no studies dealing with EO from shoots without leaves and inflorescences. The L EO was also rich in monoterpene hydrocarbons (15.26%), while I EO and SWLI EO contain these compounds in smaller quantities (8.94% and 7.85%, respectively). Sesquiterpenes were present only in two analyzed EOs, but in low amounts (2.94% in L EO and 1.19 in I EO). Thus, monoterpenes were found in higher abundance than sesquiterpenes both in L EO and I EO, which also showed Nguyen et al. (2018). On the other hand, SWLI EO lacks sesquiterpenes, while L EO lacks other compounds. Blagojević et al. (2006) documented differences between aerial parts EO and root EO, where monoterpenes (84.6%) dominated in the aerial parts EO, while aliphatic esters (64.5%) were major compounds of the root EO. In addition, a predominance of oxygenated monoterpenes (81.4-89.1%) was documented in aerial parts EO, while the root EO showed high ratios of hydrocarbon monoterpenes (43.8–55.1%) and monoterpene esters (36.6–41.5%) (Llorens-Molina & Vacas 2015).

The dominant compound in all three EOs was *trans*-thujone (in SWLI EO 23.6%, in L EO 60.4%, in I EO 46.2%). Wormwood is usually known and reported to be rich in thujone (Meschler & Howlett, 1999; Juteau et al., 2003). The concentration of *trans*-thujone was the highest in the L EO (60.4%), which is in agreement with Riahi et al. (2013). However, Judzentiene & Budiene (2010) determined higher ratios of thujone in flowers EO (5.3-10.4%) than in leaves EO (0.0-8.9%). It should be noted that for the praxis the ratio of plant parts may play an important role in reaching lower thujone levels of the drug. In our study, less thujone content was detected in inflorescences EO, thus *A. absinthium* drug should contain more flowers than leaves which was also concluded by Nguyen et al. (2019). On the other hand, it was shown that thujone is not necessarily the main compound of the essential oil of wormwood (Nguyen et al., 2017). In addition, Nguyen et al. (2017) showed that *trans*-thujone is the major compound, however, in some cases, *cis*-thujone reached comparable percentages, while no sample was found where this latter one would have been the only isomer. In this study, we documented that SWLI EO and I EO lack *cis*-thujone.

Other dominant compounds were as follows:

Table 2. Composition of EOs of different parts of *A. absinthium*

No	RI*	Compounds	%		
			SWLI[1]	L	I
1	923	*α*-Thujene	-	1.12	1.01
2	943	*α*-Fenchene	1.65	-	-
3	971	Sabinene	1.51	6.23	4.17
4	1024	*p*-Cymene	4.68	7.08	2.62
5	1031	1,8-Cineole	-	2.67	4.28
6	1059	*γ*-Terpinene	-	0.83	1.14
7	1104	Linalool	-	-	4.54
8	1110	*cis*-Thujone	-	1.12	-
9	1122	*trans*-Thujone	23.56	60.41	46.16
10	1134	*(Z)-epoxy*ocimene	2.93	15.23	5.22
11	1137	*iso*-3-Thujanol	-	0.99	-
12	1178	Terpinen-4-ol	-	1.38	2.42
13	1228	Nerol	3.51	-	-
14	1291	Lavandulyl acetate	3.49	-	-
15	1425	Lavandulyl isobutanoate	9.81	-	1.27
16	1488	*β*-Selinene	-	-	1.19
17	1491	Neryl isobutanoate	5.73	-	2.44
18	1511	Geranyl isobutanoate	11.65	-	3.15
19	1513	Lavandulyl 2-methylbutanoate	4.03	-	1.52
20	1578	Geranyl butanoate	8.94	-	7.97
21	1584	Caryophyllene oxide	-	2.94	-
22	1585	Neryl isovalerate	5.29	-	6.40
23	1603	Fatty acid ester	4.25	-	-
24	1609	Geranyl isovalerate	2.35	-	-
25	2008	Fatty acid ester	2.47	-	1.78
26	2014	Fatty acid ester	-	-	2.72
27	2082	Fatty acid ester	4.16	-	-
		Total monoterpenes	**37.85**	**97.06**	**71.57**
		Monoterpene hydrocarbons	7.85	15.26	8.94
		Oxygenated monoterpenes	30.00	81.80	62.63
		Total sesquiterepenes	**0.00**	**2.94**	**1.19**
		Sesquiterpene hydrocarbons	0.00	0.00	**1.19**
		Oxygenated sesquiterpenes	0.00	2.94	0.00
		Other	**62.15**	**0.00**	**27.24**
		Total	**100.00**	**100.00**	**100.00**
		No. of compounds	**17**	**11**	**18**

Contents of the total essential oil composition are given as percentages; -: not detected; *Linear retention index was calculated for all compounds using the following formula: LRI=100•(trs-trn)/((trn+1-trn)+100•n; Other - non terpenoid compounds, i.e. alyphatic and aromatic hydrocarbons; MS spectra of unidentified fatty acids are given in the **Appendix 1**.

[1]**SWLI** - shoots without leaves and inflorescences; **L** - leaves; **I** - inflorescences

geranyl isobutanoate and lavandulyl isobutanoate (11.65%, 9.81%, respectively) in SWLI EO; (*Z*)-epoxyocimene and *p*-cymene (15.23%, 7.08%, respectively) in L EO; and geranyl butanoate and neryl isovalerate (7.97%, 6.40% respectively) in I EO. Some constituents were specific for certain examined EO. For example, six compounds were found only in SWLI EO: α-fenchene, nerol, lavandulyl acetate, geranyl isovalerate, and two fatty acid esters; three were found only in L EO: *cis*-thujone, *iso*-3-thujanol and caryophyllene oxide; while three were found only in I EO: linalool, *β*-selinene and one fatty acid ester. Nguyen et al. (2018) also showed qualitative differences in EO profiles in *A. absinthium* thujone chemotype, e.g. geranyl-*p*-cymene, nuciferol esters and two unknown compounds were present only in the flower oil. Also, neryl-isobutanoate, neryl-isovalerate and caryophyllene oxide were much more present in flower oil than in the leaves oil (Nguyen et al., 2018). However, we have shown herein that neryl-isobutanoate was in a higher amount in SWLI EO (5.73%) than in I EO (2.44%), while L EO lacks this compound. On the other hand, the amount of neryl-isovalerate was slightly lower in SWLI than in I EO (5.29% and 6.40, respectively). Caryophyllene oxide was found only in the L EO. The relative amount of sabinene was higher in the L EO compared to I EO (6.23% and 4.17%, respectively), which is in accordance with the results obtained by Riahi et al. (2013).

Blagojević et al. (2006) showed that the main compound in the aerial parts EO of wormwood was *trans*-thujone (*β*-thujone), while *α*-fenchene was the main compound found in root oil. α-Fenchene was recorded only in SWLI EO. In addition, Llorens-Molina & Vacas (2015) documented (*Z*)-epoxyocimene, (*Z*)-chrysanthenyl acetate and linalool as the main compounds in aerial parts EO, while *β*-myrcene and *α*-fenchene were the main compounds in the root oil. (*Z*)-epoxyocimene was recorded in all three EOs, (*Z*)-chrysanthenyl acetate and *β*-myrcene were not recorded, while linalool was found only in I EO. Significant differences in EO composition were observed also between leaves and flowers. Ariño et al. (1999) reported some quantitative differences in EO profile from leaves and flowers, e.g., *cis*-epoxyocimene was found in higher ratios in leaf oil, which is in accordance with our study. Also, Ariño et al. (1999) showed that *cis*-chrysanthnyl acetate, which we have not detected, was the dominant compound in the flower oil. In addition, Riahi et al. (2013) showed that camphor was present only in flower oil but not in the leaves oil. On the other hand, bornan-2-one, was found in leaf oil, whereas flower oils were lacking in this compound. Neither camphor nor bornan-2-one have been detected in our examined EOs.

Differences in the composition of the essential oils of each part of the plant, especially in the flower heads, may have chemophenetic significance in future research, given that their components are very significant and genetically stable due to their repellent role in flower protection.

Antibacterial activity of A. absinthium EO

The results of MIC and MBC are given in **Tab. 3**. It was shown that examined EOs exhibited various degrees of antibacterial activities depending on tested bacterial strains (**Tab. 3**). In general, tested oils had moderate antibacterial activity. MIC assay indicates that the most sensitive bacterial strains were *Erwinia amylovora*, *Rathayibacter tritici*, and *Xanthomonas campestris* pv. *campestris*, while *Pseudomonas oryzihabitans* and *Pantoea alli* were the most resistant. The strongest effect was shown for SWLI EO against *E. amylovora* (MIC 0.03 mg/mL). Also, EO from L demonstrated high activity against *E. amylovora* and *R. tritici* (MIC 0.09 mg/mL). Equal activity against *X. campestris* (MIC 0.13 mg/mL) was detected for all three tested EOs.

Antibacterial activity of wormwood EO against human pathogens was documented in several studies. It was shown that *A. absinthium* EO had a significant antimicrobial effect against human pathogenic bacteria isolated from wounds and stools of patients (Mihajilov-Krstev et al., 2014) with minimal inhibitory/bactericidal concentrations ranging from <0.08 to 2.43 mg/mL and from 0.08 to 38.80 mg/mL, respectively. Riahi et al. (2015) reported that majorly Gram-positive bacterial strains showed more sensitivity against the wormwood EOs. In another study, the wormwood EO has shown very pronounced antibacterial activity, with inhibitory and bactericidal concentrations ranging from 4.72 against Gram-negative to 37.80 mg/mL against Gram-positive bacterial strains (Stanković et al., 2016). The study by Kordali et al. (2005) demonstrated by disk diffusion method that *A. absinthium* EO at 600-1200 μ/disk concentrations was active against a limited number of bacterial strains, including phytopathogens: *Curtobacterium flaccumfaciens*, *Pseudomonas syringae* pv. *maculicola*, *P.syringae* pv. *syringae* (RK-470), *Xanthomonas axanopodas* pv. *vesicatoria*, and *X. pelargonii*.

Different inhibitory effects of tested EOs may be attributed to the differences in the biological properties of the main compounds, differences in composition in the oil and their synergistic effects. This result is in agreement with Delaquis et al. (2002), who reported that the biological activity of different EOs was the result of the interaction between their total chemical compounds, mainly with additive and

Table 3. Antibacterial activity of different parts of *A. absinthium* EOs

Phytopathogenic Strains	*Artemisia* EOs (mg/mL)						Gentamicin (mg/mL)		Kanamycin (mg/ mL)	
	SWLI		L		I					
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Xanthomonas campestris pv. *campestris*	0.125	0.5	0.125	0.25	0.125	0.25	0.008	0.006	0.008	0.006
Pseudomonas oryzihabitans	1	>1	0.75	1	1	>1	0.016	0.013	0.016	0.0125
Erwinia amylovora	0.031	0.5	0.094	0.0625	0.25	0.5	0.004	0.003	0.008	0.006
Rathayibacter tritici	0.125	0.25	0.094	0.0625	-	0.031	0.008	0.006	0.003	0.025
Pantoea alli	>1	-	0.75	1	>1	-	0.006	0.025	0.006	0.025
Agrobacterium tumefaciens	0.5	1	0.75	1	0.5	1	-	>0.400	-	>0.400

- not detected in the range of tested concentrations

synergistic effects. The action mode of antimicrobial agents essentially depends on the type of the treated microorganism in relation to the structure of their cell wall and the outer membrane (Shan et al., 2007). Considering that there are a large number of different compounds in EO, it is most likely that their antibacterial activity is a result of targeting different cell components and consequently causing several types of damage (Cha et al., 2005; Sadaka et al., 2013). That could explain the broad antibacterial activity of tested *A. absinthium* EO against both Gram-negative *E. amylovora* and *X. campestris* pv. *campestris* and Gram-positive *R. tritici* were observed in the present study. It was confirmed that monoterpene ketones, α –and β-thujone, could be responsible for antibacterial potential against Gram-positive and Gram-negative bacteria (Juteau et al., 2003; Blagojević et al., 2006; Tsiri et al., 2009; Mihajilov-Krstev et al., 2014). According to these, high amounts of thujone in all tested EOs could be responsible for antibacterial activity. Also, these substances can be important in agriculture as potential agents in pest control.

Conclusions

Herein, we presented a detailed analysis of the EO composition of the *A. absinthium* different parts (shoots without leaves and inflorescences, leaves, and inflorescences) and their antimicrobial activity. *Trans*-Thujone was the dominant compound in all three EOs. On the other hand, the organoleptic characteristics, yield, number of identified components, the dominant group of compounds, the amount of the dominant compound, as well as the presence and amount of other compounds differed among the tested EOs. These differences indicate the different biological functions of different components in different parts of the plant. Moreover, the strongest effect was shown for SWLI EO, which may be in relation to the dominance of non-terpene compounds present in the EO. Although the biochemical and physiological processes behind these differences in EO profiles still await explanation, other *Artemisia* species should be included in future similar studies in order to get better insight into EO variability and antimicrobial activity of different plant parts.

Acknowledgements. Financial support was provided by the Ministry of Education, Science and Technological Development of the Republic of Serbia (451-03-68/2022-14/200178).

References

Ahamad, J. 2019: A pharmacognostic review on *Artemisia absinthium*. *International Research Journal of Pharmacy*, 10(1): 25-31.

Ariño, A., Arberas, I., Renobales, G., Arriaga, S., Domínguez, J.B. 1999: Seasonal variation in wormwood (*Artemisia absinthium* L.) essential oil composition. *Journal of Essential Oil Research*, 11: 619–622.

Basta, A., Tzakou, O., Couladis, M., Pavlović, M. 2007: Chemical composition of *Artemisia absinthium* L. from Greece. *Journal of Essential Oil Research*, 19: 316-318.

Benkhaled, A., Boudjelal, A., Napoli, E., Baali, F., Ruberto, G. 2020: Phytochemical profile,

antioxidant activity and wound healing properties of *Artemisia absinthium* essential oil. *Asian Pacific Journal of Tropical Biomedicine*, 10(11): 496.

Blagojević, P., Radulović, N., Palić, R., Stojanović, G. 2006: Chemical composition of the essential oils of Serbian wild-growing *Artemisia absinthium* and *Artemisia vulgaris*. *Journal of Agricultural and Food Chemistry*, 54(13): 4780-4789.

Bora, K.S., Sharma, A. 2011: The genus *Artemisia*: A comprehensive review. *Pharmaceutical Biology*, 49: 101-109.

Cha, J.D., Jeong, M.R., Jeong, S.I., Moon, S.E., Kim, J.Y., Kil, B.S., Song, Y.H. 2005: Chemical composition and antimicrobial activity of the essential oils of *Artemisia scoparia* and *A. capillaris*. *Planta Medica*, 71(2): 186–190.

Deans, S.G., Kennedy, A.I. 2001: *Artemisia absinthium*. In: Wright C.W. (Ed.), *Artemisia*. *1*: 79-90, CRC Press, London, UK.

Delaquis, P.J., Stanich, K., Girard, B., Mazza, G. 2002: Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *International Journal of Food Microbiology*, 74: 101–109.

European Directorate for the Quality of Medicines, European pharmacopoeia, sixth ed., Council of Europe, 2007 (ISBN: 9789287160546)

Gajić, M. 1973: *Artemisia.* In: Josifović M. (Ed.), *Flora of Serbia VII*: 122 p., SANU, Belgrade.

Isman, M.B. 2000: Plant essential oils for pest and disease management. *Crop Protection*, 19: 603-608.

Janaćković, P., Gavrilović, M., Rančić, D., Dajić-Stevanović, Z., Giweli, A.A., Marin, P.D. 2019: Comparative anatomical investigation of five *Artemisia* L. (Anthemideae, Asteraceae) species in view of taxonomy. *Brazilian Journal of Botany*, 42(1): 135-147.

Jarić, S., Mačukanović-Jocić, M., Djurdjević, L., Mitrović, M., Kostić, O., Karadžić, B., Pavlović, P. 2015: An ethnobotanical survey of traditionally used plants on Suva planina mountain (south-eastern Serbia). *Journal of ethnopharmacology*, 175: 93-108.

Joshi, R.K. 2013: Volatile composition and antimicrobial activity of the essential oil of *Artemisia absinthium* growing in Western Ghats region of North West Karnataka, India. *Pharmaceutical Biology*, 51(7): 888-892.

Judzentiene, A. 2016: Wormwood (*Artemisia absinthium* L.) oils. In: *Essential Oils in Food Preservation, Flavor and Safety*: 849-856, Academic Press.

Judzentiene, A., Budiene, J. 2010: Compositional variation in essential oils of wild *Artemisia absinthium* from Lithuania. *Journal of Essential Oil Bearing Plants*, 13(3): 275-285.

Judzentiene, A., Budiene, J., Gircyte, R., Masotti, V., Laffont-Schwob, I. 2012: Toxic activity and chemical composition of Lithuanian wormwood (*Artemisia absinthium* L.) essential oils. *Records of Natural Products*, 6(2).

Juteau, F., Jerkovic, I., Masotti, V., Milos, M., Mastelic, J., Bessière, J.M., Viano, J. 2003: Composition and antimicrobial activity of the essential oil of *Artemisia absinthium* from Croatia and France. *Planta Medica*, 69(02): 158-161.

Kordali, S., Kotan, R, Mavi, A., Cakir, A., Ala, A., Yildirim, A. 2005: Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *A.dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils. *Journal of agricultural and food chemistry*, 53(24): 9452-9458.

Llorens-Molina, J.A., Vacas, S. 2015: Seasonal variations in essential oil of aerial parts and roots of an *Artemisia absinthium* L. population from a Spanish area with supramediterranean climate (Teruel, Spain). *Journal of Essential Oil Research*, 27: 395–405.

Meschler, J. P., Howlett, A.C. 1999: Thujone exhibits low affinity for cannabinoid receptors but fails to evoke cannabimimetic responses. *Pharmacology Biochemistry and Behavior*, 62(3): 473-480.

Mihajilov-Krstev, T., Jovanović, B., Jović, J., Ilić, B., Miladinović, D., Matejić, J., Rajković, J., Đorđević, Lj., Cvetković, V., Zlatković, B. 2014: Antimicrobial, antioxidative, and insect repellent effects of *Artemisia absinthium* essential oil. *Planta medica*, 80(18): 1698-1705.

Mohammadi, A., Sani, T., Ameri, A., Imani, M., Golmakani, E., Kamali, H. 2015: Seasonal variation in the chemical composition, antioxidant activity, and total phenolic content of *Artemisia absinthium* essential oils. *Pharmacognosy Research*, 7: 329.

Msaada, K., Salem, N., Bachrouch, O., Bousselmi, S., Tammar, S., Alfaify, A., Marzouk, B. 2015: Chemical composition and antioxidant and antimicrobial activities of wormwood (*Artemisia absinthium* L.) essential oils and phenolics. *Journal of Chemistry*, 1–12.

Nguyen, H.T., Inotai, K., Radácsi, P., Tavaszi-Sárosi, S., Ladányi, M., Zámboriné-Németh, É. 2017: Morphological, phytochemical and molecular characterization of intraspecific variability of wormwood (*Artemisia absinthium* L.). *Journal of Applied Botany and Food Quality*, 90: 238-245.

Nguyen, H.T., Németh, Z.É. 2016: Sources of variability of wormwood (*Artemisia absinthium* L.) essential oil. *Journal of applied research on medicinal and aromatic plants*, 3(4): 143-150.

Nguyen, H.T., Radácsi, P., Gosztola, B., Rajhárt, P., Németh, É.Z. 2018: Accumulation and composition of essential oil due to plant development and organs in wormwood (*Artemisia absinthium* L.). *Industrial Crops and Products*, 123: 232-237.

Nguyen, H.T., Radácsi, P., Rajhárt, P., Németh, É. 2019: Variability of thujone content in essential oil due to plant development and organs from *Artemisia absinthium* L. and *Salvia officinalis* L. L. *Journal of Applied Botany and Food Quality*, 92: 100-105.

Olsen, R.W. 2000: Absinthe and γ-aminobutyric acid receptors. *Proceedings of the National Academy of Scieces*. *USA 97*, (pp. 4417–4418).

Padosch, S.A., Lachenmeier, D.W., Kröner, L.U. 2006: Absinthism: a fictitious 19th century syndrome with present impact. *Substance Abuse Treatment, Prevention, and Policy* 1(14).

Pino, J.A., Rosado, A., Fuentes, V. 1997: Chemical composition of the essential oil of *Artemisia absinthium* L. from Cuba. *Journal of Essential Oil Research*, 9(1): 87–89.

Riahi, L., Chograni, H., Elferchichi, M., Zaouali, Y., Zoghlami, N., Mliki, A. 2013: Variations in Tunisian wormwood essential oil profiles and phenolic contents between leaves and flowers and their effects on antioxidant activities. *Industrial Crops and Products*, 46: 290-296.

Riahi, L., Ghazghazi, H., Ayari, B., Aouadhi, C., Klay, I., Chograni, H., Cherif, A., Zoghlami, N. 2015: Effect of environmental conditions on chemical polymorphism and biological activities among *Artemisia absinthium* L. essential oil provenances grown in Tunisia. *Industrial Crops and Products*, 66: 96-102.

Ristivojević, P., Dimkić, I., Trifković, J., Berić, T., Vovk, I., Milojković-Opsenica, D., Stanković, S. 2016: Antimicrobial activity of Serbian propolis evaluated by means of MIC, HPTLC, bioautography and chemometrics. *Public Library of Science,* 11(6): 157-097.

Sadaka, F., Nguimjeu, C., Brachais, C.H., Vroman, I., Tighzert, L., Couvercelle, J.P. 2013: Review on antimicrobial packaging containing essential oils and their active biomolecules. *Innovative Food Science and Emerging Technologies*, 20: 350.

Shan, B., Cai, Y.Z., Brooks, J.D., Corke, H. 2007: The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts. *International Journal of Food Microbiology*, 117(1): 112–119.

Stanković, N., Mihajilov-Krstev, T., Zlatković, B., Matejić, J., Jovanović, V.S., Kocić, B., Čomić, L. 2016: Comparative study of composition, antioxidant, and antimicrobial activities of essential oils of selected aromatic plants from Balkan Peninsula. *Planta medica*, 82(07): 650-661.

Taherkhani, M. 2014: *In vitro* cytotoxic activity of the essential oil extracted from *Artemisia absinthium*. *Iranian Journal of Toxicology*, 8(26): 1152-1156.

Tsiri, D., Graikou, K., Pobłocka-Olech, L., Krauze-Baranowska, M., Spyropoulos, C., Chinou, I. 2009: Chemosystematic value of the essential oil composition of *Thuja* species cultivated in Poland — antimicrobial activity. *Molecules*, 14(11): 4707-4715.

Vieira, T.M., Dias, H.J., Medeiros, T.C., Grundmann, C.O., Groppo, M., Heleno, V.C., Martins, C.H.G., Cunha, W.R., Crotti, A.E.M., Silva, E.O. 2017: Chemical composition and antimicrobial activity of the essential oil of *Artemisia absinthium* Asteraceae leaves. *Journal of Essential Oil Bearing Plants*, 20(1): 123-131.

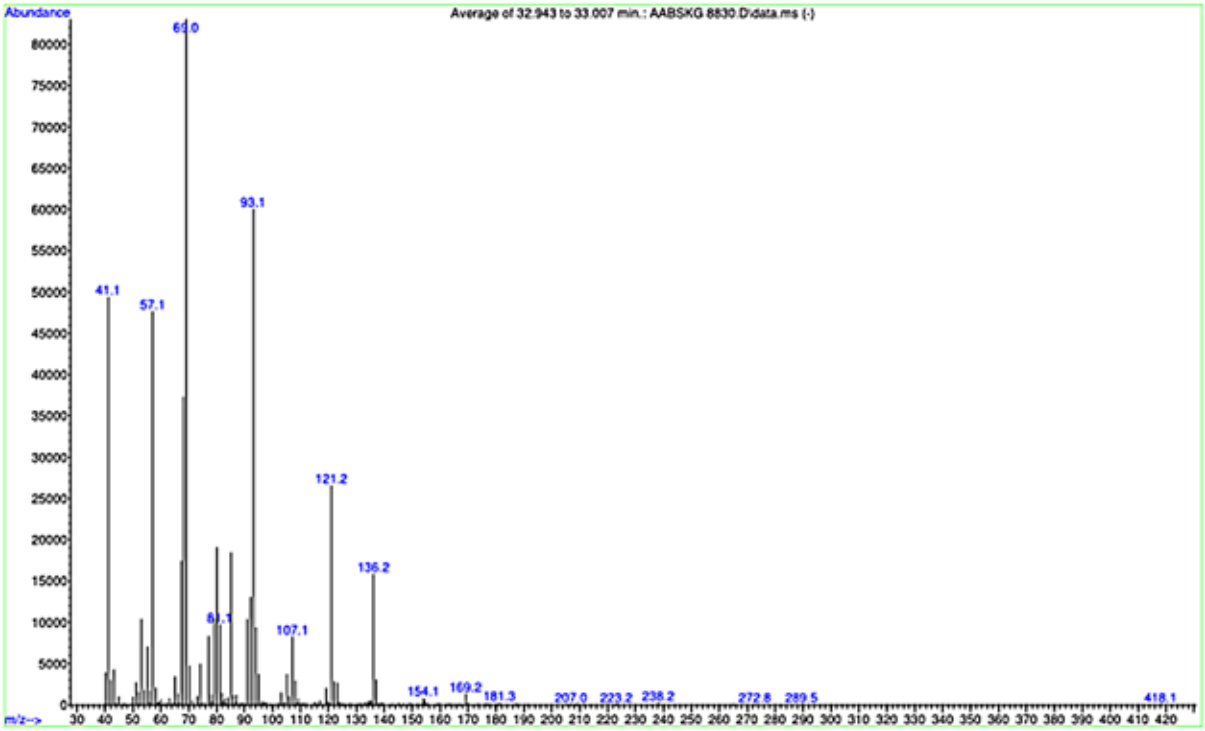
Yildiz, K., Basalan, M., Duru, O., Gokpinar, S. 2011: Antiparasitic efficiency of *Artemisia absinthium* on *Toxocara cati* in Naturally Infected Cats. *Turkish Journal of Parasitology*, 35(1): 10–14.

Živković, J., Ilić, M., Šavikin, K., Zdunić, G., Ilić, A., Stojković, D. 2020: Traditional use of medicinal plants in South-Eastern Serbia (Pčinja District): Ethnopharmacological investigation on the current status and comparison with half a century old data. *Frontiers in Pharmacology*, 11: 1020.

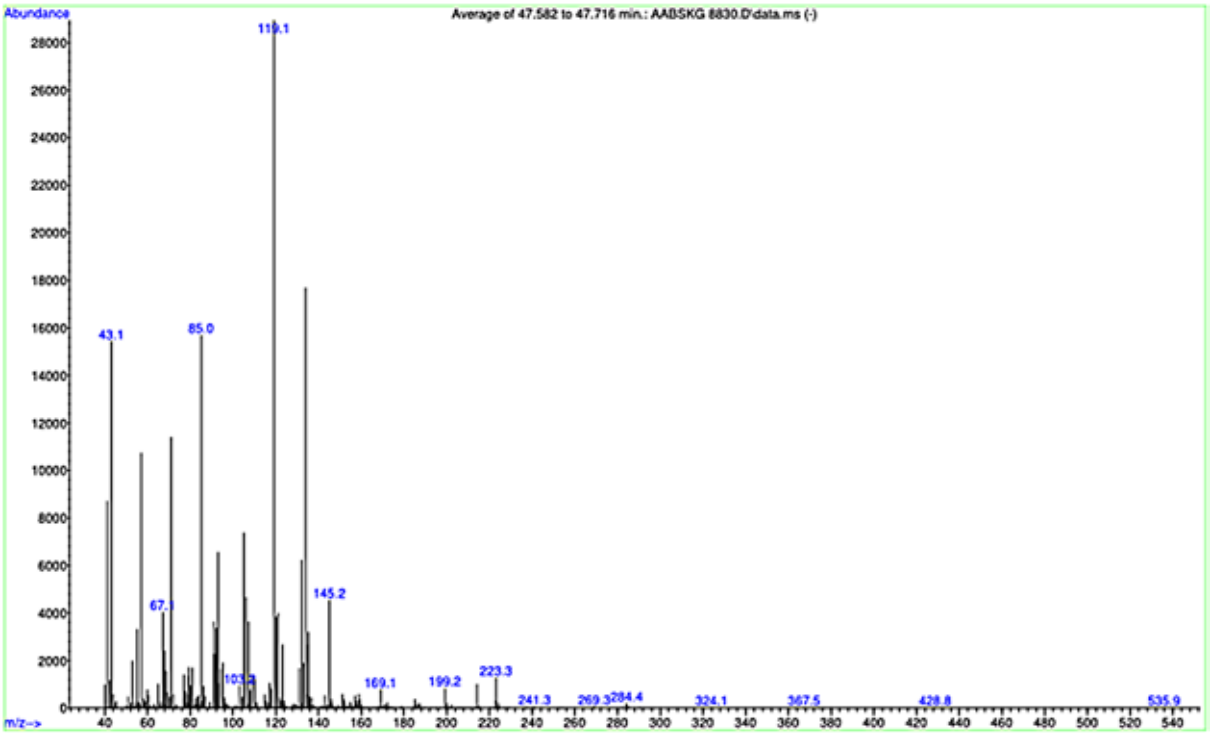
Appendix

Appendix 1. MS Spectra of unidentifies compounds from *Artemisia absinthium* essential oil

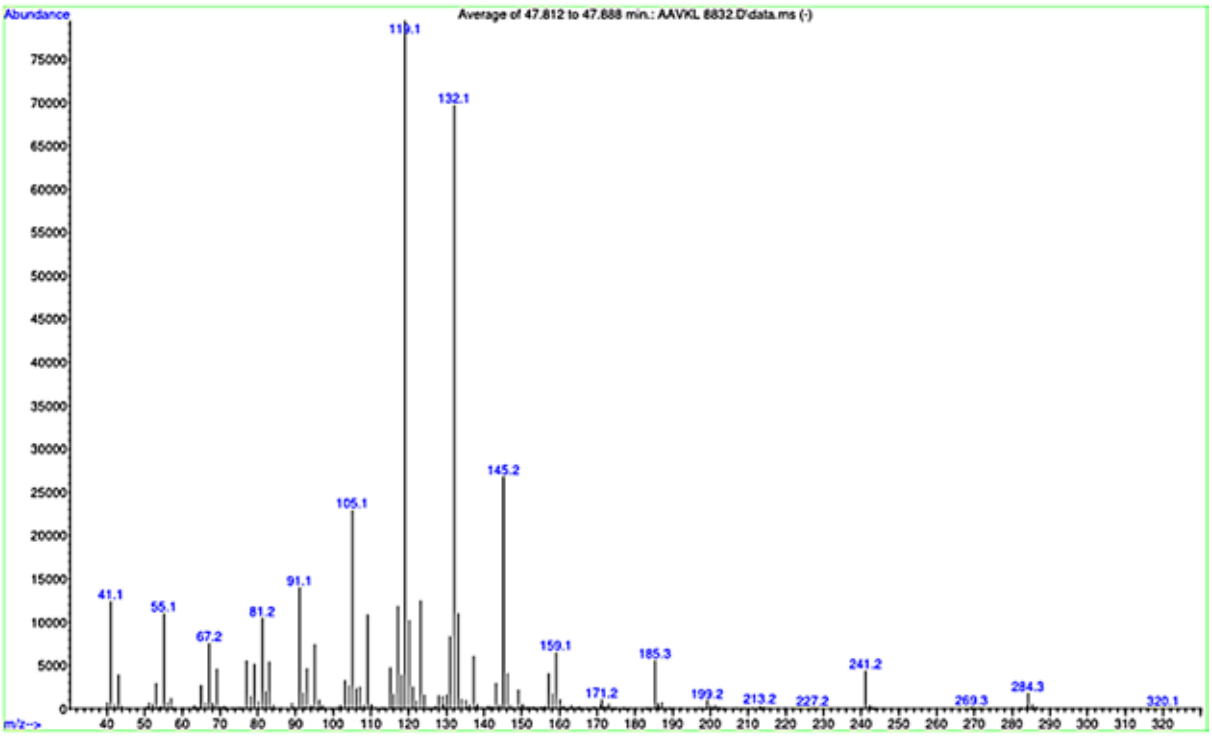
LRI1603



LRI2008



LRI2014



LRI2082