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Chemical Composition of the Essential Oils of Serbian Wild-Growing *Artemisia absinthium* and *Artemisia vulgaris*

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The chemical composition of the aerial and root essential oils, hydrodistilled from *Artemisia absinthium* L. and *Artemisia vulgaris* L. (wild-growing populations from Serbia), were studied by gas chromatography, gas chromatography−mass spectrometry, and ^{13}C nuclear magnetic resonance. During the storage of plant material under controlled conditions, a significant decrease of essential oil yields (isolated directly after drying and after 1 year of storage) and significant differences in their chemical compositions were observed. A possible mechanism for the observed oil component interconversion has been discussed. The noticeable differences in the chemical composition of the oils isolated from roots and aerial parts of *A. absinthium* and *A. vulgaris* were also correlated with the diverging biosynthetic pathways of volatiles in the respective plant organs. The antimicrobial activities against the common human pathogens of all of the isolated oils were tested according to National Committee on Clinical Laboratory Standards. The oils showed a broad spectrum of antimicrobial activity against the tested strains. Therefore, these oils can be used as flavor and fragrance ingredients.

KEYWORDS: ***Artemisia absinthium*; *Artemisia vulgaris*; essential oil; root; storage period; plant organ specification; antimicrobial activity**

INTRODUCTION

According to the Council Directive 88/388/EEC (*1*), on the approximation of the laws of the Member States relating to flavorings for use in foodstuffs and source materials for their production, the addition of thujone-containing plants was reallowed in the European Union. For this reason, an increase in the industrial consumption of *Artemisia absinthium* L. (wormwood), Asteraceae, limited in the last century as the result of absinth prohibition (*2*, *3*), could be expected. However, the *A. absinthium* plant material used in industries is still in many instances harvested from wild populations, with variable, nonstandardized thujone (both α- and β-isomers) contents (*4*). This can be seen from the numerous formerly published data on the variability of *A. absinthium* essential oil composition considered in detail in the Results and Discussion section. There are no previous reports on the composition of the essential oil isolated from *A. absinthium* and *Artemisia vulgaris* L. [locally known as "beli (white) pelin" and "crni (black) pelin", respectively] growing wild in Serbia.

In addition to the renowned wormwood application in preparation of absinth and related beverages, *A. absinthium* has been used since ancient times for medical purposes. From the ethnopharmacological point of view, *A. absinthium* has been used for its antihelmintic, stomachic, antibacterial, antifeedant, antifertility, antipyretic, cytostatic, antitumor, and antimalarial actions (*3*, *5*). In this sense, it has been reported that *A. vulgaris* (mugwort) possesses similar uses to *A. absinthium*. The rational justification of the widespread use of the mentioned herbs was assessed by testing the antibacterial and antifungal activities of the isolated oils against seven common human pathogens.

Both *A. absinthium* and *A. vulgaris* represent economically important plant species; hence, the investigation of the influence of storage time on the chemical compositions of their volatile oils deserves attention. Because both roots and aerial parts of *A. vulgaris* are used as an herbal remedy (*5*), we found it interesting to determine the differences in the chemical compositions of the oils isolated from roots and aerial parts. It is surprising that the root essential oil of *A. vulgaris*, a widespread and investigated plant species, has not previously been analyzed. It has been reported that the thujone type monoterpenoids (including thujone itself) were found in only trace amounts in *A. absinthium* root oil (*6*, *7*). The possibility of utilizing *A. absinthium* root oil in flavoring or for medical purposes provoked us to reexamine the oil of *A. absinthium* roots as well.

In light of the above-mentioned information, the aim of the present study was (i) to investigate the essential oil chemotypes of wild-growing *A. absinthium* and *A. vulgaris* from Serbia, using gas chromatography (GC), gas chromatography−mass spectrometry (GC-MS), and ^{13}C nuclear magnetic resonance (NMR) analysis; (ii) to asses their antimicrobial activity; and (iii) to clarify the influence of plant organ specification and storage on essential oil yield and composition.

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MATERIALS AND METHODS

Plant Materials and Isolation of Essential Oils. The investigation of two populations of *A. absinthium* growing spontaneously at two different locations in the southeastern Serbia was carried out. For this purpose, plant material was collected in the village Mokra (Bela Palanka) (aerial parts; June, 2002; 313 m above sea level) and at the banks of the river Nišava in the urban surroundings of Niš (whole plant; July, 2003; 199 m above sea level). *A. vulgaris* (entire herb) was gathered at Nišava riverside in the city of Niš in July, 2003.

All mentioned species were collected in the full-blooming stage. The voucher specimens were deposited at the Herbarium of the Faculty of Biology, University of Belgrade (BEOU): 16030 (*A. vulgaris*), 16033 (*A. absinthium*, Mokra), and 16034 (*A. absinthium*, Niš).

Immediately after the specimens were air-dried at room temperature (to constant moisture content), the plant material was subjected to hydrodistillation for 2.5 h using a Clevenger type apparatus. In the case where the influence of prolonged storage period was investigated, the dried herbs were additionally kept, in a sealed container, for a total of 1 year prior to oil isolation at ambient temperature (25 ± 2 °C) and without exposure to direct sunlight (*A. absinthium*, Mokra). The oils were dried over anhydrous $MgSO_4$ and kept at 4 °C until analysis.

Throughout the text, the following abbreviations have been used to designate the obtained essential oils: Aa1 and Aa2 (oils from aerial parts of *A. absinthium*, Mokra, isolated immediately after drying the plant material, and stored for 1 year, respectively); Aa3 and Aa3R (oils from aerial parts and roots of *A. absinthium* oil, Nišava riverside, respectively); and Av and AvR (oils from aerial parts and roots of *A. vulgaris*, Nišava riverside, respectively). Yields of the isolated oils (w/w of dry plant material) and the results of GC, GC-MS, and ^{13}C NMR analyses are presented in **Table 1**.

GC and GC-MS. The GC-MS analysis of the oils was performed using a Hewlett-Packard 5890 series II gas chromatograph equipped with SPB-1 (30 m × 0.25 mm i.d., 0.25 μm film thickness) and DB-5 (30 m × 0.2 mm i.d., 0.25 μm film thickness) fused silica capillary columns directly coupled to a mass selective detector MSD 5971A from the same company, which was operated in EI mode (70 eV). Helium was the carrier gas at a flow rate of 1 mL/min. The injector temperature was 250 °C, and the oven temperature was programmed as follows: held isothermal at 50 °C for 3 min, then gradually increased to 250 °C at 5 °C/min, and finally held isothermal at 250 °C for 15 min. The volume injected was 0.1 μL of the 10% solution, diluted with diethyl ether. The conditions for the GC−flame ionization detection (FID) were the same as for the GC-MS except H_2 was the carrier gas. The area percentage was obtained electronically from the GC-FID response without the use of an internal standard or correction factors. The compound content in the oils hydrodistilled from *A. absinthium* (Mokra) before and after 1 year of storage (Aa1 and Aa2 oils, respectively) was additionally expressed as the relative amount of identified components to the dry weight of plant material (mmol/100 g), and the corresponding values are given in **Table 1**.

NMR Spectroscopy of Carbon-13. ^{13}C NMR spectra of the essential oils were recorded on a Varian Mercury 300 spectrometer, operating at 75.462 MHz, equipped with a 5 mm probe in benzene-d_6 (around 50 mg in 0.5 mL of C_6D_6). Chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane as an internal standard. Parameters: pulse width, 6.0 μs (flip angle, 45°); acquisition time, 1.705 s; relaxation delay D1, 0.294 s (total recycling time 1.999 s); for 64 K data table with a spectral width of 18863.2 Hz; globally optimized alternating-phase rectangular pulses were used; digital resolution, 0.469 Hz/pt; and 5000 scans were accumulated. An exponential multiplication of the free induction decay with the line broadening of 1.0 Hz was applied before Fourier transformation.

Identification Procedure. The linear retention indices (RIs) for all of the compounds were determined by coinjection of the sample with a solution containing the homologous series of C_8-C_{25} *n*-alkanes. Individual identification of components was based on comparison of their mass spectra (MS) with those of Wiley 275 MS and MassFinder 2.3 (*8*) libraries and with those described by Adams (*9*), as well as on comparison of their RIs (*10*) with literature values (*9*, *11*) and, wherever possible, by coinjection with a reference sample (ref; see **Table 1** for the method of identification of each individual compound). The identity of some compounds was also confirmed by ^{13}C NMR in the same way as it was described in the ref *12*.

Antimicrobial Activity. The disk diffusion method according to the NCCLS (*13*) was employed for the determination of in vitro antimicrobial activity of the essential oils and available pure constituents [in 1:10 and 1:30 dilution, w/v (mg/μL)], completely following the method and using the same panel of laboratory control strains (see Results and Discussion) as previously reported in ref *12*. Standard disks of doxycycline, tiamulin, erythromycin, and nystatin (origin, Institute of Immunology and Virology "Torlak", 30 μg of the active component, 6 mm diameter) were individually used as positive controls, while the disks imbued with 50 μL of pure diethyl ether were used as negative controls. Each test was performed in triplicate and repeated three times, and results were analyzed for statistical significance.

RESULTS AND DISCUSSION

Influence of Storage. Because the increase in *A. absinthium* exploitation in the food industry can be expected, it is of great importance to establish whether significant changes in oil composition and yield during storage of wormwood occur. After 1 year of plant material storage, the oil yield dropped from 0.29 to 0.08 (%, w/w), as well as the number of detected components. With some observed exceptions, all constituents were found in reduced quantities in Aa2 oil (prolonged storage time) (**Table 1**, values in parentheses). This could be due to, at least partially, the evaporation of the oil constituents during storage. Factors such as light, temperature, and moisture greatly influence the emission of volatiles, and in general, physical properties of one particular compound determine the rate of its release into the atmosphere (*14*). If the decrease in the amount of components is only the consequence of their volatility properties, it is reasonable to expect a larger decrease in the amount for those constituents that are characterized with higher vapor pressures. Indeed, the fraction ratio of oxygenated monoterpenes that are less volatile than the parent hydrocarbons to hydrocarbon monoterpenes in Aa1 and Aa2 oils was 6.3 and 17.5, respectively, indicating a 2.8 times higher rate of emission of the hydrocarbon fraction. This relationship seems to stand also within the sesquiterpene fraction but is, as expected, less pronounced (0.6 in Aa1 and 0.7 in Aa2 oil). The general ratio of the monoterpene to the sesquiterpene fraction does not, however, follow this rule (the fraction ratio of total monoterpenes to the total sesquiterpenes is 6.1 in Aa1 oil and 21.9 in Aa2 oil). However, this simplified approach could be misleading since some monoterpenoids are less volatile than some other sesquiterpenes.

The relative amounts of certain individual components were higher in the oil isolated from plant material after 1 year of storage: *cis*- and *trans*-linalooloxides (furanoid) (**1** and **2**), 1,8-cineole (**3**), neryl 2-methylpropanoate (**4**), and geranyl 3-methylbutanoate (**5**) (**Figure 1**). This increase could be the outcome of chemical transformations of initially existing compounds. All of these transformations could be associated with certain existing metabolic pathways and connected with the action of enzymes or could be temperature, humidity, and light driven alone. Most probably, at the beginning of the drying process, both enzymatic and nonenzymatic reactions took a role in the terpenoid transformation. However, it could be expected that, as a consequence of complete plant dehydration, enzymatic transformations were stopped [enzyme inactivation in dry plant material is very probable (*15*)]. **Figures 2** and **3** show straightforward reaction pathways that could interconnect oxides of linalool (**1** and **2**) with its potential precursors: *cis*-β-epoxyocimene (**6**), linalool (**7**), or β-myrcene (**8**), components

Table 1. Composition of the *A. absinthium* and *A. vulgaris* Essential Oils

		RI[a]		*A. absinthium* yield in parentheses				*A. vulgaris*		
	compound	SPB-1	DB-5	Aa1 (0.29)	Aa2 (0.08)	Aa3 (0.26)	Aa3R (0.22)	Av (0.06)	AvR (0.04)	identification method[b]
1	hexanal[c]	769	801	*d*					0.2	RI, MS
2	santolinatriene	910	909					4.0		RI, MS
3	α-thujene	938	928	0.2 (0.33)[e]	0.6 (0.26)	0.4		1.0		RI, MS
4	α-pinene	947	940	0.2 (0.33)	0.4 (0.17)	0.8	0.6	5.9	0.1	RI, MS, ref
5	α-fenchene[c]	959	950	0.1 (0.16)	0.3 (0.13)	2.3	23.3		tr[f]	RI, MS, ^{13}C NMR
6	camphene	961	953					0.1	tr	RI, MS, ref
7	sabinene	992	976	8.1 (13.23)	3.3 (1.42)	10.8	0.2	13.7		RI, MS, ^{13}C NMR, ref
8	*β*-pinene	997	980				1.7	tr	tr	RI, MS, ref
9	*β*-myrcene	1016	994	0.9 (1.47)		0.3	2.3	1.3		RI, MS, ref
10	α-phellandrene[c]	1028	1006	0.7 (1.14)		0.5	3.4			RI, MS
11	*p*-cymene	1047	1027	1.2 (1.96)	0.5 (0.22)	6.7	4.1	tr	tr	RI, MS, ref
12	1,8-cineole	1050	1034	1.0 (1.63)	16.3 (7.01)			28.9	0.3	RI, MS, ^{13}C NMR, ref
13	limonene	1053	1039						tr	RI, MS, ref
14	*γ*-terpinene[c]	1083	1059	0.2 (0.33)				0.3		RI, MS
15	*trans*-sabinene hydrate[c]	1087	1069	0.3 (0.49)					tr	RI, MS
16	*cis*-linalooloxide (furanoid)[c]	1089	1070		1.5 (0.64)				tr	RI, MS
17	fenchone[c]	1097	1088						tr	RI, MS
18	*trans*-linalooloxide (furanoid)[c]	1100	1090		1.5 (0.64)					RI, MS
19	α-thujone	1113	1102	0.9 (1.47)	0.6 (0.26)	1.8		1.2		RI, MS, ref
20	linalool	1117	1104	4.1 (6.70)	0.2 (0.09)					RI, MS, ref
21	*β*-thujone	1123	1114	19.8 (32.33)	20.2 (8.69)	63.4		13.5		RI, MS, ^{13}C NMR, ref
22	camphor	1139	1143					1.4	0.4	RI, MS, ref
23	*cis*-*β*-epoxyocimene[c]	1141	1139	10.7 (17.47)						RI, MS, ^{13}C NMR
24	*trans*-sabinol[c]	1148	1140	2.5 (4.08)						RI, MS
25	albene[c]	1161	1152						0.6	RI, MS
26	lavandulol[c]	1167	1170	1.2 (1.96)						RI, MS
27	4-terpineol	1173	1178	1.7 (2.78)	2.3 (0.99)			2.2		RI, MS, ref
28	pulegone	1214	1207	2.8 (4.57)						RI, MS, ref
29	*trans*-sabinenehydrate acetate[c]	1244	1229					2.5		RI, MS
30	bornyl acetate	1258	1280			0.3	4.2	tr	5.0	RI, MS, ref
31	*trans*-sabinyl acetate[c]	1264	1290	8.8 (14.37)	15.5 (6.66)					RI, MS, ^{13}C NMR
32	isobornyl acetate[c]	1268	1290						tr	RI, MS
33	linalyl acetate	1270	1272			0.1	7.7			RI, MS, ^{13}C NMR, ref
34	7α-silphiperfol-5-ene[c]	1307	1300						0.3	RI, MS
35	presilphiperfol-7-ene[c]	1317	1328						0.2	RI, MS
36	silphin-1-ene[c]	1329	1351						1.0	RI, MS
37	eugenol	1346	1356						0.3	RI, MS, ref
38	linalyl propanoate[c]	1346	1363				8.2			RI, MS, ^{13}C NMR
39	isobornyl propanoate[c]	1349	1366						0.8	RI, MS
40	pethybrene[c]	1362	1371						tr	RI, MS
41	silphiperfol-6-ene[c]	1365	1375						tr	RI, MS
42	α-copaene	1365	1372					0.6		RI, MS, ref
42	modhephene[c]	1369	1385						1.2	RI, MS
43	*β*-bourbonene[c]	1374	1384					tr		RI, MS
44	α-isocomene[c]	1377	1388						4.0	RI, MS, ^{13}C NMR
45	linalyl 2-methylpropanoate[c]	1379	1378				1.0			RI, MS
46	*β*-elemene[c]	1380	1393					1.4	2.0	RI, MS
47	petasitene[c]	1385	1400						0.7	RI, MS
48	cyperene[c]	1390	1398						4.9	RI, MS
49	*β*-isocomene[c]	1395	1402						1.9	RI, MS
50	α-gurjunene[c]	1400	1409					tr	tr	RI, MS
51	linalyl butanoate[c]	1402	1414	1.2 (1.96)	1.8 (0.77)	0.7	14.4			RI, MS, ^{13}C NMR
52	α-cedrene[c]	1405	1411						0.2	RI, MS
53	*β*-caryophyllene	1408	1418	3.0 (4.90)		1.2		2.3	1.2	RI, MS, ref
54	α-santalene[c]	1410	1424						tr	RI, MS
55	α-*trans*-bergamotene[c]	1424	1426						0.4	RI, MS
56	*epi*-*β*-santalene[c]	1434	1431						0.9	RI, MS
57	α-humulene	1440	1444	0.7 (1.14)				0.5	1.6	RI, MS, ref
58	*β*-santalene[c]	1445	1462						0.8	RI, MS
59	aromadendrene[c]	1447	1434					0.9		RI, MS
60	*β*-bisabolene[c]	1462	1496						1.7	RI, MS
61	*γ*-humulene [c]	1464	1481					1.1	1.1	RI, MS
62	neryl 2-methylpropanoate[c]	1469	1475		0.5 (0.22)		2.8			RI, MS
63	(*Z*,*E*)-α-farnesene[c]	1470	1462	3.3 (5.39)	2.5 (1.08)					RI, MS
64	*β*-selinene[c]	1470	1474	0.8 (1.31)		2.9		4.7	2.0	RI, MS
65	*allo*-aromadendrene[c]	1471	1477						tr	RI, MS
66	bicyclogermacrene[c]	1477	1491						0.7	RI, MS
67	*γ*-gurjunene[c]	1476	1479					2.8		RI, MS
68	linalyl 3-methylbutanoate[c]	1484	1473	7.5 (12.25)	12.5 (5.38)	4.5	21.1			RI, MS, ^{13}C NMR
69	bornyl 3-methylbutanoate[c]	1488	1468						8.4	RI, MS, ^{13}C NMR
70	*δ*-cadinene[c]	1500	1505					1.3	4.3	RI, MS

Table 1. (Continued)

		RI[a]		*A. absinthium* yield in parentheses				*A. vulgaris*		
	compound	SPB-1	DB-5	Aa1 (0.29)	Aa2 (0.08)	Aa3 (0.26)	Aa3R (0.22)	Av (0.06)	AvR (0.04)	identification method[b]
71	*cis*-α-bisabolene[c]	1521	1504						tr	RI, MS
72	myrtenyl 3-methylbutanoate[c]	1532	1521						0.8	RI, MS
73	neryl 3-methylbutanoate[c]	1553	1535	4.4 (7.18)					0.9	RI, MS
74	neryl 2-methylbutanoate[c]	1563	1584	3.7 (6.04)					13.2	RI, MS, ^{13}C NMR
75	caryophyllene oxide	1567	1580					6.5		RI, MS, ref
76	geranyl 3-methylbutanoate[c]	1573	1582	1.5 (2.45)	12.9 (5.55)	1.1	4.0		0.6	RI, MS, ^{13}C NMR
77	geranyl 2-methylbutanoate[c]	1593	1579	0.9 (1.47)	3.3 (1.42)	0.4	0.1		tr	RI, MS
78	humulene oxide II[c]	1596	1595					1.2	2.5	RI, MS
79	β-eudesmol	1647	1650						10.0	RI, MS, ^{13}C NMR, ref
80	valeranone[c]	1665	1672						5.5	RI, MS, ^{13}C NMR
81	α-bisabolol[c]	1686	1681	0.7 (1.14)					6.0	RI, MS, ^{13}C NMR
82	chamazulene[c]	1728	1714	1.0 (1.63)						RI, MS
83	(*E*,*E*)-farnesal[c]	1735	1730	0.8 (1.31)						RI, MS
84	(*E*,*E*)-farnesyl acetate[c]	1828	1844						tr	RI, MS
85	*trans*-nerolidyl propanoate[c]	1829	1850						tr	RI, MS
86	hexahydrofarnesyl acetone[c]	1838	1843						tr	RI, MS
87	(*Z*)-nuciferyl propanoate[c]	1875	1893	1.2 (1.96)	0.3 (0.13)					RI, MS
88	methyl palmitate	1908	1910						tr	RI, MS, ref
89	ethyl palmitate	1968	1990						tr	RI, MS, ref
90	(*E*)-nuciferyl 2-methylpropanoate[c]	1992	1997	0.6 (0.98)	0.4 (0.17)					RI, MS
91	(*E*)-nuciferyl butanoate[c]	2004	2012	1.7 (2.78)	1.1 (0.47)	0.7				RI, MS
92	(*E*,*E*)-farnesyl 3-methylbutanoate[c]	2040	2058						tr	RI, MS
93	methyl linoleate	2071	2097						tr	RI, MS, ref
94	ethyl linoleate	2139	2155						tr	RI, MS, ref
	totally identified			98.4	98.5	98.9	99.1	99.3	86.7	
	no. of components			35	22	18	16	29	63	
	monoterpenes			84.6	94.2	94.1	99.1	76.0	30.5	
	hydrocarbons			11.6	5.1	21.8	35.6	26.0	0.1	
	oxygenated (without esters)			45	42.6	65.2	0.0	47.5	0.7	
	esters			28	46.5	7.1	63.5	2.5	29.7	
	sesquiterpenes			13.8	4.3	4.8	0.0	23.3	55.7	
	hydrocarbons			8.8	2.5	4.1	0.0	7.7	31.1	
	oxygenated			5.0	1.8	0.7	0.0	15.6	24	
	other			0.0	0.0	0.0	0.0	0.0	0.5	

[a] RIs relative to *n*-alkanes on SPB-1 and DB-5 capillary columns. [b] Methods: RI, identification based on RI comparison with literature data on at least one column (SBP-1, DB-5); MS, identification based on mass spectra comparison with those of Wiley 275 MS and MassFinder 2.3 (*8*) library as well as with those described by Adams (*9*); ^{13}C NMR, identification based on ^{13}C NMR spectra comparison; and ref, coinjection with an authentic sample. [c] Tentatively identified by the combination of MS and RI on two columns and/or ^{13}C NMR. [d] Compound not found. [e] The values in parentheses represent the compound content expressed in mmol/100 g of dry plant material. [f] Less then 0.1%.

found only in Aa1 oil. After initial epoxidation of linalool, the subsequent in situ conversion of the epoxide to the furanooxides was reported previously (*16*). A similar chemical connection can be devised to explain the notable increase in the amount of 1,8-cineole (1.63 mmol/100 g in Aa1 and 7.01 mmol/100 g in Aa2 oil). The heat of formation of 1,8-cineole, as compared with those of *trans*-sabinol, *trans*-sabinene hydrate, linalool, nerol, geraniol, and *cis*-β-epoxyocimene, indicates that reactions that would have 1,8-cineole as a product, and some of above-mentioned compounds as substrates, would tend toward 1,8-cineole as the thermodynamically more stable compound.

The amounts of α-thujene, α-pinene, and α-fenchene were decreased during storage. However, it was less than one might expect. A possible explanation in the case of α-pinene and α-fenchene could be found in the transformation of myrcene (*17*) or in the rearrangement reactions of sabinene (corresponding amount dramatically decreased in Aa2 oil) that would lead to the ring strain release due to the opening of the small three-member cycle. An additional explanation in the case of α-thujene could probably be sought in the transformations of *trans*-sabinene hydrate and *trans*-sabinyl acetate.

Even though the relative amounts of neryl 2-methyl propanoate (**4**) and geranyl 3-methyl butanoate (**5**) are higher in Aa2 than in Aa1 oil, the total amount of esters of acyclic monoterpenes decreased from 20.33 (Aa1 oil) to 12.33 mmol/100 g of dry plant material. The mentioned increase could be the consequence of isomerization processes of linalyl and neryl esters found in Aa1 oil.

Plant Organ Specification. Although the use of thujone-containing plants is reallowed in the European Union, there are restrictions on the maximum acceptable levels of thujones in final products for consumption (*1*). Previously reported data concerning major contributors of *A. absinthium* (collected in different countries) oils are listed in **Table 2** (*18*−*24*), and as it can be seen, high variability, not only in quantity of the major compounds but also in the corresponding class, can be observed. It is, thus, clear why it is necessary to investigate wild wormwood populations for the essential oil composition prior to their application in food and beverage production.

The main constituents found in the essential oil isolated from the aerial parts of *A. absinthium* collected at Mokra were β-thujone (19.8%), *cis*-β-epoxyocimene (10.7%), *trans*-sabinyl acetate (8.8%), sabinene (8.1%), and linalyl 3-methylbutanoate (7.5%). The oil was dominated by monoterpenes (84.6%), most of all belonging to the thujane type compounds (40.6%). The highest amounts of β-thujone (63.4%), sabinene (10.8%), and *p*-cymene (6.8%) were detected in the aerial parts oil obtained from wormwood herbs gathered at the Nišava banks (Aa3). Aa3

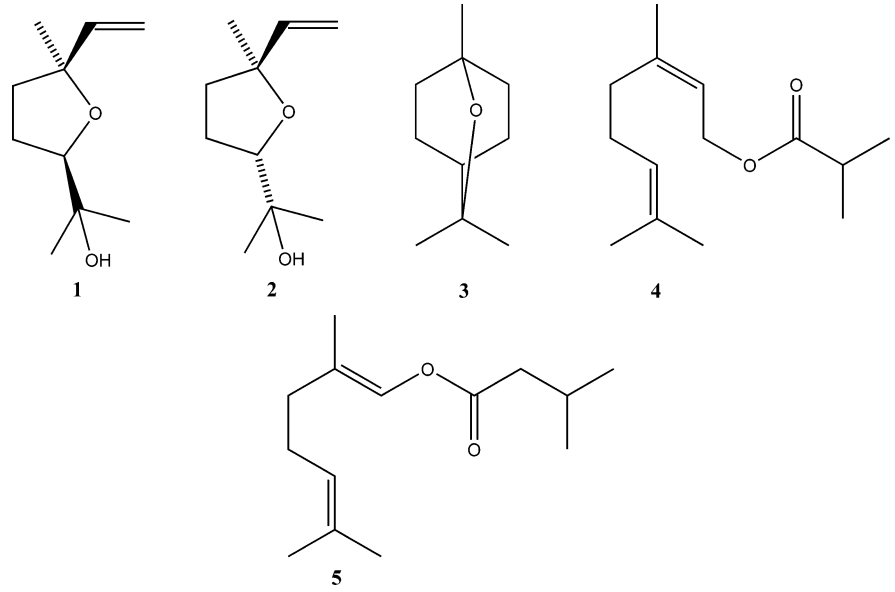

Figure 1. Monoterpenes found in the *A. absinthium* essential oil from Mokra (Aa2) with an increased amount after the storage period: **1**, *cis*-linalooloxide (furanoid); **2**, *trans*-linalooloxide (furanoid); **3**, 1,8-cineole; **4**, neryl 2-methylpropanoate; and **5**, geranyl 3-methylbutanoate.

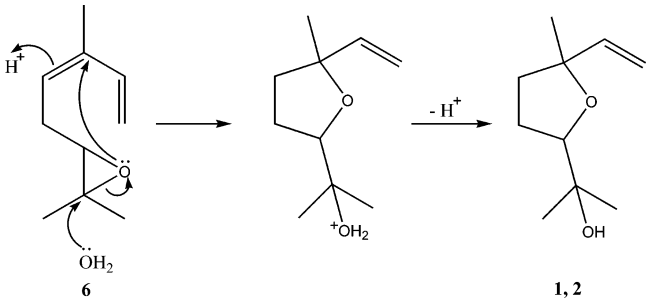

Figure 2. Possible mechanism of linalool oxides (**1** and **2**) formation from *cis*-β-epoxyocimene (**6**).

oil, similar to Aa1, is rich in monoterpenes of the thujane group (77.4%). All of the oils isolated from aerial parts of *A. absinthium* contained nuciferyl esters (**Figure 4**). These rather uncommon essential oil constituents, however, seem to be quite characteristic for species belonging to the genus *Artemisia* (*24*).

The oil isolated from aerial parts of *A. vulgaris* was characterized with a high amount of 1,8-cineole (28.9%), sabinene (13.7%), β-thujone (13.5%), and β-caryophyllene oxide (6.5%). Literature data on previously analyzed mugwort oils are summarized in **Table 3** (*21*, *23*, *25*−*29*).

As far as the food industry is concerned, every factor influencing the organoleptic properties of the oil, or its physiological action, no matter if it is genetic or environmental in nature, is of immense importance in plant selection. Having this in mind and the differences in composition of the oils isolated from aerial parts of *A. absinthium* gathered in Nišava banks and in Mokra, as well as from *A. vulgaris*, from previously reported ones (**Tables 2** and **3**), these plant populations could be considered to represent new chemotypes.

The oils obtained from *A. vulgaris* and *A. absinthium* (**Tables 3** and **2**) gathered in Egypt had α-phellandrene as the main compound (*25*). β-Myrcene, the major contributor of wormwood oil from the Republic of Bashkortostan, was also found in high amounts in mugworth oil from the same country (*23*). α-Phellandrene and β-myrcene were not found in such a significant amount in other *A. vulgaris* oils (**Table 3**). Camphor, the major component of *A. vulgaris* oil from Italy, was also one of the major contributors in the Italian *A. absinthium* oil (*21*). The resemblance in essential oil compositions of two different plant species, geographically closely related, could indicate a similar high degree of susceptibility of the two *Artemisia* species to external factors. To some extent, this applies to the populations of *A. absinthium* and *A. vulgaris* collected in Niš.

The major component of wormwood root oil was α-fenchene (23.3%), but the most interesting property of this oil was the domination of aliphatic esters. The sum of linalyl (53.4%) (*30*), bornyl (4.2%), geranyl (4.1%), and neryl (2.8%) esters represents 64.5% of the total oil. In addition to the esters, wormwood root oil contained only hydrocarbon monoterpenes [acyclic (2.3%), *p*-menthane (7.5%), pinane (0.8%), and thujane (0.2%) type skeletons] and no free (not bound in an ester) oxygenated monoterpenes and no sesquiterpenes at all. This is unusual knowing that monoterpene formation is bound to the presence of active chloroplasts, and the latter cannot be expected in underground plant organs. Contrary to the oil isolated from aerial parts of the same herb, where thujane type monoterpenes were far most prevailing (altogether 76.9% of total oil), a single thujane type compound found in root oil was sabinene but in a very small amount (0.2% of total oil). This difference could be of great importance, since thujone is related with many malicious attributes (*2*, *31*). Toxicological and dermatological studies of linalyl esters, agents that are known and widely used in fragrance and, in some cases, flavor ingredients, utilized in decorative cosmetic, fine fragrances, and other toiletries, as well as in noncosmetic products (*32*−*34*), unlike those of thujone, have not revealed that the use of these compounds would have serious effects on human health (*32*−*35*).

The composition of Aa3R root essential oil seems to be intermediate between those isolated from normal (main compound was α-fenchene) and transformed (only constituents reported were neryl esters) roots of *A. absinthium* investigated by Kennedy et al. (*6*) and is in agreement with their proposed hypothesis on the maturity of the root system as the factor governing the oil compositions. However, because they have identified only 62% of the normal and 53% of the transformed root oil components, these hypotheses (maturity of root system or translocation effects) should be taken with some reserve. It is especially difficult to accept the hypothesis based only upon translocation effects knowing that β-thujone, the component that

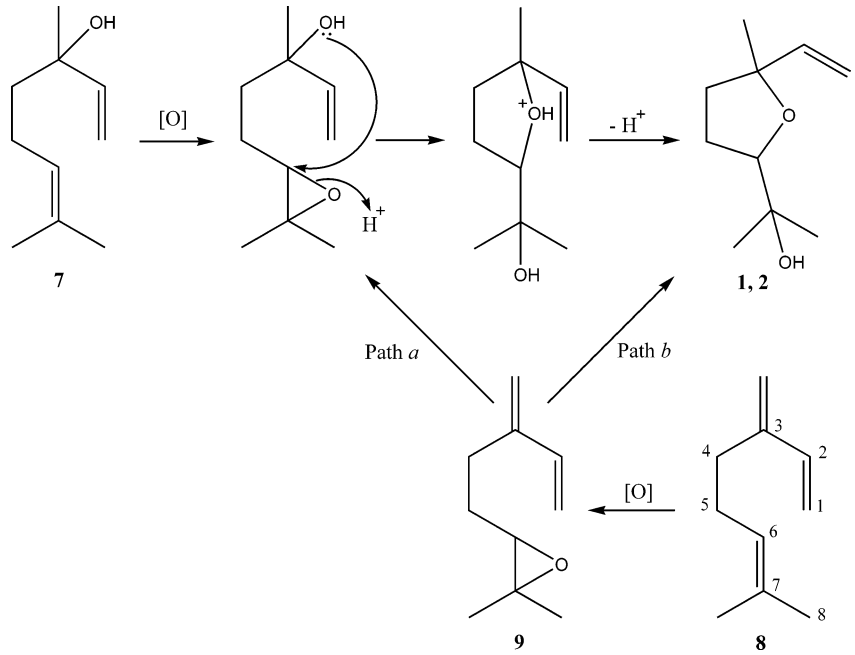

Figure 3. Possible mechanism of *cis*- and *trans*-linalooloxide (**1** and **2**) formation from linalool (**7**) and β-myrcene (**8**) [via 6,7-epoxymyrcene (**9**) as a reaction intermediate]. Path a: Markovnikov's addition of water to C3 methylenic double bond and subsequent epoxide opening by the nucleophilic attack of the formed alcohol. Path b: Epoxyde ring opening as a result of the nucleophilic attack of water in position 7 and subsequent furanoid ring closure.

Table 2. Previously Reported Data on the *A. absinthium* Essential Oil[a] Composition from Different Countries[b]

	% (w/w) in essential oil							
component	1	2	3	4	5	6	7	8
sabinene	**5.5**[c]	0.1	*d*	0.3	tr[e]	0.8–9.8	**7.4**	–
β-myrcene	0.1			1.2	tr	**6.8–20**	**33.2**	0.2
α-thujone	0.4				tr	**2.8–16**	**20.8**	0.2
linalool	**5.9**	1.6	**10.5**	**7.4**	1.7	2.1–5.1	1.5	0.2
β-thujone	**26.0**				1.3	**5.5–12.8**	**13.7**	0.5
cis-6,7-epoxyocimene	**24.1**	**30.0**		**31.1**	**25**	0–6.7		
trans-6,7-epoxyocimene	1.9	2.3		**4.0**		0–0.7		
camphor					**17**	0–0.2		1.4
terpinene-4-ol	1.0		**12**		tr	0.2–0.5	0.4	1.8
cis-chrysanthenyl acetate	1.2	**15.5**		**43.4**	**22**			0.1
bicyclo[2.2.1]hept-2-en-7-ol		**8.5**						
α-phellandrene	0.2		**50.5**		tr	0.2–0.7	0.3	
thujyl acetate						**0.4–21.3**		
sabinyl acetate	4.3				tr		6.6	
β-caryophyllene			**4.12**	0.6	1.4	1.5–4.5	0.6	
chamazulene	1.0					0.2–0.8		**17.8**
caryophyllene oxide	0.3	2.8	0.52		**10.0**			**4.3**
nuciferol butanoate								**8.2**
nuciferol propanoate								**5.1**
neryl propanoate		**3.9**				0.52–1.3[f]		

[a] Essential oils were obtained from plant material collected at the full bloom from aerial parts of plant. [b] Key: 1, Croatia (*18*); 2, France (*18*); 3, Egypt (*19*); 4, Spain (*20*); 5, Italy (*21*); 6, Siberia (*22*); 7, Republic of Bashkortostan (*23*); and 8, Turkey (*24*). [c] The four most dominant components in every oil are given in bold. [d] Component not identified. [e] Compound found in traces. [f] Other esters of nerol were present in much higher amounts.

represents up to 63.4% of the oil isolated from the aerial parts of wormwood collected at Nišava banks, was not found in the corresponding root oil at all. It is reasonable to expect that volatiles found in the aerial plant parts would be also present, in some amount, in the root system if translocation channels are present and the transport works both ways.

The main components of mugwort root oil were neryl 2-methylbutanoate (13.2%), β-eudesmol (10.0%), and bornyl

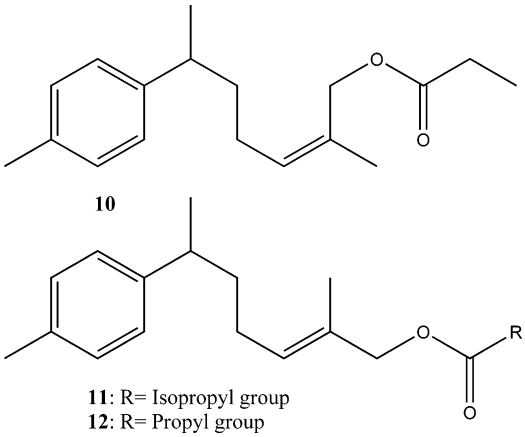

Figure 4. Esters found in the *A. absinthium* essential oils from Mokra: **10**-(*Z*)-nuciferyl propanoate, **11**-(*E*)-nuciferyl 2-methylpropanoate, and **12**-(*Z*)-nuciferyl butanoate.

3-methylbutanoate (8.4%). None of these substances was found in the oil obtained from aerial parts of this plant species. Worthy of mention is the fact that, contrary to the other examined oils, this one was characterized with a high percentage of sesquiterpenes (55.1%) and that esters prevailed in the monoterpenoid fraction (29.7%). Another interesting characteristic of the root oil was also the presence of the rather unusual compounds presented in **Figure 5**: petasitene (**13**), albene (**14**), and triquinane sesquiterpenes, 7α-silphiperfol-5-ene (**15**), silphiperfol-6-ene (**16**), modhephene (**17**), pethybrene (**18**), presilphiperfol-7-ene (**19**), silphin-1-ene (**20**), α-isocomene (**21**), and β-isocomene (**22**). Triquinane sesquiterpenes were previously also found in some other species from the *Artemisia* genus (*36*, *37*).

In the oil isolated from aerial parts of *A. vulgaris*, the co-occurrence of β-caryophyllene and caryophyllene oxide, and the total lack of triquinane sesquiterpenes, suggests that the only fate of β-caryophyllene is the oxidation to caryophyllene oxide. In the root oil, however, caryophyllene oxide was not detected,

Table 3. Previously Reported Data on the *A. vulgaris* Essential Oil[a] Composition from Different Countries[b]

component	% (w/w) in essential oil							
	1	2	3	4	5	6	7	8
α-thujene	2.1	0.8	0.4				0.2	**4.1**[c]
α-pinene	2.0	**53.7**	1.3	*d*		**15.1**	**7.6**	0.1
camphene	**9.1**	1.8	4.2				2.0	3.9
β-pinene	2.1	**7.4**	1.8				**11.7**	1.0
β-myrcene	0.1	**8.8**	1.3				1.9	
α-phellandrene	tr[e]	1.0	**17.3**			6.3		
1,8-cineole	3.9	1.0	3.6			**11.7**	**24.1**	
artemisia alcohol	tr							**4.5**
α-thujone	tr				**0.4**	8.5		1.3
trans-chrysanthenol		**13.1**						
β-thujone	0.1	0.5			**1.2**	**20.8**		
camphor	**47.7**	0.5	tr		**9.2**	**8.7**	**13.2**	**38.7**
lyratol			**15.1**					
trans-verbenol	**7.0**							
isoborneol							0.3	**8.2**
borneol	0.4	0.6			**8.9**	2.4	2.1	
α-terpineol	0.3		0.5			0.2	7.5	
verbenone	**8.6**		tr					
γ-elemene			**8.8**					
humulene			**8.8**			0.2		
isobornyl 2-methylbutyrate				**5.3**				
caryophyllene oxide	2.2		tr	**31.1**	2.3	0.6	0.3	
2-heptadecanone				**5.1**				
hexadecanoic acid				**6.3**				
trans-isoelemicin			**15.1**					

[a] Essential oils were obtained from plant material collected at the full bloom from aerial parts of the plant. [b] Key: 1, Italy (*21*); 2, Republic of Bashkortostan (*23*); 3, Egypt (*25*); 4, Cuba (*26*); 5, France (*27*); 6, Croatia (*27*); 7, Vietnam (*28*); and 8, India (*29*) (oil obtained from flowers). [c] The four most dominant components in every oil are given in bold. [d] Component not identified. [e] Compound found in traces.

but instead, considerable amounts of sesquiterpenes **15**−**22** (**Figure 5**) were present. Triquinane sesquiterpenes also originate from β-caryophyllene (*38*) and are thus the products of a biosynthetic branch that could be considered as an adversary to one resulting in caryophyllene oxide formation. This suggests that separate and distinct enzymatic pathways operate in the root system and the aerial parts of the plant.

Antimicrobial Activities. The oils and individual terpenes identified in the oils were tested at two doses (1:10 and 1:30 dilution, 50 μL), and the results of the antimicrobial assays are given in **Table 4**. Essential oils isolated from both *A. absinthium* populations, collected at Mokra and Nišava banks (Aa1 and Aa3 oils, respectively), showed a high inhibitory effect on bacterial and fungal growth, with Aa3 being slightly more active against all tested microorganisms, except in the cases of *Escherichia coli* and *Staphylococcus aureus*. This difference could be the consequence of a notably higher β-thujone content in the oil from *A. absinthium* gathered at Nišava riverside (64.4%) as compared to the oil from Mokra (19.8%). Both thujone isomers showed high antimicrobial activity (**Table 4**) corroborating this assumption, and in general, it is thought that thujones are recognized as wormwood oil active principles affecting microbial growth (*18*). However, the observed high activity of Aa1 oil could be correlated as well to the presence of additional two known active constituents, linalool and β-caryophyllene, which also showed good results in the present antimicrobial testing (**Table 4**). In most cases, *A. absinthium* (Mokra) oil with prolonged storage time (Aa2) was more active then Aa1 oil. Exceptions include the activity against *E. coli*, *Candida albicans*, and *S. aureus*. Aa2 as compared to Aa1 oil had a considerably higher content of 1,8-cineole, a well-known antimicrobial agent (*39*), although our findings did not confirm this. A higher

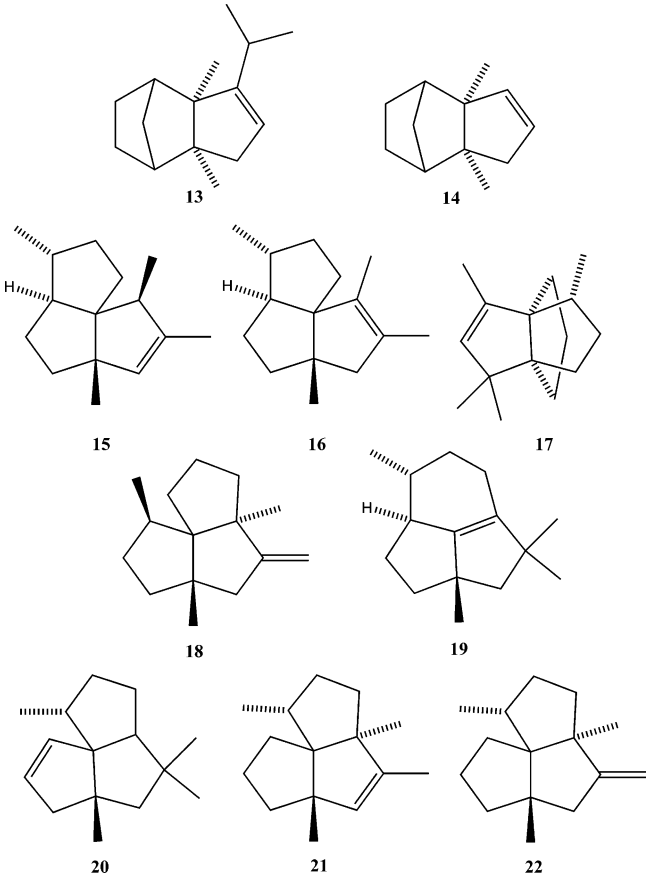

Figure 5. Some unusual compounds found in the root oil from *A. vulgaris*: **13**, petasitene; **14**, albene and triquinane sesquiterpenes; **15**, 7α-silphiperfol-5-ene; **16**, silphiperfol-5-ene; **17**, modhephene; **18**, pethybrene; **19**, presilphiperfol-7-ene; **20**, silphin-1-ene; **21**, α-isocomene; and **22**, β-isocomene.

susceptibility of *E. coli* to Aa1 as compared to Aa2 and Aa3 oils could be connected to the presence of linalool, a monoterpene alcohol apparently showing a high activity against this bacterium (**Table 4**). Although *A. absinthium* root oil (Aa3R) completely lacked thujones, it exhibited a relatively high and nonselective activity. Recent research (*40*) on the antimicrobial activity of geraniol and geranyl derivatives including esters of isobutyric, butyric, propanoic, and acetic acids suggests that the corresponding linalyl esters identified in Aa3R oil could likewise be held responsible for the observed activity of this oil. The antimicrobial action of its main components is yet to be tested, and possible synergistic effects should not be excluded. The high antimicrobial activity of this oil indicates that wormwood roots could have a potential medicinal use.

It seems that the presence of 1,8-cineole and β-thujone ensured that the oil isolated from mugworth aerial parts has a good and nonselective activity against the bacteria and fungi tested. An unexpectedly low activity of *A. vulgaris* root oil was in correlation with the absence of thujones and a rather low concentration of 1,8-cineole. Furthermore, the demonstrated static and noncidal activity of bornyl acetate (5.0%), one of the major constituents of AvR oil, is in agreement with the overall low AvR oil activity. It is reasonable to presume that bornyl 3-methylbutanoate (8.4%) would have comparable influence on microbial growth. Compounds with triquinane carbon skeletons, related to those identified in *A. vulgaris* root oil, represented in **Figure 5** (**15**, **16**, **18**, **20**, **21**, and **22**), are reported to be biologically important sesquiterpene antibiotics (*41*). Considering all of these facts, it would be interesting to investigate the

Table 4. Antimicrobial Activity[a] of the Examined Oils and Corresponding Pure Constituents

		Sample													
	micro-organism[b]	*Escherichia coli* 95		*Salmonella enteritidis* ATCC 13076		*Pseudomonas aeruginosa* ATCC 9027		*Klebsiella pneumoniae* ATCC 10031		*Staphylococcus aureus* ATCC 6538		*Candida albicans* ATCC 10231		*Aspergillus niger* ATCC 16404	
	sample	C[c]	S[d]	C	S	C	S	C	S	C	S	C	S	C	S
							1:10 dilution								
A. absinthium	Aa1	37	*e*	24		25	27	26		24	27	26		25	
	Aa2	27		30		26	40	27		24	28	25		29	
	Aa3	29		28		28		36		29		30		31	
	Aa3R	30	33	25		20	29	22	30	26	31	29		24	28
A. vulgaris	Av	32	36	25	29	31	35	26	34	26	29	40		30	31
	AvR	16	18		37	13.5	16	13.5	16	13.5	16		13.5		
							1:30 dilution								
A. absinthium	Aa1	23		15		15		17		26	28	15		16	
	Aa2	16	18	22		16	22	19		16		18		18	
	Aa3	18	20	19		17		21		19		16		17	
	Aa3R	18	24	16			17		15	17	20	14			15
A. vulgaris	Av	22.5	25	17	18	27.5		22	26	17	18	20		21	
	AvR						14								
		Standard													
	micro-organism[b]	*Escherichia coli* 95		*Salmonella enteritidis* ATCC 13076		*Pseudomonas aeruginosa* ATCC 9027		*Klebsiella pneumoniae* ATCC 10031		*Staphylococcus aureus* ATCC 6538		*Candida albicans* ATCC 10231		*Aspergillus niger* ATCC 16404	
	sample	C[c]	S[d]	C	S	C	S	C	S	C	S	C	S	C	S
							1:10 dilution								
β-caryophyllene		16		30		14	18			14					
caryophyllene oxide		18	26	25		20	24	21		20			21	21	
α-humulene															
linalool		37		34		33		27		24		40		34	
linalooloxide (furanoid)		25		25			18		16		17		21	19	
bornyl acetate			36		35		22				30		34		
camphor		38		28	32	18	31	21	29	29		22		29	
α-thujone		25		25	37	24		24	30	31		26		26	
β-thujone		24		25		23		23	31	29		27		26	
1,8-cineole			18		20		17				17		18		18
α-pinene															
p-cymene															
eugenol		34		38		36		37		31		28	39	30	39
		Antibiotics													
micro-organism[b]		*Escherichia coli* 95		*Salmonella enteritidis* ATCC 13076		*Pseudomonas aeruginosa* ATCC 9027		*Klebsiella pneumoniae* ATCC 10031		*Staphylococcus aureus* ATCC 6538		*Candida albicans* ATCC 10231		*Aspergillus niger* ATCC 16404	
sample		C[c]	S[d]	C	S	C	S	C	S	C	S	C	S	C	S
doxycycline		29		28		27.5		27		28		nt[f]		nt	
tiamulin		10		10		11		10		10		nt		nt	
erythromycin		27		24		22		24		20		nt		nt	
nystatin		nt		nt		nt		nt		nt		18		17	

[a] Antimicrobial activities are represented as the inhibition zones, mm, including the disk diameter, 6 mm. [b] All strains were obtained from the American Type Culture Collection (MD), except for the *E. coli* 95, which was acquired from the Institute of Immunology and Virology "Torlak" (Belgrade). [c] Bacterio- and fungicidal zones. [d] Bacterio- and fungistatic zones. [e] No activity observed. [f] Not tested.

antimicrobial activities of the pure terpenoids found in AvR oil, since possible antagonism and/or synergism of the oil constituents can be expected. Despite the fact that the root oil of *A. vulgaris* had shown the weakest inhibitory effect on microbial growth as compared to all other examined oils, the existence of other possible specific therapeutic properties of the mugwort root essential oil cannot be excluded.

Although, according to the data presented in **Table 4**, it can be concluded that the antimicrobial activity of some of the analyzed flavor compounds (in given concentrations) is comparable with those of well-known antibiotics such as erythromycin, it is very important to emphasize that flavors cannot be applied in the same way as antibiotics (orally) because of self-dosage limitations influenced by their toxicity, resorption properties, smell, and taste.

ACKNOWLEDGMENT

Tanja Mitrović is acknowledged for collecting *A. absinthium* plant material from Mokra.

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(30) Reviewing the available literature brought to our attention a paper of Fraternale et al. (*42*). In their work, they determined the chemical composition of *Peucedanum verticillare*. One of the components that they identified was linalyl 3-methyl butanoate, which had a RI of 1395 for this ester on a SPB-1 fused silica column. The RI for the same compound obtained in this study was 1484. Although the experimental conditions were to some extent different, we find them insufficient to cause such a large difference in the value of RIs. As the mentioned component was among the most abundant in *A. absinthium* essential oil, the confirmation of its identity was also accomplished using ^{13}C NMR spectra, in addition to the GC and GC-MS analysis. Moreover, altogether, six different linalyl esters have been found in the analyzed oils, and an excellent correspondence was established between the RIs on a DB-5 column of some of the identified linalyl esters with literature data (*43*, *44*). This strongly suggests that the identification of linalyl 3-methyl butanoate by Fraternale and co-workers (*42*) may need revision. The RI of their component better corresponds to that for linalyl 2-methyl propanoate or linalyl butanoate.
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