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Essential Oil Composition of *Ambrosia Artemisiifolia* and Its Antibacterial Activity Against Phytopathogens†

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Abstract: The composition of essential oil from aerial parts of Ambrosia *artemisiifolia* L. from Bor (Serbia) was analyzed. The essential oil was obtained by hydrodistillation and analyzed by gas chromatography (GC-FID, GC-MS). In total, 45 compounds were detected (98.49% of the total). The essential oil was dominated by monoterpene (45%) and sesquiterpene (38.51%) hydrocarbons. The principal constituents were germacrene D (25.3%), limonene (21.6%), and α-pinene (15.7%). The microdilution method was used to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the essential oil against five Gram-negative phytopathogenic strains. Essential oil exhibited strong antimicrobial activity against two *Xanthomonas campestris* strains and one referent and one natural isolate of *Ervinia amylovora*, causative agents of black rot and fire blight.

Keywords: biological control; invasive species; chemical composition; monoterpene hydrocarbons; sesquiterpene hydrocarbons

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1. Introduction

Ambrosia L. (Asteraceae, Heliantheae, Ambrosiinae) includes nearly 35–40 species [1,2] distributed mainly in America [1]. The genus comprises annual or perennial [3] anemophilous plants [4].

Ambrosia artemiisifolia L. is an annual herb native to North America [5,6], but it is widespread in many parts of the world [7]. Ragweed was introduced from North America into Europe in the 19th century [5], and it grows in the central and southern parts of the continent, usually in waste places near urban areas [8]. Nowadays, it is known in most European countries [9]. This plant is also widespread in Serbia, especially in the northern part of the country [10].

Ragweed has a strong reproductive capacity. Each plant can produce a large number of seeds and pollen, causing numerous allergic reactions [11].

Only a few previous studies focused on the analysis of the composition of the essential oil of *A. artemisiifolia* and showed that different classes of specialized metabolites are present in the essential oil. Monoterpenes and sesquiterpenes were dominant compounds [6,12].

To the best of our knowledge, there is no data regarding the antibacterial activity of *A. artemisiifolia* essential oil.

The objectives of the present study were to determine the composition of the EOs of ragweed and investigate its potential use as a biological control agent.

2. Material and Methods

2.1. Plant Material

Plant material of *A. artemisiifolia* was collected in October 2020, during the flowering period near the town Bor, in Eastern Serbia. Plants were identified using floras of Serbia and Europe [3,10]. Voucher specimens were deposited at the Herbarium of the University of Belgrade—Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac" (BEOU 17821). Standard herbarium acronym follows Index herbariorum [13].

2.2. Isolation of Essential Oil

Dried flowering aerial parts (200 g) were chopped and placed in a round-bottomed flask, and then 2l of cold distilled water was added. Hydrodistillation was performed 3 times for 3 h using the Clevenger-type apparatus, according to the procedure described in Ph. Eur. 6 [14]. The obtained oils were stored at 4 °C before the GC analyses.

The extraction yield of oil was calculated according to the equation given: $y = V/W \times 100$ where y is the oil yield (%, *w*/*w*), V is the mass of extracted plant oil (g), and W is the mass of dried plant material (g).

2.3. GC-FID and GC/MS Analyses

The GC-FID and GC/MS analyses were conducted according to the procedure described in [15].

2.4. Antibacterial Activity

2.4.1. Bacterial Strains and Growth Conditions

Antibacterial activity was tested using five Gram-negative phytopathogenic strains *Pseudomonas syringae* pv. *syringae* GSPB 1142, *Xanthomonas* pv. *campestris* NCPPB 528 and NCPPB 1144, *X. arboricola* pv. *juglandis* CFBP 2528, *Erwinia amylovora* NCPPB 683, *E. amylovora* 16–13. The bacterial strains were cultured in TY medium (composition g/L: tryptone 5, yeast extract 5, $CaCl_2 \times 2\ H_2O$ 0.9) for 48 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.

2.4.2. MIC Assay

The microdilution method [16] was used to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the *A. artemisiifolia* essential oil. Two-fold serial dilutions with TY 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. Final essential oil concentrations in the first well ranged from 0.008–2 mg/mL. Besides a negative control, a sterility control, and control for the solvent (DMSO), the antibiotics streptomycin (Sigma-Aldrich, USA) was tested as positive controls in the concentration range from 0.003–0.2 mg/mL. The final concentration of dimethyl sulfoxide (DMSO) as a solvent was 10%. All dilutions were done in duplicate, and the results are expressed in mg/mL. After reaching the final volume, 22 μL of resazurin as an indicator in the final concentration of 0.675 mg/mL was added, and the 96-well microtiter plates were incubated for 48 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.

3. Results

3.1. A. Artemisiifolia EO Composition

The yield of essential oil was 0.03%. The oil was transparent yellow, with a sharp and strong smell. The conducted GC-FID, and GC-MS analyses resulted in the detection of 45 compounds (41 identified, 4 unidentified), making on average 98.49% of the total oil. All identified compounds are listed in Table 1.

The results showed that monoterpene hydrocarbons and sesquiterpene hydrocarbons were the dominant constituents in the EO (45% and 38.51%, respectively). Oxygenated monoterpenes and oxygenated sesquiterpenes were also present, but in smaller quantities (3.42% and 11.54%, respectively). The most dominant constituents were germacrene D (25.3%), limonene (21.6%), and α-pinene (15.7%).

Table 1. Chemical constituents of the essential oil of investigated *A. artemisiifolia.*

No.	RI [1]	Compound	[%] [2]
1	903	Santolina Triene	0.10
2	929	α-Pinene	15.75
3	944	Camphene	0.33
4	969	β-Phellanderene	0.94
5	973	β-Pinene	1.77
6	987	Myrcene	4.54
7	1027	Limonene	21.59
8	1139	*trans*-Pinocarveol	0.21
9	1144	1,3,8-p-Menthatriene	0.31
10	1164	Borneol	0.63
11	1166	Ni (109,69,93,81,41)	0.30
12	1285	Bornyl Acetate	1.81
13	1290	Lavandulyl Acetate	0.46
14	1337	δ-Elemene	0.29
15	1375	α-Copaene	0.22
16	1384	β-Bourbonene	0.58
17	1390	β-Cubebene	0.27
18	1391	β-Elemene	0.33
19	1419	*(E)*-Caryophyllene	3.22
20	1429	β-Copaene	0.47
21	1435	*trans*-α-Bergamotene	0.53
22	1453	α-Humulene	1.09
23	1457	*(E)*- β-Farnesene	0.28
24	1475	β-Chamigrene	0.48
25	1482	Germacrene D	25.26
26	1484	β-Selinene	1.35
27	1496	Bicyclogermacrene	1.92
28	1508	β-Bisabolene	0.91
29	1511	Lavandulyl isovalerate	0.51
30	1515	δ-Amorphene	0.39
31	1523	δ-Cadinene	0.75
32	1556	Germacrene B	0.18
33	1559	Ni sesquiterpene (159,177,135,41,91)	0.44
34	1576	Spathulenol	1.98
35	1582	Caryophyllene oxide	3.06
36	1608	Humulene epoxide II	0.64
37	1610	β-Atlantol	0.39

38	1617	Junenol	0.92
39	1622	1,10-di-*epi*-Cubenol	0.25
40	1631	Muurola-4,10(14)-dien-1- β-ol	0.29
41	1634	Ni (246,119,105,91,187)	0.35
42	1653	α-Cadinol	0.66
43	1658	Valerianol	1.59
44	1686	Germacra-4(15),5,10(14)-trien-1-α-ol	0.81
45	1766	Ni (81,93,107,123,147)	0.35
		Total monoterpenes	48.43
		Monoterpene hydrocarbons	45.01
		Oxygenated monoterpenes	3.42
		Total sesquiterpenes	50.05
		Sesquiterpene hydrocarbons	38.51
		Oxygenated sesquiterpenes	11.54
		Unknown	1.00
		TOTAL	**98.49**

[1] The retention indices (RI) were experimentally determined using the standard method involving retention times (tR) of n-alkanes, which were injected under the same chromatographic conditions. [2] Contents are given as percentages of the total essential oil composition; Ni = not identified.

3.2. Antibacterial Activity of A. artemisiifolia EO

Tested essential oil exhibited strong antimicrobial activity against both *X. campestris* strains, and against one referent and one natural isolate of *E. amylovora.* Strains were inhibited by lower concentrations which could be designated as similar detected in the positive control of streptomycin. Moderate activity was detected against *P. syringae* pv. *syringae*, while *X. arboricola* pv. *juglandis* was the most resistant strain tested. All inhibitory and bactericidal activities of EO were below the detected inhibitory concentrations of DMSO as solvent. Results of tests on *A. artemisiifolia* oil antibacterial activity are given in Table 2.

Table 2. Antibacterial activity of *A. artemisiifolia* EO.

Phytopathogenic Strains	*Ambrosia* EO (mg/mL)		DMSO (%)		Streptomycin (mg/mL)	
	MIC	MBC	MIC	MBC	MIC	MBC
Xanthomonas pv. *campestris* NCPPB 528	0.004	0.008	1.875	2.500	0.025	0.100
Xanthomonas pv. *campestris* NCPPB 1144	0.063	0.125	0.469	0.625	0.050	0.100
Erwinia amylovora NCPPB 683	0.016	0.031	7.500	10.000	0.006	0.100
Erwinia amylovora 16–13	0.047	0.063	7.500	10.000	0.012	0.100
Pseudomonas syringae pv. *syringae* GSPB 1142	0.500	2.000	>10.000	-	0.006	>0.200
Xanthomonas arboricola pv. *juglandis* CFBP 2528	1.500	2.000	>10.000	-	0.003	0.013

- not detected in the range of tested concentrations.

4. Discussion

In the present study, the most abundant compounds were germacrene D (25.3%), limonene (21.6%), and α-pinene (15.7%). These results are congruent with the scarce literature data [6,12]. There are some differences in the relative amounts of major classes of compounds between EOs of *A. artemisiifolia* and related species. The oil of *A. artemisiifolia* is much more abundant in monoterpene hydrocarbons and sesquiterpene hydrocarbons, in contrast with *A. trifida* [17].

It was shown that significant bactericidal activity of *A. artemisiifolia* essential oil was effective even in very dilute solutions against a broad range of human opportunistic bacterial strains, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Sarcina lutea*, *Shigella flexneri* and *Salmonella enteritidis* [12]. In general, plant extracts and essential oils contain numerous compounds like sesquiterpenoids, which can exhibit antimicrobial activity [18]. Although many sesquiterpene lactones have been related to allergenic effects, some previous studies showed how isabelin, the lactone isolated from *A. artemisiifolia*, was able to inhibit soil-borne bacteria [19]. That might imply the potential of these molecules to modify the surrounding soil microbiota and associated pathogens eventually. In the present study, *A. artemisiifolia* essential oil exhibited strong antimicrobial activity against *X. campestris* and *E. amylovora* strains, causative agents of black rot and fire blight. Thus, our results indicate that essential oil produced by invasive plant *A. artemisiifolia* could be a valuable source of compounds with great potential for biological control of economically important phytopathogens.

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