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Original Scientific Paper

Phenolic profile and antimicrobial activity of leaf extracts from five *Artemisia* species (Asteraceae)

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ABSTRACT:
This study aimed to analyse and evaluate the antimicrobial activity and phenolic compounds in the leaf extracts of five *Artemisia* species (*A. alba*, *A. annua, A. campestris*, *A. pontica*, and *A. vulgaris*), of which *A. annua* is of significant medical importance. Although many *Artemisia* plants are well known from ethnobotanical and phytochemical studies, the biological activity of *Artemisia* species against phytopathogenic strains is scarcely investigated. Therefore, the presence of phenolic compounds and the antimicrobial activity of dichloromethane-methanol (1:1) leaf extracts of five *Artemisia* species against phytopathogenic fungi and bacteria were analysed. The phenolic compounds were determined by liquid chromatography mass spectrometry (LC-MS). In total, 13 phenolic compounds and quinic acid were identified and quantified. Chlorogenic acid was the dominant compound in all the samples, while the second dominant compounds were rutin in the *A. alba*, *A. campestris*, and *A. pontica* extracts, vitexin in *A. annua*, and esculin in the *A. vulgaris* extracts. Antifungal activity was tested against 12 micromycetes a using mycelial growth assay and the microdilution method. Antibacterial activity was tested against 18 bacterial strains using the well diffusion and microdilution methods. In each test, the highest activity was shown for the extracts of different *Artemisia* species. The most sensitive micromycetes were *Monillinia laxa*, *Penicillium citreonigrum*, and *Botrytis cinerea*, while *Fusarium graminearum* B1 was the most resistant. The analysed extracts showed moderate antibacterial activity only against *Xanthomonas campestris* pv. *campestris* compared with all the other tested phytopathogens. The study shows that the leaf extracts of certain *Artemisia* species contain phenolic compounds and showed moderate antimicrobial activity against some species of fungi and bacteria.

Keywords:
antibacterial activity, antifungal activity, flavonoids, phenolic acids

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INTRODUCTION

According to the latest phylogenomic analysis, the genus *Artemisia* L. (Anthemideae, Asteraceae) is divided into eight subgenera: *Dracunculus*, *Pectinata*, *Pacifica*, *Ponticae*, *Seriphidium*, *Tridentatae*, *Absinthium*, and *Artemisia* (Jiao *et al.* 2023). It comprises over 500 species (Bora & Sharma 2011), including small herbs and shrubs, mainly distributed in the northern hemisphere, with widespread therapeutic and pharmacological activities (Nigam *et al.* 2019). Phenolic compounds, flavonoids and phenolic acids, are one of the major classes of specialised metabolites isolated from different species of the genus *Artemisia* (Al-hazim & Bashra 1991; Carvalho *et al.* 2011; Ashok & Upadhyaya 2013). The most extensively phytochemically studied species is *A. annua* with around 50 flavonoids identified (Dilshad *et al.* 2016). However, several flavonoids have been found in other *Artemisia* species, i.e. *A. alba* (Trendafilova *et al.* 2018), *A. campestris* (Ghlissi *et al.* 2016), and *A. vulgaris* (Pires *et al.* 2009). Phenolic acids were also detected in all of these species (Djeridane *et al.* 2007; Jakovljević *et al.* 2020; Abate *et al.* 2021). On the other hand, only a few studies have identified phenolic compounds in *A. pontica* aerial parts (Talzhanov *et al.* 2005; Saunoriūtė *et al.* 2022).

A broad spectrum of natural phenolic compounds can be biologically active and effective in the prevention of different diseases and disorders (Soto-Hernández *et al.* 2017). Various parts of *Artemisia* taxa have been utilised as remedies in traditional herbal medicine, e.g. the leaves of *A. annua* against fever (Hayat *et al.* 2009), colds, and coughs (Hayat *et al.* 2009; Kayani *et al.* 2014), the leaves of *A. vulgaris* against jaundice (Ginko *et al.* 2022), and the aerial parts of *A. alba* and *A. campestris* against digestive system disorders (Rigat *et al.* 2007; Benderradji *et al.* 2014), etc. Some of them are also used internally as anthelmintics such as the leaves and buds of *A. campestris* (Benderradji *et al.* 2014), and the leaves of *A. vulgaris* (Hamayun *et al.* 2007; Aziz *et al.* 2016). Also, the phenolic compounds identified in various extracts of *Artemisia* species have shown antimicrobial, antioxidant (Karabegović *et al.* 2011), anti-inflammatory (Afsar *et al.* 2013), cytotoxic (Radović Jakovljević *et al.* 2019), and other properties. Chlorogenic acid has proved to be very effective against some human (Naveed *et al.* 2018) and plant bacterial pathogens (Ravn *et al.* 1989).

To date, the antimicrobial activity of *Artemisia* extracts has been intensively studied. Patwa & Das (2020) investigated the antifungal activity of the stem extracts of *A. vulgaris in vitro* against some phytopathogenic fungi from the genera *Aspergillus*, *Fusarium*, *Penicillium,* and *Colletotrichum*. Hendel *et al.* (2021) tested the antifungal activity of methanolic extracts of *A. campestris* against *Botrytis cinerea*, and *P. expansum*. Antibacterial activity has also been analysed against some human (Tharib *et al.* 1983; Patwa & Das 2020), and plant pathogen bacterial strains (Karabegović *et al.* 2011). A small number of studies investigated the effect of *Artemisia* spp. extracts on phytopathogenic bacteria (Ivanescu *et al.* 2021).

Nowadays crop loss represents a growing problem due to plant diseases caused by different insects and other plant pathogens. The overuse of artificial chemical pesticides, considered as environmental pollutants, has led to the emergence of multidrug-resistant phytopathogenic strains, giving rise to a new generation of diseases which cannot be treated with existing agents (Dimkić *et al.* 2020). In response, there has been a growing interest in research into different natural compounds as alternative agents including plant extracts and EOs.

The objective of this study was to evaluate the antimicrobial potential of dichloromethane-methanol (DCM/MeOH 1:1) leaf extracts of five *Artemisia* species: (*A. alba* Turra, *A. annua* L., *A. campestris* L., *A. pontica* L., and *A. vulgaris* L.) from Serbia against selected phytopathogenic fungi and bacteria, and to analyse their phenolic composition.

MATERIALS AND METHODS

Plant material. The sample data are listed in Table 1. The voucher specimens were deposited in the Herbarium of the Institute of Botany and Botanical Garden Jevremovac – Faculty of Biology, University of Belgrade (BEOU). The plant material was determined using professional literature (Gajić 1975). The leaves were separated and dried at room temperature for several days, then packed in paper bags and stored in dark and dry places prior to use.

Preparation of the leaf extracts. The dried leaves (10 g of each species) were powdered, and extracted with 150 mL of solvent mixture of dichloromethane and methanol (DCM:MeOH = 1:1). All the samples were ultrasonicated for 30 min in an ultrasonic bath at 25°C (first series). Each extract was filtered through Whatman No. 1 filter paper immediately after ultrasonic extraction. The filtrates were evaporated to dryness with a rotary vacuum evaporator and stored at 4°C until use. For the analyses of the metabolite profile all the prepared extracts were dissolved in MeOH at a concentration of 30 mg/mL.

The LC/MS metabolic profile. The metabolite profile of the extracts was determined by UHPLC-ESI/MS2 (Dionex Ultimate 3000 UHPLC, Thermo Scientific, San Jose, CA, USA). The phenolic compounds and quinic acid were separated and identified as previously described (Gašić *et al.* 2015). The MS detection was performed in negative mode using a triple quadrupole (QqQ) mass spectrometer (Thermo Finnigan, San Jose, CA, USA)

Table 1. The investigated *Artemisia* species

Species	Code	Locality	Coordinates	Growing stage	BEOU
Artemisia alba Turra	AALB	Svrljig, Niševac	43°27'48.75"N; 22°06'0.75"E	Flowering	17915
Artemisia annua L.	AANN	Opovo, Sakule	45°08'38"N; 20°28'06"E	Flowering	17917
Artemisia campestris L.	ACAMP	Deliblatska peščara	44°48'54.4"N; 21°03'58.7"E	Flowering	17918
Artemisia pontica L.	APONT	Zaječar	43°55'24.4"N; 22°17'46.17"E	Flowering	17453
Artemisia vulgaris L.	AV	Opovo, Sakule	45°08'38"N; 20°28'06"E	Flowering	17761

Table 2. The extraction yield of the *Artemisia* leaf extracts

Code	Yield
AALB	9.41 %
AANN	14.74 %
ACAMP	5.11 %
APONT	7.57 %
AV	7.43 %

equipped with a heated electrospray ionisation (H-ESI) source. The compounds were identified based on their chromatographic behaviour and mass spectra by comparison with standard compounds. Data acquisition was carried out using an Xcalibur® data system (Thermo Finnigan, San Jose, CA, USA). For quantitative analysis, a calibration curve for each available standard was constructed based on the MS/MS spectra. The results were expressed as mg/g of the extract.

The tested fungal isolates and growth conditions. The selected fungal isolates used in this study were: *Alternaria alstromeriae* IB1I-20, *Aspergillus parasiticus* S3-1, *Botrytis cinerea* 46, *Cladosporium cladosporioides* S1-4, *Curvularia spicifera* S2-5, *Fusarium graminearum* B1, *F. graminearum* CIK, *F. oxysporum* S4-2, *F. verticillioides* 5.1, *Monilinia laxa* M1, *Penicillium citreonigrum* BEOFB11191m, and *P. expansum* BEOFB11132m. These phytopathogenic fungi were obtained from the official culture collection of the Department for Algology, mycology, and lichenology, Institute of Botany and Botanical Garden Jevremovac – Faculty of Biology, University of Belgrade.

Prior to the study, the tested filamentous fungi were stored in cryovials with 1.5 mL of 30% glycerol at -75°C. Conidial suspensions were prepared by washing the conidia from the surface of 7-day-old potato dextrose agar (PDA) slants using a sterile saline solution (0.9% NaCl, Hemofarm hospitalogica) supplemented with 0.1% Tween 20 (v/v). The final concentration of the prepared suspensions was 1.0×10^5 CFU mL^{-1} per protocol given in Unković *et al.* (2018). Before use, the conidial suspensions (10 µL) were cultured on PDA plates to check the validity of the inocula and verify the absence of contamination.

The tested bacterial strains and growth conditions. The antibacterial activity of different extracts was tested against 18 phytopathogenic bacterial strains. The prepared leaf extracts were tested on 17 Gram-negative bacteria, including four reference strains *E. amylovora* txid665029, *P. syringae* pv. *syringae* txid317, *X. campestris* pv. *campestris* txid1357999, and txid1358009, and four natural isolates of *Pseudomonas oryzihabitans,* two of *E. amylovora, Pantoea allii* and *Xanthomonas arboricola* pv. *juglandis*, and one of *Agrobacterium tumefaciens*, *Erwinia persicina*, *Kosakonia cowanii*, *X. campestris*, and one Gram-positive strain (*Rathayibacter tritici*). The bacterial strains were cultured on Luria-Bertani agar plates (LA; composition g/L: tryptone 10, yeast extract 5, NaCl 5, agar 15) for 24 h at 30. The suspensions were prepared in a phosphate saline buffer (1 × PBS, Sigma Aldrich, USA) in the final concentration of 10^6 CFU mL^{-1}. All the reference strains used belonged to the Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Belgrade.

The antimicrobial activity assays. Antimicrobial assays were used to determine the antifungal and antibacterial biological activities: a mycelial growth assay for antifungal, a well diffusion test for antibacterial, and the microdilution method (MIC assay) for both activities. For the antifungal activity each of the tested extracts was dissolved in 5% dimethyl sulfoxide (DMSO) at a final concentration of 40 mg/mL, while for the antibacterial activity these extracts were dissolved in MeOH at the same concentration.

The mycelial growth assay. The antifungal effect of all the tested leaf extracts against selected fungi isolates was tested *in vitro*. A final volume of 100 µL of each extract dissolved in 5% DMSO (40 mg/mL) was spread on a PDA plate. A mycelial plug from the periphery of 7-day-old cultures was placed in the centre of the PDA plate. The experiment was repeated twice independently, with three replications for each fungus. The inoculated plates were incubated at 25°C and colony diameter measurements were conducted after 7 days. The effects on mycelial growth were evaluated by calculating the percentage of growth inhibition per formula: PGI% = 100(KR-R1)/KR, where KR represents the growth of the test fungus in the control, and R1 is the growth of the test fungus

Table 3. The compounds identified in the *Artemisia* leaf extracts

Compounds	Retention time (t_R), min	Molecular ion, *m/z*	MS/MS fragments, *m/z*	AALB[1]	AANN	ACAMP	APONT	AV
Quinic acid	0.87	191.02	173.01	0.639	0.098	0.706	0.383	0.107
Aesculin	4.56	339.07	177.02; 133.01	0.533	0.053	0.097	0.077	0.108
Chlorogenic acid	4.92	353.09	191.03	2.515	0.721	2.416	1.253	0.231
Caffeic acid	5.33	179.04	135.02	0.112	0.037	0.068	0.060	0.030
Rutin	6.03	609.15	301.04; 179.10	0.995	0.118	1.488	1.017	0.063
Vitexin	6.04	431.10	311.07; 283.06	0.030	0.181	nd	0.013	0.021
Isoquercetin	6.19	463.09	301.04: 179.10	0.108	0.135	0.209	0.127	0.053
Narcissin	6.45	623.16	315.05; 300.04	0.242	nd	0.233	0.036	0.022
Apigetrin	6.68	431.09	269.04; 255.03	0.024	0.025	0.015	0.017	0.018
Luteolin	7.76	285.04	241.03; 227.02	0.595	nd	0.054	0.189	nd
Quercetin	7.93	301.04	179.02; 151.00	0.113	0.076	0.039	0.049	0.044
Apigenin	8.53	269.04	225.03; 151.00	0.044	0.029	0.028	0.153	0.029
Naringenin	8.53	271.06	151.00; 107.00	0.050	0.044	nd	0.041	0.038
Hispidulin	8.80	299.06	284.05; 256.04	0.588	0.022	0.030	0.187	0.025

[1] AALB – Extract of *A. alba*. AANN – Extract of *A. annua*. ACAMP – Extract of *A. campestris*. APONT- Extract of *A. pontica*. AV – Extract of *A. vulgaris*
[2] The contents of the compounds are expressed in mg/g
"nd" – not detected

Table 4. The antifungal activity of the *Artemisia* leaf extracts determined in the mycelial growth assay

	Fungal isolates	AALB[1]	AANN	ACAMP	APONT	AV	Antracol WP 70
1	*Botrytis cinerea* 46	35.29	47.05	29.41	23.52	29.41	100
2	*Curvularia spicifera* S2-5	22.58	16.12	3.22	3.22	3.22	100
3	*Fusarium graminearum* B1	0.00	0.00	0.00	0.00	0.00	100
4	*Monillinia laxa* M1	54.83	67.74	29.03	29.03	22.58	100
5	*Fusarium graminearum* CIK	23.07	7.69	7.69	7.69	7.69	49.41
6	*Alternaria alstromeriae* IB1I-20	33.33	25.92	11.11	11.11	25.92	100
7	*Cladosporium cladosporioides* S1-4	28.57	0.00	22.85	22.85	28.57	27.27

[1] AALB – Extract of *A. alba*. AANN – Extract of *A. annua*. ACAMP – Extract of *A. campestris*. APONT- Extract of *A. pontica*. AV – Extract of *A. vulgaris*
The results are expressen in % PGI.

in the presence of the inhibitory agent (Janakiev *et al.* 2019). Fungicide Antracol WP 70 (40 mg/mL) was used as the control to evaluate the efficiency of the antifungal activity of the tested extracts.

The well diffusion test. A well diffusion test (Dimkić *et al.* 2016) was used to determine the antibacterial potential of the investigated leaf extracts. Sterile moulds were placed on the LA plates, which were used as the solid medium. Then 7 mL of LA soft agar, previously inoculated with 70 µL of the appropriate bacterial strain was spread on the LA plates. After solidification of the soft agar the moulds were removed and the leaf extracts were added to the formed wells. Two concentrations of extracts were tested: 20 µL (40 mg/mL) and 10 µL (20 mg/mL) of each extract. In both cases, MeOH was used as the negative control, while antibiotics gentamicin and kanamycin (200 µg/mL) were used as the positive control. The petri plates were incubated for 24 h at 30°C. After overnight incubation the plates were examined for zones of inhibition. The diameters of the inhibition zones were measured and expressed in mm. The sensi-

tivity of the bacteria to the tested samples was evaluated as follows: no inhibition (-), inhibition zone up to 8 mm (+), inhibition zone 8–12 mm (++), inhibition zone above 12 mm (+++).

The microdilution assay. A microdilution assay was used to determine the minimum inhibitory concentrations (MICs), minimum fungicidal concentrations (MFCs), and minimum bactericidal concentrations (MBCs) of the examined extracts. Two-fold serial dilutions in 96-well microtiter plates were performed (Stupar *et al.* 2014; Dimkić *et al.* 2020). Luria-Bertani broth (LB) was used as the medium for bacterial growth, while malt extract broth (MEB) was used for the fungal isolates. Antracol WP 70 was used as the positive control for the fungal isolates. For the bacterial strains, methanol was tested as the negative control, while antibiotics gentamicin and kanamycin were used as the positive control in the same concentration as previously described. Resazurin was used as an indicator to evaluate the bacterial cell growth. The plates were incubated for 24 h at 30°C for the antibacterial, and 72 h at 37°C for antifungal activity. The MIC value was defined as the lowest concentration without visible growth (under a binocular microscope) and without any change in the colour of resazurin. The lowest concentrations without bacterial and fungal growth overnight were defined as the MBC and MFC values (Stupar *et al.* 2014; Dimkić *et al.* 2016). The obtained results were expressed in mg/mL.

RESULTS AND DISCUSSION

Extraction yield. The extraction yield of the five investigated *Artemisia* species ranged from 5.11% to 14.74% (Table 2). The lowest extraction yield was noted for extract *A. campestris* (5.11%), followed by extract *A. vulgaris* (7.43%). The highest extraction yield was observed for the leaf extract of *A. annua* (14.74%).

Phenolic profile. All the phenolic compounds present in the investigated extracts are listed in Table 3. In total, 13 phenolic compounds and quinic acid were identified. Certain differences were exhibited between the investigated taxa in terms of composition and amounts of phenolic compounds. Chlorogenic acid was the main constituent in all the investigated extracts. The highest concentration of this phenolic acid was found in the extracts of *A. alba* and *A. campestris* (2.515 mg/g and 2.416 mg/g, respectively), while the lowest concentration was detected in *A. vulgaris* (0.231 mg/g). The second most dominant compounds were rutin in the *A. campestris, A. pontica* and *A. alba* extracts (1.488 mg/g, 1.017 mg/g, and 0.995 mg/g, respectively); vitexin in *A. annua* (0.181 mg/g), and esculin in the *A. vulgaris* extract (0.108 mg/g). The highest amounts of quinic and caffeic acid were present in the *A. alba* extract (0.639 mg/g and 0.112 mg/g, respectively). Vitexin and naringenin were not found in *A. campestris*, while luteolin and narcissin were not found in the *A. annua* extract. Luteolin was absent in the *A. vulgaris* extract.

Numerous studies have reported phenolic compounds in different extracts of the aerial parts of various *Artemisia* species (Lee *et al.* 1998; Djeridane *et al.* 2007; Huang *et al.* 2009; Pires *et al.* 2009; Ivanescu *et al.* 2010, 2021; Karabegović *et al.* 2011; Carbonara *et al.* 2012; Đorđević *et al.* 2013; Sebai *et al.* 2014; Dilshad *et al.* 2016; Saunoriūtė *et al.* 2021; Trifan *et al.* 2022). Melguizo-Melguizo *et al.* (2014) previously identified the presence of quinic and caffeic acid in the leaf extract of *A. vulgaris*, which were also documented in our study. However, protocathecuic acid, found in the *A. vulgaris* extracts (Melguizo-Melguizo *et al.* 2014), were not found in our study. Previous studies showed that the leaf extracts of *Artemisia annua* contain flavonoids including chrysoplenetin, casticin, eupatin, and artemetin (Baraldi *et al.* 2008), as well as astragalin and luteolin (Ferreira *et al.* 2010), which were not found in the present study. On the other hand, apigenin and naringenin which were detected in the current study, were also previously found in the leaf extracts of *A. annua* (Ferreira *et al.* 2010; Skowyra *et al.* 2014; Martini *et al.* 2020). Trendafilova *et al.* (2018) investigated the chemical profile of leaf methanolic extracts of *A. alba* and identified numerous flavonoids: rutin, quercetin hexoside, apigenin glucuronide, eriodictyol, luteolin, nepetin, quercetin 3-methyl ether, axillarin, apigenin, hispidulin, diosmetin, chrysoeriol, desmethoxycentaureidin, jaceosidin, centaureidin, methoxyquercetin, dimethoxyquercetin, methoxyapigenin, and dimethoxyapigenin. However, in the current study, only four flavonoids (rutin, luteolin, apigenin and hispidulin) were found in the *A. alba* leaf extract.

Antimicrobial activity. The most sensitive micromycetes in the mycelial growth assay were *M. laxa*, and *B. cinerea* (Table 4). The highest antifungal activity was shown by the *A. annua* extract against *M. laxa* (67.74% inhibition of mycelial growth), followed by the extract of *A. alba* against the same fungus (54.83% inhibition of mycelial growth). Both extracts also induced a significant reduction in the growth of *B. cinerea* (47.05%, and 35.29% inhibition of mycelial growth, respectively). The other extracts also inhibited the mycelial growth of the same isolates. The results demonstrated that the highest resistance was documented for *F. graminearum* B1 against all the tested leaf extracts. Only the *A. annua* extract exhibited no inhibitory effects on *C. cladosporioides*. Using a mycelial growth assay Carvalho *et al.* (2011) showed moderate antifungal activity of the leaf extracts of *A. annua* against *Alternaria alternata*. It is well known that chlorogenic acid inhibits the mycelial growth of phytopathogenic fungi (Martínez *et al.* 2017). Thus, the presence of this phenolic acid in the ex-

Table 5. The antifungal activity of the *Artemisia* leaf extracts determined in the microdilution assay

	Fungal isolates	AALB[1]		AANN		ACAMP		APONT		AV		Antracol WP 70	
		MIC[2]	MFC[3]	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
1	*Botrytis cinerea 46*	1.25	5	1.25	5	1.25	5	1.25	5	2.5	2.5	0.156	0.156
2	*Fusarium oxysporum* S4-2	5	20	1.25	10	5	10	10	20	20	>20	0.156	0.156
3	*Aspergillus parasiticus* S3-1	>20	>20	>20	>20	>20	>20	20	>20	>20	>20	0.156	0.156
4	*Cladosporium cladosporioides* S1-4	20	20	20	20	20	>20	>20	>20	>20	>20	2.5	2.5
5	*Penicillium expansum* BEOFB11132	20	20	20	20	20	>20	20	>20	>20	>20	0.156	0.156
6	*Fusarium verticillioides* 5.1	2.5	5	10	20	1.25	20	10	20	10	>20	0.156	0.156
7	*Penicillium citreonigrum* BEOFB11191	1.25	10	10	10	0.625	2.5	10	>20	20	>20	0.156	0.156

[1] AALB – Extract of *A. alba*. AANN – Extract of *A. annua*. ACAMP – Extract of *A. campestris*. APONT- Extract of *A. pontica*. AV – Extract of *A. vulgaris*
[2] MIC - minimal inhibitory concentration.
[3] MFC – minimal fungicidal concentration
[2.3] The MIC/MFC values are expressed in mg/mL

Table 6. The antibacterial activity of the *Artemisia* leaf extracts determined in the microdilution assay

	Bacterial strains	AALB[4]		AANN		ACAMP		APONT		AV		Gentamicin		Kanamycin	
		MIC[2]	MBC[5]	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	*Xanthomonas campestris pv. campestris* txid1358009	1.000	>2.000	0.750	>2.000	0.750	>2.000	0.500	>2.000	1.000	>2.000	0.006	0.008	0.006	0.008
2	*Erwinia amylovora* 16-13	>2.000	-	>2.000	-	>2.000	-	>2.000	-	>2.000	-	0.003	0.004	0.006	0.008

[4] AALB – extract of *A. alba* leaves. AANN – extract of *A. annua* leaves. ACAMP – extract of *A. campestris* leaves. APONT- extract of *A. pontica* leaves. AV – extract of *A. vulgaris* leaves
[2] MIC - minimal inhibitory concentration.
[5] MBC – minimal bactericidal concentration
[2.5.] The MIC/MBC values are expressed in mg/mL

tract of *A. annua* could influence this activity.

The results of the microdilution method showed that the extracts had very low antifungal activity against the tested fungal isolates (Table 5). Among the micromycetes used in this assay, *P. citreonigrum* and *B. cinerea* were the most sensitive. The best inhibitory effect was exhibited by the *A. campestris* extract against *P. citreonigrum* (MIC 0.625 mg/mL), followed by the *A. alba* extract against the same micromycete (MIC 1.25 mg/mL). All the extracts exhibited activity against *B. cinerea* with the same MIC and MFC values of 1.25 mg/mL and 5 mg/mL, respectively. Depending on the extracts, different antifungal properties were reported against *F. oxysporum*. The most effective was the *A. annua* extract (MIC 1.25 mg/mL) compared to the other tested species. Although the extracts of *A. campestris* and *A. alba* also showed weak antifungal activity against the same phytopathogen (MIC 5 mg/mL), those two extracts were more effective than those of *A. pontica* and *A. vulgaris*. Very weak antifungal activity was noted against *F. verticillioides* (the *A. campestris* extract was the most effective with MIC 1.25 mg/mL). The weakest antifungal activity of all the extracts was detected against three micromycetes: *A. parasiticus*, *C. cladosporioides* and *P. expansum*.

Well diffusion was used as an initial screening for the antimicrobial activity of the examined *Artemisia* spp. extracts against 18 bacterial strains. The results indicated that none of the tested extracts showed strong antibacterial activity (the results are not shown here). It can be concluded that all the tested bacterial strains are highly resistant to the tested extracts. It should be kept in mind that many of the antimicrobial compounds in plant extracts are relatively non-polar and do not diffuse well in agar diffusion studies (Eloff *et al.* 2019). The MIC assay was further performed to assess the antibacterial activity of the *Artemisia* spp. extracts.

After the initial screening the most sensitive bacterial strains, one referent (*X. campestris* pv. *campestris* txid1358009), and one natural isolate (*E. amylovora* 16-13) were chosen for the MIC assay. The results of the *in vitro* antibacterial activity are given in Table 6. The tested extracts showed weak antibacterial activity against *X. campestris* pv. *campestris*. On the other hand, none of the tested extracts exhibited antibacterial activity against *E. amylovora*. The extract of *A. pontica* showed the highest activity against *X. campestris* pv. *campestris* (MIC 0.500 mg/mL), while the *A. annua* and *A. campestris* extracts showed moderate antibacterial activity with the same MIC value (0.750 mg/mL). Higher antibacterial activity was shown for the *A. campestris* than the *A. vulgaris* extracts. The *A. pontica* extract exhibited the strongest activity, which may be attributed to the high amounts of chlorogenic acid and rutin. Saunoriūtė *et al.* (2021, 2022) have previously reported the presence of these phenolic compounds in *A. pontica* extracts. These two compounds showed various effects against some bacterial strains (Ravn *et al.* 1989; Orhan *et al.* 2010; Ganeshpurkar & Saluja 2017).

Lou *et al.* (2011) analysed the antibacterial activity and mechanism of action of chlorogenic acid against pathogenic bacteria. In addition to strong antibacterial activity (MIC values from 20 to 80 μg/mL), they also demonstrated that chlorogenic acid can bound and to some extent disrupt the outer membrane, which leads to irreversible permeability changes in the cell membranes and the slight leakage of nucleotides.

A large number of studies have reported the antimicrobial activity of *Artemisia* species leaf extracts against human pathogenic fungi and bacteria (Cosoveanu *et al.* 2012; Juvatkar *et al.* 2012; Massiha *et al.* 2013; Parveen *et al.* 2014; Tajehmiri *et al.* 2014; Ghareeb 2018; Alotibi & Rizwana 2019; Parameswari *et al.* 2019; Benderradji *et al.* 2021). For example, methanolic extracts of the leaves of *A. campestris* have shown significant antibacterial activity against a wide range of human pathogenic bacteria (Naili *et al.* 2010; El-Abed *et al.* 2014). Al-Moghazy *et al.* (2017) documented that the leaves of *A. vulgaris* also exhibited antibacterial activities against some human pathogenic bacterial strains. On the other hand, the antimicrobial potential of the leaf extracts of *Artemisia* species against phytopathogenic fungi and bacteria (Carvalho *et al.* 2011; Mengane & Kamble 2014; Nehad *et al.* 2017; Ma *et al.* 2019) are scarcely investigated.

Mengane & Kamble (2014) and Ma *et al.* (2019) showed that the leaf extracts of *A. annua* were highly effective against phytopathogenic fungus, *F. oxysporum*. This may be in correlation with the chemical profile of *A. annua* (Potawale *et al.* 2008; Massiha *et al.* 2013). Vitexin, the second dominant compound identified in the *A. annua* extract, could be a compound with antifungal properties (El-Khateeb 2010).

Given that plant pathogens can cause different plant diseases, posing a major constraint to crop production, the obtained results may represent guidelines for similar research regarding other *Artemisia* species as a potential source of bioactive compounds against phytopathogens. Future studies should be focused on the endophytic microbial population and their metabolites from both studied and other *Artemisia* species which can be used in biological control strategies in combination with *Artemisia* extracts against most specific phytopathogens.

CONCLUSION

In this study, a total of 14 metabolites were identified in dichlormethane:methanol (1:1) leaf extracts of five *Artemisia* species (*A. alba*, *A. annua*, *A. campestris*, *A. pontica*, and *A. vulgaris*) using liquid chromatography mass spectrometry (LC-MS). Chlorogenic acid was a principal compound. Furthermore, the antimicrobial potential of the obtained extracts against 12 micromycetes, and 18 bacterial strains was evaluated using different methods. The most sensitive micromycetes were *M. laxa*, *P. citreonigrum*, and *B. cinerea*. The extracts showed moderate antibacterial activity against *X. campestris* pv. *campestris*. Chlorogenic acid, rutin and vitexin might be responsible for such antimicrobial activity. Further research of other *Artemisia* extracts is recommended in order to develop more efficient antimicrobial agents against phytopatogenic strains.

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REZIME

Botanica SERBICA

Fenolni profil i antimikrobna aktivnost ekstrakata listova pet vrsta roda *Artemisia* (Asteraceae)

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Cilj ovog rada je analiza fenolnih jedinjenja i utvrđivanje antimikrobne aktivnosti ekstrakata listova pet vrsta roda *Artemisia* (*A. alba*, *A. annua*, *A. campestris*, *A. pontica* i *A. vulgaris*), od kojih *A. annua* ima najveći medicinski značaj. Iako su mnoge vrste roda *Artemisia* dobro istražene sa etnobotaničkog i fitohemijskog aspekta, one su slabo istražene sa aspekta biološke aktivnosti protiv fitopatogenih bakterija i gljiva. Stoga je u ovom radu analizirano prisustvo fenolnih jedinjenja i antimikrobna aktivnost ekstrakata listova pet vrsta roda *Artemisia* na fitopatogene gljive i bakterije. Fenoli su ekstrahovani pomoću smeše rastvarača dihlormetan: methanol (1:1) i analizirani pomoću tečne hromatografije sa masenom spektrometrijom (LC-MS). Ukupno je identifikovano i kvantifikovano 13 fenolnih komponenti i hininska kiselina. Hlorogena kiselina je bila dominantna komponenta u svim uzorcima. Ostale dominantne komponente bile su: rutin u ekstraktima *A. alba*, *A. campestris* i *A. pontica*; viteksin u *A. annua* i eskulin u ekstraktu *A. vulgaris.* Antifungalna aktivnost je testirana je na 12 mikromiceta korišćenjem testa inhibicije rasta micelije i mikrodilucione metode. Antibakterijska aktivnost je testirana na 18 sojeva bakterija upotrebom disk difuzione i mikrodilucione metode. U svakom testu je najveća aktivnost pokazana za ekstrakte različitih vrsta *Artemisia*. Najosetljivije mikromicete bile su: *Monillinia laxa*, *Penicillium citreonigrum* i *Botrytis cinerea*, dok je *Fusarium graminearum* B1 bila najotpornija. Analizirani ekstrakti su pokazali umerenu antibakterijsku aktivnost samo na *Xanthomonas campestris* pv. *campestris* u poređenju sa ostalim testiranim fitopatogenima. U ovoj studiji je pokazano da ekstrakti listova određenih vrsta roda *Artemisia* sadrže fenolne komponente i pokazuju umerenu antimikrobnu aktivnost na određene vrste bakterija i gljiva.

Ključne reči: antibakterijska aktivnost, antifungalna aktivnost, fenolne kiseline, flavonoidi