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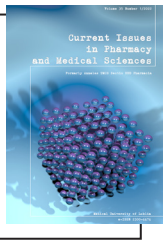

Antimicrobial activity and chemical analyses of seven *Juniperus* L. species

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ABSTRACT

The objective of this study was to present a comparative analysis of the antimicrobial activity of methanolic leaf extracts from seven *Juniperus* L. species, together with a tentative analysis of their total flavonoid and *o*-dihydroxyphenolic acids content. The chemical analyses were performed by colorimetric methods and the antimicrobial activity was assessed via broth microdilution. The studied extracts showed total content of *o*-dihydroxyphenols in the range of 26 to 34 μg of caffeic acid equivalent/mg DE and total flavonoid content of 13 to 24 μg of quercetin equivalent/mg DE. The sensitivity of Gram-positive bacteria to the studied extracts differed significantly with the following order of activity: *J. sabina* var. *balkanensis* > *J. communis* 'Laxa' > *J. formosana* > *J. pinchotii* > *J. ashei* > *J. excelsa* ≈ *J. sibirica*. Weak activity was observed for all extracts against Gram-negative bacteria and *Candida* strains. The analysis of the MBC/MIC ratio showed that the extracts exhibited bactericidal effect against Gram-negative bacteria (MBC/MIC≤4), while bactericidal or bacteriostatic action (MBC/MIC>4) was determined towards Gram-positive bacteria. Moreover, these extracts showed fungicidal (MFC/MIC≤4) or fungistatic effect (MFC/MIC>4). Best antibacterial activity was registered for the *J. sabina* var. *balkanensis* leaf extract. The multivariate data analyses were carried out by SIMCA 16 (v16.0.2) software. The hierarchical cluster analysis and principal component analysis, based on phytochemical and antimicrobial data, classified the studied juniper species into four groups: 1. *J. ashei*; 2. *J. excelsa*, *J. sibirica*; 3. *J. sabina* var. *balkanensis*, *J. communis* Laxa, *J. formosana*; 4. *J. pinchotii.*

INTRODUCTION

The antibiotic resistance of human pathogens, including bacteria and fungi such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP) and vancomycin-resistant enterococci (VRE), is a rising problem and therefore discovering and developing new antimicrobial compounds are extremely important [1]. The screening of plant extracts for their antimicrobial activity has shown that plants represent a potential source of new anti-infective compounds. The genus *Juniperus* L. comprises from 50 to 67 species depending on the taxonomy [2] and more than 220 cultivars [3]. The genus is usually divided into three distinct sections or subgenera: *Juniperus* (syn: *Oxycedrus*, 9 or 10 species), *Caryocedrus* (one species, *J. drupacea* Labill.), and *Sabina* (50 species) [4].

Juniperus extracts were reported to display antimicrobial activities against various human pathogens, including Gram-positive and Gram-negative bacteria and fungi, including yeasts [5]. Accordingly, methanol and water extracts of branches of five *Juniperus* spp. (*J. communis* L. var. *communis*, *J. communis* L. var. *saxatilis* Pall., *J. drupacea* Labill., *J. oxycedrus* L. subsp. *oxycedrus*, J. *oxycedrus* L. subsp. *macrocarpa* (Sibth. & Sm.) Ball.) showed significant antimicrobial activity only against Gram-positive bacteria, but no important antimicrobial activity was detected against Gram-negative bacteria or yeasts [6]. Significant activity of two diterpenes ((+)-ferruginol and sandaracopimeric acid) from *J. excelsa* was detected against *Bacillus subtilis*,

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Staphylococcus aureus and *Streptococcus durans* [7]. Moreover, the essential oil and ethanol extract of *J. sibirica* leaves showed strong antibacterial activity against *Salmonella typhimurium* and *Escherichia coli* [8]. The essential oils (EO) of cedarwood of *Juniperus ashei* and berries of *Juniperus communis* exhibited considerable inhibitory effects against all tested bacteria: Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*, *S. epidermis*), Gram-negative (*Citrobacter diversus*, *Escherichia coli*, *Pseudomonas aeruginosas*, *P. fluorescens*, *Salmonella abony*) except *Pseudomonas*. In addition, higher activity was observed against Gram-positive strains in comparison with Gram-negative bacteria.

GC/MS identified thujopsene, cedrol, α-cedrene and β-himachalene as main compounds in the *J. ashei* EO; and α-pinene, myrcene, limonene, β-caryophyllene, β-pinene as main components in the *J. communis* EO [9]. The methanolic leaf extract of *J. communis*, known to be rich in terpenoids and phenolics, showed activity against both antibiotic-sensitive and antibiotic-resistant *Staphylococcus aureus* [10,11]. Methanolic extracts of *J. communis* needles and branches were found to contain isocupressic acid, communic acid and deoxypodophyllotoxin, which were endorsed to be responsible for their activity against *Mycobacterium tuberculosis* [12]. The major detected substances of the essential oil of fruits and leaves of *J. sabina* were sabinene, α-pinene and myrcene, but weak or no antimicrobial activity was detected against the tested strains **(***Bacillus subtilis***,** *Candida albicans, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus***)** [13]. However, efficient antibacterial effect for the ethanol leaf extract of *J. sabina* against seven species of microorganisms (*Staphylococcus aureus*, *Bacillus subtilis*, *B. cereus*, *Listeria innocua*, *Corynebacterium xerosis*, *Rhodococcus* equi and *Pseudomonas aeruginosa)* was reported [14]. Other studies found efficient antibacterial activity of ethanolic and methanolic *J. sabina* leaf extracts against *Escherichia coli* and *Salmonella typhimurium* [15].

In this study, a tentative analysis of the chemical component and antimicrobial activity of seven *Juniperus* species (Figure 1) is presented. *J. excelsa* M. Bieb. (Greek juniper) is found in Greece, Southern Bulgaria, Crimea, Eastern Mediterranean region, Turkey, Syria, Lebanon, Jordan and in the Caucasus mountains. *Juniperus communis* L. and *Juniperus sibirica* Burgsd. **(**synonym of *Juniperus communis* var. *saxatilis* Pall.) are widely distributed at high altitudes in the mountains of the Northern Hemisphere, and *Juniperus pinchotii* Sudw. (redberry juniper) has restricted distribution in Mexico, Texas, New Mexico and Oklahoma. The native habitats of *Juniperus formosana* Hayata are in China and Taiwan, while *Juniperus ashei* J. Buchholz (mountain cedar) is native from Mexico and central Texas. In contrast, *Juniperus sabina* var. *balkanensis* R.P. Adams and A.N. Tashev is an endemic variety, found recently in the Balkan Peninsula (Albania, Bosnia-Herzegovina, Bulgaria, Croatia, Greece, Macedonia, Turkey, etc.) and Italian regions. This juniper representative was supposed to has been arisen by hybridization of *Juniperus sabina* L. and *Juniperus thurifera* L., when the natural habitats of these species have overlapped in ancient times [16]. High genetic diversity of *J. sabina* var. *balkanensis* was revealed by study of molecular and phytochemical markers [17].

The selected seven juniper representatives were found recently to exhibit best antioxidant activity among about 25 studied *Juniperus* species. High content of bioactive polyphenols, such as catechin, quercitrin, isoquercetin, as well as apigenin, protocatechuic acid, etc., were found in the corresponding leaf extracts [18]. However, comparison of the phytochemical components and bioactivities among *Juniperus* species are not widely studied, which greatly limits the application of *Juniperus* species. The objective for this study was a tentative analysis of the chemical composition and antimicrobial activity of the leaf extracts of seven *Juniperus* species, including not yet studied *J. sabina* var. *balkanensis*.

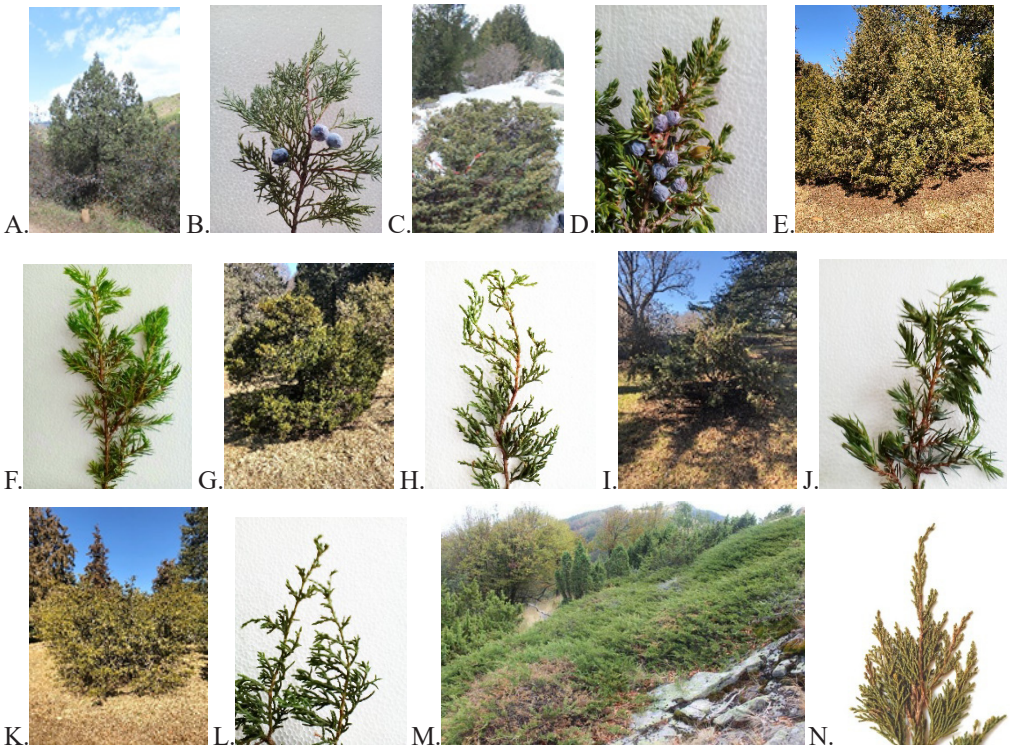


Figure 1. Habitats and specimens of the studied juniper species. A, B: *J. excelsa*; C, D: *J. sibirica*; E, F: *J. communis* 'Laxa'; G, H: *J. pinchotii*; I, J: *J. formosana*; K, L: *J. ashei*; M, N: *J. sabina* var. *balkanensis*, in the background *J. communis* columnar trees (photography by A. Tashev)

MATERIALS AND METHODS

Plant material

Specimens of the juniper representatives were collected as follows: *J. excelsa* M. Bieb. was from the reserve Tisata, the riverside of Struma, Bulgaria (SOM 174404, female, April 2017). *J. sibirica* Burgsd. was from the Vitosha mountain on the outskirts of Sofia (SOM 174401, female, April 2017); *Juniperus sabina* var. *balkanensis* R.P. Adams & A.N. Tashev was collected from peak Veikata, Eastern Rhodopes, Bulgaria (SOM 177009, male, November 2017). Voucher specimens of *J. excelsa*, *J. sibirica* and *J. sabina* var. *balkanensis* were authenticated by A.N. Tashev and were deposited in the Herbarium of the Institute of biodiversity and ecosystem research, Bulgarian Academy of Sciences. The following samples were collected in June 2017 from the Arnold Arboretum (AA), Harvard University, Boston, MA, USA, with accession summaries and original sources as follows: *J. pinchotii* Sudw. (AA 642-88*B, male, wild) – from Kiowa, Oklahoma, USA; *J. formosana* Hayata (AA 280-98*A, female, wild) – from Taiwan; *J. ashei* J. Buchholz (AA 276-86*A, male, wild) – from Oklahoma, Murray, USA; *J. communis* 'Laxa' (AA 49-66*A, male) – from the U. S. National Arboretum, Washington.

Extraction procedure

Juniper extracts were obtained by a previously published method [19]. In brief, the plant material (5 g) was ground and mixed with methanol (50 mL, 80% v/v) in an Erlenmeyer flask with a stopper. The suspension was then stirred for 1.5 h in a shaker water bath at 20°C. Afterwards, the mixture was filtered and the extract was collected. The remaining solid material was subsequently subjected to a second extraction for 1.5 h with a new portion of 80% methanol (50 mL). After filtration, the solid mass was stirred again for 1.5 h in 80% methanol (25 mL). The combined extracts were concentrated by a vacuum evaporator. During the vacuum evaporation, a chlorophyll-containing dark green oil appeared in traces amounts (2-4% yield), and was removed by decantation from the main extract. All extracts were freeze-dried (24 h, -50°C, 0.1 mbar) and kept in a freezer (-20°C) until analyses.

Chemicals

Quercetin and caffeic acid were from Sigma Aldrich, Germany. Methanol, aluminium chloride, sodium hydroxide, and hydrochloric acid were provided by Avantor Performance Materials (Poland). Arnov's reagent was from Chempur, Poland. All chemicals and solvents used were of analytical grade.

Total phenolic acid (*o*-dihydroxyphenols) content

The content of *o*-dihydroxyphenols was determined using a modified protocol, adapted to a microscale [20]. A total of 20 µL of the extract, 120 µL of ultrapure water, 20 µL HCl (10 g/L), 20 µL of Arnov reagent, and 20 µL NaOH (40 g/L) was mixed. Then, the absorbance was measured (using an Infinite Pro 200F microplate reader from Tecan Group Ltd.; Switzerland) at 490 nm with the solution containing water instead of a sample as a blank. The phenolic acid content was calculated using a standard curve plotted for caffeic acid and expressed as µg of caffeic acid per mg of dry extract.

Total flavonoid content (TFC)

TFC was determined using the aluminium chloride colorimetric method, a previously described protocol and an Infinite Pro 200F microplate reader (Tecan Group Ltd.; Switzerland) [21]. In brief, 20 µL of extract was mixed with 160 µL of methanol and 20 µL of 2% $AlCl_3$ solution. The mixture was allowed to stand for 30 min, and absorbance was measured at 430 nm. The external calibration was done using different concentrations of quercetin. The total flavonoid content was calculated from a calibration curve, and the result was expressed as µg quercetin equivalent per mg dry extract.

Antimicrobial activity assay

The antibacterial and antifungal activities of the juniper extracts were determined by applying the micro-dilution broth method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST, www.eucast.org). Mueller-Hinton broth and Mueller-Hinton broth with 5% lysed sheep blood was used for growth of non-fastidious and fastidious bacteria, respectively, or RPMI with MOPS for growth of fungi as described recently [22]. A panel of reference microorganisms from the American Type Culture Collection (ATCC) was employed: Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus feacalis* ATCC 29212, *Micrococcus luteus* ATCC 10240, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876, *Streptococcus pyogenes* ATCC 19615, *Streptococcus pneumoniae* ATCC 49619, *Streptococcus mutans* ATCC 25175); Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Salmonella Typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 9027, *Proteus mirabilis* ATCC 12453), and fungi (*Candida albicans* ATCC 10231, *Candida albicans* ATCC 2091, *Candida parapsilosis* ATCC 22019, *Candida glabrata* ATCC 90030, *Candida kruzei* ATCC 14243). The Minimal Inhibitory Concentration (MIC) was determined by the lowest concentration of the assayed agent that inhibits the microbial growth. The Minimal Bactericidal Concentration (MBC) was assessed as the lowest concentration of the tested extract that killed the bacteria, i.e. reducing the viability of the bacteria by ≥99.9%. Analogously, the antifungal activities of the plant extract were evaluated by its Minimal Fungicidal Concentration (MFC) and Minimal Inhibitory Concentration (MIC). Each experiment was repeated in triplicate. Representative data were presented.

Statistical Analysis

The multivariate data analyses were carried out using a SIMCA 16 (v16.0.2, Umetrics, Sweden). Principal component analysis (PCA) was applied for identifying similarities and differences between analysed samples. Data were scaled to unit variance and centered. Hierarchical Cluster Analysis (HCA) were employed for samples classification and predictions.

RESULTS AND DISCUSSION

Juniper leaves are known to be a source of several groups of polyphenolic compounds [23-25], such as the flavonoids and phenolic acids (*o*-dihydroxyphenol type). These compounds significantly affect an extract's bioactivity. Extracts from leaves of different juniper species were previously found to differ greatly in terms of total polyphenolic content, and individual components were analysed with targeted LC-ESI-MS [18]. A prior study indicated differences in the total polyphenol content and in the content of individual flavonoids (e.g. catechin, rutin, quercitrin, isoquercetin) and phenolic acids (e.g. protocatechuic, *p*-coumaric acid) in juniper samples. The best TPC was determined for *J. ashei* (263±24 mg gallic acid equivalents per g of dry extract) leaf extract with highest concentration of catechin, which was supposed to contribute to its best antioxidant activity.

Seven juniper species with high antioxidant activity were chosen in this study for further analysis of their total chemical composition and comparison of their antimicrobial activity. The present study notably compared the overall content of metabolites belonging to two polyphenolic classes – total flavonoid content and total content of *o*-dihydroxyphenols. The obtained results showed that the studied *Juniperus* leaf extracts had similar total content of *o*-dihydroxyphenols (26.02±0.18 to 34.25±0.95 µg of caffeic acid/mg dry extract) and total flavonoid content (TFC) (12.93±0.55 to 23.66±1.51 µg of quercetin/mg DE) (Table 1).

Table 1. Total *o*-dihydroxyphenol (µg of caffeic acid/mg dry extract) and flavonoid (TFC; µg of quercetin/mg dry extract) content of different juniper leaf extracts

Juniperus leaf extracts	*o*-dihydroxyphenols (µg /mg)	TFC (µg /mg)
J. excelsa	29.83±0.30	23.66±1.51
J. sibirica	29.52±0.38	20.99±0.31
J. communis 'Laxa'	27.90±0.15	20.17±0.45
J. pinchotii	29.86±0.28	13.78±0.33
J. formosana	28.48±0.54	23.05±0.28
J. ashei	34.25±0.95	12.93±0.55
J. sabina var. *balkanensis*	26.02±0.18	20.77±0.79

This study was also aimed at comparing the antimicrobial activity of the leaf extracts of the studied *Juniperus* species (including not yet studied *J. sabina* var. *balkanensis*) on a panel of microorganisms (known harmful pathogens or commensal microorganisms) capable of becoming highly pathogenic in immunocompromised patients and especially in hospital-acquired infections. The extracts were tested according to EUCAST guidelines, and the panel consisted of human pathogenic Gram-positive and Gram-negative bacteria and yeasts (Table 2). The obtained data were interpreted according to the criteria described by O'Donnell *et al*.: weak bioactivity (MIC>1 mg/mL), mild bioactivity (MIC = 0.501-1 mg/mL), moderate bioactivity (MIC=0.126-0.500 mg/mL), good bioactivity (MIC=0.026-0.125 mg/mL) [26].

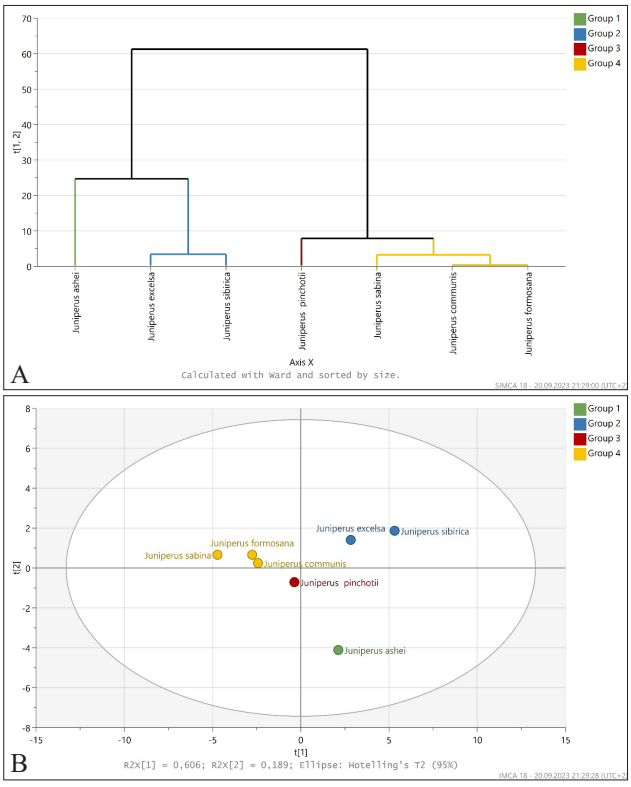
Table 2. Comparison of the minimal inhibitory concentration (MIC, mg/ml), minimum bactericidal/fungicidal concentration (MBC/MFC, mg/ml) of the assayed juniper extracts

Leaf extracts / Microorganism	*Juniperus excelsa*		*Juniperus sibirica*		*Juniperus communis* 'Laxa'		*Juniperus pinchotii*		*Juniperus formosana*		*Juniperus ashei*		*Juniperus sabina* L. *var. balkanensis*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-positive bacteria														
S. aureus ATCC 25923	0.625	2.5	1.25	1.25	0.313	0.625	0.625	0.625	0.313	0.625	0.313	0.625	0.313	0.625
S. epidermidis ATCC 12228	1.25	5	1.25	5	0.313	0.625	0.625	0.625	0.313	0.625	0.313	0.625	0.156	1.25
M. luteus ATCC 10240	0.625	1.25	0.625	1.25	0.156	0.625	0.156	0.313	0.156	0.313	0.313	0.625	0.078	0.313
E. faecalis ATCC 29212	1.25	10	2.5	10	1.25	10	1.25	1.25	0.625	1.25	0.625	0.625	0.625	10
B. subtilis ATCC 6633	1.25	1.25	2.5	2.5	0.078	0.156	0.625	0.625	0.156	0.313	1.25	1.25	0.078	0.078
B. cereus ATCC 10876	0.625	2.5	0.625	2.5	0.156	0.156	0.313	0.625	0.156	0.313	0.313	0.313	0.078	0.078
S. pyogenes ATCC 19615	5	10	10	>10	0.625	1.25	1.25	10	1.25	2.5	2.5	>10	0.313	1.25
S. pneumoniae ATCC 49619	5	5	10	>10	0.313	0.625	5	5	0.625	1.25	5	10	0.313	0.625
S. mutans ATCC 25175	10	>10	10	>10	0.625	5	5	>10	1.25	>10	10	>10	0.625	>10
Gram-negative bacteria														
S. typhimurium ATCC 14028	10	10	10	>10	10	10	10	10	10	10	10	10	5	10
E. coli ATCC 25922	10	10	10	>10	10	>10	10	10	10	10	10	10	10	10
P. mirabilis ATCC 12453	2.5	2.5	2.5	10	5	>10	5	5	2.5	2.5	2.5	5	10	10
K. pneumoniae ATCC 13883	1.25	1.25	1.25	1.25	2.5	2.5	2.5	2.5	1.25	1.25	1.25	1.25	2.5	2.5
P. aeruginosa ATCC 9027	10	10	10	>10	10	>10	10	10	10	10	5	10	5	10
Yeasts	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
C. albicans ATCC 102231	10	10	10	10	5	10	5	10	2.5	10	10	10	2.5	10
C. albicans ATCC 2091	5	10	10	10	1.25	10	5	10	0.313	10	10	10	1.25	10
C. parapsilosis ATCC 22019	5	10	10	10	2.5	10	2.5	10	1.25	10	10	10	1.25	10
C. glabrata ATCC 90030	10	10	10	>10	5	10	5	10	0.625	10	10	10	1.25	10
C. krusei ATCC 14243	10	10	10	10	5	10	10	10	2.5	10	10	10	2.5	10

Accordingly, weak activity was observed for the leaf extracts from all the tested *Juniperus* species against Gram-negative bacteria (MIC = 1.25-10 mg/mL). Weak bioactivity of these extracts was also observed against most yeast strains (MIC = 1.25-10 mg/mL), with the exception of *J. formosana* extract, which demonstrated mild or moderate activity towards *C. glabrata* ATCC 90030 (MIC=0.625 mg/mL) and *C. albicans* ATCC 2091 (MIC = 0.313 mg/mL), respectively.

Overall, the sensitivity of Gram-positive bacteria to the leaf extracts differed significantly, depending on the juniper and bacterial species. Weak or mild bioactivity was presented by *J. excelsa* and *J. sibirica* extracts (MIC = 0.625-10 mg/mL). Weak, mild or moderate bioactivity was exerted by *J. pinchotii* (0.156-5 mg/mL), *J. formosana* (MIC=0.156-1.25 mg/mL) and *J. ashei* extracts (MIC = 0.313-10 mg/mL). *J. communis* Laxa extract showed weak, mild, moderate or good bioactivity (MIC = 0.078-1.25 mg/mL), and *J. sabina* L. var. *balkanensis* presented mild, moderate or good bioactivity (MIC = 0.078-0.625 mg/mL). In this assay, according to the strength of activity against Gram-positive bacteria, the tested extracts followed the order: *J. sabina* L. var. *balkanensis* > *J. communis* Laxa > *J. formosana* Hayata > *J. pinchotii* Sudw. > *J. ashei* Buchh. > *J. excelsa* M. Bieb. ≈ *J. sibirica* Burgsd.

The extracts from the tested *Juniperus* species also exhibited bactericidal effect against Gram-negative bacteria (MBC/MIC ≤4), while bactericidal or bacteriostatic action (MBC/MIC >4) towards Gram-positive bacteria was determined. Moreover, these extracts showed fungicidal (MFC/MIC ≤4) or fungistatic effect (MFC/MIC >4).



(A) hierarchical cluster analysis; (B) component plot

Figure 2. Multivariate analysis of similarity of *Juniperus* leaf extracts

A multivariate analytical approach was used to determine and verify the variation of the bioactivity and chemical content in the studied seven *Juniperus* spp. extracts. The quantitative data from Tables 1 and 2 were used as input for the hierarchical cluster analysis (HCA) and principal component analysis (PCA). As shown in the dendrogram generated by the HCA (Fig. 2A), the results were classified into four groups: 1) *J. ashei*; 2) *J. excelsa*, *J. sibirica*; 3) *J. sabina* var. *balkanensis*, *J. communis* Laxa, *J. formosana*; 4) *J. pinchotii*, with samples connected by a short distance being more similar than those connected by a long distance.

To evaluate the accuracy of this classification, the clustering was next evaluated by PCA. Therefore, in our case, only the first two principal components (explaining 79.5% of the total variance) were retained. Interestingly, when the component plot (Figure 2B) was designed based on the first two principal components (PC1:60.6%; PC2: 18.9%), the same clustering pattern as in the HCA was observed.

CONCLUSION

The leaf extracts from seven *Juniperus* species analyzed in the present study differed greatly in their activity against Gram-positive bacteria. The most active extract was from *J. sabina* var. *balkanensis*. This juniper species can be regarded as a valuable plant material for isolation of bioactive compounds with antibacterial properties and preparation of natural antibacterial agents with pharmaceutical applications.

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REFERENCES

1. Baquero F. Threats of antibiotic resistance: an obliged reappraisal. *Int Microbiol.* 2021;24(4):499-506.
2. Adams RP. *Junipers of the world: The Genus Juniperus.* Bloomington, Indiana (USA): Trafford Publishing Co.; 2014.
3. Krussmann G. *Manual of cultivated conifers.* Portland, Oregon (USA): Timber Press; 1991.
4. Adams RP. The leaf essential oil and chemotaxonomy of *Juniperus* sect. Juniperus. *Biochem Syst Ecol.* 1998;26:637-45.
5. Afsharzadeh M, Naderinasab M, Tayarani Najaran Z, Barzin M, Emami SA. *In-vitro* antimicrobial activities of some Iranian conifers. *Iran J Pharm Res.* 2013;12(1):63-74.
6. Taviano MF, Marino A, Trovato A, Bellinghieri V, La Barbera TM, Güvenç A, et al. Antioxidant and antimicrobial activities of branches extracts of five *Juniperus* species from Turkey. *Pharm Biol.* 2011;49(10):1014-22.

7. Muhammad I, Mossa JS, El-Feraly FS. Antibacterial diterpenes from the leaves and seeds of *Juniperus excelsa* M. Bieb. *Phytother Res*. 1992;6(5):261-4.
8. Zhang Y, Wu D, Kuang S, Qing M, Ma Y, Yang T, et al. Chemical composition, antioxidant, antibacterial and cholinesterase inhibitory activities of three *Juniperus* species. *Nat Prod Res.* 2020;34:24,3531-5.
9. Wanner J, Schmidt E, Bail S, Jirovetz L, Buchbauer G, Gochev V, et al. Chemical composition and antibacterial activity of selected essential oils and some of their main compounds. *Nat Prod Commun.* 2010;5(9):1359-64.
10. Martz F, Peltola R, Fontanay S, Duval RE, Riitta JT, Stark S. Effect of latitude and altitude on the terpenoid and soluble phenolic composition of juniper (*Juniperus communis*) needles and evaluation of their antibacterial activity in the boreal zone. *J Agric Food Chem*. 2009;57(20):9575-84.
11. Rolta R, Kumar V, Sourirajan A, Upadhyay NK, Dev K. Phytocompounds of three medicinal plants (*Juniperus communis*, *Urtica dioica* and *Coleus forskohlii*) of north west himalayas increases the potency of antibacterial and antifungal antibiotics. *Plant Archives*. 2020;20:481-9.
12. Carpenter CD, O'Neill T, Picot N, Johnson JA, Robichaud GA, Webster D, et al. Anti-mycobacterial natural products from the Canadian medicinal plant *Juniperus communis*. *J Ethnopharmacol.* 2012;143(2):695-700.
13. Asili J, Emami SA, Rahimizadeh M, Fazly-Bazzaz BS, Hassanzadeh MK. Chemical and antimicrobial studies of *Juniperus sabina* L. and *Juniperus foetidissima* Willd. Essential Oils. *J Essent*. 2010;13(1): 25-36.
14. Zazharskyi VV, Davydenko PO, Kulishenko OM, Borovik IV, Kabar AM, Brygadyrenko VV. Antibacterial and fungicidal effect of ethanol extracts from *Juniperus sabina*, *Chamaecyparis lawsoniana*, *Pseudotsuga menziesii* and *Cephalotaxus harringtonia*. *Regul Mech Biosyst*. 2020;11(1): 105-9.
15. Kavaz D, El Faraj R. Investigation of composition, antioxidant, antimicrobial and cytotoxic characteristics from *Juniperus sabina* and *Ferula communis* extracts. *Nature Sci Rep.* 2023;13:7193.
16. Adams RP, Schwarzbach AE, Tashev AN. Chloroplast capture by a new variety, *Juniperus sabina* var. *balkanensis* RP. Adams and AN. Tashev, from the Balkan peninsula: A putative stabilized relictual hybrid between *J. sabina* and ancestral *J. thurifera*. *Phytologia*. 2016;98(2):100-11.
17. Rajčević N, Dodoš T, Janković S, Janaćković P, Zheljazkov VD, Marin PD. Molecular and phytochemical variability of endemic *Juniperus sabina* var. *balkanensis* from its natural range. *Diversity.* 2022;14(12):1062.
18. Olech M, Nowak R, Ivanova D, Tashev A, Boyadzhieva S, Kalotova G, et al. LC-ESI-MS/MS-MRM profiling of polyphenols and antioxidant activity evaluation of junipers of different origin. *Appl Sci*. 2020;10:8921.
19. Ivanova DI, Nedialkov PT, Tashev AN, Olech M, Nowak R, Ilieva YE, et al. Junipers of various origins as potential sources of the anticancer drug precursor Podophyllotoxin. *Molecules.* 2021;26:5179.
20. Olech M, Nowak R, Łoś R, Rzymowska J, Malm A, Chruściel K. Biological activity and composition of teas and tinctures prepared from *Rosa rugosa* Thunb. *Cent Eur J Biol.* 2012;7(1):172-82.s
21. Olech M, Łyko L, Nowak R. Influence of accelerated solvent extraction conditions on the LC-ESI-MS/MS polyphenolic profile, triterpenoid content, and antioxidant and anti-lipoxygenase activity of *Rhododendron luteum* Sweet leaves. *Antioxidants*. 2020;9:822.
22. Motyka S, Kusznierewicz B, Ekiert H, Korona-Głowniak I, Szopa A. Comparative analysis of metabolic variations, antioxidant profiles and antimicrobial activity of Salvia hispanica (Chia) seed, sprout, leaf, flower, root and herb extracts. *Molecules.* 2023;28(6):2728.
23. Seca AML, Silva AMS. Recent Progress in Medicinal Plants, In: The Chemical Composition of the *Juniperus* Genus (1970-2004). *Recent Progress in Medicinal Plants.* 2006;16: 01-522.
24. Dziedzinski M, Kobus-Cisowska J, Szymanowska D, Stuper-Szablewska K, Baranowska M. Identification of polyphenols from coniferous shoots as natural antioxidants and antimicrobial compounds. *Molecules.* 2020;25(15):3527.
25. El-Abid H, Amaral C, Cunha SC, Correia-da-Silva G, Fernandes JO, Moumni M, et al. Anti-cancer properties of hydroethanolic extracts of *Juniperus oxycedrus* L. in breast cancer cells. *J Herb Med.* 2023;37:100614.
26. O'Donnell F, Smyth TJP, Ramachandran VN, Smyth WF. A study of the antimicrobial activity of selected synthetic and naturally occurring quinolones. *Int J Antimicrob Agents*. 2010;35(1):30-8.