ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 22(1) 2023, 11–17
https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2023.4577
ORIGINAL PAPER
Accepted: 15.07.2022
Published: 24.02.2023

CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIBACTERIAL PROPERTIES OF *Juniperus excelsa* M. Bieb. LEAVES FROM TÜRKİYE

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ABSTRACT

Juniper species are especially used in traditional medicine due to their analgesic, diuretic, antibacterial, antimicrobial, anti-inflammatory, and liver-protective effects. The goal of the present study was to investigate the chemical compounds, antioxidant and antimicrobial properties of essential oil of *Juniperus excelsa* M. Bieb., a species of juniper with a large spread area in Turkey. Essential oils were extracted by the hydro-distillation method. The components of the hydro distilled oil samples were analyzed by gas chromatography-mass spectrometry (GC-MS). In the study, the essential oil was evaluated for its antibacterial activity against six bacterial strains consisting of two Gram (+) and four Gram (–) by the agar disc diffusion method. Additionally, the antioxidant properties of the oil were determined by TEAC/ABTS^{+} free radical scavenging assay. As a result, there were 27 compounds in the essential oil of *J. excelsa*. Of the 27 essential oil components identified, α-pinene (40.59%), α-cedrol (18.15%), β-myrcene (4.53%), and limonene (3.84%) were determined as the main components in total 91.54% of the essential oil. As a result, it was observed that the examined juniper essential oil showed a weak but effective antibacterial activity against all bacterial strains compared to the control agents, and also, the examined oil had low but valuable antioxidant activity.

Key words: GS-MS, bioactivity, phytochemicals, essential oil, *Juniperus excelsa*

INTRODUCTION

The genus Juniperus (Cupressaceae) includes more than 60 species common in the northern hemisphere, primarily including North America, Europe, and Asia [Stankov et al. 2020]. One of these species *Juniperus excelsa*, belonging to the family Cupressaceae, locally known as "boyluardıç" are traditionally important because of its use in folk medicine to treat abdominal spasm, asthma, diarrhea, fever, headache, gonorrhea, leucorrhoea, and is considered useful as an antihypertensive, appetizer, diuretic, carminative, stimulant, anticonvulsant [Khan et al. 2012], and used for flavor and organoleptic development and food preservatives [Gülser et al. 2012]. Extracts of fruits, leaves, and wood of these plants are being used for these purposes [Gülser et al. 2012]. Additionally, essential oils of *J. excelsa* M. Bieb. display antibacterial, antimicrobial, antioxidant, and antifungal activities, as well [Sahin Yaglioglu et al. 2020]. It grows at an altitude of 300 m at the lowest and 2300 m at the highest in Turkey [Günal 1997]. It is found individually or in groups on

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dry and Stony slopes in all of Anatolia except the Eastern Black Sea [Gülser et al. 2012]. Its natural range is the Balkans, Macedonia, Greece, and the Aegean islands in the west, the south of Bulgaria, the Northeastern and southeastern parts of Iran in the East, Crimea, the Caucasus and Anatolia, and the west of Lebanon, Syria, and Iraq in the South [Emami et al. 2011, Khan et al. 2012]. This juniper species, which is resistant to extreme growing conditions, is known to have various biochemical properties such as tannin, resin, essential oil, phenolic, and antioxidants in its different organs [Okut et al. 2018]. The considerable major components of *J. excelsa* essential oils are typically determined by their rich content of α-pinene, cedrol, sabinene [Unlu et al. 2008], α-pinene, α-cedrol [Topçu et al. 2005], carene and β-pinene [Andoğan et al. 2002]. It is undoubtedly known that the plant species that grow naturally in any variable region are directly affected by the ecological conditions of that unique geography. Although the plant species are the same, this interaction can make their essential oil compositions and, consequently, their bioactivity different. For this reason, it is very important to determine the chemical components and bioactivity of essential oils of medicinal plants of the same species grown in different geographies.

In view of the above, the main purpose of this experimental study was to determine the antibacterial, and antioxidant properties of the essential oils of *J. excelsa* M. Bieb. leaves collected in the Van Lake basin, depending on their qualitative and quantitative chemical composition.

MATERIAL AND METHOD

Plant samples

The mature leaves of *J. excelsa* M. Bieb. were care-fully collected in August from native populations that grew naturally in the fertile Altısaç village location in the Gevas District of Van, Turkey, at a certain altitude of 1730–1875 m. The plant taxonomic determination of the materials was carefully carried out by a taxono-mist, Fevzi Ozgokce, a taxonomist at the Department of Biology at Van Yuzuncu Yil University. The plant materials were then air-dried in a canopy at room tem-perature and milled into small particles. They were stored in a dry and cool environment for analysis.

Essential oil extraction

The samples were hydro-distilled for 3 h in a suitable Clevenger-type apparatus to carefully extract the essential oils in the laboratory of the Department of Field Crops in the Faculty of Agriculture, Van Yuzuncu Yil University. The essential oil extraction was carried out with three replications (3 × 100 g). The oils extracted were dried using anhydrous sodium sulfate, weighed, and stored in sealed vials at 4°C before gas chromatography/mass spectrometry (GC/MS) analyses [Khoury et al. 2014].

GC/MS analyses of essential oils

The modified method of [Sandri et al. 2007] was used to determine the chemical compounds essential oils of *J. excelsa* M. Bieb. The analysis of essential oil was carried out by gas chromatography quadrupole mass spectrometry (Shimadzu QP2010) using helium as the carrier gas at a constant linear speed of 36.5 cm/s. The column used was a TRB-WAX capillary column (30 m long × 0.25 mm I.D. × 0.25 µm film thickness) programmed starting from 60°C (5 min) to 240°C at a rate of 3°C min^{-1} and finally held for 5 min. The volume of injection was 1.0 µL, the split ratio was 1 : 50, and the temperature of injection was 240°C. The transfer was operated at 70 eV (electron Volts) ionization energy. The accurate identification of the stable components presented in Table 1 was performed using library data (Nist 27, Wiley 7, and Nist 147) and confirmed by direct comparison with standard mass spectra and by comparison of the retention time, which is one of the most significant chromatographic properties for analytical analysis, as it is the key parameter for separating, identifying, and quantifying related compounds from complex mixtures [Etxebarria et al. 2009].

Antioxidant activity

The ABTS assay was conducted according to the method of Re et al. [1999] with a slight modification. The ABTS+ radical cation was obtained by reacting the ABTS+ spare solution with the inorganic compound of formula 2.45 mM final potassium persulfate concentration. $K_2S_2O_8$ and the mixture were kept in the dark for about 12–16 hours before use. The radicals were kept in the dark continuously at room temperature for more than two days. The $ABTS^+$ concen-

tration of the stock solution was suitably diluted with distilled water to 0.70 ±0.02 absorbance at 734 nm for measurement and equilibrated at 30°C. Then, 2.95 mL of diluted fresh $ABTS^+$ solution was added to aliquots of the 5 mL essential oil sample, which was diluted with ethanol. After the addition, the absorbance reading was evaluated at 30°C exactly 6 minutes after mixing [Altunkaya et al. 2014]. Percent inhibition of absorbance was measured at 734 nm and plotted as a function of antioxidant concentration and Trolox for standard reference data. Antioxidant total activity was expressed as mM Trolox Equivalent Antioxidant Capacity (TEAC).

Antimicrobial activity

The strains of two Gram (+) bacteria (*Bacillus subtilis* and *Staphylococcus aureus* ATCC 12600) and four Gram (–) bacteria (*Escherichia coli* ATCC 11775, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa*, and *Salmonella typhimurium* ATCC 25241) were used as test microorganisms. The strains of bacteria tested were sourced from the Clinical Microbiology Department of Medicinal Faculty of Van Yuzuncu Yil University. The provided bacterial strains were cultured for 18 h at 37 ±1°C in Nutrient agar (NA, Oxoid Ltd., England). The antibacterial activity of the essential oils of *J. excelsa* was accurately determined by the agar disc-diffusion method recommended by [Altunkaya et al. 2014]. The bacterial suspensions were adjusted to 0.5 McFarland standard turbidity, which was carefully prepared as typically described by CLSI [2009]. In this comparative study, Ampicillin and Ofloxacin antibiotics were used as control agents at a dose of 25 mcg [Ponce et al. 2003, Moreira et al. 2005]. All infectious strains of bacteria were incubated in a standard oven. The tested microbial strains were carefully spread on the ideal surface of pre-prepared tryptic soy agar plates. Then, the filter paper discs in 6 mm diameter were saturated directly with the 5 µL essential oil and properly placed onto these plates. The plates were incubated for 30 necessary minutes at the appropriate temperature. After the incubation period, all resulting inhibition zone diameters were measured in millimeters with an electronic caliper with an accuracy of 1/1000. All antibacterial assays were carefully carried out with three independent replications, and the observed results were evaluated statistically at a $P < 0.05$ significance level and expressed as average

Table 1. The chemical composition of essential oils of *J. excelsa* M. Bieb.

Peak	Compound	Retention time	Ratio (%)	Peak	Compound	Retention time	Ratio (%)
1	α-pinene	3.147	40.59	15	Germacrene D	12.520	0.67
2	Camphene	3.525	1.21	16	Isocaryophyllene	12.669	0.62
3	β-pinene	4.013	2.23	17	α-camigrene	12.734	0.66
4	β-myrcene	4.728	4.53	18	Alloaromadendrene	13.081	0.85
5	Limonene	5.251	3.84	19	Germacrene B	14.007	1.58
6	γ-terpinene	5.941	1.32	20	Endo-1-bourbonanol	16.413	0.63
7	Thymene	6.320	0.70	21	Elemol	16.703	1.42
8	α-terpinolene	6.504	1.11	22	Sesquisabinene hydrate	17.064	2.07
9	β-cedrene	10.756	2.33	23	α-cedrol	17.256	18.15
10	α-fenchyl acetate	10.815	0.91	24	Trans-isoelemicin	18.160	0.80
11	Caryophyllene	11.083	0.91	25	α-cadinol	18.283	1.08
12	γ-elemene	11.564	0.59	26	Spathulenol	18.492	0.73
13	Cis-verbenol	12.073	0.99	27	Cembrene	20.615	0.50
14	Bornyl formate	12.376	0.52	In total:			91.54

values. The sensitivity to the essential was determined by the diameter of the inhibition zones: not sensitive for diameters <8 mm; sensitive for diameters 9–14 mm; very sensitive for diameters 15–19 mm and extremely sensitive for diameters >20 mm [Ponce et al. 2003, Eryigit et al. 2015].

Statistical analysis

The antibacterial measurements were performed in triplicate and analyzed according to one way completely randomized experimental design via Costat 6.3. The obtained results were presented as the mean value of the individual measurements with the corresponding standard deviation (SD), and LSD groups. Antioxidant measurements in triplicate were presented together with the mean values of the standard deviation (SD) by using Microsoft Excel.

RESULTS AND DISCUSSION

Chemical compounds of the essential oil of *J. excelsa* M. Bieb.

The oil phytochemical contents of the juniper leaves collected from native populations that grow naturally in Altınsaç village location under Van-lake Basin ecological conditions were presented in Table 1. The observation of the essential oil components typically revealed that the accurate profile of GC/MS results consisted of twenty-seven constituents, representing 91.54% of the oil. The major components of the essential oil profile were prominently represented by α-pinene (40.59%), a monoterpene, and α-cedrol (18.15%), a sesquiterpenic component. Contrary to our study, Sahin Yaglioglu et al. [2020] determined that limonene (16.9%) and Thujone (11.6%) are among the major elements. It can be said that the reason for this is that the plant organ taken as the extract is different, and the ecological conditions in which the plants grow are different. Similarly, Emami et al. [2011] declared α-pinene (32.34%) and α-cedrol (13.06%) as the main compounds in the oil of *J. excelsa* leaves. In a similar study conducted by Topçu et al. [2005], they reported that the main components in the oil of excelsa leaves were a-pinene (29.7%) and a-cedrol (25.3%). Among the minor compounds, β-myrcene (4.53%), limonene (3.84%), β-cedrane (2.33%), and β-pinene (2.23%) may be specified as the main compounds in the fraction (Tab. 1). The differences in the composition and the ratio of components of the oils reported in these studies might result from the plants' habitat, ecological conditions, or harvesting time.

Antioxidant activity

As known, there are many effective methods for accurately determining antioxidant capacities that typically differ in familiar terms of determination principles and experimental conditions [Cao and Prior 1998, Moein and Moein 2010]. In this study, the essential oil of wild *J. excelsa* leaves was subjected to screening for its possible antioxidant activity by a preferred test system described above. It has been previously reported by Emami, Abedindo and Hassanzadeh-Khayyat [2011] that alpha-pinene and limonene extracts found as major components in *J. excelsa* species essential oils do not provide higher antioxidant effects. In such investigative studies, the effectiveness of antioxidant activity is naturally determined by the main chemical components of the essential oil studied. In this in vitro study, *J. excelsa* M. M. Bieb. essential oil was found to have weak radical scavenging activity (3.5 ±1.2 mM) due to its alpha-pinene component, which was previously reported [Emami et al. 2011, Hosseinihashemi et al. 2017] to have a low antioxidant effect.

Antimicrobial activity

The results of the antibacterial assays with essential oils of *J. excelsa* M. Bieb. are summarized in Table 2 and compared in Figure 1. The essential oil obtained showed a low inhibitory activity (9.0, 11.0, 11.5, 12.0, 9.0, and 8.0 mm, respectively) on all the microorganisms tested compared to the two control antibiotics.
As shown in Table 2 and Figure 1, according to the comparative analysis, it was determined that six specific bacteria sensitive to the two synthetic antibiotics used as standard showed low but different sensitivity to the essential oil used correctly. The verifiable results of this study show that *J. excelsa* essential oil enough has moderate activity against tested positive and negative bacterial strains (Tab. 2). In profound regard to its potential effectiveness against specific bacteria, similar results were typically expressed by Unlu, Vardar-Unlu, Vural, Donmez and Cakmak [2008] and also was reported by Khoury, El Beyrouthy, Ouaini, Iriti, Eparvier and Stien [2014] only for *S. aureus* bacteria

Table 2. Antibacterial activities (inhibition zone measured in mm) of the essential oils of *J. excelsa* M. Bieb. leaves (mean ±SE)

Antibiotic/Essential oil	SA	BS	PA	EF	ST	EC
Ampicillin	21.0 ±0.47	25.0 ±0.00	22.5 ±0.24	27.0 ±0.47	23.0 ±0.40	27.0 ±0.47
Ofloxacin	25.0 ±0.43	30.0 ±0.12	27.0 ±0.47	21.0 ±0.40	25.0 ±0.37	27.0 ±0.40
Juniperus excelsa M. Bieb.	9.0 ±0.42	11.0 ±0.24	11.5 ±0.24	12.0 ±0.47	9.0 ±0.33	8.0 ±0.00

SA – *Staphylococcus aureus* ATCC 12600, BS – *Bacillus subtilis* ATCC 6051, PA – *Pseudomonas aeruginosa* ATCC 10145, EF – *Enterococcus faecalis* ATCC 29212, ST – *Salmonella typhimurium* ATCC 25241, EC – *Escherichia coli* ATCC 11775.
Sensitivity – not sensitive for diameters < 8 mm; sensitive for diameters 9–14 mm; very sensitive for diameters 15–19 mm and extremely sensitive for diameters >20 mm

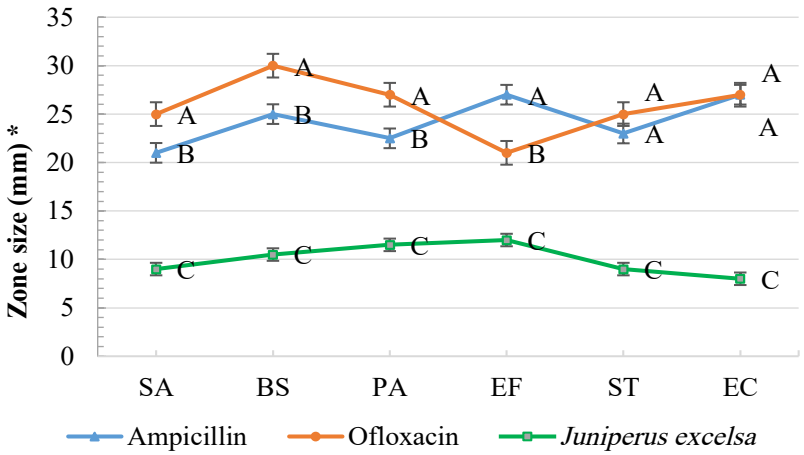

Fig. 1. Antibacterial activities of essential oils of *J. excelsa* leaves against six bacteria. * there is no difference between the means indicated by the same letter for the bacteria

strains. The data obtained in this study are consistent with the previous study, which states that α-pinene, the main component in essential oil, is a key component with low antibacterial activity [Unlu et al. 2008]. From these beneficial results, essential oils of *J. excelsa* could potentially be used as natural preservatives in nutritious food against the familiar causal agents of foodborne diseases like *B. subtilis*, *S. aureus*, and *E. coli* [Hosni et al. 2011].

CONCLUSION

It is known that there is an increasing demand for antioxidants of plant origin due to current concerns about possible adverse health effects caused by synthetic antioxidants. In this study, it was seen that there was a positive correlation between observed biological activities due to the major components such as alpha-pinene and limonene which had low antibacterial and antioxidant effects. Although the results of the present study demonstrate relatively low antioxidant and antibacterial activities for the tested essential oils obtained from leaves of *J. excelsa* species, these activities suggest the possible use of the essential oils of this plant in low concentrations for preserving food materials. Additionally, this essential oil is more suitable for use in the cosmetic industry due to the high amount of alpha-pinene because alpha-pinene is highly preferred in the cosmetics industry.

SOURCE OF FUNDING

This research received no external funding.

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