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Chemical Content and Antimicrobial Activities of Essential Oils Obtained from Plant Parts of *Juniperus excelsa* M. Bieb.

Kemijski sastav i antimikrobno djelovanje eteričnih ulja dobivenih iz dijelova biljke *Juniperus excelsa* M. Bieb.

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ABSTRACT • *This study was conducted to determine the chemical content, antibacterial, and antifungal properties of essential oil obtained from the parts of the plant called Juniperus excelsa M. Bieb. In this study, essential oils of cone, needle, and twig of J. excelsa plant, which is a naturally grown plant in Gümüşhane province, were obtained by hydrodistillation method in a Clevenger type device. Chemical composition of essential oils was determined thanks to the analysis conducted with GC-MS/FID device. Besides, antimicrobial activity tests of essential oils were decided in contrast to 23 different microorganisms with the disc diffusion method. As a result of the essential oil analysis of J. excelsa, the percentage of essential oil yield in cones, needles, and twigs was found as 5.88 %, 2.00 %, and 0.62 %, respectively. α-pinene was confirmed to be the most abundant main compound found in the essential oils of cones, needles, and twigs. As a result of the essential oil analysis of the cone, needle, and twig of J. excelsa species, it was revealed that monoterpenes were the most abundant chemical class in terms of percentage. In the antimicrobial activity test performed on the essential oils of J. excelsa plant parts, it was found that cones, needles, and twigs have a strong antimicrobial effect against Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Escherichia coli O157:H7, Listeria monocytogenes, Salmonella enteritidis, Salmonella typhimurium, Staphylococcus aureus, Candida albicans, Penicillum expansum, Saccharomyces cerevisiae bacteria, yeast, and molds.*

KEYWORDS *antimicrobial activity, chemical content, GC-MS; essential oil, Juniperus excelsa M. Bieb.*

SAŽETAK • *Ovo je istraživanje provedeno kako bi se utvrdio kemijski sastav te antibakterijska i antifungalna svojstva eteričnog ulja dobivenoga iz dijelova biljke Juniperus excelsa M. Bieb. Za potrebe ove studije eterična ulja češera, iglica i grančica biljke J. excelsa, koja je prirodno uzgojena u pokrajini Gümüşhane, dobivena su metodom hidrodestilacije u uređaju tipa Clevenger. Kemijski sastav eteričnih ulja određen je analizom na uređaju GC-MS/FID. Osim toga, disk-difuzijskom metodom ispitano je antimikrobno djelovanje eteričnih ulja na 23 različita mikroorganizma. Analizom eteričnog ulja biljke J. excelsa utvrđeno je da je postotak prinosa eteričnog ulja u češerima, iglicama i grančicama bio (redom) 5,88 % 2,00 % i 0,62 %. Najzastupljeniji glavni spoj pronađen u eteričnim uljima češera, iglica i grančica jest α-pinen. Rezultat analize eteričnih ulja češera, iglica i grančica biljke J. excelsa pokazao je da su postotno najzastupljenija kemijska klasa monoterpeni. U ispitivanju antimikrobne*

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aktivnosti provedenome na eteričnim uljima dijelova biljke J. excelsa ustanovljeno je da češeri, iglice i grančice imaju snažno antimikrobno djelovanje na bakterije, kvasce i plijesni Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Escherichia coli O157:H7, Listeria monocytogenes, Salmonella enteritidis, Salmonella typhimurium, Staphylococcus aureus, Candida albicans, Penicillum expansum i Saccharomyces cerevisiae.

KLJUČNE RIJEČI: *antimikrobna aktivnost, kemijski sastav, GC-MS, eterično ulje, Juniperus excelsa M. Bieb.*

1 INTRODUCTION
1. UVOD

Forests are home to biodiversity of terrestrial ecosystems, and they play a main role in maintaining this biodiversity. Forests do not only meet important human functions such as shelter and food, but they also have important economic value based on products such as wood, food, fiber medicine, etc. Moreover, the fact that forests are home to medicinal and aromatic plants, whose value has greatly increased in recent years, adds additional importance to our forests (Başaran, 2012; Deniz *et al.,* 2014). Considering the flora of Türkiye in terms of its location, it is understood that 11466 plant species are distributed across 7 geographical regions. Related studies demonstrate that 3649 of these plants are endemic, 1700 of them have medicinal properties, and 500 of these plants have medicinal and aromatic properties (Fidan *et al.,* 2011; OGM, 2020). Türkiye is a country rich in coniferous forests due to its geographical location. About half of the country's total forest area consists of coniferous trees (Tumen *et al.,* 2009).

Plants are important potential sources of phytochemicals (Baltacı *et al.,* 2022a). Natural herbal products have been utilized as raw materials in hundreds of industrial products (Öz *et al.,* 2015). The biochemical ingredients found in plants are directly related to the medicinal and aromatic properties of plants. The fact that plants contain various volatile components is a special sign that the medicinal and aromatic properties of plants are quite high (Faydaoğlu and Sürücüoğlu, 2011). Essential oils are described as natural, volatile, complex compounds obtained from the essence of medicinal aromatic plants as secondary metabolites. In order to obtain essential oils, parts of the plant like flowers, needles (leaves), seeds, roots, stems, bark, wood can be utilized separately or used as a whole without parting. In order to determine the essential oil content of the plants, certain parts of the plants must go through the dry or wet distillation process. Following this process, methods such as GC and GC-MS are used to determine essential oil components (Başer, 2010; Baltacı *et al.,* 2022b). Other sectors such as medicine, food, and cosmetics use of essential oils as primary raw materials. These valuable oils have analgesic, wound healing, calming, bactericidal, and fungicidal, refreshing, stress-reducing, mind-opening, and sedative effects. Furthermore, many essential oils have strong antimicrobial properties. In the literature, a great number of studies can be found on the determination of the chemical composition of essential oils obtained from plants, and the determination of antioxidant and antimicrobial properties of these oils (Üçüncü *et al.,* 2010; Fidan *et al.,* 2022).

Yaglioglu *et al.* (2020) stated that the genus *Juniperus* (Cupressaceae) is represented by 8 taxa in Türkiye and approximately 68 species in the world, and that Juniperus species have been used in folk medicine since ancient times. *Juniperus excelsa* M. Bieb. is a tree species that spreads from the Eastern Mediterranean to the Caucasus, Greece, Macedonia, Türkiye, Iran, Afghanistan, and Pakistan. It has been reported that *J. excelsa* is the most dominant species among forests formed by juniper species with a distribution rate of 82 % in Türkiye (OGM, 2014; Saruhan, 2022). *J. excelsa* is one of the evergreen tree species that can grow up to 25 m, and its trunk diameter can be up to 2.5 m. The color of the trunk shell is gray-brown, and the trunk shell, which is in the form of cracked strips towards the length of the trunk, is brown in color when the tree is young, while the color of the shell turns gray when the tree ages (Gültekin and Gültekin, 2006). Juniper cone oil has been reported to be sedative, antiseptic, and analgesic, and is known to cure many other ailments such as jaundice, eczema, and tuberculosis (Khajjak *et al.,* 2012). Kakar *et al.* (2017) reported that *Juniperus* species are characterized by large amounts of essential oil in needles and cones, as well as in seeds and twigs.

J. excelsa (Crimean Juniper) species have been the focus of much research both around the world and in Türkiye due to their characteristics. Among different studies conducted in Türkiye, Topçu *et al.* (2005) investigated essential oil components of *J. excelsa* needles, Ünlü *et al.* (2008) looked into the composition of essential oils obtained from cones, and Nadir *et al.* (2013) examined the chemical composition of needle and cone essential oils. Lesjak *et al.* (2017) evaluated the bioactivity and chemical profile of the needles, and cones of *J.excelsa* in their study. Sela *et al.* (2015) carried out a study to determine the yield, chemical composition, and antimicrobial activity of the essential oils of the cones and needles of *J. excelsa*. In their study, Emami *et al.* (2011) examined antioxidant activity of essential oils obtained from different parts of the *Juniperus excelsa* M. Bieb. *subsp. excelsa* tree. Hojjati *et al.* (2019) reported a comparison of the leaf essential oil composition of ten populations of the *Juniperus excelsa* complex found in Iran.

No study was found on the chemical composition of the essential oils of cones, needles, and twigs of *Juniperus excelsa* grown in Gümüşhane region of Türkiye, nor were their antimicrobial properties investigated. The intent of this study was to contribute to the understanding of the medicinal importance of the plant by determining the chemical composition and antimicrobial properties of essential oils obtained from *J. excelsa* plant parts and by comparing it with other studies.

2 MATERIALS AND METHODS
2. MATERIJALI I METODE

2.1 Plant materials
2.1. Biljni materijali

Cone, needle, and twig samples of the *J. excelsa* tree, which is the focus of the study, were gathered from the natural areas with no human activities in the central Hacıemin District of Gümüşhane province. Identification of plants species was performed by Prof. Dr. Sefa AKBULUT, Faculty Member of Department of Forestry Engineering, Faculty of Forestry at Karadeniz Technical University (Kato Herbarium No:19555). The samples were collected by hand on September 13, 2020 (autumn season) from the above region and dried in the shady and airy environment by mixing at regular intervals. Cone, needle, and twig samples were stored in a cool, dry place out of the sun until analysis. For analysis, the samples were ground and treated in the same way. The area where *J. excelsa* cone, needle, and twig samples were collected is shown in Figure 1.

2.2 Methods
2.2. Metode

2.2.1 Extraction of essential oils
2.2.1. Ekstrakcija eteričnih ulja

Essential oils were obtained by hydrodistillation process. Samples of 100 grams from dried cones, needles, and twigs of *J. excelsa* species were collected, and ground. 100 g of homogenized cones, needles, and twigs samples were weighed, respectively, in a 2000 mL round Clevenger device flask, by adding 1000 mL of distilled water. It was done with a modified Clevenger apparatus with +4 ℃ cooler for 4 hours. 2 mL of n-hexane was placed in the collection part of the Clevenger device. The temperature of the cooler was set to +4.0 ℃. Essential oils were collected by boiling at a low temperature for 4 hours (Öz *et al.,* 2021). Percentage yields of essential oils by weight were calculated for cone, needle, and twig, respectively. The essential oils obtained were put into colored bottles in n-hexane in GC quality and stored with the lid closed. Percentage of yields for essential oils by weight were calculated separately for cones, needles, and twigs (w/w) (Djordjevic *et al.,* 2021).

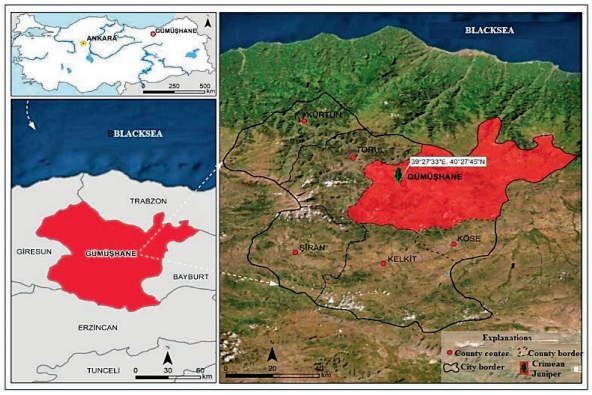

Figure 1 The area where *J. excelsa* cone, needle, and twig samples were gathered
Slika 1. Područje na kojemu su prikupljeni uzorci češera, iglica i grančica biljke *J. excelsa*

$$Yield\ \% = \frac{(Amount\ of\ extracted\ essential\ oil(g))}{(Amount\ of\ dry\ plant\ material)} \text{x}100 \quad (1)$$

2.2.2 Analysis of volatile oils components with GC-MS/FID
2.2.2. Analiza hlapljivih organskih spojeva uređajem GC-MS/FID

The volatile oils obtained by hydrodistillation in the Clevenger apparatus were filtered as dissolved in hexane and then placed in the autosampler by putting them in dark colored bottles. Essential oil component analyses were carried out in the Gas Chromatography Mass Detector-Flame Ionization Detector (GC-MS/FID) (GC-FID Agilent-7890A, MS Agilent 5975C model). HP-5MS model nonpolar capillary column (30 m × 0.32 mm, film thickness 0.25 µm) was used for analysis. The injections were applied in splitless mode at 240 °C using helium as the carrier gas with a flow rate of 1 mL / min. 1 µL volatile oil solution in hexane (GC class) was injected, and was initially stored at 60 °C for 2 minutes, and then spectra were taken by raising to 240 °C with an increase of 3 °C/min. After the volatile compounds were separated on a gas chromatography column, mass spectra of each compound were obtained in the mass spectrophotometer one by one. The identification of components of essential oils was performed based on a comparison of retention indices (*RI*) with reference to a homologous series of n-alkanes (C_6-C_{32}), under identical experimental conditions. The mass spectrum of each component was identified through the structure clarification by comparing with the reference compounds of the NIST and Willey libraries, as well as by making a comparison of their retention time either with retention times of authentic compounds or with the literature data (Adams, 2007).

2.2.3 Determination of antimicrobial activity
2.2.3. Određivanje antimikrobne aktivnosti

The antimicrobial activities of essential oils extracted from *J. excelsa* plant parts were carried out in accordance with the disc diffusion method (Matuschek *et al.,*

2014). Essential oil samples were prepared by dissolving them in hexane. According to the method, the samples must be in different concentrations depending on their essential oil densities; values were determined against 23 different microorganisms activated using cones (100 ppm, 50 ppm, 25 ppm), needles (100 ppm, 50 ppm, 25 ppm), and twigs (6240 ppm, 1000 ppm, 500 ppm).

The disc diffusion method was performed in two stages: the preparation of microorganisms and the preparation of samples. All test microorganisms were obtained from Gümüşhane University, Faculty of Engineering, and Natural Sciences, Department of Food Engineering. For this purpose, bacteria were used for antibacterial analysis in Nutrient Broth medium after 24 hours of first activation at 36 ºC, and after 18 hours of second activation at 36 ºC. After the second activation, the prepared sterile Nutrient Agar was smeared on the nutrient media with the swap method. For antifungal analysis, yeasts and molds were used in Malt Extract Broth medium at 27 ºC for 48 hours after the first activation followed by a second activation for 24 hours. The yeasts and molds which had been activated twice were smeared on Malt Extract Agar with the swap method, as was the case with bacteria, and prepared for analysis. The obtained essential oils were absorbed into sterile antimicrobial discs with 20 µL and placed on the previously prepared petri dishes. Petri dishes containing bacteria were incubated for 24 hours at 36 ºC, and petri dishes containing yeast and mold were incubated for 48 hours at 27 ºC. When the specified time expired, the transparent zones around the discs were measured in millimeters, and antimicrobial activity results were specified.

3 RESULTS AND DISCUSSION
3. REZULTATI I RASPRAVA

3.1 The amount of obtained essential oil
3.1. Količina dobivenoga eteričnog ulja

Essential oils were obtained as a result of the hydrodistillation process applied to 100 g of dried cones, needles, and twigs. As a result of the analysis, it was found that the amount of essential oil obtained from the cones was 5883.4 mg with a yield of 5.88 % (w/w), the amount of essential oil obtained from the needles was 1988.7 mg with a yield of 2.00 % (w/w), and the amount of essential oil obtained from the twigs was 0.62 % (w/w) with a yield of 618.9 mg. When the amount and percentage of essential oil detected in cones, needles, and twigs were compared, it was decided that the amount and percentage of essential oil in the cones were higher.

Al Hafi *et al.* (2015) reported that the highest yields of essential oils were obtained by Clevenger from *J. excelsa* cone needles and twigs (1.20-2.50 %), followed by needles (1.00-1.50 %), and twigs (0.20-0.50 %). Asili *et al.* (2008), as a result of their study to determine the essential oil in the cones and needles of *J. excelsa*, indicated that the essential oil yields were 1.66 % (v/w) from cones and 1.50 % (v/w) from needles. Furthermore, in a different study carried out in Macedonia, Sela *et al.* (2015) pointed out that the essential oil yields obtained from the needles of this species were between 0.89 % and 1.39 %. In his study, Öncel (2016) obtained the highest amount of essential oil from cones of 1.69 %. It was reported that the amount of essential oil was found to be 1.04 % from the needles, and 0.45 % from the twigs. The literature shows that the results of the present study are compatible with other studies of the same topic and it is supposed that small differences seen may be caused by the method applied, collection time, and genetic factors.

3.2 GC/MS and GC/FID analysis results of essential oil components obtained from cones, needles, and twigs
3.2. Rezultati GC/MS i GC/FID analize komponenata eteričnog ulja dobivenoga iz češera, iglica i grančica

On account of GC-MS/FID analysis, the structure of 93 compounds from the cones of *J. excelsa*, 113 compounds from the needles, and 111 compounds from the twigs were identified. When the essential oil samples are compared in terms of the number of compounds, the number of compounds identified in needle essential oils is seen to be higher than the number of compounds identified in cone and twig essential oils. The results of GC-MS/FID analysis of essential oils obtained from the cones, needles, and twigs of *J. excelsa* are presented in Table 1.

When all components in essential oils obtained from cones were studied, it was determined that the main compounds were *α*-pinene (85.78 %), cedrol (2.62 %), and *β*-myrcene (2.11 %) (Table 1). When all the components in the essential oils obtained from the needles were examined, the main compounds were found to be α-pinene (67.52 %), cedrol (7.98 %), and *β*-myrcene (1.65 %) (Table 1). When all the components in the essential oils obtained from the twigs were examined, it was decided that the main compounds were *α*-pinene (69.92 %), cedrol (2.49 %), and Germacrene B (2.47 %) (Table 1).

Al Hafi *et al.* (2015) stated that the main component detected in essential oils obtained from the needles, twigs, and cones of *J. excelsa* was α-pinene in cones (86.80-95.20 %), in needles (30.60-68.80 %), and in twigs (78.30-89.80 %). In their study on the composition of the essential oil obtained from the cones of the same species, Ünlü *et al.* (2008) found that the essential oil consists of 44 components and that α-pinene (55.50 %), α-cedrol (7.70 %), and sabinene

Table 1 Results of chemical constituents of volatile oil obtained from plant parts (cones, needles, and twigs) of J. excelsa
Tablica 1. Rezultati analize kemijskog sastava hlapljivog ulja dobivenoga iz biljnih dijelova (*češera*, iglica i grančica) *J. excelsa*

No	RI	LRI	Compounds / *Spojevi*	Percent composition / *Postotni udio*		
				JeC	JeL	JeT
1	706	700	Heptane	0.02	0.04	0.06
2	725	725	Cyclohexylmethane	0.03	0.03	0.06
3	802	802	Hexanal	0.02	0.03	0.01
4	825	832	Methyl hexyl ether		0.05	
5	851	850	2-Hexenal		0.09	
6	923	923	Tricyclene	0.20	0.18	0.20
7	931	931	*α*-Thujene			0.06
8	946	946	***α*-Pinene**	85.78	67.52	69.92
9	951	951	*α*-Fenchene			0.08
10	958	958	Camphene	0.37	0.44	0.20
11	961	961	Verbenene	0.02	0.03	0.03
12	975	975	Sabinene	0.27	0.06	0.59
13	982	982	*β*-Pinene	0.87	0.79	1.16
14	997	997	***β*-Myrcene**	2.11	1.65	
15	1007	1007	*α*-Phellandrene	0.02	0.02	0.02
16	1011	1011	3-Carene	0.01	0.76	0.26
17	1019	1019	*α*-Terpinene	0.05	0.04	0.05
18	1027	1027	*o*-Cymene	0.06	0.12	0.09
19	1033	1033	Limonene	0.79	1.49	0.72
20	1034	1034	Eucalyptol	0.01		0.01
21	1038	1038	*cis*-*β*-Ocimene	0.01	0.01	0.02
22	1039	1039	*trans*-*β*-Ocimene	0.01	0.03	
23	1047	1054	Butyric acid, isopentyl ester		0.03	
24	1061	1061	*γ*-Terpinene	0.48	0.44	0.13
25	1069	1069	Sabinene hydrate	0.06	0.02	0.02
26	1091	1091	Terpinolene	0.84	0.72	1.35
27	1100	1100	*trans*-Sabinene hydrate	0.05		
28	1102	1102	Linalool		0.16	0.02
29	1104	1104	Nonanal			0.03
30	1106	1106	Solusterol		0.07	
31	1109	1107	Umbellulol	0.01	0.01	
32	1113	1113	*p*-Mentha-1,5,8-triene	0.01		0.01
33	1115	1115	Fenchol	0.01	0.08	0.01
34	1118	1118	3-Methyl-3-butenyl isovalerate		0.03	
35	1128	1128	*α*-Campholenal	0.02	0.05	0.13
36	1137	1137	1-Terpinenol	0.01	0.01	0.01
37	1141	1141	L-Pinocarveol	0.05	0.06	0.12
38	1144	1144	*trans*-Verbenol	0.03	0.03	0.03
39	1147	1147	Camphor	0.16	0.27	0.12
40	1151	1151	*α*-Phellandren-8-ol	0.01	0.03	0.05
41	1160	1149	*cis*-*p*-Ment-2,8-dien-1-ol		0.03	
42	1163	1163	Pinocamphone	0.01	0.05	0.02
43	1165	1165	Pinocarvone	0.01	0.02	0.03
44	1169	1169	Borneol	0.12	0.11	0.15
45	1175	1177	(*E,E*)-1,3,5-Undecatriene	0.01	0.01	
46	1180	1180	Terpinen-4-ol	0.08	0.04	0.13
47	1188	1188	p-Cymen-8-ol	0.01	0.02	0.02
48	1193	1193	*α*-Terpineol	0.05	0.05	0.16
49	1195	1191	*trans*-Undec-4-enal		0.05	
50	1199	1199	Myrtenol	0.03	0.03	0.09
51	1206	1212	Homomyrtenol	0.01	0.02	0.02
52	1212	1204	Levoverbenone	0.02	0.04	0.03
53	1222	1222	*trans*-Carveol	0.02	0.03	0.03
54	1243	1243	Hexyl isovalerate		0.07	0.02
55	1246	1246	*p*-Cymene-2-ol methyl ether	0.01		0.04

Table 1 (Continuation)
Tablica 1. (Nastavak)

No	RI	LRI	Compounds / *Spojevi*	Percent composition / *Postotni udio*		
				JeC	JeL	JeT
56	1259	1259	Piperitone	0.01	0.05	0.01
57	1263	1257	Myrtanol	0.01		0.03
58	1289	1289	*L*-bornyl acetate	0.29	0.19	0.21
59	1295	1295	(*E,E*)-2,4-Decadienal		0.03	0.03
60	1342	1342	*δ*-Elemene	0.12	0.42	0.70
61	1353	1353	*α*-Cubebene		0.01	0.01
62	1376	1376	Ylangene		0.01	0.01
63	1380	1380	*α*-Copaene		0.08	0.03
64	1384	1385	*α*-Funebrene		0.01	0.01
65	1388	1387	Hexanoic acid hexyl ester		0.06	
66	1389	1389	2-epi-*α*-Funebrene			0.03
67	1397	1397	*β*-Elemene	0.12	0.34	0.34
68	1410	1410	*α*-Cedrene	0.03	1.13	0.04
69	1419	1415	(+)-*β*-Funebrene	0.28		
70	1421	1421	*β*-Cedrene		0.84	0.12
71	1428	1428	Caryophyllene	0.32	0.76	0.73
72	1439	1445	Elixene	0.31		1.45
73	1452	1447	Alloaromadendrene		0.02	0.01
74	1459	1458	*cis*-*β*-Farnesene			0.06
75	1461	1461	Humulene		0.35	
76	1471	1469	*α*-Elemene			0.02
77	1473	1474	Acoradien	0.02	0.06	0.01
78	1478	1483	*γ*-Cadinene		0.03	
79	1481	1481	*γ*-Muurolene	0.02		0.40
80	1484	1484	*α*-Amorphene	0.01	0.27	
81	1494	1494	*β*-Selinene		0.14	0.22
82	1488	1488	Germakren D	0.31	0.99	0.72
83	1496	1493	*β*-Cadinene			0.05
84	1498	1497	*δ*-Selinene		0.12	
85	1501	1501	*α*-Farnesene	0.05		
86	1503	1503	*α*-Selinene	0.04	0.31	0.25
87	1507	1507	*α*-Muurolene	0.02	0.22	0.11
88	1514	1514	*β*-Bisabolene	0.27	0.14	
89	1523	1523	*γ*-Cadinene	0.18	0.83	
90	1533	1533	*δ*-Cadinene	0.09	1.24	0.74
91	1535	1535	Germacrene B	0.48	1.50	2.47
92	1538	1538	*γ*-Bisabolene	0.06	0.10	0.04
93	1541	1539	Cadine-1,4-diene		0.08	
94	1542	1532	*γ*-Selinene		0.29	0.18
95	1544	1542	Cubenene	0.02	0.05	0.04
96	1551	1551	Selina-3,7(11)-dien	0.02	0.11	0.15
97	1557	1557	Elemol	0.19	0.50	0.71
98	1572	1576	Selin-4,7(11)-diene		0.07	
99	1584	1584	Salvial-4(14)-en-1-one			0.26
100	1586	1586	Spatulenol	0.14	0.91	0.36
101	1593	1593	Caryophyllene oxide	0.03	0.05	0.10
102	1596	1596	Globulol		0.02	0.02
103	1599	1599	Epiglobulol			0.16
104	1600	1600	Humulene epoxide	0.16		
105	1602	1601	Viridiflorol		0.51	
106	1617	1612	Isoaromadendrene epoxide			0.05
107	1625	1625	Cedrol	2.62	7.98	2.49
108	1629	1629	Caryophylla-4(12),8(13)-dien-5α-ol	0.02	0.23	
109	1642	1642	*γ*-Eudesmol	0.01	0.17	0.22
110	1653	1653	*τ*-Muurolol	0.02	1.17	0.49

Table 1 (Continuation)
Tablica 1. (Nastavak)

No	RI	LRI	Compounds / *Spojevi*	Percent composition / *Postotni udio*		
				JeC	JeL	JeT
111	1659	1659	*β*-Eudesmol	0.03	0.10	0.62
112	1664	1664	*β*-Selinenol	0.05	0.22	1.03
113	1674	1674	Caryophyllenol-II	0.01	0.08	0.11
114	1679	1674	Eudesma-4(15),7-dien-1-b-ol		0.03	
115	1688	1688	Cinnamyl valerate		0.04	
116	1689	1689	8-Cedren-13-ol	0.01	0.03	0.09
117	1702	1702	14-Hydroxy-α-humulene	0.15	0.50	0.63
118	1707	1709	Juniper camphor		0.04	0.04
119	1713	1714	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-2-ol	0.01	0.03	0.05
120	1719	1720	epi-α-Bisabol-1-one		0.02	
121	1726	1724	*β*-Santalol			0.08
122	1756	1748	1,10-seco-1-hydroxycalamenen-10-one			0.04
123	1776	1776	*α*-Muurolene-14-ol		0.02	
124	1778	1775	Isovalencenol			0.05
125	1785	1781	*cis*-Lanceol	0.01	0.01	0.06
126	1940	1933	(*E,E*)-5,9-Farnesyl acetone	0.01		0.01
127	1975	1975	Sandaracopimaradiene	0.02	0.01	0.03
128	1991	1991	Manoyl oxide	0.02	0.20	0.13
129	2004	2005	Epimanoyl oxide			0.31
130	2023	2023	Kaurene	0.04	0.01	0.40
131	2032	2032	Kaur-16-ene	0.07	0.01	0.03
132	2068	2069	Dehydroabietan		0.01	0.17
133	2094	2080	2-Phenylethyl geranoate	0.39	0.07	0.04
134	2148	2134	1-Adamantanecarboxylic acid, 3-methylphenyl ester	0.02		0.04
135	2162	2163	Dodecyl benzoate	0.04		0.01
136	2199	2199	*p*-Methoxybenzoic acid, 2-isopropoxyphenyl ester	0.02	0.03	1.31
137	2234	2227	Pimara-7,15-dien-3-one		0.07	2.18
138	2246	2252	Methyl sandaracopimarate		0.01	0.05
139	2285	2288	Neoabietal			0.06
140	2319	2319	Totarol	0.04	0.10	0.19
141	2336	2336	Ferruginol	0.01		1.85
142	2403	2391	Abietinol	0.01	0.01	

RI: Retention indices calculated against C_6-C_{32} n-alkanes on HP 5MS column / *indeksi retencije izračunani u odnosu prema C_6-C_{32} n-alkanima na HP 5MS koloni;* LRI: Retention Indices reported in Literature (Adams, 2007; Wiley and NIST) / *indeksi retencije navedeni u literaturi* (Adams, 2007.; Wiley and NIST); JeL: *Juniperus excelsa* needles / *iglice Juniperus excelsa*; JeC: *Juniperus excelsa* cones / *češeri Juniperus excelsa*; JeT: *Juniperus excelsa* twigs / *JeT: grančice Juniperus excelsa*

(3.50 %) were the main components, as a result of the GC-MS analysis. Lesjak *et al.* (2017) reported that the main components of essential oils obtained from *J. excelsa* cones are α-pinene (77.00 %), cedrol (8.00 %), and limonene (6.00 %), while the main components of the essential oil obtained from the needles are α-pinene (31.00 %), cedrol (37.00 %), and limonene (15.00 %). Gülsoy and Merdin (2017) identified 41 different components in the needles in their study. They determined the essential oil in the needles of *J.excelsa* and its properties. They confirmed that α-pinene (81.28 %) was the main component among these components, followed by myrcene (5.19 %), and limonene (4.52 %). In another study, α-pinene (36.00 %), β-pinene (30.20 %), limonene (12.60 %), β-phellandrene (3.90 %) were specified as the main components of the needles of *J. excelsa* (Nadir *et al.,* 2013). There are some differences between the results of this study and the studies in the literature, although they are similar in general. The chemical classification of the compounds detected in *J. excelsa* cone, needle, and twig essential oils is shown in Table 2.

After the amounts of essential oil components obtained from cones were examined, the most common compound class was found to be monoterpenes with 91.90 % (17 compounds), followed by sesquiterpenoids with 3.47 % (16 compounds), and sesquiterpenes with 2.77 % (20 compounds). It is understood

Table 2 Chemical classification of compounds detected in *J. excelsa* cone, needle, and twig essential oils
Tablica 2. Kemijska klasifikacija spojeva otkrivenih u eteričnim uljima *češera*, iglica i grančica *J. excelsa*

Chemical classification *Kemijska klasifikacija*	**Number of compounds in cones** *Broj spojeva u češerima*	**% amount** *Postotna količina*, %	**Number of compounds in needles** *Broj spojeva u iglicama*	**% amount** *Postotni udio*, %	**Number of compounds in twigs** *Broj spojeva u grančicama*	**% amount** *Postotni udio*, %
Aldehydes	2	0.04	5	0.25	5	0.26
Alcohols	1	0.01	1	0.08	1	0.01
Esters	5	0.76	10	0.60	7	1.68
Ethers	1	0.01	1	0.05	1	0.04
Hydrocarbons	3	0.06	3	0.08	2	0.12
Ketones					1	0.04
Monoterpenes	17	91.90	16	74.32	18	74.90
Monoterpenoids	21	0.77	20	1.06	19	1.09
Sesquiterpenes	20	2.77	29	10.52	27	8.94
Sesquiterpenoids	16	3.47	20	12.62	21	7.63
Diterpene	3	0.13	4	0.04	5	0.95
Diterpenoids	4	0.08	4	0.38	4	4.34
Total	93	100	113	100	111	100

that the total ratio of other compounds was quite low with 1.86 %. Considering the amounts of essential oil components obtained from the needles, the most common compound class was monoterpenes with 74.32 % (16 compounds), followed by sesquiterpenoids with 12.62 % (20 compounds), and sesquiterpenes with 10.52 % (29 compounds). The total ratio of other compounds was found to be very low with 2.54 %. Regarding the amounts of essential oil components obtained from the twigs, the most common compound class was found to be monoterpenes with 74.90 % (18 compounds), followed by sesquiterpenes with 8.94 % (27 compounds), and sesquiterpenoids with 7.63 % (21 compounds). The total ratio of other compounds was found to be 8.53 %.

3.3 Antimicrobial test results of essential oil extracts
3.3. Rezultati antimikrobnih ispitivanja ekstrakata eteričnih ulja

The results of antimicrobial activity tests of pine cone, needle, and twig essential oil samples are presented in Table 3.

Essential oil obtained from cones was detected to have a strong antibacterial effect against *Aeromonas hydrophila*, *Enterococcus faecalis*, *Escherichia coli* O157:H7, *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica* from gram-negative bacteria; and *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus,* and *Listeria monocytogenes* from gram-positive bacteria. It should be noted that cone essential oils show activity in 15 of 23 different test microorganisms analyzed, being effective at different concentrations. Essential oil obtained from needles was found to have a strong antibacterial effect against *Escherichia coli*, *Escherichia coli* O157:H7, *Salmonella enteritidis*, *Klebsiella pneumoniae*, *Yersinia enterocolitica,* and *Enterococcus faecalis* from gram-negative bacteria, and *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis,* and *Listeria monocytogenes* from gram-positive bacteria. Needle essential oils were found to show activity in 13 of 23 different test microorganisms analyzed. Essential oil from twigs was found to have a strong antibacterial effect against *Escherichia coli* O157:H7, *Escherichia coli*, *Salmonella typhimurium*, *Salmonella enteritidis,* and *Enterococcus faecalis* from gram-negative bacteria, and *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus subtilis,* and *Listeria monocytogenes* from gram-positive bacteria. It was observed that twig essential oils did not demonstrate antifungal activity against analyzed yeast and mold fungi.

In a study, in which the antimicrobial activity of *J. excelsa* essential oils is evaluated clinically and against gram-positive and gram-negative bacterial strains that cause foodborne illness, while the antimicrobial activity of essential oils against gram-negative bacteria is moderate, it has been stated that gram-positives examined in essential oil of both needles and cones are more effective in growth inhibition (Lesjak *et al.,* 2017). Moein *et al.* (2010) reported in their study that the most sensitive bacteria of the essential oil of *J. excelsa* are *Staphylococcus epidermidis,* and *Pseudomonas aeruginosa*. In their study, Asili *et al.* (2008) investigated the antimicrobial activities of essential oil obtained from the cones and needles of *J. excelsa*, and it showed antimicrobial activity against *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus*. Ünlü *et al.* (2008) stated that Crimean Juniper cones showed

Table 3 Results of antimicrobial activity test conducted on cone, needle, and twig essential oils of *J. excelsa*
Tablica 3. Rezultati ispitivanja antimikrobnog djelovanja eteričnih ulja *češera*, iglica i grančica *J. excelsa*

	Cones / *Češeri*			**Needles** / *Iglice*			**Twigs** / *Grančice*			**Penisilin G (10 mg)**
	100 ppm	50 ppm	25 ppm	100 ppm	50 ppm	25 ppm	6240 ppm	1000 ppm	500 ppm	
Gram-negative bacteria *Gram-negativne bakterije*	**Diameter of inhibition zones** / *Promjer zona inhibicije*, mm									
Aeromonas hydrophila	16.75±0.10	12.45±0.10	6.15±0.10	-	-	-	-	-	-	34±0.01
Enterococcus faecalis	14.50±0.10	10.35±0.10	5.15±0.10	8.75±0.10	-	-	11.50±0.10	5.50±0.10	-	32±0.01
Escherichia coli O157:H7	14.45±0.10	9.50±0.10	4.60±0.10	16.50±0.10	9.75±0.10	-	16.35±0.10	10.65±0.10	5.60±0.10	34±0.01
Escherichia coli	15.55±0.10	7.80±0.10	-	16.70±0.10	10.25±0.10	-	15.40±0.10	9.80±0.10	4.50±0.10	34±0.01
Salmonella typhimurium	16.80±0.10	9.75±0.10	-	-	-	-	15.70±0.10	8.70±0.10	-	34±0.01
Klebsiella pneumoniae	10.50±0.10	5.10±0.10	-	10.50±0.10	-	-	-	-	-	30±0.01
Pseudomonas aeruginosa	15.50±0.10	8.45±0.10	-	-	-	-	-	-	-	30±0.01
Yersinia enterocolitica	14.50±0.10	8.90±0.10	-	8.90±0.10	-	-	-	-	-	30±0.01
Salmonella enteritidis	-	-	-	15.60±0.10	9.85±0.10	-	14.10±0,10	8.40±0.10	-	34±0.01
Proteus vulgaris	-	-	-	-	-	-	-	-	-	30±0.01
Vibrio parahaemolyticus	-	-	-	-	-	-	-	-	-	26±0.01
Shigella flexneri	-	-	-	-	-	-	-	-	-	30±0.01
Gram-positive bacteria *Gram-pozitivne bakterije*										
Bacillus cereus	15.30±0.10	9.50±0.10	4.30±0.10	15.40±0.10	9.70±0.10	-	16.40±0.10	10.10±0.10	5.45±0.10	30±0.01
Bacillus subtilis	14.60±0.10	8.90±0.10	-	10.50±0.10	5.50±0.10	-	15.50±0.10	9.40±0.10	-	34±0.01
Staphylococcus aureus	14.50±0.10	8.50±0.10	-	15.50±0.10	9.85±0.10	-	16.50±0.10	10.75±0.10	5.35±0.10	38±0.01
Listeria monocytogenes	12.70±0.10	6.90±0.10	-	9.70±0.10	-	-	15.70±0.10	8.90±0.10	-	30±0.01
Micrococcus luteus	-	-	-	-	-	-	-	-	-	28±0.01
Yeast-Molds *Kvasac – Plijesni*										
Candida albicans	15.50±0,10	10.70±0.10	-	8.90±0.10	-	-	-	-	-	22±0.01
Saccharomyces cerevisiae	12.30±0,10	7.30±0.10	-	6.50±0.10	-	-	-	-	-	14±0.01
Penicillum expansum	10.70±0,10	5.65±0.10	-	6.75±0.10	-	-	-	-	-	16±0.01
Fusarium oxysporum	-	-	-	-	-	-	-	-	-	20±0.01
Aspergillus flavus	-	-	-	-	-	-	-	-	-	25±0.01
Zygosaccharomyces bailii	-	-	-	-	-	-	-	-	-	10±0.01

(-): No activity, Penisilin G (10 mg) was used as the standard for bacteria, yeast, and molds / (-): *nema aktivnosti, penicilin G (10 mg) uzet je kao standard za bakterije, kvasce i plijesni*

strong activity against *Clostridium perfingens* bacteria in terms of antimicrobial activity, while it showed weaker activity against *Staphylococus aureus*, *Streptococus pvogenes*, *Candida albicans*, *Streptococus ppneumoniae*, *Mycobacterium smegnatis,* and *Candida crusei*. The results of the present study and these studies in the literature are parallel, and it is understood that the essential oils obtained from the plant parts of *J. excelsa* have a strong antimicrobial effect.

4 CONCLUSIONS
4. ZAKLJUČAK

Within the scope of this study, the amount of essential oil, and chemical components, antibacterial and antifungal properties of the cones, needles, and twigs of *J.uniperus excelsa* were examined. In this study, 93 compounds were identified from the cone part, 113 compounds from the needle part, and 111 compounds from the twig part of the plant. As a result of the essential oil analyses of the plant parts, it was seen that the essential oil ratio of the cones was higher than the others, and the number of compounds in the needles was found to be higher. The most abundant main compound in essential oils was found to be α-pinene with (85.78 % in cones), (67.52 % in needles), and (69.92 % in twigs) in all three parts of the plant. In terms of chemical classification of essential oils, the most abundant compound class by percentage in all three parts of the plant was found to be monoterpenes with (91.90 % in cones), (74.32 % in needles), and (74.90 % in twigs). Essential oils obtained from *J. excelsa* plant parts have the highest sensitivity against *B. cereus*, *B. subtilis*, *E. faecalis*, *E. coli*, *E. coli* O157:H7, *L. monocytogenes*, *S. enteritidis*, *S. typhimurium*, *S. aureus*, *C. albicans*, *P. expansum*, *S. cerevisiae*, and they can replace synthetic antibiotics against diseases caused by these organisms.

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