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COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *Juniperus excelsa* ESSENTIAL OIL

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The genus *Juniperus* (Cupressaceae) consists of 55 species, all of which occur throughout the northern hemisphere of the world [1]; eigth species of them grow in Turkey [2]. Evergreen shrubs and trees in this conifer genus are slow growing and long lived. Various species of juniper are used medicinally with a range of applications from antiseptic to diuretic [3, 4]. *Juniperus excelsa* M. Bieb. is a medium-sized shrub or tree up to 20 m. This species was divided into 2 subspecies (subsp. *excelsa* and subsp. *polycarpos*) by Farjon: one with a distribution in southeastern Europe, the Crimea, and mainly southern Turkey to Lebanon; the other a more continental element extending from North Turkey to Kirgizistan and Pakistan [5]. Subspecies *excelsa* is the subject of this paper. This subspecies is widespread in Turkey but most common in South Anatolia, in dry rocky slopes in hills and mountains at between 150–2700 m, often forming the tree line in the Taurus mountains [5]. It is locally known as "boylu ardic" tall juniper in Turkey [6].

The chemical composition of the leaf and wood essential oils from *Juniperus excelsa* were previously reported [7–9]. In the literature, there are a number of reports on the composition of the essential oil from berries of *Juniperus* species and their antimicrobial activities [10–13]. Hexane and methanol extracts of *J. excelsa* were reported to demonstrate antimicrobial activity against microorganisms, including *Mycobacterium tuberculosis* [14]. The antimicrobial activity of *J. excelsa* essential oil against three standard bacterial strains and the yeast *Saccharomyces cerevisiae* have been reported [15]. The objective of this study was to determine the *in vitro* antimicrobial activity of the essential oil of the berries of *J. excelsa* and its main component, α-pinene, against clinically important microorganisms, including an anaerobic bacterium and the pathogenic yeasts.

GS/MS analysis of *J. excelsa* essential oil resulted in the identification of forty-four constituents [16], representing 91.3% of the oil, as shown in Table 1. The major components were α-pinene (55.5%), α-cedrol (7.7%), sabinene (3.5%), and verbenone (2.4%). Similarly, Topcu et al. reported α-pinene (34.0%) and α-cedrol (12.3%) as the major components in the essential oil from berries of *J. excelsa* [17]. In another study, the main constituents were found to be α-pinene (29.1%) and carene (29.1%) [15]. The differences in the content of the oils might result from geographical origin, edaphic factors, or harvesting time.

Antimicrobial chemotherapy has not achieved the much required success in the eradication of microbial infections because of the antimicrobial resistance developed by most pathogenic microorganisms. The antimicrobial properties of essential oils derived from many plants are under extensive study. The assays were performed as described elsewhere [18]. The results of this study shows that *J. excelsa* essential oil has a strong activity against the anaerobic bacterium *Clostridium perfingens* while exhibiting moderate activity against *Staphylococus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Mycobacterium smegmatis*, *Candida albicans*, and *Candida krusei* (Table 2). The data presented here are consistent with the previous studies which demostrated that α-pinene is a slightly active component [18]. The MICs of the main component of the essential oil, α-pinene, were also determined in parallel experiments, proving that this constituent is responsible for the antimicrobial activity, at least against *C. perfringens* and yeasts.

Each plant extract and essential oil contain complex mixtures of volatile and non-volatile compounds, and little is known about the effect of interactions between individual constituents on antimicrobial activity. Interactions between these components and known antibiotics may also lead to additive, synergistic, or antagonistic effects.

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TABLE 1. Chemical Composition of the Essential Oil from *Juniperus excelsa*

Compound[a]	R_t[b]	%	Compound[a]	R_t[b]	%
Toluene	5.108	0.03	1-Camphor	25.300	0.28
Tricyclene	12.150	0.31	*trans*-(-)-Pinocarveol	25.400	0.88
α-Thujene	12.508	0.11	Limonene oxide	26.772	1.58
α-Pinene	12.770	55.53	Isopinocamphene	27.728	0.37
Camphene	13.555	0.63	Terpinen-4-ol	28.500	1.09
Sabinene	15.067	3.55	Myrtenal	28.780	0.61
β-Pinene	15.183	1.05	Verbenone	29.800	2.45
Triene cycloheptane	16.142	0.27	Carvone	29.910	0.10
1,3,5-trismethylene			Endobornylacetate	36.200	0.50
β-Myrcene	16.418	1.30	α-Cububene	41.441	0.06
p-Mentha(1,5,8)triene	16.825	0.48	Longipinene	44.983	1.58
o-Allyl toluene	17.250	0.02	α-Cedrene	45.210	0.61
δ-3-Carene	17.500	0.23	*trans*-Caryophyllene	45.983	0.06
α-Terpinene	18.000	0.32	Junipene	46.327	0.23
m-Cymene	18.185	1.69	Naphthalene	49.292	0.32
d,l-Limonene	18.910	1.44	α-Chamigrene	50.233	0.18
p-Cymene	18.968	0.06	α-Muurolene	50.497	0.10
γ-Terpinene	20.667	0.78	Germacrene-B	51.050	0.40
cis-Sabinene hydrate	21.203	0.28	γ-Cadinene	51.625	0.27
α-Pinene oxide	21.780	0.30	α-Cedrol	54.495	7.75
α-Terpinolene	21.908	0.73	Kaur-16-ene	66.873	0.50
3-Thujanone	23.200	0.54	Total		91.35
α-Campholene aldehyde	24.500	1.70			

[a]Compounds listed in order of elution from a HP-5 MS column. [b]Retention time (as minutes).

TABLE 2. Antimicrobial Activity of the *Juniperus excelsa* Essential Oil and Its Main Component α-Pinene

Microorganism	Essential oil		α-Pinene	
	DD[a]	MIC[b]	DD	MIC
Staphylococcus aureus ATCC 29213	12	9.00	8	>72.00
Streptococcus pyogenes ATCC 19615	14	4.50	N.a.	>72.00
Streptococcus pneumoniae ATCC 49619	13	4.50	8	36.00
Moraxella catarrhalis ATCC *49143*	N.a.	>72.00	N.a.	>72.00
Bacillus cereus ATCC 11778	9	18.00	10	>72.00
Listeria monocytogenes F 1483	9	36.00	N.t.	N.t.
Listeria ivanovii F 4084	10	18.00	N.t.	N.t.
Clostridium perfringens KUKENS	30	2.25	31	2.25
Mycobacterium smegmatis CMM 2067	14	18.00	N.a.	72.00
Acinetobacter lwoffii ATCC 19002	N.a.	>72.00	N.a.	>72.00
Enterobacter aerogenes ATCC 13043	N.a.	>72.00	N.a.	>72.00
Escherichia coli ATCC 25922	N.a.	>72.00	N.a.	72.00
Klebsiella pneumoniae ATCC 13883	N.a.	>72.00	N.a.	>72.00
Proteus mirabilis ATCC 7002	N.a.	>72.00	N.a.	>72.00
Pseudomonas aeruginosa ATCC 27853	N.a.	>72.00	N.a.	>72.00
Salmonella typhimurium ATCC 14028	N.a.	>72.00	N.a.	>72.00
Candida albicans ATCC 10239	14	36.00	30	4.50
Candida krusei ATCC 6258	14	36.00	>40	0.60

[a]DD, disc diffusion method. Diameter of inhibition zone (mm) including disk diameter of 6 mm. [b]MIC, minimum inhibitory concentration. Values given as mg/mL for the essential oils and as mg/mL for antibiotics. [c]The MIC of antibiotics for *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*: amikacin - 2.00; 2.00; 1.00, ciprofloxacin - 0.25, 0.015, 0.25 respectively. N.a.: not active. N.t.: not tested.

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