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RESEARCH ARTICLE

Anticandidal, Antibacterial Properties and Composition of the Essential Oils of *Juniperus excelsa* Bieb. subsp. *excelsa* Growing in Eskişehir, Turkey.

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Abstract

The genus *Juniperus* L. belongs to the Cupressaceae family, representing about 70 species all over the world. *Juniperus* L. is represented in Turkey by 7 species and 14 taxa. *Juniperus excelsa* Bieb. has two subspecies – subsp. *excelsa* ''Boz ardıç, Boylu ardıç Adi Ardıç, Çerkem'' and subsp. *polycarpos* (K.Koch) Takht. "Daltaban ardıcı''– in Turkey. Leaves of the *Juniperus excelsa* subsp. *excelsa* were water distilled and the oil was studied by GC and GC/MS. Main components in the essential oil were found to be β-Thujone (28.3%), Terpinen-4-ol (10.9%), Sabinene (9.3%) and limonene (7.2%), resp.

Key words: *Juniperus excelsa* subsp. *excelsa*, Cupressaceae, Essential oil, β-Thujone, Terpinen-4-ol, Sabinene

Introduction

The genus *Juniperus* L. belongs to the Cupressaceae family, representing about 70 species all over the world, and widely distributed throughout the forests of the temperate and cold regions of the northern Hemisphere, from the far north to the Mediterranean. *Juniperus* L. is represented in Turkey by 7 species and 14 taxa. *Juniperus excelsa* Bieb. has two subspecies – subsp. *excelsa* ''Boz ardıç, Boylu ardıç Adi Ardıç, Çerkem'' and subsp. *polycarpos* (K.Koch) Takht. "Daltaban ardıcı''– in Turkey (Coode and Cullen, 1965; Guner et al., 2012).

Crimean Juniper (*Juniperus excelsa*), is widely distributed in the Anatolian mountains, also found in Lebanon, Iran, Syria, and Greece (Avci and Bilir, 2014). *Juniperus* species are used due to antiseptic effect of their various parts in medicine, cosmetic and food sectors. Furthermore, their cones are used in animal husbandry due to their carbohydrate and oil contents in Turkey (Yaltırık and Efe, 2000; Baytop, 1977).

The oils obtained from cones (berries), leaves and wood of the species have been historically used for various diseases ranging from leprosy, typhoid to tape worm; thus, *Juniperus* spp. are well known in herbal medicine today as antifungal, disinfectant, insect repellant properties and supports the arteries, the heart, the kidneys, the lungs (Uçar and Balaban, 2002; Avci and Bilir, 2014). Various species of juniper are used medicinally with a range of applications from antiseptic to diuretic (Unlu et al., 2008; Baytop, 1999).

In a previous report for *Juniperus excelsa* subsp. *excelsa*; α-pinene (32.3-47.6%), α-cedrol (13.1-12.0%), myrcene (5.4-5.9%) and limonene (4.4-4.5%) were reported as main constituents of the leaf and fruit oils,

resp. Essential oils were found to have weak antimicrobial activity against *Candida albicans* and *Staphylococcus aureus* (Asili et al., 2008).

α-pinene (46.1%), *epi*-cedrol (9.7%) and β-pinene (4.4%) were reported as main constituents of the berries of *Juniperus excelsa* Bieb. subsp. *excelsa* oil (Atas et al., 2012).

Materials and Methods

Plant Material

Juniperus excelsa subsp. *excelsa* was collected from Eskisehir: Türkmendag in Turkey on 20 February 2015. Voucher specimens are kept at the Herbarium of Anadolu University (ESSE), Eskisehir, Turkey (ESSE: 14943). The plant material was identified by one of us (SK).

Isolation of the Essential Oils

Leaves of the plant were water distilled for 3 h using a Clevenger-type apparatus to yield 0.2% essential oil on moisture-free basis. The oil was stored at 4°C in the dark until analysed.

GC and GC/MS Analyses

The oils were analyzed by Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) using an Agilent GC-MSD system (Mass Selective Detector-MSD).

GC-MS analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey). Innowax FSC column (60m x 0.25mm, 0.25mm film thickness) was used with helium as carrier gas (0.8 mL/min.). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted 40:1. The injector temperature was at 250°C. The interphase temperature was at 280°C. MS were taken at 70 eV. Mass range was from m/z 35 to 450.

GC analysis

GC analyses were performed using an Agilent 6890N GC system. FID temperature was set to 300°C and the same operational conditions were applied in triplicate and the same column used in GC–MS analyses was employed. Simultaneous auto injection was carried out to obtain equivalent retention times. Relative percentages of the separated compounds were calculated from integration of the peak areas in the GC-FID chromatograms.

Identification of the volatile compounds

The components of essential oils were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Adams Library (Adams, 2007), MassFinder Library (Hochmuth, 2008), Wiley GC/MS Library (McLafferty & Stauffer, 1989) and confirmed by comparison of their retention indices. These identifications were accomplished by comparison of retention times with authentic samples or by comparison of their relative retention index (RRI) to a series of *n*-alkanes. Alkanes (C8-C29) were used as reference points in the calculation of relative retention indices (RRI) (Curvers et al. 1985). Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The analysis results are expressed as mean percentage ± standard deviation (SD) (*n* = 3) as listed in Table 1.

Antimicrobial Assay

Anticandidal and antibacterial effects of the essential oil of *J. excelsa* subsp. *excelsa* were screened by using partly modified CLSI (formerly NCCLS) micro dilution broth methods M27-A2 (CLSI, 2006) and M7-A7 (CLSI, 2002) respectively. All microdilution broth tests were performed by using sterile 96 U-shaped multiwell microdilution plates (L.P. Italiana) in laminar flow cabinet. Furthermore, in the M27-A2 method, *C. krusei* (ATCC® 6258) and *C. parapsilosis* (ATCC® 22019) were used as quality control strains. Amphotericin-B and Ketoconazole were used as antifungal agents while the ampicillin and chloramphenicol were used as antibacterial agents.

Results and Discussion

The present work investigated the composition of the essential oil of *Juniperus excelsa* subsp. *excelsa*, Eskisehir in Turkey. Leaves were hydrodistilled for 3h using a Clevenger-type apparatus in 0.2 % yield. The oil was analyzed by GC and GC-MS.

Fifty-six compounds were identified in the essential oil representing 93.1% of the total oil, β-thujone (28.3 %), terpinen-4-ol (10.9 %) and sabinene (9.3 %) were found to be the major compounds (Table1).

The essential oil showed moderate inhibitory effects against all tested microorganisms between the concentrations of 250 to 2000 µg/mL except for *Candida zeylanoides* which was inhibited by the EO at the concentration of the 62.5 µg/mL. In a previous antimicrobial study on the *J. excelsa* leaf essential oil, reported that moderate inhibitory effects of the oil on the tested pathogenic bacteria strains were obtained and no inhibition on *C. albicans* was observed, similar to our results (Sela, 2015).

Table 1. Composition of the essential oils of *Juniperus excelsa* subsp. *excelsa* leaves

RRI	Compounds	%
1032	α-Pinene	1.5 ± 0.0
1035	α-Thujene	0.4 ± 0.0
1132	Sabinene	9.3 ± 0.1
1174	Myrcene	1.3 ± 0.0
1188	α-Terpinene	1.8 ± 0.1
1203	Limonene	7.2 ± 0.1
1210	β -Phellandrene	0.1 ± 0.0
1241	Butyl-2-methyl butyrate	0.1 ± 0.0
1255	γ-Terpinene	3.5 ± 0.1
1259	Butyl-3-methyl butyrate	0.4 ± 0.0
1280	*p*-Cymene	0.6 ± 0.1
1285	Isoamyl isovalerate	0.2 ± 0.0
1290	Terpinolene	1.0 ± 0.0
1299	2-Methyl butyl isovalerate	0.7 ± 0.1
1379	3-Methyl-3-butenyl isovalerate	1.1 ± 0.1
1437	α-Thujone	3.6 ± 0.1
1451	β-Thujone	28.3 ± 0.2
1468	3-Methyl butyl hexanoate	0.2 ± 0.0
1474	*trans*-Sabinene hydrate	1.0 ± 0.1
1499	Campholenal	0.2 ± 0.0
1553	Linalool	2.1 ± 0.0
1556	*cis*-Sabinene hydrate	0.6 ± 0.0

1570	8,9-Limonene epoxide-II	0.1 ± 0.0
1571	*trans*-*p*-Menth-2-en-1-ol	0.8 ± 0.0
1580	Acetoxy linalool oxide	0.2 ± 0.0
1591	β-Funebrene	0.2 ± 0.0
1604	Isobornyl acetate	0.1 ± 0.0
1611	Terpinen-4-ol	10.9 ± 0.2
1617	Hexyl hexanoate	0.1 ± 0.1
1628	Terpinen-4-yl acetate	0.1 ± 0.0
1638	*cis*-*p*-Menth-2-en-1-ol	0.7 ± 0.1
1651	Sabinaketone	0.3 ± 0.0
1658	Sabinyl acetate	3.0 ± 0.1
1689	*trans*-Piperitol	0.5 ± 0.0
1700	*p*-Mentha-1,8-dien-4-ol	0.2 ± 0.0
1706	α-Terpineol	0.5 ± 0.1
1726	Germacrene D	0.1 ± 0.0
1747	*p*-Mentha-1,5-dien-8-ol	0.1 ± 0.0
1757	Carvone	0.4 ± 0.1
1758	*cis*-Piperitol	0.3 ± 0.0
1772	Citronellol	0.2 ± 0.1
1802	Cumin aldehyde	0.1 ± 0.0
1814	*p*-Mentha-1,5-dien-7-ol	0.7 ± 0.0
1827	(*E*,*E*)-2,4-Decadienal	0.1 ± 0.0
1845	*trans*-Carveol	0.5 ± 0.0
1853	Ethyl dodecanoate	0.1 ± 0.0
1882	*cis*-Carveol	0.2 ± 0.0
2057	*p*-Mentha-1,4-dien-7-ol	0.5 ± 0.1
2113	Cumin alcohol	0.1 ± 0.0
2148	Cedrol	4.6 ± 0.1
2255	α-Cadinol	0.1 ± 0.0
2287	Sandaracopimaradiene	0.1 ± 0.0
2503	Dodecanoic acid	1.3 ± 0.3
2524	Abietatriene	0.1 ± 0.0
2696	Tetradecanoic acid	0.3 ± 0.1
2931	Hexadecanoic acid	0.3 ± 0.1

RRI; Relative retention indices calculated against n-alkanes
%; calculated from the FID chromatograms and expressed as mean ± SD (*n* = 3).

Table 2. MICs (µg/mL) of the *Juniperus excelsa* subsp. *excelsa* against *Candida* species

	Resource	JEO	St-1	St-2
C. albicans	ATCC 90028	1000	0.12	0.01
C. albicans	ATCC 10231	2000	0.5	0.06
C. glabrata	ATCC 66032	500	0.06	0.06
C. krusei	ATCC 6258	1000	0.12	0.25
C. parapsilosis	ATCC 22019	1000	0.12	0.06
C. tropicalis	ATCC 750	2000	0.25	0.06
C. zeylanoides	NRRL Y-1774	**62,5**	0.5	0.06

JEO: Juniperus essential oil, St1: Amphotericin-B, St2: Ketoconazole

Table 3. MICs (µg/mL) of the *Juniperus excelsa* subsp. *excelsa* against Bacteria species

	Resource	JEO	St1	St2
Bacillus cereus	NRRL B-3711	250	1	8
Bacillus subtilis	NRRL B-4378	250	0.5	2
Salmonella typhimurium	ATCC 14028	2000	1	2
Staphylococcus aureus	ATCC 43300	250	0.5	16
E. coli O157:H7	RSSK 234	2000	2	2
Pseudomonas aeruginosa	ATCC 10145	2000	32	32
Listeria monocytogenes	ATCC 19111	500	8	2
Staphylococcus epidermidis	ATCC 14990	500	0.5	2

JEO: *Juniperus* essential oil, St1: Ampicillin, St2: Chloramphenicol

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