

Full Length Research Paper

Chemical composition and antibacterial activity of two *Juniperus* species essential oils

E. Ehsani^{1*}, K. Akbari², M. Teimouri³ and A. Khadem⁴

¹Department of Microbiology, Faculty of Science, Islamic Azad University, Karaj, Iran.

²National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.

³Research Institute of Forests and Rangelands, Tehran, Iran.

⁴Department of Animal Sciences, Faculty of Animal and Aquatic Sciences, Sari Agricultural Sciences and Natural Resources University, Sari, Iran.

Accepted 21 August, 2012

Nowadays, the overdose use of antibiotics and the increase of microbial resistances have made researchers to carry out more studies about the new drugs with new chemical composition. In order to produce these new drugs, different species of special plants can be useful by scientists and researchers. Base on this, the leaves and fruits of *Juniperus excelsa* and *Juniperus horizontalis* were collected from Iranian National Botanic Garden. Essential oils were isolated by hydro-distillation method. The essential oil components were analyzed by gas chromatography–mass spectrometry (GC-MS). The yields of the leaves and fruits of *J. excelsa* and *J. horizontalis* essential oils were 0/79 to 4/15% and 1/083 to 2/7%, respectively. There were 15 and 27 components in the essential oil of *J. excelsa* and *J. horizontalis*, respectively. The major compound in the essential oils obtained from the leaves and fruits of *J. excelsa* were α -pinene (79/95 and 89/49%, respectively). The main compound in the essential oils obtained from the leaves and fruits of *J. horizontalis* were sabinene (30/21 and 38%, respectively). In addition, bornyl acetate (10/66%) and delta-cadinene (3/79%) were identified as major components in the essential oil of the leaves obtained from *J. horizontalis*. Juniper essential oils were evaluated for the antibacterial activity against thirteen bacterial species by disk diffusion and micro dilution method. Juniper essential oils showed more antibacterial activities against Gram-positive as compared to Gram-negative bacteria species. The antibacterial activity of essential oils may be related to presence of α - pinene, limonene, and sabinene which are known to have antibacterial properties.

Key words: Antibacterial activity, gas chromatography–mass spectrometry (GC-MS), essential oil, *Juniperus excelsa*, *Juniperus horizontalis*.

INTRODUCTION

In recent years, scientists are concerned with the increasing microbial resistance against antimicrobial agents due to the indiscriminate use of commercial antibiotics (Service, 1995; Mukherjee et al., 2002). Synthetic chemicals which are widely used against these micro-organisms unfortunately develop resistance to most of the antibiotics; in addition, some antibiotics

occasionally cause allergic reaction and immunity suppression (Knobloch et al., 1989). In many cases, people are turning to synthetic drugs, but the vast majority of them are turning to natural products. Antibacterial and antifungal properties of essential oil as well as of oil constituents are documented (Knobloch et al., 1989; Pepelnjak et al., 2003). Essential oils or their constituents are used as antimicrobial agents for food preservatives, in clinical microbiology or in pharmaceutical preparations. Screening for antimicrobial activity has been the subject of many investigations, and

*Corresponding author: E-mail: el.ehsani@gmail.com.

oils with very potent antibacterial and antifungal activity could be promising agents for the future extensive research and *in vivo* examination. The use of essential oils is less damaging to the human health, because they have generally low toxic and they do not have side effects (Isman, 2000; Misra and Pavlovstathis, 1997).

Among such oils are the essential oils from juniper. *Juniperus* is the second most diverse genus of the conifers. The genus *Juniperus* belonging to Cupresseceae family contains more than 60 species. It is widespread in temperate regions of the northern hemisphere including Europe, Asia, and North America (Adams, 2008). *Juniperus excelsa* is native to Iranian flora that grows on mountainous regions (Assadi, 1998). But *Juniperus horizontalis* is non endemic, although, it has been planted in many parks and gardens.

The potential applications of *Juniperus* oils include aromatherapy, mood scents, scent masks, soaps and candles, cosmetics and fragrances, lotions and remedies (Yesenofski, 1996). In addition, *Juniperus* species are used for treatment of hyperglycemia, tuberculosis, bronchitis, pneumonia, ulcers, intestinal worms, to heal wounds, and cure liver diseases in traditional medicine (Burits et al., 2001; Loizzo et al., 2007).

The chemical composition of the leaf and wood essential oils from *Juniperus excelsa* were previously reported (Adams, 1990a, 1990b). There are a number of reports on the composition of the essential oil from berries of *Juniperus* species and their antimicrobial activities (Angioni et al., 2003; Cosentino et al., 2003; Filipowicz et al., 2003). Topcu et al. (1999) reported antimicrobial activity of hexane and methanol extracts of *J. excelsa* against *Mycobacterium tuberculosis*. The antimicrobial activity of *J. excelsa* essential oil against three standard bacterial strains and *Saccharomyces cerevisiae* were reported by Aridogan et al. (2002).

The *J. excelsa* essential oil showed a strong antimicrobial activity against the anaerobic bacterium *Clostridium perfringens*, while exhibiting moderate activity against *Staphylococcus aureus*, *Staphylococcus pyogenes*, and *Candida albicans* (Unlu et al., 2008). However, as far as our literature survey could ascertain, there was no report on the antimicrobial activity of *J. horizontalis*.

The aim of this study was to identify the chemical composition of the oils of *J. excelsa* and *J. horizontalis* leaves and fruits and their antibacterial activity in an attempt to contribute to their use as alternative products for microbial control and food preservation.

MATERIALS AND METHODS

Plant and oil isolation

The leaves and fruits of *J. excelsa* and *J. horizontalis* were collected from the Research Institute of Forests and Rangelands, located in Tehran, Iran. Collected plant materials were air-dried at room temperature. The dried parts of the plants were crushed to

small particles. The samples (about 80 g) were hydro distilled for 2 to 2.5 h in a Clevenger-type apparatus to produce the oils. Three distillations were performed for each sample and mixed for analysis. The obtained oils were dried over anhydrous sodium sulfate and stored in sealed vials at 4°C before analysis.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m × 0.25 mm, film thickness 0.25 mm, J&W Scientific Corp). Oven temperature was 50 to 260°C at a rate of 4°C min⁻¹. Transfer line temperature was 270°C, Helium was used as the carrier gas with a linear velocity of 31.5 cm/s, split ratio was 1:60, ionization energy was 70 eV (electron Volts), scan time was 1 s, and mass range was 40 to 300 amu (Resolution).

Identification of compounds

The constituents were identified by comparison of their mass spectra with those in a computer library (LIBR-TR and Wiley-5 lib.) or with authentic compounds. The identification was confirmed by comparison of their retention indices either with those of authentic compounds or with data in the literature (Adams, 1995).

Antibacterial screening

Microorganisms

Thirteen bacterial species were used to test the antibacterial activity of juniper essential oils. Microorganisms were obtained from the Microbial Collection of Microbiology Department, Faculty of Science, Karaj Azad University, Iran. The microorganisms were *Bacillus anthracis*, *Bacillus cereus*, *Bacillus subtilis*, *S. aureus*, *Staphylococcus epidermidis*, *Citrobacter freundii*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Enterobacter aerogenes*, and *Shigella dysenteriae*.

Disc diffusion method

Disc diffusion method was used to determine of antibacterial activities of the essential oils in question (Lesueur et al., 2007). Paper discs (6 mm diameter) were impregnated with 30 µl of the oil dissolved in dimethyl sulfoxide (DMSO) (final concentration of 5, 10, and 20% v/w) and transferred onto the Mueller-Hinton agar plates which had been surface spread with 0.5 ml of bacterial suspension adjusted to 3 × 10⁸ cfu/ml (1 Mac-Farland's). The DMSO did not have antimicrobial activity and was used as negative control. Commercial standard antibiotics (tetracycline (30 µg/disk) and gentamicin (30 µg/disk)) were used as positive controls. After incubation at 37 ± 1°C for 24 h, the diameter of inhibition zones was measured in millimeters. Tests were carried out in triplicate. Sensitivity of the bacterial species to the oils was determined comparing the sizes of inhibitory zones (Murray et al., 1995).

Determination of minimum inhibitory and bactericidal concentration (MIC and MBC) by micro dilution method

The minimal inhibitory values were determined by using a 48-well microtiter plates (Murray et al., 1995). The bacterial suspension was adjusted with sterile saline to a concentration of 3 × 10⁸ cfu/ml. The essential oils were diluted to the highest concentration (256 µg/ml)

Table 1. Constituents (Area %) of the oil of *J. excelsa* and *J. horizontalis*.

Compound	Leave % (<i>J. excelsa</i>)	Fruit % (<i>J. excelsa</i>)	Leave % (<i>J. horizontalis</i>)	Fruit % (<i>J. horizontalis</i>)	RI
Alpha-pinene	79.956	89.493	1.986	1.925	940
Beta-pinene	0.803	1.531	-	-	981
Myrcene	2.289	2.609	8.776	22.587	992
Delta-3-carene	7.548	-	-	-	1012
p-Cymene	1.172	0.408	1.658	0.858	1026
Limonene	1.361	1.254	26.265	27.814	1032
e-Caryophyllene	0.824	0.378	1.529	1.666	1418
Gamma-cadinene	0.949	-	1.585	-	1514
Delta-cadinene	0.740	-	3.799	0.676	1525
Germacrene B	4.364	2.174	1.213	0.686	1557
Camphene	-	0.475	0.403	-	954
Sabinene	-	0.283	30.211	37.998	976
Terpinolene	-	0.777	-	-	1088
Bornyl acetate	-	0.365	10.665	-	1286
Germacrene D	-	0.260	0.595	0.554	1481
Alpha-thujene	-	-	0.868	1.022	931
Gamma-terpinene	-	-	-	0.422	1063
p-mentha-2,4(8)-dinene	-	-	-	0.944	1087
Linalool	-	-	0.979	0.641	1098
Terpinene-4-ol	-	-	1.934	1.274	1143
Epi-bicyclosesquiphllandrene	-	-	1.676	0.940	1576
Tricyclene	-	-	0.381	-	926
Camphor	-	-	1.320	-	1143
Methyl citronellate	-	-	1.000	-	1261
Alpha-muurolene	-	-	1.101	-	1500
Delta-cadinol	-	-	1.321	-	1645
Alpha-eudesmol	-	-	0.748	-	1654

to be tested, and then serial doubling dilutions of each oil was performed over the range of 8 to 256 µg/ml. The microplates were incubated for 24 h at 37°C. The lowest concentrations without visible growth was defined as MIC. MBCs were determined by serial sub-cultivation of 100 µl into plates containing Mueller-Hinton agar and further incubation for 24 h at 37°C. The lowest concentration with no visible growth was defined as the MBC, indicating 99/5% killing of the original inoculums.

RESULTS

In this study, the essential oil composition of *J. excelsa* and *J. horizontalis* leaves and fruits and their antibacterial activity was determined. The chemical compositions of investigated essential oils are presented in Table 1. The yield of *J. excelsa* leaves oil is 0/8% (v/w). The main components in *J. excelsa* leaf oil are α-pinene (79/95%), delta-3-carene (7/55%), and germacrene B (4/36%). The yield of *J. excelsa* fruit oil is 4/15% (v/w). The main components *J. excelsa* fruits oil is α-pinene (89/49%). The amount of germacrene B (2.17%) was less as compared to the leaves oil (4.36%).

The yield of *J. horizontalis* leave oil is 1/1% (v/w). The main components are sabinene (30/21%), limonene (26/26%), bornyl acetate (10/66%), and myrcene (8/77%) in *J. horizontalis* leaves oil. The yield of *J. horizontalis* fruits oil is 2/7% (v/w). The main components are sabinene (37/99%), limonene (27/81%), and myrcene (22/58%) in *J. horizontalis* fruits oil.

The results of antibacterial activity of the *J. excelsa* essential oil by the disk diffusion method are presented in Table 2. All tested Gram-positive bacteria species are sensitive to *J. excelsa* leaves and fruits of essential oil at 20% concentration with the inhibition zones ranging from 13/6 ± 0/58 to 25/3 ± 4/7 mm and 9 to 15/6 ± 3/8 mm, respectively. The inhibition zone for Gram-negative by *J. excelsa* leaves and fruits essential oil at 20% concentration ranged from 6 to 12 mm and 6 to 11 mm, respectively. According to Table 3, all Gram-positive bacteria species tested are sensitive to *J. horizontalis* leaves and fruits essential oil at 20% concentration with the inhibition zones ranging from 17 ± 1/73 to 25 ± 2/31 mm. and 11 ± 1 mm to 17 ± 3/46, respectively. Gram-

Table 2. Antibacterial activity of leave and fruit from *Juniperus excelsa* against the bacterial strains based on disc-diffusion method (mm).

Bacteria	Leave			Fruit			Tetracycline	Gentamicin
	1/5	1/10	1/20	1/5	1/10	1/20		
<i>S. aureus</i>	17 ± 1	13.6 ± 1.53	13.6 ± 2.51	9	10 ± 1.73	8.3 ± 2.51	27.3 ± 4.16	-
<i>S. epidermidis</i>	25.3 ± 4.7	21.3 ± 4.04	14 ± 1	10.6 ± 2.51	9.6 ± 0.58	8.6 ± 2.31	28 ± 2	-
<i>B. anthracis</i>	25 ± 1	20.6 ± 2.51	16.3 ± 1.52	15.6 ± 3.8	11.6 ± 0.58	10.6 ± 1.15	29 ± 1	-
<i>B. cereus</i>	19 ± 1	13.6 ± 1.15	12	11.6 ± 1.15	11.6 ± 1.89	13 ± 6	19.6 ± 0.58	-
<i>B. subtilis</i>	17.3 ± 0.58	14.6 ± 1.53	13 ± 1.73	13.3 ± 3.21	11.6 ± 2.1	10.3 ± 0.58	27.3 ± 2.5	-
<i>L. monocytogenes</i>	13.6 ± 0.58	12.6 ± 0.58	11.3 ± 1.15	13.6 ± 3.05	11.6 ± 2.3	11.3 ± 0.58	22.6 ± 1.52	-
<i>E. coli</i>	6	6	6	11	10 ± 1	11 ± 1.15	-	10.6 ± 0.58
<i>P. aeruginosa</i>	10.6 ± 0.58	10	10.3 ± 0.058	9 ± 2.64	7.3 ± 1.53	8.3 ± 2.1	-	13 ± 1
<i>K. pneumoniae</i>	9 ± 1	10.6 ± 1.15	8.6 ± 1.152	6 ± 1.15	7 ± 1.73	6	-	10 ± 1
<i>S. dysenteriae</i>	12	11	10	7 ± 1.73	8.6 ± 2.31	6	-	10.3 ± 1.15
<i>S. marcescens</i>	10	10.3 ± 0.58	10 ± 1	9 ± 2.64	9 ± 3	9 ± 2.64	-	14.3 ± 0.58
<i>C. freundii</i>	12	10 ± 3.46	8.6 ± 2.51	9.3 ± 3.05	9.6 ± 1.52	9.3 ± 0.57	-	9.3 ± 0.58
<i>E. aerogenes</i>	7.6 ± 2.9	7 ± 1.73	6	6	6	6	-	13

Tetracycline for gram positive bacteria and Gentamicin for gram negative bacteria.

Table 3. Antibacterial activity of leave and fruit from *J. horizontalis* against the bacterial strains based on disc-diffusion method (mm).

Bacteria	Leave			Fruit			Tetracycline	Gentamicin
	1/5	1/10	1/20	1/5	1/10	1/20		
<i>S. aureus</i>	13.3 ± 4.9	12.3 ± 3	9 ± 1.52	11 ± 1	10.3 ± 0.58	10.3 ± 0.58	27.3 ± 4.16	-
<i>S. epidermidis</i>	19 ± 1.73	16.6 ± 1.52	14.6 ± 3.21	11.3 ± 1.52	11.3 ± 1.52	10.3 ± 0.58	28 ± 2	-
<i>B. anthracis</i>	23/3 ± 2	18 ± 0.58	16.3 ± 2.08	17 ± 3.46	13.6 ± 3.21	12.3 ± 2.31	29 ± 1	-
<i>B. cereus</i>	17 ± 1.73	16 ± 1	16 ± 2.64	13.6 ± 3.21	11 ± 1.73	10 ± 1	19.6 ± 0.58	-
<i>B. subtilis</i>	25 ± 2.31	22.3 ± 1.73	19 ± 2.64	17 ± 1	12.6 ± 1.52	12.6 ± 2.51	27.3 ± 2.5	-
<i>L. monocytogenes</i>	19 ± 1.15	18.3 ± 2.31	15.3 ± 5.3	13.3 ± 3.21	11.3 ± 0.58	11.6 ± 0.58	22.6 ± 1.52	-
<i>E. coli</i>	11.3 ± 0.58	14 ± 1.73	11.3 ± 1.52	11.3 ± 1.15	11 ± 1	10.3 ± 1.15	-	10.6 ± 0.58
<i>P. aeruginosa</i>	10.6 ± 1.15	10.6 ± 3.51	9.6 ± 2.51	10 ± 1.15	9.3 ± 3.51	10 ± 2.51	-	13 ± 1
<i>K. pneumoniae</i>	10 ± 1	9.5 ± 2.12	9 ± 1	10	10	8.6 ± 2.31	-	10 ± 1
<i>S. dysenteriae</i>	12.6 ± 0.58	13.6 ± 1.52	12.3 ± 1.52	11.3 ± 0.58	10.3 ± 1.15	10.3 ± 0.58	-	10.3 ± 1.15
<i>S. marcescens</i>	11.3 ± 1.52	12 ± 1	10.3 ± 2.08	11	10.6 ± 0.58	8.6 ± 2.31	-	14.3 ± 0.58
<i>C. freundii</i>	11.3 ± 0.58	11 ± 1	9.6 ± 0.58	10.3 ± 1.53	10.6 ± 0.58	9.6 ± 0.58	-	9.3 ± 0.58
<i>E. aerogenes</i>	12	11 ± 1	10	10.6 ± 1.15	9.6 ± 1.15	10.3 ± 1.15	-	13

Tetracycline for Gram-positive bacteria and gentamicin for Gram-negative bacteria.

Table 4. The MIC and MBC values of leaves and fruits from *J. excelsa* and *J. horizontalis* against the microorganism tested in microdilution assay.

Bacteria	MIC l.e	MBC l.e	MIC f.e	MBC f.e	MIC l.h	MBC l.h	MIC f.h	MBC f.h
<i>S. aureus</i>	64	128	32	>256	64	64	128	>256
<i>S. epidermidis</i>	64	128	64	256	64	64	128	>256
<i>B. anthracis</i>	32	64	16	64	64	64	128	256
<i>B. cereus</i>	64	256	64	256	128	256	128	>256
<i>B. subtilis</i>	64	64	32	128	64	64	128	256
<i>L. monocytogenes</i>	128	256	128	256	64	256	128	>256
<i>E. coli</i>	256	>256	256	>256	32	32	256	256
<i>P. aeruginosa</i>	128	256	256	>256	256	256	128	>256
<i>K. pneumoniae</i>	128	>256	256	>256	128	256	256	>256
<i>S. dysenteriae</i>	128	256	256	>256	128	128	256	>256
<i>S. marcescens</i>	128	>256	256	>256	256	256	128	>256
<i>C. freundii</i>	256	>256	256	>256	256	>256	256	>256
<i>E. aerogenes</i>	256	>256	256	>256	256	256	256	256

MIC: minimal inhibition concentration (µg/ml); MBC: minimal bactericidal concentration (µg/ml). l.e: Leave of *J. excelsa*, f.e: fruit of *J. excelsa*, l.h: leave of *J. horizontalis*, f.h: fruit of *J. horizontalis*.

negative bacteria species have inhibition zones from 10 ± 1 to $12/6 \pm 0/58$ mm and 10 to $11/3 \pm 0/58$ mm by *J. horizontalis* leaves and fruits essential oil at 20% concentration, smaller as compared to Gram-positive bacteria species.

The results of the antibacterial activity of the *J. excelsa* and *J. horizontalis* essential oil by the dilution method are given in Table 4. MIC for the bacteria species ranged from 32 to 256 µg/ml and 16 to 256 µg/ml for *J. excelsa* leaves and fruits essential oil, respectively. The lower MIC values were found for the tested Gram-positive bacteria as compared to the tested Gram-negative bacteria. The lowest MIC belonged to *B. anthracis* with 32 and 16 µg/ml values for leaves and fruits essential oil of *J. excelsa*, respectively. MIC ranged from 64 to greater than 256 µg/ml for *J. excelsa* leaves and fruits essential oil. Gram-positive bacteria showed the lower MBC. The lowest MBC belonged to *B. anthracis* with 64 µg/ml value.

As shown in Table 4, MIC ranged from 32 to 256 µg/ml and 128 to 256 µg/ml for *J. horizontalis* leaves and fruits essential oil, respectively. In Table 4, all Gram-positive bacteria had lower MIC except *E. coli*. MBC ranged from 32 to greater than 256 µg/ml for *J. horizontalis* leaves and fruits essential oil. Gram-positive bacteria showed the lowest MBC.

DISCUSSION

In this study, the essential oil compositions of *J. excelsa* and *J. horizontalis* leaves and fruits were collected from Iranian National Botanic Garden and were analyzed by GC-MS.

α-Pinene as major components in both leaf and fruit oil of *J. excelsa* was reported by Adams (2011). In contrast

to our results, Narasimhachari and von Rudloff (1961) reported that alpha-cedrene, thujopsene, cuparene, cedrol, and widdrol are determined as the main components of *J. horizontalis* essential oil. These differences in essential oil composition may be related to the climatic and geographical condition of growth.

Our results indicated more susceptibility of the Gram-positive bacteria which have been reported by others (Ouattara et al., 1997; Shelef et al., 1980). The weak antibacterial activity against Gram-negative bacteria was related to the presence of an outer membrane (Mann et al., 2000; Gaunt et al., 2005). Hydrophilic polysaccharide chains act as a barrier to hydrophobic essential oils and do not allow them to enter and have antibacterial activity. In addition, the resistance in the Gram-negative bacteria may be related to the possible resistance genes on plasmids (Shelef et al., 1980) that may inactivate essential oil components with antimicrobial potential.

Leaves of *J. excelsa* and *J. horizontalis* showed more antibacterial activity as compared to the fruits. According to MBC and MIC, the leaves essential oil of *J. horizontalis* exhibited better antimicrobial activity against all tested bacteria except *C. freundii*. More activity of *J. horizontalis* essential oil may be related to more amounts of different components such as limonene and α-pinene. The antibacterial activity of limonene (Bevilacqua et al., 2010) and sabinene (Sandra et al., 2007) has been evaluated. Terpene hydrocarbons (including α-pinene) which are known to possess strong antibacterial activities (Aligianis et al., 2001; Couladis et al., 2000; Unlu et al., 2008).

All terpene hydrocarbons are antiseptic, anti-inflammatory, and antibacterial. They are also pain killers, sedative, stimulants, and media for the excommunication of excrete mucus (Barjaktarevic et al., 2005). In addition, terpenes retard the retention of toxins in human

organisms, they increase the abstraction of aggregated toxic material from the veins and liver and act as antispasmodic agents (media for mitigating convulsions) (Damnjanovic, 2000). Certainly, α -pinene is an acute antiseptic and it was found to act as a rubefaciens, while cadinene, caryophyllene, terpinene, and sabinene pronounced anti-inflammatory and antibacterial properties (Damnjanovic, 2000). Myrcene acts as a sedative, an anti-inflammatory agent and as a pain-killer for peripheral organs (Damnjanovic, 2000). Furthermore, myrcene stimulates the recovery of liver and it is known as a strong anti-inflammatory substance (Damnjanovic, 2000). Monoterpenes hydrocarbons, such as sabinene (Sokovic et al., 2004; Staniszewska et al., 2005; Skocibusic and Bezic, 2004; Delaquis et al., 2002; Jirovetz et al., 2003) also showed antimicrobial properties that appear to have strong to moderate antibacterial activity against Gram-positive bacteria and against pathogenic fungi, but in general, weaker activity was observed against Gram-negative bacteria (Tepe et al., 2005; Haznedaroglu et al., 2001). Some studies indicate that whole essential oils have a higher antibacterial activity than the combination of the major isolated components, indicating that minor components are critical to the activity, probably by producing a synergistic effect (Burt, 2004; Mastelic et al., 2005). For example, myrcene synergizes the antibiotic potency of other essential oil components (Onawunmi et al., 1984). Toxic effects on membrane structure and function have been generally used to explain the antimicrobial action of essential oils and their monoterpenoid components. In fact, as a result of their lipophilic character, monoterpenes will preferentially partition from an aqueous phase into membrane structures (Sikkema et al., 1994, 1995). This result in membrane expansion, increased membrane fluidity and permeability, disturbance of membrane-embedded proteins, inhibition of respiration, and alteration of ion transport processes. Andrews et al. (1980) studied the toxic effects of α -pinene and some other terpenes produced by the Douglas fir on some *Bacillus* strains and on *S. cerevisiae*. It was shown that α -pinene, limonene, camphene, and isobornyl acetate inhibited microorganisms. The presence of such terpenes in the diet of these insects was found to strongly influence the infectivity of *Bacillus thuringiensis* spores. α -pinene destroyed the cellular integrity and inhibited respiratory activity in yeast mitochondria. Helander et al. (1998) described the effects of selected essential oil components on outer membrane permeability in Gram-negative bacteria, evidencing that monoterpene uptake is also determined by the permeability of the outer envelope of the target microorganism. However, specific mechanisms involved in the antimicrobial action of monoterpenes remain poorly characterized.

The antimicrobial efficacy of three monoterpenes (linalyl acetate, menthol and thymol) was examined (Domenico et al., 2005). The results were related to the

relative lipophilicity and water solubility of the compounds examined. The antimicrobial effect of three monoterpenes may result from a perturbation of the lipid fraction of microorganism plasma membrane, resulting in alterations of membrane permeability and in leakage of intracellular materials. This effect also seems to be dependent on lipid composition and net surface charge of microbial membranes. Furthermore, the drugs might cross the cell membranes, penetrating into the interior of the cell and interacting with intracellular sites critical for antibacterial activity (Domenico et al., 2005).

Conclusion

The results of this work showed that the *J. excelsa* and *J. horizontalis* essential oils possess antibacterial properties (mainly *J. horizontalis* leave essential oil, because of its more components like limonene, myrcene, and sabinene). This showed their potential to be use as natural antibacterial agents to treat infectious diseases and to preserve food. Furthermore, the development of natural antibacterial agents will help to decrease negative effects (pollution in environment, resistance) of synthetic chemicals and drugs.

ACKNOWLEDGEMENTS

The authors thank RIFR for providing support and Dr. Mirza for doing GC/MS analysis.

REFERENCES

- Adams RP (1990a). The volatile leaf oils of *Juniperus excelsa* M-Bieb. From a native stand in Greece versus *J. excelsa* cultivated at Kew, London. J. Essent. Oil Res. 2:45-48.
- Adams RP (1990b). *Juniperus procera* of east Africa: volatile leaf oil composition and putative relationship to *J. excelsa*. Biochem. Syst. Ecol. 18:207-210.
- Adams RP (1995). Identification of Essential Oil Components by Gas Chromatography/ Mass Spectroscopy. Allured Publ. Corp Carol Stream, IL.
- Adams RP (2008). The Junipers of the World: The Genus *Juniperus*, 2nd ed. Trafford Publ., Victoria, BC, Canada.
- Adams RP (2011). Junipers of the world, The genus *Juniperus*. 3rd Edition. Trafford Publishing. p. 428.
- Aligianis N, Kalpoutzakis E, Chinou IB (2001). Composition and antibacterial activity of essential oils of five taxa of Sideritis from Greece. J. Agric. Food Chem. 49(2):811-815.
- Andrews RE, Parks LW, Spence KD (1980). Some effects of Douglas fir terpenes on certain microorganisms. Appl Environ. Microbiol. 40: 301-304.
- Angioni A, Bara A, Russo T, Coroneo V, Dessi S, Cabras P (2003). Chemical composition of the essential oils of *Juniperus* from ripe and unripe berries and leaves and their antimicrobial activity. J. Agric. Food Chem. 51:3073-3078.
- Aridogan BC, Baydar H, Kaya S, Demirci M, Ozbasar D, Mumcu E (2002). Antimicrobial activity and chemical composition of some essential oils. Arch. Pharm. Res. 25:860-864.
- Assadi M (1998). Flora of Iran, No. 19-22, Pinaceae, Taxaceae, Cupressaceae and Ephedraceae. Technical Publication of Research Institute of Forests and Rangelands, Iran.

- Barjaktarevic B, Sovilj M, Knez Z (2005). Chemical composition of *Juniperus communis* L. fruits supercritical CO₂ extracts: dependence on pressure and extraction time. *J. Agric. Food Chem.* 53:2630-2636.
- Burits M, Asres K, Bucar F (2001). The antioxidant activity of the essential oils of *Artemisia afra*, *Artemisia abyssinica* and *Juniperus procera*. *Phytother. Res.* 15:103108.
- Bevilacqua A, Corbo MR, Sinigaglia M (2010). *In vitro* evaluation of the antimicrobial activity of eugenol, limonene, and citrus extract against bacteria and yeasts, representative of the spoiling microflora of fruit juices. *J. Food Prot.* 3(5):888-894.
- Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *Int. J. Food Microbiol.* 94:223-253.
- Cosentino S, Barra A, Pisano B, Cabizza M, Pirisi FM, Palmas F (2003). Composition and antimicrobial properties of Sardinian *Juniperus* essential oils against foodborne pathogens and spoilage microorganisms. *J. Food Prot.* 66:1288-1291.
- Couladis M, Tanimandis A, Tzakuo O (2000). Essential oils of *Phlomis lanta* growing in Greece: Chemical composition and antibacterial activity. *Planta Med.* 66:670-672.
- Damjanovic BM (2000). M.Sc. Thesis, Faculty of Technology and Metallurgy, Belgrade.
- Delaquis PJ, Stanich K, Girard B, Mazza G (2002). Antimicrobial activity of individual mixed fractions of dill, Cilantro, coriander and eucalyptus essential oils. *Int. J. Food Microbiol.* 74:101-109.
- Domenico T, Castelli F, Sarpietro M, Venuti V, Cristani M, Daniele C, Saija A, Mazzanti G, Bisignano G (2005). Mechanisms of antibacterial action of three monoterpenes. *Antimicrob. Agents Chemother.* 49(6):2474-2478.
- Filipowicz N, Kaminski M, Kurlenda J, Asztemborska M, Ochocka JR (2003). Antibacterial and antifungal activity of juniper berry oil and its selected components. *Phytother. Res.* 17:227-231.
- Gaunt LF, Higgins SC, Hughes JF (2005). Interaction of air ions and bactericidal vapours to control micro-organisms. *J. Appl. Microbiol.* 99:1324-1329.
- Haznedaroglu MZ, Karabay U, Zeybek U (2001). Antibacterial Activity of *Salvia tomentosa* Essential Oil. *Fitoterapia* 72:829-831.
- Helander IM, Alakomi HL, Latva-kala K, Mattila-Sandholm T, Pol I, Smid EJ, Gorris GM, Wright A (1998). Characterization of the action of selected essential oil components on Gram-negative bacteria. *J. Agric. Food Chem.* 46:3590-3595.
- Isman MB (2000). Plant essential oils for pest and disease management. *Crop Prot.* 19:603-608.
- Jirovetz L, Buchbauer G, Stoyanova AS, Georgiev EV, Damianova ST (2003). Composition, quality control, and antimicrobial activity of the essential oil of long-time stored dill (*Anethum graveolens* L.) seeds from Bulgaria. *J. Agric. Food Chem.* 51:3854-3857.
- Knobloch K, Pauli A, Iberl B, Weigand H, Weis N (1989). Antibacterial and antifungal properties of essential oil components. *J. Essent. Oil Res.* 1:119-128.
- Lesueur D, Rocca Serra de D, Bighelli A, Hoi TM, Ban NK, Thai TH, Casanova J (2007). Chemical composition and antibacterial activity of the essential oil of *Michelia faveolata* Merrill ex Dandy from Vietnam. *Flavour Fragr. J.* 22:317-321.
- Loizzo MR, Tundis R, Conforti F, Saab AM, Statti GA, Menichini F (2007). Comparative chemical composition, antioxidant and hypoglycaemic activities of *Juniperus oxycedrus* ssp. *oxycedrus* L. berry and wood oils from Lebanon. *Food Chem.* 105:572-578.
- Mann CM, Cox SD, Markham JL (2000). The outer membrane of *Pseudomonas aeruginosa* NCTC6749 contributes to its tolerance to the essential oil of *Melaleuca alternifolia* (tea tree oil). *Lett. Appl. Microbiol.* 30:294-297.
- Mastelic J, Politeo O, Jerkovic I, Radosevic N (2005). Composition and antimicrobial activity of *Helichrysum italicum* essential oil and its terpene and terpenoid fractions. *Chem. Nat. Compd.* 41:35-40.
- Misra G, Pavlovstathis SG (1997). Biodegradation kinetics of monoterpenes in liquid soil-slurry systems. *Appl. Microbiol. Biotechnol.* 7:572-577.
- Mukherjee PK, Saritha GS, Suresh B (2002). Antimicrobial potential of two different *Hypericum* species available in India. *Phytother. Res.* 16:692-695.
- Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover RH (1995). *Manual of Clinical Microbiology*, ASM, Washington, Dc. vol. 6.
- Narasimhachari N, von Rudloff E (1961). The chemical composition of the wood and bark extractives of *Juniperus horizontalis* Monech. *Can. J. Chem.* 39:2572-2581.
- Onawunmi GO, Yisak WA, Ogunlana EO (1984). Antibacterial constituents in the essential oil of *Cymbopogon citratus* (DC.) Stapf. *J. Ethnopharmacol.* 12(3):279-286.
- Ouattara B, Simard RE, Holley RA, Piette GJP, Begin A (1997). Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. *Int. J. Food Microbiol.* 37:155-162.
- Pepelnjak S, Kosalec I, Kaloera Z, Kutrak D (2003). Natural Antimicrobials from Croatian Plants, in *Plant-derived Antimicrobials – Current Trends and Future Prospects* (Eds. M. Rai, D. Mares), Haworth Press, New York. pp. 41-84.
- Sandra BG, Svetomir M, Suzana D, Aleksandar M, Dejan S (2007). Antimicrobial activity of the essential oil and different fractions of *Juniperus communis* L. and a comparison with some commercial antibiotics. *J. Serb. Chem. Soc.* 72(4):311-320.
- Service RF (1995). Antibiotics that resist resistance. *Science* 270:724-727.
- Shelf LA, Naglik OA, Bogen DW (1980). Sensitivity of some common food-borne bacteria to the spices sage, rosemary and all spice. *J. Food Sci.* 45:1042-1044.
- Sikkema J, de Bont JAM, Poolman B (1994). Interactions of cyclic hydrocarbons with biological membranes. *J. Biol. Chem.* 269:8022-8028.
- Sikkema J, de Bont JAM, Poolman B (1995). Mechanism of membrane toxicity of hydrocarbons. *Microbiol. Rev.* 59:201-222.
- Skocibusic M, Bezic N (2004). Phytochemical analysis and *in vitro* antimicrobial activity of two *Satureja* species essential oils. *Phytother. Res.* 18:967-970.
- Sokovic MD, Ristic M, Grubisic D (2004). Chemical composition and antifungal activity of the essential oil from *Juniperus excelsa* berries. *Pharm. Biol.* 42:328-331.
- Staniszewska M, Kula J, Wieczorkiewicz M, Kusewicz D (2005). Essential Oils of Wild and Cultivated Carrots – the Chemical Composition and Antimicrobial Activity. *J. Essent. Oil Res.* 17:579-583.
- Tepe B, Sokmen M, Sokmen A, Daferera D, Polissiou M (2005). Antimicrobial and antioxidant activities of the essential oil and various extracts of *Cyclotrichium origanifolium* (Labill.) Manden. and Scheng. *J. Food Eng.* 69:335-334.
- Topcu G, Erenler R, Cakmak O, Johansson CB, Celik C, Chai HB, Pezzuto JM (1999). Diterpenes from the berries of *Juniperus excelsa*. *Phytochemistry* 50:1195-1199.
- Unlu M, Varder-Unlu G, Vural N, Donmez E, Cakmak O (2008). Composition and antimicrobial activity of *Juniperus excelsa* essential oil. *Chem. Nat. Compd.* 44:129-131.
- Yesenofski J (1996). Juniper oil distillation and marketing project western juniper commercialization program. In: Final Report, Ver 2. The Confederated Tribes of the Warm Springs Reservation of Oregon. Business and Economic Development Branch. <http://juniper.orst.edu/wjoils.htm>.