Antimikrobni učinak eteričnog ulja borovice (Juniperus communis L., Cupressaceae)

Pepeljnjak, Stjepan; Kosalec, Ivan; Kalođera, Zdenka; Blažević, Nikola

Acta Pharm. 55 (2005) 417–422 Short communication

Antimicrobial activity of juniper berry essential oil (*Juniperus communis* L., *Cupressaceae*)

STJEPAN PEPELJNJAK[1]*
IVAN KOSALEC[1]
ZDENKA KALOĐERA[2]
NIKOLA BLAŽEVIĆ[3]

[1] *Department of Microbiology Faculty of Pharmacy and Biochemistry University of Zagreb, Zagreb, Croatia*

[2] *Department of Pharmacognosy Faculty of Pharmacy and Biochemistry University of Zagreb, Zagreb, Croatia*

[3] *Ireks-Aroma, Zagreb, Croatia*

Received February 25, 2005
Accepted October 4, 2005

Juniper essential oil (Juniperi aetheroleum) was obtained from the juniper berry, and the GC/MS analysis showed that the main compounds in the oil were α-pinene (29.17%) and β-pinene (17.84%), sabinene (13.55%), limonene (5.52%), and mircene (0.33%). Juniper essential oil was evaluated for the antimicrobial activity against sixteen bacterial species, seven yeast-like fungi, three yeast and four dermatophyte strains. Juniper essential oil showed similar bactericidal activities against Gram-positive and Gram-negative bacterial species, with *MIC* values between 8 and 70% (*V/V*), as well as a strong fungicidal activity against yeasts, yeast-like fungi and dermatophytes, with *MIC* values below 10% (*V/V*). The strongest fungicidal activity was recorded against *Candida* spp. (*MIC* from 0.78 to 2%, *V/V*) and dermatophytes (from 0.39 to 2%, *V/V*).

Keywords: *Juniperus communis*, juniper, essential oil, antibacterial, antifungal activity

Antibacterial and antifungal properties of essential oil as well as of oil constituents are well documented (1, 2). Essential oils or some of their constituents have found applications 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 oils with very potent antibacterial and antifungal activity could be promising agents for the future more extensive research and *in vivo* examination. Among such oils are the essential oils from juniper berries (*Juniperus communis* L., *Cupressaceae*). The plant juniper is wide-spread in Croatia and it grows in temperate regions of Europe, Asia, and North America. Juniper berries (female cones) are used commercially for the preparation of essential oil, gin, and as a spice (3).

The essential oil of juniper berry has diuretic properties, gastrointestinal irritant and antiseptic properties. The diuretic action of juniper is primarily due to its essential oil, which contains terpinen-4-ol (4). The content of essential oil in the berry ranges from 0.5 to 2.5% (*V/m*), and its main compounds are terpen hydrocarbons such as α- and β-pinene, myrcene, sabinene, thujone, limonene, *etc*. Oil also contains sesquiterpene hydrocarbons (caryophyllene, cadinene, elemene) and terpen alcohols (terpinen-4-ol) (4, 5).

* Correspondence, e-mail: spep33@yahoo.com

The use of juniper berry has been approved for dyspepsia and, with other plants for bladder and kidney conditions. The berry has digestive, stomachic, and antirheumatic properties.

Because of these antiseptic properties, we decided to investigate the antimicrobial activity of the essential oil from juniper berry (Juniperi aetheroleum) against some Gram-positive and Gram-negative bacterial species as well as against fungi.

EXPERIMENTAL

Plant material

Essential oil of dried juniper berry (*Juniper communis* L.) was obtained from Bioaromatica d.o.o. (Croatia)

Gas-chromatography/mass spectrometry (GC/MS) analysis

The GC/MS analysis were carried out with a Pye Unicam PU 4550 GC apparatus (Pye Unicam, UK), equipped with a FID coupled to a PU 4810 integrator, column: WCOT fused silica CP-Sil 8CB (length 25 m x 0.32 mm *i. d.*, coating thickness 0.13 μm). Working conditions: injector temperature 220 °C, detector temperature 250 °C, oven temperature program 60 °C (1 min), 60–240 °C at 4 °C min^{-1}, carried gas H_2, 0.3 mL min^{-1}, split ratio 1:50. GC/MS analyses were performed on a Shimadzu QP 1000-GC-MS-EI 70 eV instrument (Shimadzu, Japan) connected to a data station. The components were identified by comparing their retention times to those of authentic samples, as well as by comparing their mass spectra with those of Wiley and NBS Libraries described by Masada (6). Quantitative data were obtained by the peak normalization technique using integrated FID response.

Microorganisms

Sixteen bacterial species, seven yeast-like fungi, three yeast and four dermatophyte species were used to test the antimicrobial activity of juniper essential oils.

Microorganisms used were from the Collection of Microorganisms of the Department of Microbiology, Faculty of Pharmacy and Biochemistry, Zagreb. The microorganisms included bacterial species *Bacillus cereus* ATCC 11778, *Bacillus subtilis* NCTC 8236, *Micrococcus flavus* MFBF, *Micrococcus luteus* ATCC 8341, *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* MFBF, *Staphylococcus epidermidis* MFBF, *Enterococcus faecalis* MFBF, *Serratia* spp. MFBF, *Citrobacter freundii* MFBF, *Salmonella enteritidis* MFBF, *Proteus mirabilis* MFBF, *Shigella sonnei* MFBF, *Klebsiella oxytoca* MFBF, *Escherichia coli* MFBF, *Yersinia enterocolitica* MFBF; yeasts and yeast-like fungi: *Candida albicans* MFBF, *C. krusei* MFBF, *C. tropicalis* MFBF, *C. parapsilosis* MFBF, *C. glabrata* MFBF, *C. kefyr* MFBF, *C. lusitaniae* MFBF, *Cryptococcus neoformans* MFBF, *Geotrichum candidum* MFBF, *Hansenula anomala* MFBF and dermatophytes *Microsporum gypseum* MFBF, *M. canis* MFBF, *Trichophyton mentagrophytes* MFBF, and *T. rubrum* MFBF.

Antimicrobial activity – diffusion and dilution methods

Antimicrobial activity by the diffusion method was determined using the methods described in *European Pharmacopoeia* (7). Inoculum was prepared with fresh cultures of bacterial strains, cultured on tryptic-soy agar (Merck, Germany) for 18 h at 37 ± 1°C with physiological saline, with 3 x 10^6 cells mL^{-1}. Inoculum density was compared with MacFarland's standard solution of $BaSO_4$ (0.1 mL of 1% $BaCl_2$ + 9.9 mL of 1% H_2SO_4) (8). Yeasts and dermatophytes were cultivated on Sabouraud 2% (*m/V*) dextrose agar (Biolife, Italy) with the addition of 50 mg L^{-1} chloramphenicol (Sigma, Germany) for 5 days for yeasts and 10 days for dermatophytes at 25 ± 1 °C. One mL of inoculum was mixed with 22 ± 5 mL of Müller-Hinton agar (Merck, Germany) for bacterial strains, and the same amount of Sabouraud agar for fungi. After cooling the inoculated agars at room temperature for 25 min, holes (d = 6 mm) were made with stainless steel cylinders. 40 μL of juniper essential oil was dropped into holes. In order to accelerate diffusion of the essential oil into agar, plates were incubated at 4 °C for 1 h and were then incubated at 37 ± 1 °C for 18 h under aerobic conditions. The diameter of the inhibition zone around each hole was measured and recorded.

Minimum inhibitory concentration (*MIC*) and minimum microbicidal concentration (*MMC*) were determined by the broth twofold macro dilution method in Müller-Hinton broth (Merck, Germany) for bacterial strains and in Sabouraud 2% (*m/V*) broth (Biolife, Italy) for fungal strains, as described by Pepeljnjak *et al*. (2) *MIC* was defined as the lowest concentration of juniper essential oil that allows no more than 20% growth of the bacteria, which is seen as the decreased number of colonies after removing the loop with 10 μL of each dilution on tryptic-soy agar and incubation at 37 ± 1 °C for 18 h. *MMC* was defined as the lowest concentration of the juniper essential oil that allows no growth of microorganisms.

RESULTS AND DISCUSSION

The preliminary GC/MS analysis of the juniper essential oil showed that there were approximately 35 compounds present in the essential oil with α-pinene (29.17%), β-pinene (17.84%), sabinene (13.55%), limonene (5.52%) and mircene (0.33%) as the most abundant ones.

The results of antimicrobial activity of the juniper essential oil by the diffusion method are presented in Table I. All Gram-positive bacterial species tested are sensitive to the juniper essential oil with the inhibition zones ranging from 10 mm to 16 mm.

The juniper essential oil possesses bactericidal activity against six of the eight strains tested, with *Citrobacter freundii* and *Escherichia coli* resistant to the essential oil. Susceptible Gram-negative bacterial strains have inhibition zones from 8 mm to 17 mm, smaller compared to Gram-positive bacterial strains.

All yeasts and yeast-like fungi were sensitive to the juniper essential oil, and the inhibition zones varied from 8 to 17 mm. Among the dermatophyte species tested, only *Microsporum canis* was resistant, while *M. gypseum* and *Trychophyton* spp. showed inhibition zones between 10 and 14 mm.

Table I. Antimicrobial activity of the juniper essential oil by the diffusion method

		Inhibition zones (mm)
Gram-positive bacterial species	*Bacillus cereus* ATCC 11778	16
	Bacillus subtilis NCTC 8236	10
	Micrococcus flavus MFBF	15
	Micrococcus luteus ATCC 9341	12
	Staphylococcus aureus ATCC 6538	11
	Staphylococcus aureus MFBF	14
	Staphylococcus epidermidis MFBF	13
	Enterococcus faecalis MFBF	11
Gram-negative bacterial species	*Serratia* spp. MFBF	8
	Citrobacter freundii MFBF	0
	Salmonella enteritidis MFBF	8
	Proteus mirabilis MFBF	13
	Shigella sonnei MFBF	17
	Klebsiella oxytoca MFBF	16
	Escherichia coli MFBF	0
	Yersinia enterocolitica MFBF	AE^{ø}
Yeasts and yeast-like fungi	*Candida albicans* MFBF	9
	Candida krusei MFBF	11
	Candida tropicalis MFBF	10
	Candida parapsilosis MFBF	17
	Candida glabrata MFBF	11
	Candida kefyr MFBF	10
	Candida lusitaniae MFBF	15
	Cryptococcus neoformans MFBF	8
	Geotrichum candidum MFBF	10
	Hansenula anomala MFBF	8
Dermatophytes	*Microsporum gypseum* MFBF	11
	Microsporum canis MFBF	0
	Trichophyton mentagrophytes MFBF	10
	Trichophyton rubrum MFBF	14

ATCC – American Type Culture Collection, Rockville, USA.
NCTC – National Collection of Type Cultures, London, Great Britain.
MFBF – number of strains from the Collection of microorganisms of the Department of Microbiology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia.
AE^{ø} – microbicidal influence of aerosol.

The results of the antimicrobial activity of the juniper essential oil by the dilution method are given in Table II. Minimum inhibitory concentrations for the bacterial species ranged from 18 to 70%, and the lowest *MIC* values were found for the fungal strains tested. All fungi showed *MIC* below 10%, and *Candida* spp. had the lowest *MIC* values (*C. kefyr* 0.78%, *C. albicans* 1%, *C. krusei* 2% and *C. tropicalis* 3.12%, respectively). Yeasts of the *Cryptococcus neoformans* and *Hansenula anomala* species had *MIC* values of 8 and 10%. Dermatophytes were very sensitive to the juniper berry essential oil, and *MIC* values ranged from 0.39 (against *T. rubrum* strain) and 2% against the *Microsporum gypseum* strain.

Table II. Antimicrobial activity of the juniper essential oil by the dilution method

	Microorganisms tested	*MIC* (%, *V/V*)	*MMC* (%, *V/V*)
Bacterial species	*Bacillus cereus* ATCC 11778	8	12.5
	Bacillus subtilis NCTC 8236	50	55
	Sarcina flava MFBF	15	25
	Staphylococcus aureus ATCC 6538	40	50
	Staphylococcus aureus MFBF	15	25
	Staphylococcus epidermidis MFBF	40	50
	Citrobacter freundii MFBF	60	65
	Salmonella enteritidis MFBF	70	75
	Shigella sonnei MFBF	55	60
	Klebsiella oxytoca MFBF	70	75
	Yersinia enterocolitica MFBF	40	50
Fungal species	*Candida albicans* MFBF	1	1.56
	Candida krusei MFBF	2	3.12
	Candida tropicalis MFBF	3.12	6.25
	Candida kefyr MFBF	0.78	1.56
	Cryptococcus neoformans MFBF	10	12.5
	Hansenula anomala MFBF	8	12.5
	Microsporum gypseum MFBF	2	3.12
	Trichophyton mentagrophytes MFBF	1	1.56
	Trichophyton rubrum MFBF	0.39	0.78

For symbols see Table I.

Our results on the antifungal activity of the juniper essential oil are similar to the results of Hammer *et al*. (9), who found a *MIC* against *C. albicans* of 2% (*V/V*). The same group of authors found low *MIC* values against *Staphylococcus aureus* and *Salmonella typhimurium* (2%, *V/V*), in contrast to our high values for this strains. Differences in *MIC* values are probably due to the different percentage of essential oil constituents.

CONCLUSIONS

In this study, the antimicrobial activity of the essential oil of juniper berries was determined. The oil showed activity against all eight Gram-positive bacterial strains, but two Gram-negative strains (*Citrobacter freundii* and *Escherichia coli*) were resistant among the eight Gram-negative bacterial strains tested. *MIC* for the bacterial strains were very high, ranging from 8 to 70% (*V/V*) of essential oil. Yeasts, yeast-like fungi and dermatophytes were found to be very sensitive to the juniper essential oil, with *MIC* values below 10% (*V/V*).

The lowest values of *MIC* of the essential oil against fungal strains indicate that the main compounds present in the oil-terpen hydrocarbons (pinenes, sabinene, mircene and limonene) had a stronger antifungal than antibacterial activity.

Acknowledgements. – We thank Boris Filipaj (Bioaromatica, Zagreb) for the donation of the juniper essential oil.

REFERENCES

1. K. Knobloch, A. Pauli, B. Iberl, H. Weigand and N. Weis, Antibacterial and antifungal properties of essential oil components, *J. Essent. Oil Res.* **1** (1989) 119–128.
2. S. Pepeljnjak, I. Kosalec, Z. Kalođera and D. Kuštrak, *Natural Antimycotics from Croatian Plants*, in *Plant-derived Antimycotics – Current Trends and Future Prospects* (Eds. M. Rai, D. Mares), Haworth Press, New York 2003, pp. 41–84.
3. *Herbal Medicine – Expanded Commission E Monographs* (Eds. M. Blumenthal, A. Goldberg and J. Brinckmann), American Botanical Council, Austin 2000, 178–181.
4. R. Hänsel, O. Sticher and E. Steinegger, *Pharmakognosie – Phytopharmazie*, 6th ed., Springer Verlag, Berlin 1999, pp. 860–861.
5. A. Y. Leung and S. Foster, *Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics*, 2nd ed., John Wiley & Sons, New York 1996.
6. Y. Masada, *Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry*, John Wiley & Sons, New York 1976.
7. *European Pharmacopoeia*, 4th ed., Council of Europe, Strasbourg 2001, pp. 93–99.
8. J. F. MacFaddin, *Biochemical Tests for Identification of Medical Bacteria*, 3rd ed., Lippincott Williams & Wilkins, Philadelphia 2000, pp. 825–826.
9. K. A. Hammer, C. F. Carson and T. V. Riley, Antimicrobial activity of essential oils and other plant extracts, *J. Appl. Microbiol.* **86** (1999) 985–990.

S A Ž E T A K

Antimikrobni učinak eteričnog ulja borovice (*Juniperus communis* L., *Cupressaceae*)

STJEPAN PEPELJNJAK, IVAN KOSALEC, ZDENKA KALOĐERA i NIKOLA BLAŽEVIĆ

Eterično ulje borovice (Juniperi aetheroleum) dobiveno je iz plodova (bobica *Juniperus communis* L., *Cupressaceae*) GC/MS analizom dokazane su glavne sastavnice α-pinen (29,17%) i β-pinen (17,84%), sabinen (13,55%), limonen (5,52%) i mircen (0,33%). Ispitan je antimikrobni učinak eteričnog ulja borovice na 16 bakterijskih vrsta, 7 kvasaca i kvascima sličnim gljivicama, te 4 vrste dermatofita. Eterično ulje borovice pokazuje slični baktericidan učinak na Gram-pozitivne i Gram-negativne bakterijske vrste s *MIK* vrijednostima između 8 i 70% (*V/V*). Ulje pokazuje snažni protugljivični učinak s *MIK* vrijednostima ispod 10% (*V/V*). Najjača protugljivična aktivnost uočena je na vrste roda *Candida*, s *MIK* vrijednostima između 0,78 i 2% (*V/V*) i na dermatofite s *MIK* vrijednostima između 0,39 i 2% (V/V).

Ključne riječi: *Juniperus communis*, borovica, kleka, eterično ulje, protubakterijsko, protugljivično djelovanje

Farmaceutsko-biokemijski fakultet Sveučilišta u Zagrebu, Zagreb

Irex-Aroma, Zagreb