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Review

The antioxidant and antimicrobial activity of essential oils against *Pseudomonas* spp. isolated from fishMiroslava Kačániová^{a,b,*}, Margarita Terentjeva^c, Nenad Vukovic^d, Czeslaw Puchalski^b, Shubhadeep Roychoudhury^e, Simona Kunová^f, Alina Klūga^c, Marián Tokár^g, Maciej Kluz^h, Eva Ivanišová^g^a Department of Microbiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic^b Department of Bioenergy and Food Technology, Faculty of Biology and Agriculture, University of Rzeszow, Zelwerowicza St. 4, 35-601 Rzeszow, Poland^c Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine, Latvia University of Agriculture, K. Helmaņa iela 8, LV-3004, Jelgava, Latvia^d University of Kragujevac, Faculty of Science, Department of Chemistry, P.O. Box 12, Serbia^e Department of Life Science and Bioinformatics, Assam University, 788 011 Silchar, India^f Department of Food Hygiene and Safety, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic^g Department of Storing and Processing Plant Products, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic^h Faculty of Biology and Agriculture, University of Rzeszow, Department of Microbiology and Biotechnology, Zelwerowicza St. 4, 35-601 Rzeszow, Poland

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ABSTRACT

Natural products of plant origin, which include essential oils (EO) could be used as a growth inhibitor of pathogenic and spoilage microflora in food. The objective of this study was to determine the antibacterial and antioxidant activity of 21 EO against 10 *Pseudomonas* species isolated from freshwater fish. The chemical composition of EO was determined by gas chromatography/mass spectrometry. The disc diffusion method and detection of minimum inhibitory concentration (MIC) were used for the determination of the antimicrobial activity. All the EO tested exhibited antimicrobial activity, however, *Cinnamomum zeylanicum* EO was the most effective against *Pseudomonas* spp. both according to the disc diffusion and MIC methods. The EOs of *Cymbopogon nardus*, *Origanum vulgare*, *Foeniculum vulgare* and *Thymus serpyllum* showed the highest antioxidant activity of 93.86 µg, 83.47 µg, 76.74 µg and 74.28 µg TEAC/mL. Application of EO could be an effective tool for inhibition of growth of *Pseudomonas* spp. on fish.

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1. Introduction

Essential oils (EOs) are aromatic and volatile liquids, which contain a mixture of organic compounds extracted from plant material. EOs possess a strong and generally pleasant flavour (Burt, 2004), therefore they are widely used in the cosmetic and food industry. Since the EOs exhibit antimicrobial and antioxidant properties as food additives, the research on the impact on food nutritional and microbiological properties have been intensified during the past decade. Studies on the effect of the EOs against a wide range of microorganisms, including pathogenic and food spoilage microflora, are among the most perspective for a safe food production (Trombetta et al., 2005).

The mode of action of EOs has not been completely understood yet and the main effect of EOs could be linked to the chemical compounds naturally present in EOs bearing plants (Burt, 2004; Cox and Markham, 2007). However, the antimicrobial activity of EOs depends on the composition and plant synergy showing that the chemical composition of the EOs is of great importance (Bajpai et al., 2012). The degree of antimicrobial activity exhibited by the EOs may influence their ability to penetrate through bacterial membranes and display the inhibitory activity on the functional properties of the cell (Bajpai et al., 2012; Fisher and Phillips, 2009; Guinoiseau et al., 2010). The phenolic compounds of EOs also elicit an antimicrobial response against foodborne pathogens by altering the microbial cell permeability, damaging cytoplasmic membranes, interfering with cellular energy (ATP) generation system and disruption of the proton motive force which result in the inhibition of the functional properties and the leakage of the internal cellular contents (Bajpai et al., 2012; Friedly et al., 2009).

Antibacterial properties shared by the EOs allowed to identify the effect on commensal and pathogenic microorganisms as an alternative to antimicrobial agent application. The extensive use of antibiotics in intensive food animal production has resulted in the emergence of resistance among food-borne pathogens, opportunistic pathogens and commensal flora. The resistant microflora has significantly contributed to the development of antibiotic resistance in humans with the EOs to be safe for the environment and consumers and with the ability to potentially inhibit the resistant bacteria (Heuer et al., 2009).

Pseudomonas spp are a genus of Gram-negative bacteria ubiquitous in the environment. The genus consists of species with human and animal health significance, particularly *Pseudomonas aeruginosa* is an opportunistic human pathogen while other *Pseudomonas* representatives can cause an infection in plants and insects (Stead, 1992). Some species of *Pseudomonas* exhibit the plant growth promoting and pathogen-suppressing properties and may be considered for use in biological control and bioremediation (Keel et al., 1996). *Pseudomonas* spp. are metabolically versatile, and hence, they were widely isolated from the natural environment, including water. *Pseudomonas* species were frequently associated with fish and the bacteria have been isolated from skin, gills and intestines. Despite the bacterial flora of the fish reflect the microbial population of the aquatic habitat influenced by the bacterial load in the water and salinity, the *Pseudomonas* spp. can comprise a

predominating part of fish microflora (Cahill, 1990). *Pseudomonas* could cause fish infection and contribute to the spoilage processes of freshly caught and processed fish (Tripathy et al., 2007). Studies on the effect of the EO on *Pseudomonas* spp. isolated from freshly caught fish from natural environment are still limited. Furthermore, *Pseudomonas* are inherently resistant to various antimicrobial agents (EUCAST, 2015) but the aquatic environment is a source of diverse microflora. The application of EOs for inhibition of *Pseudomonas* spp. growth could be an effective tool to alter bacterial growth, therefore studies on the comprehensive evaluation of the inhibitory effects of the EO on the microflora of freshwater fish are needed. The aims of the present study were (i) to determine the antioxidant activity of the EOs, and (ii) to evaluate the antimicrobial effect of 21 EOs against *Pseudomonas* spp. isolated from freshwater fish.

2. Material and methods

2.1. The samples of the EO

The original essential oils of 21 plants were used: *Lavandula angustifolia* Mill., *Cinnamomum zeylanicum* Nees. (C. verum J. S. Presl.), *Pinus montana*, *Mentha piperita* L., *Foeniculum vulgare* Mill., *Pinus sylvestris*, *Satureja hortensis* L., *Origanum vulgare* L., *Pimpinella anisum*, *Rosmarinus officinalis* L., *Salvia officinalis* L., *Abies alba* Mill., *Citrus aurantium* var. dulce, *Citrus sinensis* (L.) Osbeck., *Cymbopogon nardus*, *Mentha spicata* var. crispa, *Thymus vulgaris* L., *Carvum carvi*, *Thymus serpyllum*, *Ocimum basilicum*, *Coriandrum sativum*. All the EO were produced in Slovakia (samples No. 1–13 in Calendula a.s., Nova Lubovna and samples No. 14–21 in Hanus, Nitra). All tested samples were stored in the dark at 4 °C.

2.2. Productions of samples of the EO and analysis of their chemical compositions

A classical methodology for large-scale production of EOs was applied. The EOs were obtained with the distillation apparatus of two types specifically designed for aromatic and medicinal plants. Distillation equipment consisted of the main distillatory unit, steam condenser, steam boiler and apparatus for improving of the water quality. The used apparatus were of type HV-3000 with height and width of 5250 and 2180 mm and container for 200–250 kg of dried or 400 to 500 kg of fresh matter of plant material; and the type HV-300 with height and width of 3400 and 1300 mm and container for 40–50 kg of dry or 100–120 kg of fresh matter of plant material.

2.3. Qualitative and quantitative analysis of the EOs with GC/GC-MS

Analyses were carried out in an Agilent Technologies (Santa Clara, CA) 6890 N gas chromatograph fitted with an HP-5MS fused silica column (5% phenylmethyl polysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 µm, Agilent Technologies), interfaced with an Agilent Technologies mass-selective detector 5975B operated by

Table 1
Chemical composition of the investigated essential oils (S1–S10).

Compound	RI ^b	Sample concentration (% g/100 g) ^a									
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Camphene	933	/	/	8.19 ± 0.23	/	/	15.51 ± 0.37	/	/	/	10.13 ± 0.08
α-Pinene	939	/	/	21.26 ± 0.98	/	/	26.15 ± 0.87	/	/	/	15.65 ± 0.09
β-Pinene	978	/	/	6.98 ± 0.08	/	/	9.65 ± 0.11	/	/	/	4.56 ± 0.03
3-Octanone	984	2.41 ± 0.16	/	/	/	/	/	/	/	/	/
1,4-Cineole	1016	2.16 ± 0.13	2.89 ± 0.08	/	7.55 ± 0.08	/	/	/	/	/	21.26 ± 0.19
α-Terpinene	1017	/	/	/	/	/	/	2.65 ± 0.01	/	/	/
p-cymene	1027	/	/	/	/	/	/	2.29 ± 0.02	/	/	13.28 ± 0.11
limonene	1030	0.87 ± 0.06	/	3.25 ± 0.03	2.11 ± 0.02	/	7.23 ± 0.05	/	/	/	/
1,8-Cineole	1046	/	/	/	1.56 ± 0.07	/	/	/	/	/	/
γ-Terpinene	1056	/	/	/	/	/	/	32.11 ± 1.87	/	/	/
Linalool	1104	39.31 ± 1.56	6.11 ± 0.09	/	/	/	/	/	/	/	/
Camphor	1149	0.93 ± 0.05	/	/	/	/	/	/	/	/	/
Menthone	1150	/	/	/	27.29 ± 0.23	/	/	/	/	/	/
Isopulegol	1156	/	/	/	0.21 ± 0.01	/	/	/	/	/	/
Isomenthone	1165	/	/	/	9.11 ± 0.11	/	/	/	/	/	/
Borneol	1166	/	/	/	/	/	/	/	/	/	1.98 ± 0.09
Menthofuran	1168	/	/	/	6.65 ± 0.08	/	/	/	/	/	/
Lavandulol	1169	0.11 ± 0.02	/	/	/	/	/	/	/	/	/
Menthol	1170	/	/	/	28.56 ± 0.56	/	/	/	/	/	/
Terpinen-4-ol	1172	4.98 ± 0.07	/	/	/	/	/	/	/	/	/
α-Terpineol	1187	1.89 ± 0.05	/	/	/	/	/	/	/	/	2.49 ± 0.01
α-Phellandrene	1202	/	/	7.69 ± 0.08	/	/	9.56 ± 0.16	/	/	/	/
Pulegol	1213	/	/	/	2.98 ± 0.08	/	/	/	/	/	/
Carvone	1242	/	/	/	1.18 ± 0.05	/	/	/	/	/	/
Linalyl acetate	1253	37.68 ± 1.69	/	/	/	/	/	/	/	/	/
(E)-cinnamaldehyde	1266	/	63.21 ± 1.89	/	/	/	/	/	/	/	/
Anethole	1284	/	/	/	/	24.98 ± 0.89	/	/	/	63.25 ± 2.01	/
Bornyl acetate	1289	/	/	8.94 ± 0.13	/	/	14.59 ± 0.13	/	/	/	1.91 ± 0.06
Lavandulyl acetate	1292	0.19 ± 0.01	/	/	/	/	/	/	/	/	/
Safrole	1293	/	0.49 ± 0.05	/	/	/	/	/	/	/	/
Menthyl acetate	1297	/	/	/	9.37 ± 0.09	/	/	/	/	/	/
Carvacrol	1317	/	/	/	/	/	/	41.23 ± 1.59	43.26 ± 1.78	/	/
Eugenol	1373	/	7.45 ± 0.11	/	/	/	/	/	/	/	/
β-caryophyllene	1417	/	4.11 ± 0.19	/	/	/	/	/	/	/	/
Coumarin	1432	/	0.51 ± 0.03	/	/	/	/	/	/	/	/
4-methoxycinnamaldehyde	1569	/	1.36 ± 0.09	/	/	/	/	/	/	/	/
Benzyl benzoate	1753	/	1.29 ± 0.03	/	/	/	/	/	/	/	/

^a Values are given as mean value ± SD of three independent experiments.

^b RI-exp; S1- *L. angustifolia* -flowers; S2- *C. zeylanicum* -crust; S3- *P. mugo* -needles; S4- *M. piperita* -leaves; S5- *F. vulgare* -dried fruit; S6- *P. sylvestris* -needles; S7- *S. hortensis* -aerial parts; S8- *O. vulgare* -herb; S9- *P. anisum* -fruits; S10- *R. officinalis* -herb.

Table 2
Chemical composition of the investigated essential oils (S11-S21).

Compound	RI ^b	Sample concentration (% g/100 g) ^a										
		S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21
Camphene	933	/	13.29 ± 0.08	/	/	/	/	/	/	2.21 ± 0.02	/	/
α-Pinene	939	6.59 ± 0.03	3.05 ± 0.01	/	0.78 ± 0.01	/	/	/	/	3.28 ± 0.03	/	2.25 ± 0.01
Sabinene	973	/	/	1.68 ± 0.03	0.97 ± 0.03	/	/	/	/	/	/	/
β-Pinene	978	/	/	/	0.25 ± 0.01	/	/	/	/	0.49 ± 0.01	/	/
β-Myrcene	992	/	/	2.68 ± 0.01	2.68 ± 0.01	/	/	/	/	/	/	2.23 ± 0.01
Octanal	1004	/	/	/	0.31 ± 0.01	/	/	/	/	/	/	/
1,4-Cineole	1016	10.10 ± 0.08	/	/	/	/	/	/	/	/	/	/
α-Terpinene	1017	/	1.11 ± 0.01	/	/	/	/	/	/	14.58 ± 0.09	/	/
<i>p</i> -cymene	1027	/	/	/	/	/	/	21.15 ± 0.19	/	/	/	/
Limonene	1030	/	/	74.35 ± 2.23	87.89 ± 2.21	0.97 ± 0.01	3.23 ± 0.01	/	21.12 ± 0.91	/	/	/
Linalool	1104	/	/	/	0.64 ± 0.01	/	/	/	/	/	/	59.11 ± 1.19
α-Thujone	1105	23.28 ± 0.12	/	/	/	/	/	/	/	/	/	/
β-Thujone	1110	4.33 ± 0.03	/	/	/	/	/	/	/	/	/	/
Camphor	1149	13.29 ± 0.09	/	/	/	/	/	/	/	/	/	/
Citronellal	1158	/	/	/	/	16.18 ± 0.08	/	/	/	/	/	/
Borneol	1166	/	1.49 ± 0.02	/	/	/	/	/	/	/	/	/
Estragole	1201	/	/	/	/	/	/	/	/	/	61.53 ± 2.23	/
Decanal	1208	/	/	/	0.09 ± 0.01	/	/	/	/	/	/	/
Nerol	1229	/	/	/	0.23 ± 0.01	54.12 ± 1.45	/	/	/	/	/	/
Carvone	1231	/	/	/	/	/	34.22 ± 1.21	/	69.54 ± 1.16	/	/	/
Neral	1235	/	/	/	0.09 ± 0.01	/	/	/	/	/	/	/
Citronellal	1236	/	/	/	/	2.11 ± 0.01	/	/	/	/	/	/
Bornyl acetate	1289	/	23.29 ± 0.13	/	/	/	/	/	/	/	/	/
thymol	1295	/	/	/	/	/	/	41.67 ± 1.12	/	31.29 ± 1.13	/	/
Carvacrol	1317	/	/	/	/	/	/	2.16 ± 0.03	/	5.11 ± 0.08	/	/
Eugenol	1373	5.02 ± 0.01	/	/	/	/	/	/	/	/	/	/
α-Caryophyllene	1455	2.79 ± 0.02	/	/	/	/	/	/	/	/	/	/
Valencene	1495	/	/	/	0.47 ± 0.03	/	/	/	/	/	/	/

^a Values are given as mean value ± SD of three independent experiments.

^b RI-exp; S11- *S. officinalis* -leaves; S12- *A. alba* -needles; S13- *C. aurantium* -pericarp; S14- *C. sinensis* -pericarp; S15- *C. nardus* -leaves; S16- *M. spicata* -leaves; S17- *T. vulgaris* -herb; S18- *C. carvi* -fruits; S19- *T. serpyllum* -leaves; S20- *O. basilicum* -leaves; S21- *C. sativum* -dried fruit.

HP Enhanced ChemStation software (Agilent Technologies). Analytical conditions were as follows: oven temperature programmed at 50 °C with an increase of 5 °C/min to 280 °C; injection of 1 µL (10% hexane solution); split ratio 1:50.0; carrier gas, helium at 1.0 mL/min; injector and transfer line temperatures of 250 °C and 280 °C, respectively; MS source temperature 230 °C; MS quadrupole temperature 150 °C; mass scan range, 35–550 amu at 70 eV. GC analyses were performed on an Agilent model 6890 N gas chromatograph with a flame ionization detector using an HP-5MS column. The chromatographic conditions were the same as for GC/MS analyses.

The constituents of the essential oils were identified by comparing their retention times with available standards, RI (retention indices) values relative to those of C₆–C₃₀ n-alkanes and their mass spectral fragmentation pattern with those reported in literature (Adams, 2007) and stored in the MS library (Wiley7Nist) incorporated in the HP Enhanced ChemStation software.

Quantification of constituents of EOs were performed by using reference standards (3-octanone, octanal, decanal, p-cimene, estragole, benzyl benzoate, thymol, eugenol, anethole, trans-cinnamaldehyde, coumarin, α-pinene, β-pinene, α-terpinene, α-terpineol, terpinen-4-ol, (-)-menthone, menthylacetate, menthofuran, borneol, bornyl acetate, limonene, α-thujone, β-myrcene, 1,4-cineole, (+/-)-citronelol, neral, geraniol, isopulegol, sabinene, carvone, carvacrol, (+/-)-linalool, linalyl acetate, valencene, camphor, camphene, caryophyllene, α-phellandrene, (+/-)-lavandulyl acetate and (+/-)-lavandulol). Pure compounds were obtained from Sigma-Aldrich (Steinheim, Germany) and Extrasynthese (Genay, France).

In accordance to previously published procedure (Kowalski, 2008), the quantitative analysis was performed by means of the internal standard addition method (alkanes C₁₂ and C₁₉). Briefly, samples of essential oils were diluted one thousand times with n-hexane in order to obtain 1 mL of solutions. Then, 1 mg of n-dodecane and 1 mg of n-nonadecane were added to each sample of investigated diluted oils. Prepared samples were subjected to GC/MS and GC/FID examinations, with the fact that quantitative analysis were performed by using calibration curves for available standards within the concentration range 0.03–80%. Semi-quantification: saffrole from calibration curve of eugenol, trans-2-metoxycinnamaldehyde from calibration curve of trans-cinnamaldehyde, isomenthone from calibration curve of menthone, pulegol from calibration curve of isopulegol, γ-terpinene from calibration curve of α-terpinene, β-thujone from calibration curve of α-thujone, α-caryophyllene from calibration curve of β-caryophyllene, citronelal from calibration curve of citronelol.

The chemical composition of EOs is summarized in Tables 1 and Table 2.

2.4. Origin of *Pseudomonas* spp.

Freshly caught freshwater fish were used for isolation of *Pseudomonas* spp. *Pseudomonas* were confirmed with the MALDI TOF MS Biotyper (Bruker, Germany) and the following species were isolated: *Pseudomonas agglomerans*, *P. antarctica*, *P. brassicacearum*, *P. frederiksbergensis*, *P. koreensis*, *P. lundensis*, *P. mandelii*, *P. proteolytica*, *P. synxantha*, *P. veronii*. Isolates were cultivated on Mueller Hinton Agar (MHA, Merck, Germany). Bacterial culture was enriched in the Mueller Hinton Broth (MHB, Merck, Germany) at 37 °C for 24 h before the antimicrobial susceptibility and EOs antimicrobial activity tests.

2.5. Antibiotic susceptibility testing of *Pseudomonas* spp.

The antibiotic susceptibility was tested by disc diffusion method. A suspension of the *Pseudomonas* spp. in MHB was plated

out onto MHA, then, the appropriate antimicrobial discs were placed on the agar surface. Inoculated agars were incubated at 37 °C for 24 h. *Pseudomonas* spp. cultures were tested against ampicillin (10 mcg), gentamicin (10 mcg), imipenem (10 mcg) and meropenem (10 mcg) (Oxoid, UK). The results were interpreted according to the EUCAST, 2015.

2.6. Detection of antimicrobial activity of the EOs

Detection of antimicrobial activity of EOs was carried out with the agar disc diffusion method and detection of the minimum inhibitory concentration of EOs.

For the agar disc diffusion method, an aliquot of 0.1 mL of bacterial suspension in MHB was spread onto MHA. Then, the filter paper discs of 6 mm in diameter were impregnated with 15 µL of the EOs and placed on the MHA surface. Inoculated MHA plates were kept at 4 °C for 2 h and incubated aerobically at 37 °C for 24 h. The diameters of the inhibition zones were measured in mm after incubation. Each test was repeated twice.

For the detection of minimum inhibitory activity of the EO, a test oil solution was prepared in 10% aqueous dimethyl sulphoxide (DMSO, Penta, Prague, Czech Republic). Geometric dilutions from 0.75 to 100 µg/mL of the EOs in a 96-well microtitre plate were prepared. One growth control well (MHB + Tween 80) and one sterility control well (MHB + Tween 80 + test oil) were included in each assessment. The plates were incubated aerobically at 37 °C for 24 h. The presence of a white “pellet” on the well bottom indicated on the bacterial growth.

Pseudomonas spp. growth was evaluated after incubation by measuring the well absorbance at 450 nm (Biotek EL808 with shaker, Biotek Instruments, USA). Measurements were undertaken before and after the experiment and the difference between the measurements was described as growth. Measurement error was 0.05 of values from absorbance. Each test was done in eight replicates for a higher accuracy of the MICs of used EOs.

2.7. Detection of free radical scavenging activity

Free radical scavenging activity of samples was measured with 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sánchez-Moreno et al., 1998). The sample of 0.4 mL was mixed with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL methanol). The absorbance of the reaction mixture was detected with a spectrophotometer (Jenway 6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetra methylchroman-2-carboxylic acid) (10–100 mg/L; R² = 0.989) was used as a standard and the results were expressed in µg/mL Trolox equivalents.

Table 3
Antibiotic susceptibility of *Pseudomonas* species isolated from the freshwater fish.

Pseudomonas species	Antimicrobial agent			
	AMP	GMC	IPM	MPM
<i>Pseudomonas agglomerans</i>	R	I	S	R
<i>Pseudomonas antarctica</i>	R	R	R	R
<i>Pseudomonas brassicacearum</i>	R	I	R	R
<i>Pseudomonas frederiksbergensis</i>	R	R	R	R
<i>Pseudomonas koreensis</i>	R	R	S	R
<i>Pseudomonas lundensis</i>	R	I	S	R
<i>Pseudomonas mandelii</i>	R	R	R	R
<i>Pseudomonas proteolytica</i>	R	R	R	R
<i>Pseudomonas synxantha</i>	S	I	S	R
<i>Pseudomonas veronii</i>	R	R	R	R

S: susceptible, I: intermediate susceptibility, R: resistant, AMP-ampicillin, GMC-gentamicin, IPM-imipenem, MPM-meropenem.

Table 4
Antimicrobial activity of the 21 essential oils against *Pseudomonas* spp. with agar disc diffusion in mm.

Essential oil	<i>P. agglomerans</i>	<i>P. antarctica</i>	<i>P. brassicacearum</i>	<i>P. frederiksbergensis</i>	<i>P. koreensis</i>
<i>Lavandula angustifolia</i> Mill.	3.00 ± 1.00	6.00 ± 1.00	4.00 ± 1.00	10.33 ± 1.53	3.67 ± 0.58
<i>Cinnamomum zeylanicum</i> L.	10.00 ± 1.00	12.33 ± 2.52 ^a	15.00 ± 2.00 ^a	13.67 ± 1.53 ^a	12.67 ± 1.15 ^a
<i>Pinus mugo</i> Turra	5.00 ± 0.00	4.67 ± 0.58	2.67 ± 0.58	2.33 ± 0.58	4.67 ± 0.58
<i>Mentha piperita</i> L.	4.33 ± 0.58	7.00 ± 2.00	4.67 ± 0.58	4.67 ± 0.58	7.33 ± 0.58
<i>Foeniculum vulgare</i> Mill.	4.66 ± 0.58	4.00 ± 0.57	3.33 ± 0.58	4.33 ± 0.58	2.67 ± 0.58
<i>Pinus sylvestris</i> L.	4.33 ± 0.58	2.33 ± 0.57	7.67 ± 1.15	2.67 ± 1.15	3.67 ± 0.58
<i>Satureja hortensis</i> L.	2.33 ± 0.58	7.66 ± 1.53	4.67 ± 0.58	3.67 ± 0.58	4.67 ± 0.58
<i>Origanum vulgare</i> L.	4.33 ± 0.58	9.00 ± 1.00	4.33 ± 0.58	7.00 ± 1.00	4.33 ± 0.58
<i>Pimpinella anisum</i> L.	2.33 ± 0.58	8.67 ± 0.58	5.33 ± 0.58	4.33 ± 0.58	2.67 ± 0.58
<i>Rosmarinus officinalis</i> L.	4.67 ± 0.58	10.00 ± 1.00	12.33 ± 1.53	11.00 ± 1.00	5.33 ± 0.58
<i>Salvia officinalis</i> L.	4.33 ± 0.58	3.00 ± 1.00	3.33 ± 0.58	5.33 ± 0.58	4.67 ± 0.58
<i>Abies alba</i> Mill.	7.33 ± 0.58	3.00 ± 1.00	4.33 ± 0.58	14.33 ± 0.58	5.33 ± 0.58
<i>Citrus aurantium</i> var. <i>dulce</i> L.	4.33 ± 0.58	3.00 ± 1.00	5.00 ± 1.00	4.67 ± 1.15	2.33 ± 0.58
<i>Citrus sinensis</i> L. Osbeck.	2.00 ± 1.00	5.33 ± 0.58	4.33 ± 0.58	7.33 ± 0.58	4.67 ± 0.58
<i>Cymbopogon nardus</i> L.	4.66 ± 0.58	5.00 ± 1.00	3.33 ± 0.58	4.67 ± 0.58	4.33 ± 0.57
<i>Mentha spicata</i> var. <i>crispa</i> L.	6.67 ± 1.53	5.33 ± 0.57	7.33 ± 0.58	4.33 ± 0.58	4.33 ± 0.58
<i>Thymus vulgaris</i> L.	9.67 ± 1.53 ^c	5.67 ± 1.53	4.33 ± 0.58	4.67 ± 0.58	8.00 ± 1.00
<i>Carvum carvi</i> L.	4.67 ± 0.58	5.00 ± 1.00	2.33 ± 0.58	2.33 ± 0.58	4.67 ± 0.58
<i>Thymus serpyllum</i> L.	4.33 ± 0.58	7.33 ± 0.58	3.00 ± 1.00	4.33 ± 0.58	4.67 ± 0.58
<i>Ocimum basilicum</i> L.	6.00 ± 1.00	4.33 ± 0.58	12.67 ± 1.15	5.33 ± 0.58	2.67 ± 0.58
<i>Coriandrum sativum</i> L.	4.33 ± 0.58	4.33 ± 0.58	5.33 ± 0.58	3.67 ± 0.58	2.67 ± 1.15
	<i>P. lundensis</i>	<i>P. mandelii</i>	<i>P. proteolytica</i>	<i>P. synxantha</i>	<i>P. veronii</i>
<i>Lavandula angustifolia</i> Mill.	2.67 ± 1.15	3.33 ± 0.58	3.67 ± 0.57	2.67 ± 0.58	2.67 ± 0.58
<i>Cinnamomum zeylanicum</i> L.	12.33 ± 1.53	12.67 ± 1.15 ^a	13.33 ± 1.15 ^a	9.67 ± 0.58 ^a	11.33 ± 0.58
<i>Pinus mugo</i> Turra	4.67 ± 0.58	3.33 ± 0.58	5.33 ± 0.58	4.67 ± 0.58	2.33 ± 0.58
<i>Mentha piperita</i> L.	8.67 ± 0.58	4.33 ± 0.58	3.66 ± 0.58	4.67 ± 0.58	5.33 ± 0.58
<i>Foeniculum vulgare</i> Mill.	4.66 ± 0.58	4.66 ± 0.58	7.67 ± 0.58	4.33 ± 0.58	2.33 ± 0.58
<i>Pinus sylvestris</i> L.	7.67 ± 1.15	2.67 ± 0.58	2.33 ± 0.58	2.67 ± 0.57	14.67 ± 0.58 ^c
<i>Satureja hortensis</i> L.	4.33 ± 0.58	4.33 ± 0.58	2.67 ± 0.58	2.33 ± 0.58	5.33 ± 0.58
<i>Origanum vulgare</i> L.	12.33 ± 1.53 ^b	7.67 ± 1.15	4.33 ± 0.58	5.33 ± 0.58	13.00 ± 1.00
<i>Pimpinella anisum</i> L.	2.33 ± 0.58	2.33 ± 0.58	3.67 ± 0.58	2.33 ± 0.58	4.33 ± 0.58
<i>Rosmarinus officinalis</i> L.	2.00 ± 0.00	8.00 ± 1.00	4.67 ± 0.58	4.33 ± 0.58	4.33 ± 0.58
<i>Salvia officinalis</i> L.	3.67 ± 0.58	2.33 ± 0.58	4.33 ± 0.58	2.30 ± 0.57	2.33 ± 0.58
<i>Abies alba</i> Mill.	3.33 ± 0.58	2.67 ± 0.58	4.66 ± 0.58	2.33 ± 0.58	1.67 ± 0.58
<i>Citrus aurantium</i> var. <i>dulce</i> L.	3.00 ± 1.00	3.66 ± 1.52	4.67 ± 0.58	2.66 ± 0.58	4.67 ± 0.58
<i>Citrus sinensis</i> L. Osbeck.	5.00 ± 1.00	3.33 ± 1.52	2.67 ± 0.57	4.33 ± 1.15	2.66 ± 0.58
<i>Cymbopogon nardus</i> L.	5.00 ± 1.00	4.33 ± 0.58	4.67 ± 0.58	2.67 ± 0.58	3.33 ± 0.58
<i>Mentha spicata</i> var. <i>crispa</i> L.	2.33 ± 0.58	4.00 ± 1.00	4.67 ± 0.58	2.33 ± 0.58	5.33 ± 0.58
<i>Thymus vulgaris</i> L.	7.67 ± 0.58	4.00 ± 0.00	2.67 ± 0.58	8.33 ± 0.58	1.67 ± 0.58
<i>Carvum carvi</i> L.	2.33 ± 0.58	4.00 ± 1.00	6.00 ± 1.00	2.33 ± 0.57	1.33 ± 0.58
<i>Thymus serpyllum</i> L.	3.67 ± 0.58	2.33 ± 0.58	4.67 ± 0.58	4.33 ± 0.58	2.00 ± 0.00
<i>Ocimum basilicum</i> L.	3.33 ± 0.58	2.33 ± 0.58	5.33 ± 0.58	2.67 ± 0.58	9.00 ± 1.00
<i>Coriandrum sativum</i> L.	2.67 ± 0.58	2.33 ± 0.58	2.67 ± 0.58	1.33 ± 0.58	2.33 ± 0.58

^a The EO of *Cinnamomum zeylanicum* L. was the most effective against *P. antarctica*, *P. brassicacearum*, *P. frederiksbergensis*, *P. koreensis*, *P. mandelii*, *P. proteolytica* and *P. synxantha* ($P < 0.001$).

^b There were no differences in antimicrobial activity of the EOs of *Cinnamomum zeylanicum* L and *Thymus serpyllum* L against *P. agglomerans* ($P < 0.001$).

^c The EO of *Pinus sylvestris* L was the most active against *P. veronii* ($P < 0.001$).

2.8. Statistical analysis

The basic variation (disc diffusion method) was from obtained data by using the statistical programme Statgraphic and the Tukey HSD test for the comparison of the antimicrobial activity of the 21 EOs. The parameters calculated alongside with the basic variation were: average, standard deviation, minimum, maximum coefficient of variation and the frequency of size of the inhibition zones.

3. Results and discussion

3.1. Antibiotic susceptibility testing

Pseudomonas antarctica, *P. frederiksbergensis*, *P. mandelii*, *P. proteolytica* and *P. veronii* were resistant to all the antimicrobial agents tested that comprised 50% of all bacterial cultures tested (Table 3). *Pseudomonas synxantha* was the most sensitive to application of antimicrobial agents and exhibited the sensitivity to ampicillin and imipenem, intermediate susceptibility to gentamicin and resistance to meropenem. All the *Pseudomonas* were resistant to meropenem (100%) while 4 out of 10 were resistant to imipenem

(40%). Resistance against the ampicillin and gentamicin comprised 10% and 40%, respectively.

The present study revealed the high proportion of resistant strains among the *Pseudomonas* spp. isolated originated from fish. *Pseudomonas* spp., including *P. aeruginosa*, is naturally resistant to many antibiotics (Tadeu et al., 2000) with only few of antimicrobial agents were found to be effective against *Pseudomonas*. Fluoroquinolones, gentamicin and imipenem were described among the most effective but against all the *Pseudomonas* species. The high efficiency of gentamicin on *Pseudomonas* spp. animal isolates was confirmed. Also meropenem, imipenem, ciprofloxacin, ticarcillin and mezlocillin were described as the antimicrobials with high activity against environmental isolates of *Pseudomonas* spp. (Tadeu et al., 2000). The present study showed the high prevalence of the imipenem-, meropenem-, gentamicin- and ampicillin-resistant strains the presence of the large proportion of antibiotic resistant *Pseudomonas* spp. strains in the aquatic environment.

3.2. Antimicrobial activity of EOs detected by the disc diffusion method

The results on the antibacterial activity of 21 EOs tested by the disc diffusion method varied at great extent (Table 4). The majority

Table 5
Antimicrobial activity of 21 essential oils expressed as the minimum inhibitory concentration (MIC) in $\mu\text{L}/\text{mL}$ against *Pseudomonas* spp.

Essential oil	<i>P. agglomerans</i>		<i>P. antarctica</i>		<i>P. brassicacearum</i>		<i>P. frederiksbergensis</i>		<i>P. koreensis</i>	
	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90
<i>Lavandula angustifolia</i> Mill.	25.00	50.00	50.00	100.00	50.00	100.00	12.50	25.00	12.50	25.00
<i>Cinnamomum zeylanicum</i> L.	3.125	6.25	6.25	12.50	3.125	6.25	6.25	12.50	12.50	25.00
<i>Pinus mugo</i> Turra	6.25	21.50	12.50	25.00	6.25	12.50	12.50	25.00	6.25	12.50
<i>Mentha piperita</i> L.	12.50	25.00	12.50	25.00	25.00	50.00	25.00	50.00	12.50	25.00
<i>Foeniculum vulgare</i> Mill.	12.50	25.00	12.50	50.00	25.00	50.00	12.50	25.00	25.00	50.00
<i>Pinus sylvestris</i> L.	12.50	25.00	12.50	50.00	25.00	50.00	12.50	25.00	25.00	50.00
<i>Satureja hortensis</i> L.	12.50	50.00	6.25	12.50	12.50	50.00	12.50	25.00	25.00	50.00
<i>Origanum vulgare</i> L.	6.25	21.50	12.50	25.00	6.25	12.50	12.50	25.00	6.25	12.50
<i>Pimpinella anisum</i> L.	12.50	25.00	12.50	50.00	25.00	50.00	12.50	25.00	25.00	50.00
<i>Rosmarinus officinalis</i> L.	25.00	50.00	50.00	100.00	50.00	100.00	12.50	25.00	12.50	25.00
<i>Salvia officinalis</i> L.	25.00	50.00	50.00	100.00	50.00	100.00	12.50	25.00	12.50	25.00
<i>Abies alba</i> Mill.	6.25	21.50	12.50	25.00	6.25	12.50	12.50	25.00	6.25	12.50
<i>Citrus aurantium</i> var. <i>dulce</i> L.	25.00	50.00	50.00	100.00	25.00	50.00	50.00	100.00	12.50	25.00
<i>Citrus sinensis</i> L. Osbeck.	25.00	50.00	50.00	100.00	25.00	50.00	50.00	100.00	25.00	50.00
<i>Cymbopogon nardus</i> L.	25.00	50.00	50.00	100.00	50.00	100.00	12.50	25.00	12.50	25.00
<i>Mentha spicata</i> var. <i>crispa</i> L.	25.00	50.00	50.00	100.00	50.00	100.00	12.50	25.00	12.50	25.00
<i>Thymus vulgaris</i> L.	6.25	21.50	12.50	25.00	6.25	12.50	12.50	25.00	6.25	12.50
<i>Carum carvi</i> L.	25.00	50.00	50.00	100.00	50.00	100.00	12.50	25.00	12.50	25.00
<i>Thymus serpyllum</i> L.	25.00	50.00	50.00	100.00	50.00	100.00	12.50	25.00	12.50	25.00
<i>Ocimum basilicum</i> L.	12.50	25.00	12.50	50.00	25.00	50.00	12.50	25.00	25.00	50.00
<i>Coriandrum sativum</i> L.	12.50	25.00	12.50	50.00	25.00	50.00	12.50	25.00	25.00	50.00
	<i>P. lundensis</i>		<i>P. mandelii</i>		<i>P. proteolytica</i>		<i>P. synxantha</i>		<i>P. veronii</i>	
	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90
<i>Lavandula angustifolia</i> Mill.	12.50	25.00	25.00	50.00	25.00	50.00	12.50	25.00	12.50	25.00
<i>Cinnamomum zeylanicum</i> L.	3.125	6.25	6.25	12.50	3.125	6.25	6.25	12.50	12.50	25.00
<i>Pinus mugo</i> Turra	6.25	21.50	6.25	12.50	6.25	12.50	12.50	25.00	6.25	12.50
<i>Mentha piperita</i> L.	12.50	25.00	25.00	50.00	25.00	50.00	12.50	25.00	12.50	25.00
<i>Foeniculum vulgare</i> Mill.	12.50	25.00	25.00	50.00	25.00	50.00	12.50	25.00	12.50	25.00
<i>Pinus sylvestris</i> L.	6.25	21.50	6.25	12.50	6.25	12.50	12.50	25.00	6.25	12.50
<i>Satureja hortensis</i> L.	12.50	25.00	25.00	50.00	25.00	50.00	12.50	25.00	12.50	25.00
<i>Origanum vulgare</i> L.	12.50	50.00	6.25	12.50	12.50	25.00	12.50	50.00	12.50	50.00
<i>Pimpinella anisum</i> L.	25.00	50.00	25.00	50.00	12.50	25.00	25.00	50.00	25.00	50.00
<i>Rosmarinus officinalis</i> L.	12.50	50.00	6.25	12.50	12.50	25.00	12.50	50.00	12.50	50.00
<i>Salvia officinalis</i> L.	12.50	50.00	6.25	12.50	12.50	25.00	12.50	50.00	12.50	50.00
<i>Abies alba</i> Mill.	6.25	21.50	6.25	12.50	6.25	12.50	12.50	25.00	6.25	12.50
<i>Citrus aurantium</i> var. <i>dulce</i> L.	50.00	100.00	50.00	100.00	50.00	100.00	50.00	100.00	50.00	100.00
<i>Citrus sinensis</i> L. Osbeck.	50.00	100.00	25.00	50.00	50.00	100.00	50.00	100.00	50.00	100.00
<i>Cymbopogon nardus</i> L.	25.00	50.00	25.00	50.00	12.50	25.00	25.00	50.00	12.50	25.00
<i>Mentha spicata</i> var. <i>crispa</i> L.	12.50	50.00	6.25	12.50	12.50	25.00	12.50	50.00	12.50	50.00
<i>Thymus vulgaris</i> L.	12.50	50.00	6.25	12.50	12.50	25.00	12.50	50.00	12.50	50.00
<i>Carum carvi</i> L.	25.00	50.00	25.00	50.00	12.50	25.00	25.00	50.00	25.00	50.00
<i>Thymus serpyllum</i> L.	12.50	50.00	6.25	12.50	12.50	25.00	12.50	50.00	12.50	50.00
<i>Ocimum basilicum</i> L.	12.50	50.00	6.25	12.50	12.50	25.00	12.50	50.00	12.50	50.00
<i>Coriandrum sativum</i> L.	6.25	12.50	6.25	12.50	12.50	25.00	12.50	25.00	12.50	25.00

of the *Pseudomonas* spp. was sensitive to all EOs were applied. *Cinnamomum zeylanicum* EO was the most effective against seven *Pseudomonas* species, including *P. agglomerans*, *P. antarctica*, *P. brassicacearum*, *P. koreensis*, *P. mandelii*, *P. proteolytica* and *P. synxantha*. The most sensitive among *Pseudomonas* spp. to *Cinnamomum zeylanicum* EO was *P. brassicacearum* with the inhibition zone of 15.00 ± 2.00 mm. *P. frederiksbergensis* was the most sensitive to *Abies alba* Mill. EO (14.33 ± 0.58 mm) while *P. veronii* was the most sensitive to *Pinus sylvestris* L. EO (14.67 ± 0.58 mm). There were no differences between the sensitivity of *P. lundensis* to the EOs activity of *Cinnamomum zeylanicum* and *Origanum vulgare* L. (12.33 ± 1.53 mm, $P \geq 0.001$). There were significant differences ($P \leq 0.001$) between the antimicrobial activity of 18 EOs on *Pseudomonas* spp. growth for other three – *Citrus sinensis*, *Cymbopogon nardus* and *Cinnamomum zeylanicum* differences were not significant ($P > 0.001$).

A broad variation in antimicrobial properties of the EOs was reported by of Mith et al. (2014). The EOs of *Cinnamomum cassia*, *C. verum*, *Origanum compactum*, *O. heracleoticum*, *Thymus capitatus* and *T. vulgaris thymoliferum* showed strong antimicrobial activity against the tested bacteria, whereas *Cymbopogon flexuosus* EO showed strong activity against Gram-positive bacteria only. In con-

trast, *Kaempferia galanga* EOs did not exhibit the antimicrobial activity against any of the tested bacterial strains. In general, Gram-positive bacteria were found to be more sensitive to EOs or antibacterial compounds than Gram-negative bacteria because of the differences in cell structure, which may retain the entry of hydrophobic compounds in the cell (Burt, 2004; Cox and Markham, 2007; Dorman and Deans, 2000). Our results revealed that the EOs could be effective against Gram-negative *Pseudomonas* spp.

3.3. Antimicrobial activity of EOs detected by identification minimum inhibitory concentration

The best antimicrobial activity was exhibited by *Cinnamomum zeylanicum* EO against six *Pseudomonas* species, including *P. agglomerans*, *P. brassicacearum*, *P. frederiksbergensis*, *P. lundensis*, *P. proteolytica* and *P. synxantha* and our findings were in agreement with the results obtained by the disc diffusion method. The MIC of *Cinnamomum zeylanicum* EOs ranged from MIC50 of 3.125 and MIC90 of 6.25 to MIC50 of 6.25 and MIC90 of $12.50 \mu\text{L}/\text{mL}$. There were no differences between the antimicrobial activity of *Cinnamomum zeylanicum* and *Satureja hortensis* on the growth

of *P. antarctica* (6.25 $\mu\text{L}/\text{mL}$, $P \geq 0.001$) and of EOs of *Pinus mugo*, *Pinus sylvestris* and *Abies alba* on *P. veronii* (6.25 $\mu\text{L}/\text{mL}$, $P \geq 0.001$). *P. koreensis* was the most sensitive to 4 EOs (*Pinus mugo* Turra, *Origanum vulgare*, *Abies alba*, *Thymus vulgaris*) with MIC50 of 6.25 and MIC90 of 12.50 $\mu\text{L}/\text{mL}$. *P. mandelii* was the most sensitive to 12 EOs (*Cinnamomum zeylanicum*, *Pinus mugo* Turra, *Pinus sylvestris*, *Origanum vulgare*, *Rosmarinus officinalis*, *Salvia officinalis*, *Abies alba*, *Mentha spicata* var. *crispa*, *Thymus vulgaris*, *Thymus serpyllum*, *Ocimum basilicum*, *Coriandrum sativum*) with MIC50 of 6.25 and MIC90 of 12.50 $\mu\text{L}/\text{mL}$. Minimal inhibitory concentration (MIC) of 21 EOs is summarized in Table 5.

The present study showed that the application of EOs was effective in inhibition of *Pseudomonas* spp. in freshwater fish. *Pseudomonas* spp. are an important part of spoilage microflora, which alter the shelf-life and the quality of fish. The EOs was affective against the spoilage microflora for prolongation of the shelf-life of freshwater fish (Harpaz et al., 2003). The studies on the effect of treatment of *Pseudomonas* spp. with EO originated from freshwater fish are limited. However, the *Pseudomonas* spp. of freshwater fish were found to be the specific spoilage microorganisms and the activity of *Pseudomonas* spp. in fish results in rapid deterioration of the product. Therefore, the EOs activity differs from those reported from meat and another kind of products. It might be explained by different composition and percentage content of active constituents in EOs (Bozin et al., 2006), species, subspecies or variety of plants, geographical locations, harvesting, drying and extraction methods (Burt, 2004; Di Cesare et al., 2003; Hussain et al., 2008; Sarac and Ugur, 2008). Methods used to assess the antimicrobial activity, bacterial strains and their sensitivity, volume of inoculum, incubation time, and temperature may also be related to the variation in the experimental results (Burt, 2004; Bozin et al., 2006).

3.4. Antioxidant activity

The highest antioxidant activity (Table 6) was observed in *Cymbopogon nardus* (93.86 $\mu\text{g TEAC}/\text{mL}$), *Origanum vulgare* (83.47 $\mu\text{g TEAC}/\text{mL}$), *Foeniculum vulgare* (76.74 $\mu\text{g TEAC}/\text{mL}$) and *Thymus serpyllum* (74.28 $\mu\text{g TEAC}/\text{mL}$). In comparison, the antioxidant capacity of *Cymbopogon citrates* with DPPH in Vázquez-Briones et al. (2015) study was 44.06 ± 0.20 mg TEAC per 100 mL, equivalent to 55.57% of inhibition. The major compound of *Cymbopogon* oil is

citral, which possesses various useful bioactivities and one of these is an anti-clastogenic effect in nickel chloride-treated mouse micronucleus system. Citral-caused inhibition of micronuclei formation and enhanced the superoxide scavenging activity were thought to be responsible for the anti-clastogenic effects of citral (Rabbani et al., 2006). Some other compounds such as geraniol and limonene have also been correlated with different types of bioactivities. Ganjewala (2009) reported that EOs from *Cymbopogon* spp. showed scavenging of free radicals and anti-acetylcholine esterase activity proving that the EOs share strong antioxidant properties.

Strong antioxidant activity was also detected in EOs of *Origanum vulgare* and *Thymus serpyllum*. The main compounds of these EOs are thymol and carvacrol. The metabolic pathway for the carvacrol and thymol (Table 1) formation begins with the autoxidation of γ -terpinene to *p*-cymene and the subsequent hydroxylation to thymol (Alizadeh, 2013). Ruberto and Baratta (2000) confirmed that thymol and carvacrol molecules are indeed responsible for the antioxidant activity of many thymol- and carvacrol-containing EOs. Strong antioxidant activity was exhibited by the EO from *Foeniculum vulgare* also showed. Yoshioka and Tamada (2005) revealed that *Foeniculum vulgare* EO provided an inhibitory activity against platelet aggregation induced by ADP, arachidonic acid and collagen in guinea pig plasma. Similar findings were reported for aggregation of rabbit platelets. The biological activity of herbal EOs alongside with their antimicrobial activity influences the naturally occurring *Pseudomonas* spp. of freshwater fish, therefore the possible application of EO in aquaculture and food industry could be considered.

4. Conclusions

In conclusion, the EOs of EO-bearing plants used in the present study revealed a significant antimicrobial activity on the *Pseudomonas* spp. originated from freshwater fish with *Cinnamomum zeylanicum* to be the most effective. The results of the present study suggest that the EO is a potential source of natural antibacterial agents and may be used as natural compounds with anti-pseudomonal activity to improve the microbiological quality of freshly caught freshwater fish. The highest antioxidant activity was observed for *Cymbopogon nardus*, *Origanum vulgare*, *Foeniculum vulgare* and *Thymus serpyllum*.

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Table 6

Antioxidant activity of essential oils expressed as μg Trolox equivalent antioxidant capacity per mL of sample.

Essential oil	Antioxidant activity ($\mu\text{g TEAC}/\text{mL}$)
<i>Lavandula angustifolia</i> Mill.	54.76 \pm 0.38
<i>Cinnamomum zeylanicum</i> L.	55.60 \pm 2.79
<i>Pinus mugo</i> Turra	30.37 \pm 2.63
<i>Mentha piperita</i> L.	59.56 \pm 2.75
<i>Foeniculum vulgare</i> Mill.	76.74 \pm 0.45
<i>Pinus sylvestris</i> L.	45.81 \pm 1.13
<i>Satureja hortensis</i> L.	60.10 \pm 1.18
<i>Origanum vulgare</i> L.	83.47 \pm 1.10
<i>Pimpinella anisum</i> L.	28.45 \pm 3.44
<i>Rosmarinus officinalis</i> L.	42.08 \pm 0.68
<i>Salvia officinalis</i> L.	43.82 \pm 0.54
<i>Abies alba</i> Mill.	7.72 \pm 0.45
<i>Citrus aurantium</i> var. <i>dulce</i> L.	48.03 \pm 0.99
<i>Citrus sinensis</i> L. Osbeck.	66.65 \pm 3.58
<i>Cymbopogon nardus</i> L.	93.86 \pm 0.25
<i>Mentha spicata</i> var. <i>crispa</i> L.	55.18 \pm 1.88
<i>Thymus vulgaris</i> L.	65.45 \pm 1.09
<i>Carvum carvi</i> L.	17.88 \pm 0.81
<i>Thymus serpyllum</i> L.	74.28 \pm 1.09
<i>Ocimum basilicum</i> L.	67.07 \pm 0.47
<i>Coriandrum sativum</i> L.	39.38 \pm 0.75

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