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Composition and antibacterial activities of essential oils of seven *Ocimum* taxa

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ABSTRACT

GC/MS was used to identify compounds of essential oils from seven *Ocimum* taxa (*O. americanum* L., *O. basilicum* L., *O. campechianum* Mill., *O.* x *citriodorum* Vis., *O. kilimandscharicum* Baker ex Gürke and three botanical varieties and cultivars of *Ocimum basilicum* L.: 'Genovese', var. *difforme* and var. *purpurascens*). Preliminary screening of their antibacterial activity was done against a number of common pathogens (*Enterococcus faecalis*, *Enterococus faecium*, *Escherichia coli* 0157:H7, *Listeria monocytogenes*, *Listeria ivanovii*, *Proteus vulgaris*, *Staphylococcus aureus*, *Staphylococcus epidermis*) using the filter paper disc agar diffusion technique, while further analyses were done by modification of the disc diffusion method. A broad variation in the antibacterial properties of investigated essential oils was observed. *E. coli* 0157:H7 was inhibited by *O. basilicum* 'Genovese' essential oil, while *Ocimum americanum* and *Ocimum* x *citriodorum* essential oils were the most effective against *Enterococcus faecalis*, *Enterococcus faecium*, *P. vulgaris*, *S. aureus* and *S. epidermis*.

1. Introduction

Essential oils from aromatic and medicinal plants have been known to possess biological activity, notably antibacterial, antifungal and antioxidant activities (Baratta, Dorman, & Deans, 1998). Biological activity of essential oils depends on their chemical composition determined by genotype and influenced by environmental and agronomic conditions (Marotti, Dellacecca, Piccaglia, & Giovanelli, 1993). The genus *Ocimum* L. (*Lamiaceae*), collectively called basil, has long been acclaimed for its diversity as a source of essential oils, its flavour and delicacy as a spice, and its beauty and fragrance as an ornamental (Simon, Quinn, & Murray, 1990). Basil is used in traditional ceremonial rituals and as medicine, and contains biologically active constituents that are antimicrobial (Lachowicz et al., 1998; Thoppil, Tajo, & Minija, 1998), insecticidal (Umerie, Anaso, & Anyasoro, 1998), nematocidal (Vieira & Simon, 2000), fungistatic and antioxidant (Politeo, Jukic, & Milos, 2006). These properties can frequently be attributed to predominant essential oil constituents, such as methyl chavicol, eugenol, linalool, camphor and methyl cinnamate (Simon et al., 1990).

The problem of preserving food products is becoming more complex, due to the fact that the new products being introduced in the market require an ever longer shelf life and a higher degree of protection against pathogenic microorganisms (Marino, Bersani, & Comi, 2001). There is therefore a great interest for new methods of making food safe which have a natural or green image. One such possibility is the use of essential oils as antibacterial additives (Burt, 2004). Most studies investigating the action of whole essential oils against food spoilage organisms and foodborne pathogens agree that, generally, essential oils are slightly more active against Gram-positive than Gram-negative bacteria (Burt, 2004). The most interesting area of application of essential oils is the inhibition of growth and reduction in number of the more serious foodborne pathogens such as *Salmonella* spp. *Escherichia coli* 0157:H7 and *Listeria monocytogenes* (Burt, 2004). Until now, there is no standard method for studying the susceptibility of microorganisms to essential oils. The antimicrobial activity of different plant extracts and pure compounds can be measured using numerous *in vitro* assays e.g., agar or broth dilution methods as well as disk diffusion methods (Carson, Cookson, Farrelly, & Riley, 1995), the drop diffusion methods (Hili, Evans, & Veness, 1997), microtiter plates dilution method, and impedance (Marino et al., 2001). Each of these methods is based on a specific characteristic, such as the growth inhibition and changes in conductance. The agar disc diffusion method is a very popular and easy to use *in vitro* method for measuring anti-

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microbial activity that gives repeatable results (Kim, Marshall, & Wei, 1995).

Basil is rich in essential oil and has been the subject of numerous chemical studies (Grayer et al., 1996), but to the best of our knowledge, comparative antimicrobial (“*in vitro*” assay) and chemical composition studies of different *Ocimum* species are lacking. The present study was undertaken with the objective of identifying possible “candidates”, from seven different essential oils, that can be used in the food industry.

2. Materials and methods

2.1. Plant material

Research was carried out on seven *Ocimum* taxa, including five *Ocimum* species (*O. americanum* L., *O. basilicum* L., *O. champechianum* Mill., *O.* x *citriodorum* Vis., *O. kilimandscharicum* Baker ex Gürke) and three botanical varieties and cultivars of *Ocimum basilicum* (‘Genovese’, var. *purpurascens* and var. *difforme*). All investigated species, botanical varieties and cultivars, as well as their country of origin, are listed in Table 1. Seeds were obtained from the Collection of medicinal and aromatic plants of the Department of Seed Science and Technology, Faculty of Agriculture, University of Zagreb, Croatia. Seeds of all accessions were grown in a sterilized soil mix in a greenhouse. The seedlings were transplanted in the field. All the samples were collected during the flowering phase, and air dried leaves and flowers were used for isolation of essential oils.

2.2. Isolation of essential oils

One hundred grams of plant material and 500 ml water were placed in a Clevenger type apparatus. The essential oil was isolated by hydrodistillation for 3 h. The obtained essential oil was separated, dried over anhydrous sodium sulphate, and stored under argon in a sealed vial, at −20 °C before usage. The voucher specimen of the basil essential oil was deposited in the Laboratory of Biochemistry and Food Chemistry, Faculty of Chemical Technology, Split, Croatia.

2.3. Gas chromatography–mass spectrometry

The analyses of the volatile compounds were run on a Hewlett Packard GC–MS system (GC 5890 series II; MSD 5971A, Hewlett Packard, Vienna, Austria). Two columns of different polarity were used: a HP-101 column (Methyl silicone fluid, Hewlett Packard; 25 m × 0.2 mm i.d., film thickness 0.2 μm) and a HP-FFAP column (Free Fatty Acid Phase, Hewlett Packard; 50 m × 0.32 mm i.d., film thickness 0.52 μm). Oven temperature was programmed as following: isothermal at 70 °C for 4 min, then increased to 180 °C, at a rate of 4 °C/min and subsequently held isothermal for 15 min (for HP-FFAP column); isothermal at 70 °C for 2 min, then increased to 200 °C, at a rate of 3 °C/min and held isothermal for 15 min (for HP-101 column). The carrier gas was helium (1 ml/min). The injection port temperature was 250 °C and the detector temperature was 280 °C. Ionization of the sample components was performed in the EI mode (70 eV). Injected volume was 1 μl. The linear retention indices for all the compounds were determined by co-injection of the samples with a solution containing the homologous series of C_{12}–C_{30} *n*-alkanes (Van Den Dool & Kratz, 1963). The individual constituents were identified by their identical retention indices, referring to compounds known from literature data (Adams, 1995) and also by comparing their mass spectra with spectra of, either, the known compounds or with those stored in the Wiley mass spectral database (Hewlett Packard, Vienna, Austria).

2.4. Bacterial strains

Cultures of Gram-positive bacteria, *Staphylococcus aureus*, *Staphylococcus epidermis*, *L. monocytogenes*, *Listeria ivanovii*, *E. faecalis*, *E. faecium* and Gram-negative bacteria, *E. coli* O157:H7 and *Proteus mirabilis*, were obtained from the collection at the Istituto di Ispezione degli Alimenti di Origine Animale at the University of Milan, and were maintained on Brain Heart Infusion Agar (Oxoid, Milan, Italy) at 4 °C.

2.5. Determination of antibacterial activity

The agar disc diffusion method was employed for the screening of antimicrobial activities of basil essential oils. The test was performed in sterile Petri dishes (90 mm diameter) containing BHI agar medium (Oxoid, Milan, Italy). Briefly, suspension in BHI of the tested microorganism (0.1 ml of 10^7 CFU/ml) was spread on the BHI agar. The oils absorbed on sterile paper discs (5 μl per disc of 6 mm diameter) were placed on the surface of the media previously inoculated (1 μg per Petri dish). One filter paper disc was placed per Petri dish in order to avoid a possible additive activity. Every dish was sealed with laboratory film to avoid evaporation and then incubated at 37 °C for 24 h, followed by the measurement of the zone diameter of the inhibition expressed in mm. All tests were performed in triplicate. The scale of measurement was the following: >15 mm zone of inhibition was strongly inhibitory; 10–15 mm zone of inhibition was moderately inhibitory and <10 mm was not inhibitory.

The most active essential oils were selected for further study by a modification of the disc – diffusion method. Briefly, suspension in BHI of the tested microorganism (0.1 ml of 10^7 CFU/ml) was spread on the BHI agar. Different concentrations (100, 200, 300, 400 and 500 ppm) of oils absorbed on sterile paper discs (5 μl per disc of 6 mm diameter) were placed on the surface of the media previously inoculated. One filter paper disc was placed per Petri dish in order to avoid a possible additive activity. Every dish was sealed with laboratory film to avoid evaporation and then incubated at 37 °C for 24 h, followed by the measurement of the zone diameter of the inhibition expressed in mm. All tests were performed in triplicate. Minimum inhibitory concentrations (MICs) were recorded for the most active essential oils.

Table 1
Accession number, species, taxon and country of origin of basil accessions included in the analysis.

No.	Accession no.[a]	Species	Taxon	Country of origin
1	MAP00594	*O. americanum*	–	Germany
2	MAP00294	*O. basilicum*	‘Genovese’	Croatia
3	MAP00559	*O. basilicum*	var. *difforme*	Italy
4	MAP00335	*O. basilicum*	var. *purpurascens*	Russia
5	MAP01624	*O. campechianum*	–	Germany
6	MAP00156	*O.* x *citriodorum*	–	Turkey
7	MAP01636	*O. kilimandscharicum*	–	Germany

[a] Accession number from The Collection of Medicinal and Aromatic Plants, Zagreb, Croatia available at: cpgr.agr.hr.

Table 2
Chemical composition of the essential oils (% total peak area) of seven basil taxa (*O. basilicum* 'Genovese', *O. basilicum* var. *difforme*, *O. basilicum* var. *purpurascens*, *O. americanum*, *O.* x *citriodorum*, *O. campechianum*, *O. kilimandscharicum*).

Identified compound	*RI*[a] *HP-FFAP*	*RI*[a] *HP-101*	*O. americanum*	*O. basilicum* 'Genovese'	*O. basilicum* var. *difforme*	*O. basilicum* var. *purpurascens*	*O. campechianum*	*O.* x *citriodorum*	*O. kilimandscharicum*
α-Pinene		932	–	0.23	0.20	–	0.63	–	0.79
β-Pinene		986	–	0.56	0.49	–	–	–	–
Sabinene		990	–	–	0.15	–	0.25	–	–
β-Myrcene	1207	998	–	0.63	0.36	–	0.40	0.26	1.82
α-Phellandrene	1212	1017	–	–	–	–	–	–	0.42
α-Terpinene	1225	1020	–	–	0.15	–	–	–	0.62
Limonene	1240	1005	–	–	0.32	–	–	–	9.46
1,8-Cineole	1249	1006	–	7.23	6.17	–	20.31	0.88	14.63
γ-Terpinene	1278	1072	–	–	0.39	–	–	–	1.49
trans-β-Ocimene	1280	1036	–	–	0.16	–	0.59	–	–
δ-3-Carene	1281	1025	–	–	–	–	–	–	0.65
p-Cymene	1296	1036	–	–	0.17	–	–	–	0.43
6-Methyl-5-hepten-2-one	1367	–	1.25	–	–	–	–	1.14	–
α-Thujone	1413	1113	–	–	–	0.36	–	–	–
Fenchone	1414	–	0.90	–	–	–	–	–	–
cis-Linalool oxide	1454	1059	1.01	0.38	0.16	–	–	–	–
trans-Linalool oxide	1481	1073	0.94	0.27	0.15	–	–	–	–
Bicycloelemene	1495	–	–	–	–	–	1.14	–	–
Octyl acetate	1497	–	–	–	–	–	–	1.10	–
Camphor	1530	1109	–	0.49	0.60	–	–	–	56.97
Linalool	1557	1092	12.15	66.40	20.82	–	5.13	7.20	0.35
α-Terpinolene	1558	1097	–	–	–	–	1.15	0.44	1.58
Octanol	1564	991	0.31	–	–	–	–	–	–
Bornyl acetate	1588	1252	–	0.93	0.33	–	–	–	–
α-Farnesene	1597	–	1.52	–	–	–	–	1.71	–
α-Bergamotene	1598	1407	–	7.96	6.84	–	–	0.36	–
β-Elemene	1603	1364	–	1.00	–	–	11.10	–	–
Camphene hydrate	1604	–	–	–	–	–	–	–	0.40
Terpinen-4-ol	1609	1164	–	–	2.43	–	–	–	6.59
β-Caryophyllene	1613	1385	1.96	0.46	–	0.75	14.00	2.13	–
α-Cubebene	–	1465	–	–	0.14	–	–	–	–
γ-Elemene	1651	–	–	–	–	–	1.74	–	–
Alloaromadendrene	1661	1450	–	0.22	0.12	–	1.14	–	–
Estragole	1679	1177	0.65	–	47.52	94.57	–	1.12	0.30
α-Humulene	1682	1417	–	–	–	–	2.68	–	–
β-Farnesene	1693	1452			0.33				
β-Cubebene	–	1490	–	1.10	0.23	–	–	–	–
Neral	1700	1231	20.16	–	–	–	–	21.80	–
α-Terpineol	1701	1176	1.37	0.82	0.76	–	1.54	0.67	1.56
trans-Anethole	1702	1283	–	–	–	0.23	–	–	–
Borneol	1707	–	–	–	–	–	–	–	t
Germacrene D	1719	1471	–	0.37	0.64	0.49	–	–	1.22
Calarene	1723	–			t				
Neoalloocimene	1724	–	–	–	0.22	–	–	–	–
Neryl acetate	1732	1373	1.06	–	–	–	–	–	–
β-Selinene	1733	1495	–	–	–	–	2.71	–	–
β-Bisabolene	1738	–	–	–	–	0.50	–	–	–
Bicyclogermacrene	1746	–	–	–	0.21	–	–	–	–
Geranial	1757	1270	28.58	–	–	–	–	31.21	–
Geranyl acetate	1760	1396	0.32	–	–	–	–	0.86	–
Citronellol	1768	–	0.25	–	–	–	–	–	–
α-Amorphene	1771	1489	–	1.59	1.21	–	–	–	–
α-Bisabolene	1784	1506	2.04	–	–	1.12	–	1.87	–
Nerol	1812	1237	7.15	–	–	–	–	14.58	–
Germacrene B	1844	–	–	–	–	–	1.43	–	–
Geraniol	1859	1294	0.58	–	–	–	–	5.09	–
(Z) Methyl cinnamate	1926	1281	–	–	0.14	–	–	–	–
Caryophyllene oxide	2001	1576	7.12	–	–	–	8.25	3.69	–
Nor-copaanone	2013	–	0.17	–	–	–	–	–	–
Methyl eugenol	2025	1378	–	0.31	0.13	1.23	2.91	–	–
Cadina-1,4-diene	2069	–	–	0.18	–	–	–	–	–
(E) Methyl cinnamate	2094	1364	–	–	1.21	–	–	–	–
Spathulenol	2131	1580	–	0.52	0.26	–	7.79	–	–
eugenol	2177	1368	0.57	8.26	–	0.43	5.53	0.83	–
α-Cadinol	2263	1614			6.80				
2-Methyl-2-propenoic acid	2322	–	0.79	–	–	–	–	–	–
Methyl crotonate	2394	–	2.42	–	–	–	–	–	–
Chavicol	2434	–	–	–	–	0.31	–	–	–
Benzoic acid	2570	–	–	–	–	–	–	2.96	–
Total			93.27	99.91	99.8	99.99	90.42	99.90	98.85

–, not identified.
t, trace (<0.1%).
[a] Retention indices relative to C_{12}–C_{30} *n*-alkanes on polar HP-FFAP and apolar HP-101 column.

2.6. Data analysis

Principal Component Analysis (PCA) was applied in order to examine the interrelationships between seven basil taxa and their essential oil constituents. Eigenvalues and eigenvectors were calculated using a correlation matrix among 69 chemical compounds as input, and the three-dimensional PCA biplot, including both taxa and compounds, was generated using NTSYS-pc 2.1 (Rohlf, 2000).

3. Results

3.1. Chemical composition of the essential oils

Using GC/MS analyses a total of 69 volatiles were identified as constituents of investigated essential oils across seven basil accessions. In the essential oil of *O. basilicum* var. *difforme* we were able to identify the highest number of different compounds (32 compounds), and in *O. basilicum* var. *purpurascens* the lowest (10 compounds). In each accession, identified compounds represent more than 90% of the total oil (Table 2), while compounds representing more than 10% of the total oil (calculated as% peak area) were considered as major.

Table 3
Pearson correlation coefficients between nine major compounds of basil essential oils and scores of the first three principal components.

Label	Major compound	Principal components		
		PC1	PC2	PC3
C01	1,8-Cineole	0.85	0.35	0.26
C02	Camphor	0.46	−0.53	0.70
C03	Caryophyllene	0.48	0.86	0.10
C04	Estragol	0.04	−0.25	−0.39
C05	Geranial	−0.87	0.15	0.29
C06	Linalool	−0.02	−0.13	−0.67
C07	Neral	−0.87	0.15	0.29
C08	Nerol	−0.74	0.12	0.23
C09	β-Elemene	0.49	0.86	0.05
	Eigenvalue	18.69	15.77	12.76
	% of variance	26.32	22.21	17.97
	Cumulative%	26.32	48.53	66.50

The results obtained from Principal Component Analysis (PCA) revealed the existence of a high chemical variability within the essential oils of seven basil taxa. The first three principal components explained 66% of total variance and revealed a clear differentiation among the taxa along axes (Table 3). Fig. 1 shows the relative position of both taxa and compounds in a three-dimensional continuous space defined by the first three principal components (PC). The first PC, which accounted for 26% of total variance, was positively correlated with 1,8-cineole (0.85), a major compound found in essential oils of *Ocimum campechianum* and *Ocimum kilimandscharicum*, and negatively with neral (−0.87), geranial (−0.87), and nerol (−0.74), major compounds found in essential oils of *Ocimum americanum* and *O.* x *citriodorum*. Caryophyllene and β-elemene, major compounds of *O. campechianum*, showed high correlations with the second PC (0.86 in both cases), explaining 22% of total variance. The third PC accounting for an additional 17% of total variance, was positively correlated with camphor (0.70) found in *O. kilimandscharicum*, and negatively with linalool (−0.67), a major compound found in essential oils of *O. basilicum* 'Genovese', *O. basilicum* var. *difforme* and *O. americanum*. Only in *O. kilimandscharicum* oil we have identified a high concentration of limonene (9.46%) and terpinen-4-ol (6.59%). It is interesting to note that in *O. basilicum* var. *purpurascens* estragol represented 94.57% of the identified compounds. In addition, 29 more constituents of essential oils were identified in concentrations higher than 1%, and 23 further compounds were found in

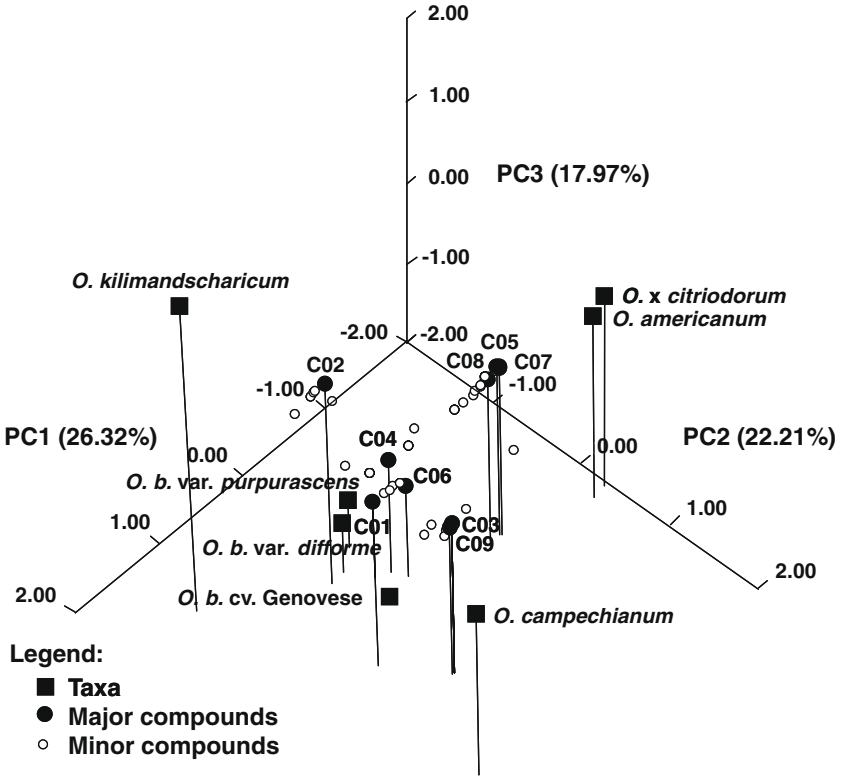

Fig. 1. Biplot of three principal components based on 69 chemical compounds identified in essential oils of seven basil taxa (*O. basilicum* 'Genovese', *O. basilicum* var. *difforme, O. basilicum* var. *purpurascens, O. americanum, O.* x *citriodorum, O. campechianum, O. kilimandscharicum*). Major compounds: C01 – 1,8-cineole; C02 – camphor; C03 – caryophyllene; C04 – estragol; C05 – geranial; C06 – linalool; C07 – neral; C08 – nerol; C09 – β-elemene.

concentrations less than 1%. Linalool and α-terpineol were identified in all samples except for the oil of *O. basilicum* var. *purpurascens*.

3.2. Antibacterial activity

A broad variation in the antibacterial properties of investigated essential oils was observed (Table 4). The results indicated in Table 4 represent the zone of inhibition including the diameter (6 mm) of the paper disk. The greatest effectiveness was achieved by the essential oils from *O. americanum*, *O. basilicum* 'Genovese' and *O.* x *citriodorum*. The essential oil of *O. basilicum* var. *purpurascens* showed the weakest antimicrobial activity, while the essential oils of *O. basilicum* var. *difforme*, *O. campechianum* and *O. kilimandscharicum* also weakly inhibited the development of bacteria.

More precise data on the antimicrobial properties of three selected essential oils were obtained through a modification of the disc diffusion method. *E. coli* 0157:H7 was inhibited by *O. basilicum* 'Genovese' essential oil, minimum inhibitory concentration being 400 ppm. *O. americanum* and *O.* x *citriodorum* essential oils were the most effective against *E. faecalis*, *E. faecium*, *Proteus vulgaris*, *S. aureus* and *S. epidermis* (Fig. 2), with MIC values ranging from 200 to 300 ppm for *O.* x *citriodorum*, and from 200 to 500 ppm for *O. americanum* essential oil. The growth of *L. ivanovi* was inhibited by *O.* x *citriodorum* essential oil, but not by *O. americanum* oil, considering investigated concentrations.

4. Discussion

The chemical composition of the essential oils of the studied *Ocimum* taxa varies greatly. Considering that all of the plants were cultivated under the same conditions the impact of the ecological factors on essential oil composition variability has been excluded.

Literature reveals that about 45 compounds are found in volatile oils of *O. basilicum* with the major compounds being linalool, eugenol, methyl chavicol, methyl eugenol, geraniol, geranial and neral (Grayer et al., 1996). The essential oil of *O. basilicum* 'Genovese' has been well studied (Suppakul, Miltz, Sonneveld, & Bigger, 2003) and the concentration and composition in our study is similar to other studies (Hussain, Anwar, Hussain Sherazi, & Przybylski, 2008; Zheljazkov, Callahan, & Canterll, 2008; Zheljazkov, Canterll, Tekwani, & Khan, 2008). The investigated *O. basilicum* 'Genovese' belongs to the linalool–eugenol chemotype (Grayer et al., 1996; Zheljazkov et al., 2008). The oil of *O. basilicum* var. *purpurascens* represents a true estragol chemotype suspected to be carcinogenic and genotoxic (Heberer et al., 2007; Tricker & Preussmann, 1990). *O. basilicum* var. *difforme* represent estragol–linalool chemotype while *O. campechianum* essential oil has the highest concentration of 1,8-cineole (eucalyptol) of the investigated plants. Eucalyptol is used in flavourings, fragrances and cosmetics. Camphor represents the major compound of the essential oils of *O. kilimandscharicum*. In this oil we have also identified limonene, one of the most common terpenes in nature. It is a major constituent in

Table 4
Zones of growth inhibition (mm) showing antibacterial activity of *Ocimum* essential oils (diameter of the zone of inhibition includes paper disk diameter, 6 mm), (Mean ± S.D.).

Bacterial species	*Ocimum* species						
	O. americanum	*O. basilicum 'Genovese'*	*O. basilicum* var. *difforme*	*O. basilicum* var. *purpurascens*	*O. campechianum*	*O.* x *citriodorum*	*O. kilimandscharicum*
L. monocytogenes	12.00 ± 0.00	11.00 ± 1.00	8.00 ± 0.00	6.00 ± 0.00	8.00 ± 0.00	14.00 ± 0.00	6.00 ± 0.00
L. ivanovii	13.67 ± 1.53	13.00 ± 1.00	9.00 ± 1.00	6.00 ± 0.00	12.67 ± 0.58	20.00 ± 0.00	8.00 ± 0.00
E. faecalis	15.67 ± 1.53	11.67 ± 0.58	10.00 ± 0.00	6.00 ± 0.00	10.00 ± 0.00	16.00 ± 1.00	6.00 ± 0.00
E. faecium	15.67 ± 1.53	10.67 ± 0.58	7.67 ± 0.58	6.00 ± 0.00	9.00 ± 0.00	15.67 ± 0.58	6.00 ± 0.00
P. vulgaris	17.67 ± 0.58	11.33 ± 1.15	9.67 ± 0.58	6.00 ± 0.00	11.33 ± 1.15	22.00 ± 0.00	6.00 ± 0.00
S. aureus	33.33 ± 2.89	10.00 ± 0.00	9.67 ± 1.53	7.67 ± 0.58	11.67 ± 0.58	36.33 ± 1.15	6.00 ± 0.00
S. epidermis	22.67 ± 0.58	13.00 ± 0.00	10.00 ± 0.00	8.00 ± 0.00	11.00 ± 0.00	30.00 ± 0.00	6.00 ± 0.00
E. coli 0157:H7	10.67 ± 1.53	17.67 ± 0.58	10.67 ± 0.58	7.00 ± 0.00	11.67 ± 0.58	10.00 ± 0.00	8.33 ± 0.58

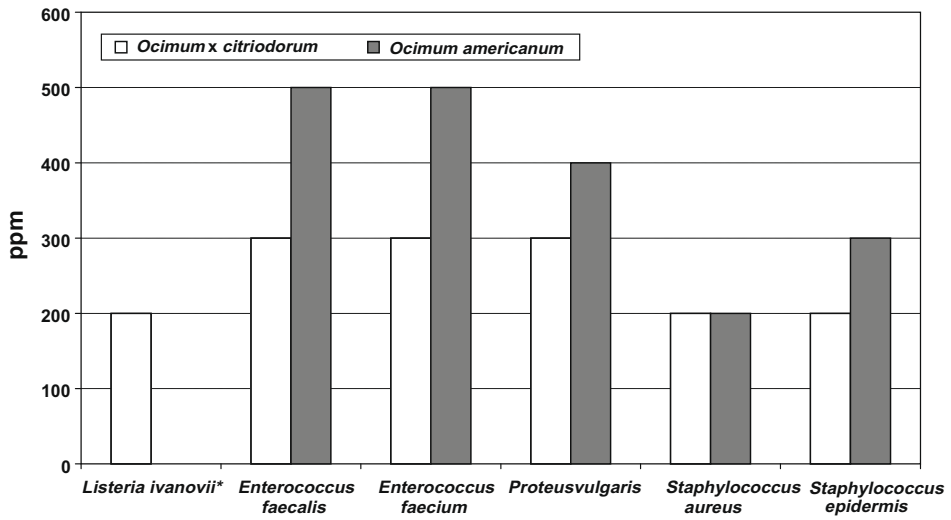

Fig. 2. The minimum inhibitory concentrations (MICs) of *O. americanum* and *O.* x *citriodorum* essential oils on the selected pathogens. * *O. americanum* oil showed no inhibitory effect on *L. ivanovii* at investigated concentrations.

several citrus oils (orange, lemon, mandarin, lime and grapefruit), eucalyptol (e.g., essential oil of laurel, *Luarus nobilis* L.) and terpinen-4-ol, the main component of the essential oil of tea tree (*Melaleuca alternifolia* L.), and suppresses inflammatory mediator production by activated human monocytes. A geranial (E isomer of citral), and neral (Z isomer of citral) as well as three monoterpenoid alcohol linalool, nerol and geraniol were the major compounds of *O. americanum* and *O.* x *citriodorum* essential oils. Grayer et al. (1996) also found citral (=geranial + neral) to be a major constituent in *O.* x *citriodorum*. Similar chemical profiles of *O. americanum* and *O.* x *citriodorum* could be explained by their genetic similarity. *O. americanum* is considered to be the most probable parental species of *O.* x *citriodorum* (Paton & Putievsky, 1996).

Concerning the antibacterial activity of *O. basilicum* accessions, only *O. basilicum* 'Genovese' showed high antimicrobial activity against *E. coli* 0157:H7. These data are not in agreement with Hussain et al. (2008). Using the same antimicrobial method, they have shown that *O. basilicum* oil was effective against *S. aureus* and *Bacillus subtilis*. Nevertheless, in agreement with our results are those of Suppakul et al. (2003) who reported that all films containing linalool or methyl chavicol showed positive antimicrobial activity against *E. coli* in the agar disc diffusion test, but not against *S. aureus* and *Saccharomyces cerevisiae*.

O. americanum and *O.* x *citriodorum* essential oils showed the highest antibacterial activity. Dalleau, Cateau, Bergès, Berjeaud, and Imbert (2008) demonstrated the influence of geraniol on the growth of three *Candida* species. Concentrations over 100 ppm of citral and thermal treatment longer than 16 min allowed a 90% probability of stability for soft drinks bottles inoculated with 10^5 *S. cerevisiae* CFU/bottle (Belletti et al., 2007), but citral reduced only 17% airborne microbes (Sato, Krist, & Buchbauer, 2006).

The variation of the antimicrobial activity could be correlated to chemical composition variability (Burt, 2004). Some studies have concluded that whole essential oils have a greater antibacterial activity than the major components mixed (Gill, Delaquis, Russo, & Holley, 2002), indicating that minor components are critical for the activity and may have a synergistic effect. In the presented work, it is possible to notice that the specific composition of the *O. americanum* and *O.* x *citrodiorum*, as well as *O. basilicum* 'Genovese' oil, could have an important antimicrobial role. The obtained results are considered encouraging, opening the possibility of using the *O. americanum*, *O. basilicum* 'Genovese' and *O.* x *citriodorum* essential oils, or some of their components, as natural food preservatives. Further work is necessary to explore the efficacy and palatability of suitable concentrations of these essential oils in foods, bearing in mind also the possible interactions of essential oil with food ingredients.

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