ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 9:1 (2015) 62-75

records of natural products

Chemical Composition, Antioxidant and Antimicrobial Activities of the Essential Oils of Twelve *Ocimum basilicum* L. Cultivars Grown in Serbia

Damir Beatović[1], Dijana Krstić-Milošević[2*], Snežana Trifunović[3], Jovana Šiljegović[2], Jasmina Glamočlija[2], Mihailo Ristić[4] and Slavica Jelačić[1]

[1] *Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia*
[2] *Institute for Biologica lResearch 'Siniša Stanković', University of Belgrade, Bulevar Despota Stefana 142, 11000 Belgrade, Serbia*
[3] *Faculty of Chemistry, University of Belgrade, StudentskiTrg 16, 11000 Belgrade, Serbia*
[4] *Institute for Medicinal Plant Research 'Josif Pančić', Tadeuša Košćuška 1, 11000 Belgrade, Serbia*

(Received August 24, 2012; Revised October 22, 2013; Accepted June 20, 2014)

Abstract This study evaluated the chemical composition, antioxidant and antimicrobial activities of the essential oils of twelve *Ocimum basilicum* L. cultivars grown in Serbia. The essential oils were obtained by hydrodistillation and analyzed by gas chromatography-flame ionization detection and GC-mass spectrometry. The oil yields ranged from 0.65 to 1.90 %. A total of 75 compounds were identified as constituents of analyzed essential oils. GC/MS analyses revealed that a majority of the examined basil cultivars belonged to the "linalool chemotype". The results of the DPPH assay showed a very high antioxidant capacity of the basil oils, which was especially high for Blue Spice cultivar (IC_{50} = 0.03 µg/mL). Significant antimicrobial activity was shown for all the tested oils. Essential oil of Compact cultivar was the most active against the bacterium *Micrococcus flavus,* with an MIC value of 0.009 µg/mL while Osmin cultivar exhibited the strongest antifungal activity with MIC values ranging from 0.08-1.07 µg/mL. All the examined essential oils showed a 10- to 100-fold greater ability to inhibit fungal growth compared to commercial antifungal agents.

Keywords: Basil cultivars; Essential oil; DPPH; Antibacterial activity; Antifungal activity.

1. Introduction

The genus *Ocimum* L. (Lamiaceae) consists of 30-160 species, distributed throughout the tropical regions of Asia, Africa and Central and South America [1-2]. Large numbers of species and varieties, differences in essential oil composition and complex taxonomy are characteristic of the genus *Ocimum* [3-5]. *Ocimum basilicum* L. (common basil) is widely used in the culinary arts and in the food processing industry. Basil is known as digestive stimulant. It is used as an aroma to improve the taste, smell and digestibility of food [2]. Secondary metabolites from *Ocimum* species possess exceptional biological activity and have antioxidant [6-8] and antimicrobial [9-12], bactericidal [13-14], repellent [15-16], anticonvulsant [17], chemopreventive and radioprotective effects [18-19].

Basil essential oils contain a broad array of chemical compounds depending on variations in chemotypes, flower and leaf colours, aroma and particularly the origin of the plant. Moreover, the aromatic and morphological character of plants is greatly influenced by environmental conditions and

* Corresponding author: E-mail address: dijana@ibiss.bg.ac.rs; Fax: +381-11-2761433

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 09/23/2014 EISSN: 1307-6167

agronomic techniques [12-20]. The chemical composition of essential oils of *Ocimum* species has been well studied. As prevalent components many basil essential oils contained monotherpene derivatives (camphor, limonene, 1,8-cineole, linalool, geraniol) and phenylpropanoid derivatives (eugenol, methyleugenol, chavicol, estragole, methyl-cinnamate) [4, 12, 21, 22]. Different chemotypes of basil have been recognised based on the predominant essential oil constituents (*e.g.* linalool, methyl chavicol, methyl cinnamate, methyl eugenol, eugenol) [22-23].

Basil is cultivated throughout Serbia, mostly in home gardens. Through many years of systematic collection, a large number of cultivars have been compiled. The collection process has introduced many new cultivars and has enlarged the number of basil accessions in our gene bank [24].

There are only few data regarding the chemical characterisation of essential oils of the basil cultivars grown in Serbia. Characterization of the volatile composition of essential oils of some Lamiaceae species is reported by Božin et al.; however, these authors did not specify which cultivar of basil has been analysed [25]. Recently, Beatović et al. determined the chemical composition of the essential oil of *Ocimum sanctum* L. grown in Serbia [26]. To our knowledge these are only reports about basil cultivated in Serbia.

This paper presents the chemical composition of the essential oils of twelve basil cultivars grown in Serbia in order to provide data for basic research needs and for practical use. The purpose of our study was to determine the essential oil chemotypes of these cultivars using classification system proposed by Lawrence [22] and Grayer [23], to estimate their radical-scavenging capacity using the DPPH method, as well as to evaluate their ability to inhibit the growth of eight bacterial strains and seven micromycetes which are known pathogens in humans, plants and animals.

2. Materials and Methods

2.1. Plant material and cultivation conditions

The seeds of twelve *Ocimum basilicum* L. cultivars were planted in the greenhouse in March 2009, in polystyrene containers with 40 holes, and stuffed with commercial substrate [27]. Seedling production continued for 48 days. In May 2009, nursery plants were transplanted into the experimental field in a randomised complete block design with four replicates in a 50x50 cm model at Surčin, Serbia (44°49' N, 20°17' S, altitude 96 m, degraded chernozem). Harvesting of the plants was performed at the full flowering stage in June 2009. Plants were air-dried in the shade, then packed and stored.

Seeds of *O. basilicum* cultivars were obtained from the Institute for Crop Sciences of the Faculty of Agriculture in Belgrade and the Plant Gene Bank of Serbia where plants were designated and deposited under DB codes (Table 1).

2.2. Essential oil isolation

The essential oils of the aerial parts of *O. basilicum* cultivars were obtained by hydrodistillation (for 2 h) using a Clevenger type apparatus, according to the standard procedure reported in the Yugoslav Pharmacopeia IV [28]. Oil samples were dried over anhydrous sodium sulphate, filtered and stored at 4°C prior to chemical analyses, antioxidant and antimicrobial analyses.

2.3. Analysis of essential oils

GC/FID analyses of the oils were carried out on an HP-5890 Series II GC apparatus [Hewlett-Packard, Waldbronn (Germany)], equipped with a split-splitless injector, an automatic liquid sampler (ALS), attached to an HP-5 column (25 m x 0.32 mm, 0.52 µm film thickness) and fitted to the flame ionisation detector (FID). The carrier gas flow rate (H_2) was 1 mL /min, the split ratio 1:30, the injector and detector temperatures were 250°C and 300°C, respectively, while the column temperature was linearly programmed from 40 - 260°C (at a rate of 4°C min^{-1}). The solutions of essential oil samples in ethanol (10 µL/mL) were consecutively injected by ALS (1 µL, split mode). The area percent reports obtained by GC/FID were used as the basis for quantification purposes. The same analytical conditions as those mentioned for GC/FID were employed for gas chromatography/mass spectrometry (GC/MS) analysis, using an HP G 1800C Series II GCD system [Hewlett-Packard, Palo Alto, CA (USA)] and an HP-5MS (30 m x 0.25 mm, 0.25 µm film thickness) column. Instead of

hydrogen, helium was used as the carrier gas. The transfer line was heated at 260°C. The mass spectra were acquired in EI mode (70 eV), in the *m/z* range of 40-450. The sample solutions in ethanol (10 μL/mL) were injected by ALS (1 μL, split mode). The components of the oil were identified by matching their mass spectra and retention indices with those obtained from authentic samples and/or NIST/Wiley spectra libraries, using different types of searches (PBM/NIST/AMDIS) and literature data [29-30].

2.4. Radical scavenging activity

The free radical scavenging activity of the essential oils of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical was carried out according to a previously described procedure [31], with some modifications. The antiradical capacity of each essential oil was evaluated using a serial dilution of the sample, in order to obtain a large spectrum of sample concentrations. Essential oils were dissolved in methanol (500 μL) and mixed with 500 μL of a 150 μM DPPH solution. The resulting mixture (1 mL) was shaken vigorously and held for 20 minutes at room temperature in the dark. Thereafter, the absorbance of the samples was measured spectrophotometrically at 517 nm. All tests were performed in triplicate, with Trolox and ascorbic acid as positive controls.

The percentage of inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = [(A_0-A_1)/A_0] \times 100$$

where A_0 is the absorbance of the control solution (blank without samples) and A_1 is the absorbance of the samples.

2.5. Statistical analysis

All calculations of the data from DPPH assay and statistical analysis were performed by Prism 4.0 for Windows (GraphPad Soft. Inc., USA). The IC_{50} values (concentration of tested compounds inducing 50% inhibition of DPPH radical) were calculated by linear regression plots where the abscissa represented the concentration of the tested samples and the ordinate represented the average percent of inhibition activity from three separate tests. Data are presented as mean ± standard deviation (SD).

2.6. Antimicrobial activity

2.6.1. Microorganisms and culture conditions

For the bioassays, eight bacterial species were used, including Gram (+) bacterial species: *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), *Staphylococcus aureus* (ATCC 6538) and *Enterococcus faecalis* and Gram (-) species: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311) and *Listeria monocytogenes* (NCTC 7973). For the antifungal bioassays, the following fungi were used: *Aspergillus fumigatus* (plant isolate), *Aspergillus niger* (ATCC 11730), *Aspergillus versicolor* (ATCC 11730), *Aspergillus ochraceus* (ATCC 12066), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112) and *Trichoderma viride* (IAM 5061). All of the organisms tested were from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stankovic", Belgrade, Serbia. Bacteria were maintained on Mueller–Hinton agar (MHA) and micromycetes were maintained on malt agar (MA). The cultures were stored at +4°C and subcultured once a month [32].

2.6.2. Microdilution method

In order to evaluate the antimicrobial activity of the isolated essential oils, a modified microdilution technique was used [33-35]. Bacterial species were cultured overnight at 37°C in a tryptic soy broth (TSB) medium. The fungal spores were washed from the surface of MA plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The bacterial and fungal cell suspensions were adjusted with sterile saline to a concentration of 1.0×10^5 in a final volume of 100 μL per well. The inocula were stored at +4°C for further use. Dilutions of the inocula were cultured on solid MHA medium for bacteria and solid MA medium for fungi to verify the absence of contamination and to check the validity of the inoculum.

The determination of minimum inhibitory concentrations (MICs) was performed by a serial dilution technique using 96-well microtitre plates. The investigated essential oils were added in TSB medium (bacteria) or TSB malt medium (fungi) with inoculum. The microplates were incubated for 48 h at 37°C for bacteria or 5 days at 25°C for fungi. The lowest concentration without visible growth (determined using a binocular microscope) was defined as the MIC.

The minimum bactericidal (MBCs) and minimum fungicidal concentrations (MFCs) were determined by serial sub-cultivation of a 2 µL sample into microtitre plates containing 100 µL of broth per well and further incubation for 48 h at 37°C or 5 days at 25°C, respectively. The lowest concentration with no visible growth was defined as the MBC/MFC, indicating 99.5% killing of the original inoculum. Streptomycin and ampicillin (0.1–2 mg/mL) were used as reference antibacterial agents and bifonazole and ketoconazole (0.1–2 mg/mL) as the positive controls for antifungal activity. Commercial antibiotics and fungicides were dissolved in 5% DMSO (dimethyl sulphoxide).

3. Results and Discussion

3.1. Essential oil analysis

All extracted essential oils exhibited a light yellow colour. The essential oil content in the dried aerial parts ranged from 0.65% in Blue Spice and Fino Verde cultivars to 1.90% in Purple Opal cultivar (Table 1). Literature data show differences in essential oil content of *Ocimum* species. Politeo et al. found that the yield of essential oils from the aerial parts of *O. basilicum* was 0.62% [36], while basil grown in Serbia and Montenegro had an essential oil yield of 0.37% [25]. Salles et al. reported the amount of essential oil obtained from five *Ocimum* species varied from 0.5% to 3.5% [6]. Furthermore, in four *Ocimum* species grown in Tanzania [37], the essential oil yields ranged from 0.5% to 4%. Great variations in the essential oil content of *O. basilicum* across geographic regions might be attributed to variable agroclimatic conditions and different agronomic techniques for cultivating [12, 20]. Given these facts, our results are in accordance with previously published reports on basil oils.

Table 1. *Ocimum basilicum* L.cultivars data and essential oils yield

Cultivar (genotype)	Voucher specimen number	Oil yield (%)	Chemotype
Blue Spice	DB-009	0.65	Bisabolene
Fino Verde	DB-003	0.65	Linalool
Holandjanin	DB-004	0.68	Linalool
Compact	DB-005	0.85	Linalool
Genovese	DB-001	0.92	Linalool
Purple Opal	DB-010	1.90	Linalool
Lattuga	DB-002	0.73	Linalool
Osmin	DB-012	1.00	Linalool
Cinnamon	DB-006	0.95	Linalool-Methyl cinnamate
Purple Ruffles	DB-011	1.10	Linalool
Lime	DB-007	0.85	Geranial-Neral
Siam Queen	DB-008	1.05	Methyl chavicol

Table 2 shows the chemical components of the twelve analysed basil essential oils. A total of 75 compounds were identified as constituents of the essential oils. Fino Verde and Holandjanin were the cultivars with the highest number of essential oil components (63 and 62, respectively). The oxygenated monoterpenes were predominant in eight basil cultivars (41.3 - 73.2%), with linalool as the main constituent (30.3 - 58.6%). According to chemotype classification system proposed by Lawrence [22] and Grayer [23], which is based on the prevalent aromatic or the components major than 20%, these cultivars belonged to the "linalool chemotype" (Table 1). The essential oil from the Cinnamon cultivar was characterised by an almost equal content of linalool and *trans*-methyl cinnanamate (31.8% and 31.4%, respectively) and could be classified as the "linalool-methyl cinnanamate" subtype. The three remaining basil cultivars completely differed in their essential oil composition from those described as the "linalool chemotype". The essential oil extracted from the

Siam Queen cultivar was characterised by a high content of methyl chavicol (83.6%), which defined its chemotype as "methyl chavicol". Blue Spice essential oil contained sesquiterpene β-bisabolene as the main compound (23.8%). The Lime essential oil composition differed from the others in terms of its geranial, neral, nerol, β-caryophyllene and β-caryophyllene oxide content at 16.1, 12.8, 5.8, 11.0 and 7.0%, respectively. Therefore, these two cultivars could not be classified into existing chemotypes.

Our results show that the majority of the basil cultivars growing in Serbia belonged to the "linalool chemotype". This agrees with the chemotaxonomic classification of *O. basilicum* based on geographical origins, as linalool and methyl chavicol are the main constituents in European basil cultivars [2, 22, 23]. Similar findings were reported by Telci et al. [38]. They have been identified seven different chemotypes in 18 basil landraces from Turkey and majority were characterized by high linalool contents. Also, linalool was found as dominant constituent in essential oils in nine basil accessions from Italy [4].

3.2. Radical scavenging activity

The antioxidant activity of the essential oils was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable DPPH as reagent. These results, presented in Table 3, reveal the variability in antioxidant capacity of the examined essential oils. Of the twelve tested samples, only Lime and Siam Queen did not demonstrate antioxidant activity. All the remaining oils exhibited activity with an IC_{50} similar to that of ascorbic acid (2.8 μg/mL) or higher. The Blue Spice cultivar had the highest antioxidant activity with IC_{50} = 0.03 μg/mL.

Table 3. DPPH radical-scavenging activity of tested essential oils

Samples	DPPH radical scavenging activity IC_{50} (μg/mL)
Blue Spice	0.03 ± 0.01
Fino Verde	0.79 ± 0.15
Holandjanin	0.85 ± 0.08
Compact	1.01 ± 0.14
Genovese	1.24 ± 0.08
Purple Opal	1.26 ± 0.15
Lattuga	1.59 ± 0.14
Osmin	1.88 ± 0.09
Cinnamon	2.83 ± 0.18
PurpleRuffles	6.80 ± 0.23
Lime	> 100
Siam Queen	> 100
Ascorbic acid	2.80 ± 0.21
Trolox	3.49 ± 0.11

Considering the structural characteristics of the most abundant compounds of the examined oils, and in the light of results of Ruberto and Baratta on the antioxidant activity of 98 common essential oil components [39], we concluded that the radical scavenging capacity of these essential oils could not be attributed to only one component. The antioxidant capacity of the oils correlated with the major proportion of volatile components in the oils and to compounds possessing a phenolic ring [6]. The antioxidant effect of the most active oil, Blue Spice, as well as the essential oils from Fino Verde, Holandjanin and Osmin, was greatly influenced by the presence of the monoterpene phenol eugenol, known to exhibit antioxidant properties [36, 39 - 41]. Politeo et al. reported that antioxidant capacity of total essential oil is due only or mainly to the presence of eugenol [36].

Table 2. The chemical composition (%) of essential oils of *Ocimum basilicum* L. cultivars

#	Constituents	RIE[a]	RIL[b]	Essential oils											
				Genovese	Lattuga	Fino Verde	Holandjanin	Compact	Cinnamon	Lime	Siam Queen	Blu Spice	Purple Opal	Purple Ruffles	Osmin
1	ethyl 2-methyl butyrate	864	864	-	-	-	-	-	-	-	-	0.2	-	-	-
2	ethyl isovalerate	866	867	-	-	-	-	-	-	-	-	1.4	-	-	-
3	α-thujene	932	924	-	-	0.05	0.05	0.1	-	-	-	-	-	-	-
4	α-pinene	934	932	0.4	0.2	0.2	0.2	0.4	0.1	0.1	0.1	0.5	0.2	0.4	0.4
5	Camphene	n/a	946	0.1	-	0.1	0.1	0.1	-	-	0.1	-	-	-	0.1
6	Sabinene	974	969	0.4	0.2	0.1	0.1	0.3	0.05	-	0.1	0.2	0.2	0.5	0.4
7	β-pinene	977	974	0.9	0.5	0.3	0.3	0.5	0.1	0.1	0.2	1.3	0.6	1.0	0.9
8	Myrcene	994	988	0.9	0.3	0.4	0.3	0.3	0.1	0.6	0.2	0.2	0.6	1.4	1.0
9	α -terpinene	1017	1014	0.05	0.1	0.1	0.1	0.1	0.1	-	-	-	0.05	0.1	0.1
10	*p*-cymene	1026	1020	-	0.1	0.2	0.2	0.2	0.1	-	-	-	-	-	0.03
11	limonene	1030	1024	0.3	0.3	0.3	0.3	0.5	0.1	0.2	0.2	0.3	0.2	0.4	0.5
12	1,8-cineole	1032	1026	9.3	6.4	3.2	3.4	4.5	1.8	0.1	2.0	14.3	5.9	10.2	9.2
13	*trans*-β-ocimene	1051	1044	0.2	0.3	0.3	0.3	-	0.3	0.2	1.3	1.0	-	-	0.03
14	γ-terpinene	1060	1054	0.1	0.2	0.2	0.2	0.4	0.2	-	-	0.1	0.1	0.1	0.1
15	*cis*-sabinene hydrate	1070	1065	0.1	0.1	0.2	0.2	0.3	0.1	0.2	-	0.3	0.1	0.1	0.1
16	*cis*-linalool oxide (furanoid)	1076	1067	0.1	0.1	0.1	0.1	-	0.1	0.1	-	-	-	-	0.05
17	Fenchone	1090	1083	0.2	0.2	0.2	0.3	0.3	0.2	1.0	0.1		0.3	0.5	1.2
18	**Linalool**	1105	1095	**50.4**	**30.3**	**54.9**	**53.3**	**34.2**	**31.8**	11.5	0.7	0.2	**41.2**	**32.4**	**58.6**
19	octen-3-yl acetate	1117	1111	0.1	-	0.1	0.2	0.1	-	0.1	-	-	0.1	0.1	0.1
20	4-acetyl-1-methylcyclohexene	1135	-	-	-	-	-	-	-	0.1	-	0.6	-	-	-
21	*trans*-myroxide	1147	1140	0.2	-	0.2	0.4	-	-	0.6	0.05	0.2	-	-	-
22	camphor	1146	1141	0.8	0.8	0.7	0.7	0.5	0.3	0.3	1.8	-	-	0.2	1.1
23	Borneol	1168	1165	-	-	-	-	-	-	0.4	-	-	-	-	-
24	δ-terpineol	1171	1162	0.4	0.3	0.2	0.4	0.4	0.1	0.5	0.3	0.3	0.2	0.3	0.3
25	terpinen-4-ol	1181	1174	0.3	1.7	2.2	1.9	2.7	1.2	0.7	0.07	0.3	0.2	0.3	0.2
26	α-terpineol	1195	1186	1.2	0.9	0.7	0.7	0.8	0.3	1.2	0.3	0.9	1.0	1.4	1.3
27	**methyl chavicol** (estragole)	1203	1195	0.2	16.3	0.1	-	-	3.1	0.3	**83.6**	11.8	0.1	16.0	0.4
28	octanol acetate	1217	1211	0.2	0.2	0.3	0.2	0.1	-	0.2	-	-	0.2	-	-
29	fenchyl acetate	1223	1218	-	-	-	-	-	-	-	-	-	0.4	0.5	0.5

30	Nerol	1232	1227	-	-	0.1	-	-	0.1	5.9	-	-		-	0.04
31	Neral	1244	1235	-	-	0.05	0.1	-	-	12.8	-	-	0.1	-	-
32	chavicol	1268	1254	0.1	-	1.6	0.5	0.1	0.05	0.5	-	0.2	0.05	-	0.7
33	geranial	1274	1264	-	-	0.05	0.1	-	-	16.1	-	-	0.1	-	-
34	bornyl acetate	1289	1287	0.8	0.4	1.1	1.5	1.5	0.3	-	0.1	-	-	0.1	0.3
35	*cis*-methyl cinnamate	1310	1299	-	-	-	-	-	2.9	-	-	-	-	-	-
36	exo-2-hydroxycineole acetate	1347	1354	0.1	0.1	0.1	0.1	0.1	0.1	-	-	-	0.1	0.1	0.1
37	eugenol	1363	1356	2.5	0.9	4.5	4.3	3.6	1.2	0.8	-	16.2	1.6	0.8	2.4
38	neryl acetate	1370	1359	-	-	1.4	0.5	-	-	-	-	-	-	0.1	0.2
39	α-copaene	1378	1374	0.2	0.2	0.2	0.3	0.2	-	0.2	-	-	0.2	0.2	0.2
40	*trans*-methyl cinnamate	1387	1376	0.1	0.1	0.1	0.1	0.2	31.4	1.1	-	0.1	0.2	0.1	0.1
41	β-elemene	1395	1389	1.4	1.3	1.3	2.0	3.0	2.0	0.7	0.2	0.2	2.1	2.3	1.8
42	methyl eugenol	1410	1403	0.1	0.1	0.2	0.1	0.2	0.1	-	0.2	0.1	0.1	0.1	0.6
43	sesquithujene	1418	1405	0.1	0.2	0.1	0.1	0.2	-	0.1	0.05	0.1	0.3	0.2	-
44	β-caryophyllene	1420	1417	0.3	0.3	0.2	0.4	0.4	0.3	11.0	0.3	0.9	1.2	1.4	1.6
45	α-*trans*-bergamotene	1439	1432	6.6	13.5	4.7	2.9	14.1	1.0	4.3	4.4	3.2	19.3	10.6	1.0
46	α-guaiene	1441	1437	0.8	1.0	0.7	1.3	1.6	0.9	0.5	0.2	1.0	1.5	1.3	0.9
47	α-humulene	1456	1452	0.7	0.6	0.7	0.7	1.1	0.6	0.2	0.2	1.2	0.7	0.9	1.0
48	*trans*-β-farnesene	1461	1454	0.9	1.2	0.8	1.3	1.1	0.6	1.3	0.1	1.3	1.3	1.4	0.6
49	*cis*-muurola-4(14),5-diene	1466	1465	0.7	0.8	0.7	0.8	1.2	0.7	-	0.1	-	0.8	0.7	0.3
50	10-*epi*-β-acoradiene	1469	1474	0.1	0.2	0.1	0.2	0.3	0.2	-	-	-	-	0.2	0.1
51	germacrene D	1484	1484	3.1	4.1	2.6	5.1	6.2	4.0	3.5	0.4	1.2	4.1	3.3	2.8
52	*trans*-muurola-4(14),5-diene	1488	1493	0.1	0.2	0.1	0.2	0.3	0.1	0.2	-	-	-	0.2	0.1
53	bicyclogermacrene	1499	1500	0.9	0.9	0.8	1.0	2.4	1.5	-	0.1	-	2.4	1.6	1.2
54	**β-bisabolene**	1512	1505	-	-	-	-	-	-	0.6	-	**23.8**	1.6	-	-
55	α-bulnesene (δ-guaiene)	1509	1509	1.1	1.0	1.0	1.7	2.0	1.4	-	0.1	-	2.2	1.5	1.2
56	γ-cadinene	1517	1513	3.2	3.3	2.8	2.8	3.4	2.6	-	-	-	1.3	1.8	1.5
57	*trans*-calamenene	1524	1521	-	0.5	-	-	1.0	-	-	1.0	0.2	-	0.8	0.3
58	β-sesquiphellandrene	1526	1521	0.4	0.5	0.4	0.5	-	0.3	0.6	0.1	0.2	0.4	-	-
59	*trans*-α-bisabolene	1545	1530	0.1	0.1	0.1	0.1	0.2	0.1	8.9	-	7.0	-	-	0.04
60	salviadienol	1557	1545	-	0.2	-	-	-	-	0.3	0.3	0.2	-	-	-
61	*trans*-nerolidol	1566	1561	0.1	0.1	0.1	0.12	0.6	0.3	-	-	-	0.3	0.3	0.1

62	maaliol	1569	1566	0.1	-	0.1	0.1	0.1	-	0.2	-	0.2	-	-	-
63	spathulenol	1580	1577	0.6	0.4	0.6	0.4	0.4	0.4	-	0.1	-	0.3	0.2	0.3
64	caryophyllene oxide	1584	1582	0.1	0.2	-	0.1	-	0.1	7.0	0.2	2.3	0.2	0.1	0.1
65	guaia-6,10(14)-diene-4β-ol	1614	1610	-	-	-	-	-	-	0.1	0.05	0.3	-	-	-
66	1,10-di-epi-cubenol	1617	1618	0.8	1.0	0.8	0.8	0.9	0.8	0.9	0.3	3.0	0.6	0.5	0.4
67	α-muurolol	1644	1644	5.2	5.6	4.9	4.9	5.9	4.7	0.3	-	0.4	3.9	3.1	2.7
68	β-eudesmol	1652	1649	0.5	0.6	0.4	0.5	0.4	0.4	0.7	0.1	0.2	0.4	0.4	0.3
69	α-cadinol	1657	1652	0.1	0.2	0.1	0.1	0.2	0.1	0.4	-	0.9	0.2	-	-
70	aromadendran-12-ol[c]	1666	1654	0.1	0.1	0.05	0.05	0.1	-	0.3	-	0.5	-	-	-
71	aromadendran-14-ol[c]	1673	1679	0.1	-	0.1	0.1	0.04	-	0.2	-	0.2	-	-	0.04
72	α-bisabolol	1690	1685	0.1	-	0.1	0.1	-	-	0.2	-	0.3	-	-	-
73	*cis*-lanceol	1752	1760	-	-	0.1	0.1	-	0.1	1.1	-	-	-	-	-
74	*trans*-phytol	2115	2112	0.3	0.1	0.3	0.2	-	0.2	0.2	-	0.1	0.3	0.1	0.1
75	*trans*-phytol acetate	2222	2218	0.1	-	-	-	-	0.1	0.2	-	0.1	0.2	-	0.1
	Total			**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
	Total compounds : 75			58	53	63	62	52	52	53	37	47	49	47	55
	Monoterpene hydrocarbons			3.3	2.2	2.3	2.3	2.9	1.1	1.3	2.2	3.7	2.0	3.8	3.4
	Oxygenated monoterpens			**64.1**	**41.3**	**65.6**	**63.6**	**45.4**	36.4	**51.5**	5.5	16.5	**49.6**	**46.1**	**73.2**
	Sesquiterpene hydrocarbons			20.9	30.1	17.5	21.3	38.8	16.5	32.1	7.4	**40.3**	39.4	28.3	14.6
	Oxygenated sesquiterpens			7.9	8.6	7.2	7.3	8.6	6.8	11.7	1.1	8.1	6.0	4.6	4.1
	Aromatic compounds			3.0	17.4	6.6	5.0	4.0	**38.9**	2.7	**83.8**	28.5	2.1	17.0	4.4
	Others			0.7	0.4	0.8	0.5	0.2	0.3	0.8	-	2.5	0.8	0.1	0.3

[a]RIE = Retention index experimentally determined (AMDIS), [b] RIL = Retention index – literature data (Adams, 2007), [c] tentative identification

Its mechanism of action relies on the donation of a hydrogen atom from the phenolic hydroxyl group to the "stable" free radical DPPH. To illustrate this point, the essential oil from Siam Queen, which did not demonstrate antioxidant activity, mainly contained methyl chavicol, which is structurally very similar to eugenol, but lacks an OH group. This difference confirms the importance of the phenolic OH group with respect to the scavenging activity of some aromatic essential oil constituents. The monoterpene hydrocarbon trans-α-bisabolene evidently contributed to Blue Spice's high antioxidant capacity, due to the presence of a strongly activated methylene group (between two double bonds). This is a key structural feature of some essential oil components with strong antioxidant activity, such as γ-terpinene and terpinolene, as well as some easily oxidised compounds, including unsaturated hydrocarbons and fatty acids [39].

The main component of the eight essential oils with remarkable antioxidant activity was linalool. This compound has previously shown contradictory results, despite the presence of the allylic alcohol functional group, which has been described as being very important for oxygenated monoterpene antioxidant activity [40]. However, the synergistic action of different chemicals in such complex natural samples as essential oils is the most likely mechanism by which biological activity is exerted.

3.3. Antimicrobial activity

The essential oils of the examined basil cultivars were tested against a panel of selected Gram positive and Gram negative bacteria as well as fungi (Tables 4 and 5).

All tested oils exhibited significant antibacterial activity against the investigated bacterial species, but at different levels (Table 4). Minimal inhibitory concentrations were between 0.009 - 23.48μg/mL, while the minimal bactericidal concentrations ranged from 0.28 to 135μg/mL. For commercial antibiotic streptomycin, these values were 1.25 - 10 μg/mL (MIC) and 2.5 - 25 μg/mL (MBC). Ampicillin showed an MIC and MBC of 100 μg/mL and 150 μg/mL, respectively. The most sensitive bacteria were *E. coli* and *S. typhimurium* with MIC values 0.18 - 5.40 μg/mL for *E. coli* and 0.28 - 5.40 μg/mL for *S. typhimurium* andwith MBC values of 0.35-25 μg/mL for *E. coli* and 0.57 - 22.5 μg/mL for *S. typhimurium*. The most resistant bacterium was *L. monocytogenes* with an MIC 2.70 - 23.48 μg/mL and an MBC of 5.40 - 135 μg/mL.

Of the twelve tested oils, the strongest effect on all the tested bacteria was exhibited by the essential oil of the Lime cultivar. However, Compact and Blue Spice cultivar oils were even more active against a few bacteria (Table 4). The lowest inhibitory concentration of 0.009 μg/mL was shown by Compact cultivar oil against pathogenic *M. flavus*. The highest bactericidal effect was demonstrated by Blue Spice and Lattuga cultivar oils against *B. cereus*, with MBC values of 0.57 and 0.67 μg/mL, respectively. The most prominent antibacterial activity was seen with the essential oil of Blue Spice against *Ps. aeruginosa* (MIC of 0.11μg/mL; MBC of 0.28μg/mL) Blue Spice cultivar oil (with the exception of *L. monocytogenes*). Compact, Lime and Lattuga cultivars showed almost the same or greater inhibitory activity than streptomycin. All the essential oils possessed much better antibacterial activity than ampicillin, with activity at a 100-fold lower concentration in some cases (Table 4).

Table 4. Antibacterial activity (MIC and MBC μg/mL) of essential oils from *O. basilicum* cultivars

Bacteria		*S. aureus*	*B. cereus*	*L. monocytogenes*	*M. flavus*	*Ps. aeruginosa*	*S. typhimurium*	*E. coli*	*En. faecalis*
Essential oils									
Siam queen	MIC	4.50	1.25	9.05	4.50	2.25	1.25	1.25	9.05
	MBC	9.05	4.50	45.25	9.05	22.6	4.50	4.50	22.6
Blu spice	MIC	9.00	0.11	9.00	0.11	0.11	0.28	0.28	9.00
	MBC	9.00	0.57	135.00	9.00	0.28	0.57	0.57	22.50
Holanđanin	MIC	2.75	0.14	5.49	2.20	2.75	0.28	0.28	5.49
	MBC	5.49	4.39	10.96	4.39	5.49	4.39	4.39	10.96
Cinnamon	MIC	11.74	4.70	23.48	4.70	11.74	4.70	4.70	11.74
	MBC	23.48	9.39	46.95	9.39	23.48	9.39	9.39	23.48
Finoverde	MIC	5.40	5.40	2.70	5.40	2.70	5.40	5.40	5.40
	MBC	10.80	10.80	5.40	10.80	5.40	10.80	10.80	10.80
Genovese	MIC	5.39	5.39	5.39	5.39	5.39	5.39	5.39	10.78
	MBC	10.78	10.78	10.78	10.78	10.78	10.78	10.78	21.57
Osmin	MIC	10.77	0.67	10.77	0.34	0.67	0.34	0.34	5.34
	MBC	21.55	1.34	21.55	10.77	13.45	0.67	0.67	10.77
Purple opal	MIC	10.81	10.81	10.81	10.81	0.67	0.67	0.67	10.81
	MBC	21.63	21.63	21.63	21.63	1.38	1.38	1.38	21.63
Purple ruffles	MIC	10.91	10.91	21.83	0.35	5.46	0.68	0.68	21.83
	MBC	21.83	21.83	43.65	21.83	21.83	21.83	21.83	21.83
Compact	MIC	5.50	0.35	11.00	0.009	11.0	0.35	0.18	11.00
	MBC	11.00	0.68	22.00	11.00	22.00	0.68	0.35	22.00
Lime	MIC	0.68	0.18	5.48	2.71	0.34	0.34	0.34	5.48
	MBC	21.90	0.34	10.80	21.9	0.68	0.68	0.68	21.90
Lattuga	MIC	5.43	0.14	10.85	5.43	0.34	0.34	0.34	5.43
	MBC	10.85	0.67	21.7	10.85	0.67	0.67	0.67	10.85
Streptomycin	MIC	10.00	1.25	10.00	1.25	1.25	1.25	5.00	2.50
	MBC	25.00	2.50	25.00	2.50	2.50	2.50	10.00	5.00
Ampicillin	MIC	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	MBC	150.00	150.00	150.00	150.00	150.00	150.00	150.00	150.00

Table 5. Antifungal activity (MIC and MBC μg/mL) of essential oils from *O. basilicum* cultivars

Fungi		*A. ochraceus*	*A. versicolor*	*A. niger*	*A. fumigatus*	*T. viride*	*P. ochrochloron*	*P. funiculosum*
Cultivar essential oils								
Siam queen	MIC	0.57	2.26	2.26	2.26	2.25	1.13	2.26
	MBC	0.57	4.53	4.53	4.53	4.53	2.26	4.53
Blu spice	MIC	2.25	2.25	5.00	5.00	2.25	2.25	2.25
	MBC	2.25	9.00	9.00	9.00	4.50	4.50	9.00
Holandjanin	MIC	1.37	0.68	1.37	0.68	1.37	0.68	1.37
	MBC	2.74	5.48	2.74	5.48	2.74	2.74	5.48
Cinnamon	MIC	1.17	0.59	1.17	0.59	1.17	1.17	1.17
	MBC	2.38	4.70	2.38	2.38	2.38	2.38	2.38
Finoverde	MIC	1.35	0.67	2.70	1.35	1.35	1.35	2.70
	MBC	2.70	10.80	27.00	2.70	2.70	5.40	5.40
Genovese	MIC	1.07	0.53	1.07	1.07	1.07	0.53	1.07
	MBC	2.15	8.60	4.30	2.15	2.15	2.15	4.30
Osmin	MIC	0.08	0.27	1.07	0.53	0.53	0.27	0.53
	MBC	0.14	4.31	4.31	2.16	2.16	2.16	4.31
Purple opal	MIC	1.08	0.53	0.93	0.53	1.08	0.53	1.08
	MBC	1.08	4.33	4.33	2.17	2.17	4.33	4.33
Purple ruffles	MIC	1.09	0.55	1.09	1.09	1.09	0.55	2.18
	MBC	1.09	4.37	8.73	4.37	2.18	4.37	4.37
Compact	MIC	0.13	0.55	1.10	1.10	1.10	1.10	2.20
	MBC	0.27	8.81	4.40	2.20	2.20	4.40	4.40
Lime	MIC	1.08	0.54	1.08	0.54	1.08	0.54	1.08
	MBC	2.17	4.34	2.17	1.08	2.17	2.17	2.17
Lattuga	MIC	0.26	0.54	1.08	1.08	1.08	0.54	1.08
	MBC	0.26	8.68	8.68	4.34	2.17	4.34	4.34
Ketoconazole	MIC	10.00	25.00	50.00	25.00	25.00	10.00	10.00
	MBC	25.00	50.00	150.00	50.00	100.00	25.00	25.00

All the examined essential oils showed a great ability to inhibit fungal growth, which was 10- to 100-fold higher than the commercial antifungal agents ketoconazole and bifonazole (Table 5). Minimal inhibitory concentrations (fungistatic) of essential oils varied with the fungal species, ranging between 0.08 - 5.00 μg/mL, while fungicidal concentrations ranged from 0.14 to 27.00 μg/mL. Ketoconazole showed fungistatic activity at 10 - 50 μg/mL and a fungicidal effect at 25 - 150 μg/mL, while bifonazole showed an MIC and MFC of 100 - 200 μg/mL and 200 - 250 μg/mL, respectively. The best antifungal activity was exhibited by the basil oil of Osmin cultivar, with MIC values from 0.08-1.07 μg/mL and MFC values 0.14 - 4.31 μg/mL. The essential oil from Fino Verde cultivar showed the weakest antifungal activity with an MFC of 27.00 μg/mL against *A. niger*. The most sensitive fungus to the tested essential oils was found to be *A. ochraceus* (MIC values 0.08 - 2.25 μg/mL and MFC 0.14 - 2.74 μg/mL), while *A. niger* was the most resistant with MIC values of 0.93 - 5.00 μg/mL and MFC from 2.17 - 27.00 μg/mL. Generally, fungi were more sensitive than bacteria to the effect of the essential oils.

Two of tested oils had the same dominant components, but in moderately different ratios: Holandjanin (linalool 53.3%, germacrene D 5.1%, α-muurulol 4.9%, eugenol 4.3% and 1,8-cineole

3.4%) and Osmin (linalool 58.6%, 1,8-cineole 9.2%, germacrene D 2.8%, α-muurulol 2.7% and eugenol 2.4%). Their antibacterial activities were very different, suggesting that oil activity is not only a consequence of its composition, but is a result of a very specific ratio of components which is actually synergistic or antagonistic. A confirmation of this is that both of these oils, with high amounts of linalool which has been shown to be an antimicrobial agent, exhibited weaker activity than the oils with lower amounts of this compound [9, 12].

The essential oils of *O.basilicum*, as indicated in the Introduction, have previously been studied for their antimicrobial activities. The differences in these results in comparison to the literature were not only influenced by the composition of the oils, but also by the methods used, which has been thoroughly reviewed by Suppakul et al. [9]. For example, in a study by Opalchenova et al., the results indicated excellent inhibitory activity against multidrug-resistant bacterial strains of *Staphylococcus*, *Pseudomonas* and *Enterococcus* [13], while the results obtained by Runyoro et al., using the agar dilution technique, showed very weak antibacterial activity of basil oil [37]. Božin et al. tested the essential oil of basil from Serbia; however, they did not mention which cultivar they used. Their results, obtained by using 13 bacteria and 6 fungi, showed high antibacterial and significant antifungal activity [25]. In our study, the essential oils from all 12 tested basil cultivars showed better antifungal than antibacterial activity.

Further investigations should focus on basil cultivars with the highest activities and their potential applications in the food industry.

Acknowledgments

The authors acknowledge their gratitude to the Ministry of Education and Science of Serbia for financial support, projects numbers 46001,173032, 173015 and 172053.

References

[1] A. Paton, R.M. Harley and M.M. Harley (1999). Ocimum: An overview of classification and relationships, In: Basil the genus Ocimum, ed: Harwood Academic Publishers, Amsterdam, The Nederland, pp.1-38.

[2] J.E. Simon, J. Quinn and R.G. Murray (1990). Basil: A source of essential oils, In: Advances in new crops, ed: Timber Press, Portland, OR, pp. 484-489

[3] L. Jirovetz, G. Buchbauer, M. P. Shafi and M. M. Kaniampady (2003). Chemo-taxonomical analysis of the essential aroma compounds of four different *Ocimum* species from southern India, *Eur. Food Res. Technol.* **217** (2), 120-124.

[4] M. Labra, M. Miele, B. Ledda, F. Grassi, M. Mazzei and F. Sala (2004). Morphological characterization, essential oil composition and DNA genotyping of *Ocimum basilicum* cultivars, *Plant Sci*. **167**, 725-731

[5] O. Makri and S. Kintzios (2007). *Ocimum* spp. (Basil): Botany, cultivation, pharmaceutical properties, and biotechnology, *J. Herbs Spices Med. Plants* **13**, 123-150

[6] M. T. Salles Trevisan, M. G. Vasconcelos Silva, B. Pfundstein, B. Spiegelhalder and R. Wyn Owen (2006). Characterization of the volatile pattern and antioxidant capacity of essential oils from different species of the genus *Ocimum*, *J. Agric. Food. Chem.* **54**, 4378-4382

[7] F. L. Hakkim, G. Arivazhagan and R. Boopathy (2008). Antioxidant property of selected *Ocimum* species and their secondary metabolite content, *J. Med. Plants Res.* **2 (9)**, 250-257.

[8] E. M. Kwee and E. D. Niemeyer (2011). Variations in phenolic composition and antioxidant properties among 15 basil (*Ocimum basilicum*) cultivars, *Food Chem.* **128**, 1044-1050.

[9] P. Suppakul, J. Miltz, K. Sonneveld and S.W. Bigger (2003). Antimicrobial properties of basil and its possible application in food packaging, *J. Agric. Food. Chem.* **51**, 3197-3207.

[10] A. K. Annand, M. Mohan, Z. Haider and A. Sharma (2011). Essential oil composition and antimicrobial activity of three *Ocimum* species from Uttarakhand (India), *Int. J. Pharm. Pharm. Sci.* **3**, 223-225

[11] K. Carović-Stanko, S. Orlić, O. Politeo, F. Strikić, A. Kolak, M. Miloš and Z. Šatović (2010). Composition and antibacterial activities of essential oils of seven *Ocimum* taxa, *Food Chem.* **119**, 196-201

[12] A.I. Hussain, F. Anwar, S.T.H. Sherazi and R. Przybylski (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*O.basilicum*) essential oils depend on seasonal variations, *Food Chem.* **108**, 986-995.

[13] G. Opalchenova and D. Obreshkova (2003). Comparative studies on the activity of basil - an essential oil from *Ocimum basilicum* L.-against multidrug resistant clinical isolates of the genera *Staphylococcus*, *Enterococcus* and *Pseudomonas*, by using different test methods, *J. Microbiol. Meth.* **54**, 105-110.

[14] M. A. Haniff, M. Y. Al-Maskari, A. Al-Maskari, A. Al-Shukaili, A. Y. Al-Maskari and J. N. Sabahi (2011). Essential oil composition, antimicrobial and antioxidant activities of unexplored Omani basil, *J. Med. Plants Res.* **5 (5)**, 751-757.

[15] M.J. Pascual-Villalobos and M.C. Ballesta-Acosta (2003). Chemical variation in an *Ocimum basilicum* germplasm collection and activity of the essential oils on *Callosobruchus maculates*, *Biochem. Sys. Ecol.* **31**, 673–679.

[16] L.S. Nerio, J. Olivero-Verbel and E. Stashenko (2010). Repellent activity of essential oils: A review, *Bioresource Technol.* **101**, 372-378.

[17] C. M. M. Freire, M. O. M. Marques and M. Costa (2006). Effects of seasonal variation on the central nervous system activity of *Ocimum gratissimum* L. essential oil, *J. Ethnopharmacol*, **105**, 161–166.

[18] Gajula, M. Verghese, J. Boateng, L.T. Walker, L. Schackelford, S.R. Mentreddy and S. Cedric (2009). Determination, of tothal phenolic, flavonoids and antioxidant and chemopreventive potential of basil (*Ocimum basilicum* L. and *Ocimum tenuiflorum* L.), *Int. J. Cancer.* **5**,130-143.

[19] M. Venkataraman, L. Jang-Choon , A. Kwang Seok, L. Hyo-Jung, L. Hyo-Jeong, L. Eun-Ok, S. Bum Sang, J. Hee Jae, K. Jin Sung, K. Dae Keun, C. Seung-Hoon , A. Kyoo-Seok and K. Sung-Hoon (2009). *Ocimum sanctum* induces apoptosis in A549 Lung cancer cells and suppresses the in vivo growth of Lewis Lung carcinoma cells, *Phytother. Res.* **23**, 1385–1391.

[20] R. Piccaglia, M. Marroti and G.C. Galletti (1991).Characterization of essential oils from *Saturea montana* L. chemotype grown in Northen Italy, *J. Essent. Oil. Res.* **3,** 147-152.

[21] A. Viña and E. Murillo (2003). Essential oil composition from twelve varieties of basil (Ocimum spp) grown in Columbia, *J. Braz. Chem. Soc.* **14 (5)**, 744-749.

[22] B.M. Lawrence (1992). Chemical components of *Labiatae* oils and their exploitation, In: Advances in Labiate Science, ed: Royal Botanic Gardens, Kew, UK, pp. 399-436

[23] R.J. Grayer, G.C. Kite, F.J. Goldstone, S.E. Bryan, A. Paton and E. Putievsky (1996). Intraspecific taxonomy and essential oil chemotypes in Sweet basil, *Ocimum basilicum. Phytochemistry* **43,** 1033-1039.

[24] D. Beatović, S. Jelačić and N. Menković (2008). Collectioning and evaluation of basil genotypes (*Ocimum basilicum* L.) in Serbia, *Agro-knowledge Journal* **9 (1)**, 35-41.

[25] B. Božin, N. Mimica-Dukić, N. Simin and G. Anackov (2006). Characterization of the volatile composition of essential oils of some Lamiaceae species and the antimicrobial and antioxidant activities of the entire oils, *J. Agric. Food. Chem.* **54**, 1822-1828.

[26] D. Beatović, S. Jelačić, Č. Oparnica, D. Krstić-Milošević, J. Glamočlija, M. Ristić and J. Šiljegović (2013). Chemical composition, antioxidative and antimicrobial activity of essential oil of *Ocimum sanctum* L, *Hem Ind* **67 (3)**, 427-435.

[27] D. Beatović, A. Vujošević, S. Jelačić and N. Lakić (2006). Development of a basil seedling production model- a choice of containers, *J. Agric. Sci.* **67**, 103-109.

[28] Yugoslav Pharmacopeia Ph. Yug. IV (1984). National Institute for Health Protection. Belgrade, Serbia, pp. 126-127

[29] R.P. Adams (2007). Identification of essential oils components by gas chromatography/mass spectrometry (4^{th} ed.). Carol Stream. IL, USA: Allured Publishing Corp

[30] D. Hochmuth (2006). MassFinder 3: GC/MS visualization, interpretation and library, In: Administration mass spectral library "Trepaenned and related constituents of essential oils", 1999-2006, Hamburg, Germany

[31] M. Cuendet, K. Hostettmann and O. Potterat (1997). Iridoid glucosides with free radical scavenging properties from *Fagraea blumei. Helv. Chim. Acta*, **80,** 1144-1152.
[32] C. Booth (1971). Fungal culture media, In: Methods in microbiology, ed. J.R. Norris and D.W. Ribbons. London & New York: Academic Press, pp. 49–94.
[33] H. Hanel and W. Raether (1988). A more sophisticated method of determining the fungicidal effect of water-insoluble preparations with a cell harvester, using miconazole as an example, *Mycoses.* **31**, 148-154.
[34] A. Espinel-Ingroff (2001). Comparasion of the E-test with the NCCLS M38-P method for antifungal susceptibility testing of common and emerging pathogenic filamentous fungi, *J. Clin. Microbiol.* **39**, 1360-1367.
[35] K.D. Daouk, M.S. Dagher and J.E. Sattout (1995). Antifungal activity of the essential oil of *Origanum syriacum* L, *J. Food. Protect*. **58**, 1147-1149.
[36] O. Politeo, M. Jukic and M. Milos (2007). Chemical composition and antioxidant capacity of free volatile aglycones from basil (*Ocimum basilicum* L.) compared with its essential oil, *Food Chem.* **101**, 379-385.
[37] D. Runyoro, O. Nagassapa, K. Vagionas, N. Aligiannis, K. Graikou and I. Chinou (2010). Chemical composition and antimicrobial activity of the essential oils of four *Ocimum* species growing in Tanzania, *Food Chem.* **119**, 311-316.
[38] I. Telci, E. Bayram, G. Yilmaz and B. Avici (2006). Variability in essential oil composition of Turkish basils (Ocimum basilicum L.). *Biochem. Syst. Ecol*. **34**, 489-497.
[39] G. Ruberto and M.T. Baratta (2000). Antioxidant activity of selected essential oil components in two lipid model systems, *Food Chem.* **69**, 167-174.
[40] H.R. Juliani and J.E. Simon (2002). Antioxidant activity of basil, In: Trends in new crops and new uses, ed: ASHS Press, Alexandria, pp.575-579.
[41] J.S. Dambolena, M.P. Zunino, A.G. López, H.R. Rubinstein, J.A. Zygadlo, J.W. Mwangi, G.N. Thoithi, I.O. Kibwage, J.M. Mwalukumbi and S.T. Kariuki (2010). Essential oils composition of *Ocimum basilicum* L. and *Ocimum gratissimum* L. from Kenya and their inhibitory effects on growth and fumonisin production by *Fusarium verticillioides*, *Innov. Food. Sci. Emerg. Technol.* **11**, 410-414.