Journal of Essential Oil Bearing Plants

ISSN: 0972-060X (Print) 0976-5026 (Online) Journal homepage: http://www.tandfonline.com/loi/teop20

Chemical Composition and Antimicrobial Activity of Essential Oils of Ocimum basilicum, Ocimum canum and Ocimum gratissimum in Function of Harvesting Time

Hasika Mith, Eléonore Yayi-Ladékan, Salomé Dosso Sika Kpoviessi, Innocent Yaou Bokossa, Mansourou Moudachirou, Georges Daube & Antoine Clinquart

To cite this article: Hasika Mith, Eléonore Yayi-Ladékan, Salomé Dosso Sika Kpoviessi, Innocent Yaou Bokossa, Mansourou Moudachirou, Georges Daube & Antoine Clinquart (2016) Chemical Composition and Antimicrobial Activity of Essential Oils of Ocimum basilicum, Ocimum canum and Ocimum gratissimum in Function of Harvesting Time, Journal of Essential Oil Bearing Plants, 19:6, 1413-1425, DOI: 10.1080/0972060X.2014.890076

To link to this article: http://dx.doi.org/10.1080/0972060X.2014.890076

Published online: 30 Nov 2016.

Submit your article to this journal

Article views: 21

View related articles

View Crossmark data

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=teop20

Download by: [University of Liege] **Date:** 05 February 2017, At: 10:34

Taylor & Francis
Taylor & Francis Group

ISSN Print: 0972-060X
ISSN Online: 0976-5026

Chemical Composition and Antimicrobial Activity of Essential Oils of *Ocimum basilicum*, *Ocimum canum* and *Ocimum gratissimum* in Function of Harvesting Time

Hasika Mith [1,5]*, Eléonore Yayi-Ladékan [2], Salomé Dosso Sika Kpoviessi [3], Innocent Yaou Bokossa [4], Mansourou Moudachirou [2], Georges Daube [1], Antoine Clinquart [1]

[1] University of Liège, FARAH, Avenue de Cureghem 10 (B43bis), Quartier Vallée 2, B-4000 Liège, Belgium
[2] University of Abomey-Calavi, Laboratory of Pharmacognosy and Essential Oils, ISBA, Champ de Foire, BP 188 Cotonou, Benin
[3] University of Abomey-Calavi, Faculty of Sciences and Techniques, Laboratory of Physic and Synthesis Organic Chemistry, 01B526 Cotonou, Benin
[4] University of Abomey-Calavi, Faculty of Sciences and Techniques, Laboratory of Microbiology, Cotonou, Benin
[5] Institute of Technology of Cambodia, Department of Chemical Engineering and Food Technology, Russian Federation Blvd., Phnom Penh, Cambodia

Received 29 June 2014; accepted in revised form 16 December 2014

Abstract: The chemical composition of essential oils obtained by hydrodistillation from aerial parts of *Ocimum basilicum*, *Ocimum canum* and *Ocimum gratissimum* from Benin as affected by harvesting time, were analyzed by GC-FID (Gas chromatography-Flame ionization detector) and GC-MS (Gas chromatography-Mass spectrometry). Based on the composition analysis, major components were as follows: estragol (43.0 - 44.7 %) and linalool (24.6 -29.8 %) in *O. basilicum* oils; carvacrol (12.0 -30.8 %) and p-cymene (19.5 -26.2 %) in *O. canum* oils; thymol (28.3-37.7 %) and γ-terpinene (12.5-19.3 %) in *O. gratissimum* oils. Disc diffusion and broth microdilution assays were used to evaluate the antibacterial activity of essential oils and their main components against two foodborne bacteria, *Listeria monocytogenes* and *Salmonella enterica* serotype Typhimurium. The tested oils and their components exhibited notable antimicrobial activities against *L. monocytogenes* and *S.* Typhimurium. The *O. canum* and *O. gratissimum* oils collected at 7h and 19h showed significant higher activities against *L. monocytogenes* and *S.* Typhimurium (MICs and MBCs 0.34 - 2.5 µL/mL) ($p < 0.05$), whereas *O. basilicum* showed lower activity (MICs and MBCs 2.0 - 8.0 µL/mL) at any daytime of harvest, the weakest being at 19h (MIC and MBC 12.0 - 32.0 µL/mL). The daytime of harvest can influence the composition of oils and their activities on bacteria.

Key words: Essential oil, *Ocimum basilicum*, *Ocimum canum*, *Ocimum gratissimum*, antimicrobial activity, chemical composition.

Introduction

At least 75 - 95 % of the world populations of developing countries still rely on traditional medicines for treatments, a major part of them involving the use of plant extracts or their bioactive constituents as reported by World Health Organization [1]. The inadequate use of antibiotics caused multiresistance of *Salmonella* spp., which is the

*Corresponding author (Hasika Mith)
E-mail: < hasika_mith@yahoo.com, hasika@itc.edu.kh >

© 2016, Har Krishan Bhalla & Sons

second most commonly reported food poisoning bacterium [2]. Foodborne *Listeria monocytogenes* is also an important pathogen due to the severity of infection with high mortality rate. The diseases, salmonellosis and listeriosis, caused by these pathogens induce serious public health and economic concerns for societies in developing countries and even developed countries [3,4]. Therefore, there is a need for natural alternatives. Essential oils, extracts or secondary metabolites from medicinal plants have been found to possess promising biological activities [5-8].

The genus *Ocimum*, belonging to the *Lamiaceae* family, contains approximately 50-150 species and is found in tropical and subtropical regions of Asia, Africa, and Central and South America [9]. Some important species of the genus *Ocimum* such as *O. basilicum* (sweet basil), *O. americanum* (syn. *O. canum*, hoary basil) and *O. gratissimum* (African basil) have been widely used as medicinal plants, culinary herbs and insect-controlling agents. The essential oils obtained by hydrodistillation or steam distillation from these aromatic and medicinal herbs were shown to possess good antimicrobial activities against a wide range of microorganisms [7,9-12] and also antioxidant activities [10,13].

The biological activity of the essential oils depends on their chemical compositions, which could be influenced by several factors such as agronomic conditions [14], genotypes of plants [15], drying methods [16], extraction methods [17], geographical locations [18] and harvest seasons [10]. However, the effects of different daytimes of harvest on their chemical composition could not be neglected. Previous works have reported the influence of harvesting time on chemical characteristics of some essential oils from the *Lamiaceae* family [19,20].

To make the collected products more valuable, further investigation on biological acti-vity needs to be considered. Therefore, the aim of this study was to determine the chemical composition and the antimicrobial activity of essential oils obtained from three *Ocimum* species: *Ocimum basilicum*, *O. canum* and *O. gratissimum* against *L. monocytogenes* and *S.* Typhimurium as a function of harvesting time.

Experimental

Plant materials

The fresh leaves of *Ocimum basilicum* L., *Ocimum canum* Sims and *Ocimum gratissimum* L. were collected at Abomey-Calavi in the south of Benin. The samples were harvested at 7h, 10h, 12h30, 16h and 19h on the same specimen. The plant species were identified by the National Herbarium of the University of Abomey-Calavi, Benin, where the three voucher specimen AA6375/HNB (*O. basilicum*), AA6378/HNB (*O. canum*) and AA6381/HNB (*O. gratissimum*) were deposited. The leaves were air dried for three days under laboratory conditions. The dried samples were distributed in batches of 100 grams in transparent bags, doubled by black bags and stored in boxes until extraction.

Extraction of essential oils

The air dried samples were subjected to hydrodistillation for three hours in an improved Clevenger-type apparatus [21]. The extraction of each sample was carried out in triplicate. Each essential oil was dried over anhydrous sodium sulfate and preserved in an amber vial at 4°C until GC-FID, GC-MS and biological analysis. The essential oils yields were calculated taking into account the dried vegetable.

Gas chromatography-flame ionization detector (GC-FID)

The GC-FID analysis was adapted from the work of Kpoviessi *et al.*, [22]. It was carried out on a FOCUS GC (Thermo Finnigan, USA) using the following operating conditions: CP Wax 52 CB capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm) (Agilent Technologies, USA); injection mode: splitless; injection volume: 1 µL (dilution with tert-butyl methyl ether); split flow: 10 ml/min; splitless time: 0.80 min; injector temperature: 260°C; oven temperature was programmed as following: 50°C to 250°C at 6°C/min and held for 5 min; the carrier gas was helium with a constant flow of 1.2 mL/min; FID detector temperature was: 260 °C. Data were recorded and treated with the ChromCard software (Thermo Scientific, USA).

Gas chromatography-mass spectrometry (GC-MS)

MS identification of compounds was realised on a TRACE GC 2000 series (ThermoQuest, USA), equipped with an autosampler AS2000 ThermoQuest [22]. The GC system was interfaced to a Trace MS mass spectrometer (ThermoQuest) operating in the electronic impact ionisation mode at 70 eV. The same CP Wax 52 CB capillary column was used for the separation using the same gradient and injection parameters as for the GC-FID method. The transfer line and the MS source were set at 260°C. Data were recorded and analyzed with Xcalibur 1.1 software (ThermoQuest). The mass spectra were analyzed and compared using reference standards and NIST/EPA/NIH library.

Individual components of the volatile oils were identified by comparison of their retention times with those of reference standards, computer matching of their MS spectra with commercial EI-MS spectra library or home-made mass spectra library made from pure substances and components of known oils [22] and confirmed by comparison of the GC retention indices (RI) (determined from the retention times of a series of *n*-alkanes "C7-C28" mixture) [23]. The Kovats indices (KI) calculated were in agreement with those reported by Adams [24]. Quantification (expressed as percentages) was carried out by the normalization procedure using peak areas obtained by FID.

Bacteria

The two foodborne pathogenic bacteria used in the present study were Gram-positive *Listeria monocytogenes* NCTC 11994 and Gram-negative *Salmonella* Typhimurium ATCC 14028. The test bacteria were maintained on Plate Count Agar (PCA) (Oxoid, UK) at 4°C, and freshly cultured by transferring into Brain Heart Infusion (BHI) (Oxoid, UK) broth and incubated at 37°C before use.

Paper disc diffusion assay

The antibacterial activity of the essential oils and linalool, carvacrol and thymol (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), the main constituents of these oils, was determined by paper disc diffusion method. A volume of 20 µL of each sample was impregnated into the paper disc of 6 mm diameter (Biomérieux, France), and then placed on the surface of prepared Mueller Hinton agar (Oxoid, Germany) plated with 200 µL of 10^6 cfu/mL inoculum. Three standard antibiotics: ampicillin (10 µg/disc), chloramphenicol (30 µg/disc) and streptomycin (10 µg/disc) (Biomérieux, France) were used as reference controls for the tested bacteria. The plates were then incubated at 37°C for 24h. The antibacterial activity was evaluated by measuring the diameter of inhibitory zones in millimeters using digital caliper Top Craft (Globaltronics GmbH & Co. KG, Germany) and results were expressed as means of five determinations.

Broth microdilution assay

Determinations of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were carried out by broth microdilution assay with 96-well microtiter plates against *L. monocytogenes* and *S.* Typhimurium. This assay was adapted from Bouhdid, Abrini, Zhiri, Espuny and Manresa [25] with some modifications. Briefly, a volume of 100 µL of Mueller-Hinton broth (MHB) (Oxoid, UK) containing 0.5 % (v/v) of Tween 20 was distributed from the second to the 12th well of each line of a 96 well microtiter plate. The solution of essential oils from *Ocimum* species was prepared in MHB containing Tween 20 to reach a final concentration of 64 µL/mL. A volume of 200 µL of these solutions was added to the first well of each microtiter line. Serial two-fold dilutions were carried out horizontally from the 1st to 10th column and the broth exceeding 100 µL was discarded from wells of the 10th column. The 11th and 12th columns were used as blank control (MHB) and bacterial growth control, respectively, because no essential oil was added. Then, 100 µl of bacterial suspension were added to each well to reach a final concentration of approximately 5×10^4 cfu/mL. The final concentrations of essential oils ranged from 0.063 to 32 µL/mL. The microtiter plate was incubated at 37°C for 18h. After incubation, 10 µl of resazurin (0.01 %) in water were added into each well to

assess bacterial growth. After incubation at 37°C for 5 h, the MIC was defined as the lowest concentration of essential oils that prevented a change in resazurin color. A color change from blue resazurin to pink resorufin was indicator of bacterial growth [26]. Aliquots of 10 µL from the wells remaining blue were subcultured onto PCA (plate count agar) plates and incubated at 37°C for 24 h. The MBC was determined as the minimum concentration where no growth or only one colony was present on the PCA plate after incubation. In order to assure that the used concentrations of essential oils do not cause a change in resazurin color, a control was also carried out. The experiment was repeated on four different wells.

Statistical analysis

The data of each experiment are presented as mean values ± standard deviation of five and four determinations for paper disc diffusion and MIC/MBC analysis, respectively. Statistical analysis of the data was performed by analysis of variance (ANOVA) using Statistical Analysis System (SAS) software version 9.1. Tukey test (inhibition diameters) and t-test (MIC/MBC) were used to evaluate the differences between the daytime of harvest. Means values were considered as significantly different when $p < 0.05$.

Results and discussion

ield and chemical composition of essential oils

The oils' yields of all three *Ocimum* species collected at different hours of the day ranged between 0.57 and 0.75 %, 1.69 and 1.78 %, and 0.78 and 1.80 % for *O. basilicum*, *O. canum* and *O. gratissimum*, respectively (Table 1). The fluctuation of essential oil content in *O. basilicum* was observed during daytime of harvest with the minimum oil content at 10h (0.57 %), while oil yield of *O. canum* were likely stable. On the other hand, *O. gratissimum* contained low oil content in the morning (0.78 %), the oil content increased under the sunlight and reached its maximum at 12h30 (1.80 %) before slightly declining. It shows that daytime of harvest may have an effect on the essential oil yield of each species of *Ocimum*. Overall, the essential oil yields of *O. basilicum* in the present study were comparable to those of Verma *et al*., [27] and Hussain *et al*., [10]. Those obtained from *O. canum* in this study were much higher than those reported by Yayi *et al*., [18] and Verma *et al*., [27] (0.1-0.30 %) for the same species, but comparable to those of Burkina Faso and Comoro Islands (1.0-2.2 %) [28-30]. *O. gratissimum* contained higher oil yields than those reported by Kpoviessi *et al*., [31] and Verma *et al*., [27] but these were obtained by hydrodistillation from fresh leaves (0.40-0.78 %). Dambolena *et al*., [12] reported yields of *O. gratissimum* essential oils from plants grown in Sagana and Yatta regions in Kenya to be 0.2 % and 2.9 %. However, our results were in agreement with previous findings of Yayi *et al*., [20] who reported oil yield variation, ranging from 0.3 to 1.8 %.

The chemical compositions obtained from GC-FID and GC-MS analysis of the essential oils of *O. basilicum*, *O. canum* and *O. gratissimum* from Benin, collected at different daytimes, are presented in Table 1. A total of 47 compounds were identified from which 25 compounds were found in *O. basilicum* oil, 26 in *O. canum* oil and 27 for *O. gratissimum* oil.

First, the results show that the essential oil of *O. basilicum* contains mainly estragol (or methyl chavicol), linalool and 1,8-cineole. Therefore, this plant belongs to the methyl chavicol/linalool/1,8-cineole chemotype, which was also identified in previous works [5,7,27,32]. These essential oils did not contain compounds such as camphor and *E*-myroxide, which were previously reported as main constituents in plants from other countries [9,12]. Other chemotypes of *O. basilicum* found in different regions in the world are: methyl chavicol [27,33], linalool [12], linalool/methyl chavicol [34,35], linalool/methyl cinnamate [27], linalool/citral [27] linalool/eugenol [7,27], eugenol/methyl eugenol [16], 1,8-cineole [9] and citral [12]. In the present study, it is noticed that the main component (estragol 43.0 - 44.7 %) showed almost no variation at the different daytimes of harvest. On the other hand, there were changes in the percentages of other components such as linalool (24.6 - 30.5 %), 1,8-cineole (8.2 - 12.8 %), β-bergamotene (1.3 - 3.5 %) and τ-cadinol (1.3 - 3.6 %). Moreover, the contents of linalool and 1,8-cineole were the lowest in the sample collected at 19h.

Table 1. Yields and chemical composition (expressed in %w/w) of the essential oils obtained from aerial parts of *O. basilicum*, *O. canum* and *O. gratissimum* as function of harvesting time

Components	KI*	*O. basilicum*					*O. canum*				*O. gratissimum*				
		7h	10h	12h30	16h	19h	7h	10h	16h	19h	7h	10h	12h30	16h	19h
Monoterpene hydrocarbons															
α-Pinene	932	0.45	0.34	0.51	0.46	0.58	7.94	6.41	6.67	5.64	4.55	6.27	6.81	5.05	4.58
Camphene	948	0.05	0.05	0.07	0.05	0.06	0.21	0.16	0.18	0.13	0.12	0.14	0.15	0.11	0.10
Sabinene	972	0.30	0.24	0.35	0.31	0.25	1.14	1.07	1.32	0.89	0.16	0.14	-	0.54	0.52
β-Pinene	977	0.83	0.69	0.94	0.80	1.02	0.50	0.51	0.56	0.36	3.79	5.45	5.48	4.76	4.33
β-Myrcene	989	0.80	0.63	0.82	0.83	0.92	5.92	4.40	6.11	4.62	0.16	0.20	0.22	0.18	0.16
2-Carene	991	-	-	-	-	-	3.66	3.69	4.26	3.17	2.71	4.24	4.60	4.14	3.78
α-Phellandrene	1003	-	-	-	-	-	0.42	0.40	0.55	0.35	-	-	-	-	-
3-Carene	1009	-	-	-	-	-	10.70	8.26	9.87	10.60	-	-	-	-	-
p-Cymene	1026	-	-	-	-	-	22.80	19.50	26.20	18.10	13.60	10.9	18.00	12.10	13.30
4-Carene	1028	0.14	0.09	0.15	0.14	0.12	0.35	0.16	0.13	0.26	0.28	0.32	0.38	0.30	0.31
β-Limonene	1031	1.67	1.32	1.66	1.45	1.97	1.27	1.28	1.75	1.09	0.80	1.14	1.23	0.91	0.92
cis-Ocimene	1040	0.05	0.03	0.07	0.07	0.05	0.35	0.26	0.34	0.28	0.23	0.41	0.36	0.28	0.24
trans-Ocimene	1050	-	-	-	-	-	0.23	Tr	0.18	0.21	-	-	-	-	-
γ-Terpinene	1059	0.08	0.05	0.08	0.07	0.05	-	-	-	-	12.50	19.1	18.6	19.3	18.1
4-Methylstyrene	1069	-	-	-	-	-	-	-	-	-	1.36	1.78	1.68	1.46	1.42
p-α-Dimestyl Styrene	1088	-	-	-	-	-	2.00	0.63	1.38	1.72	-	-	-	-	-
Oxygenated monoterpenes															
1,8-Cineole	1033	9.67	10.40	10.90	12.80	8.22	-	-	-	-	0.33	0.39	0.39	0.35	0.31
Linalool	1100	27.60	29.80	28.70	30.50	24.60	tr	0.20	0.03	0.61	-	-	-	-	-
Camphor	1149	-	-	-	-	-	-	-	-	-	0.16	0.09	0.12	0.09	0.10
Umbellulone	1170	-	-	-	-	-	0.29	0.08	0.06	0.26	0.16	0.18	0.12	0.12	0.12
Borneol	1173	-	-	-	-	-	-	-	-	-	0.52	0.30	0.46	0.33	0.45
Terpinen-4-ol	1183	0.33	0.34	0.28	0.29	0.37	1.08	0.34	0.64	0.94	2.15	1.50	1.90	1.42	1.80
p-Cymene-8-ol	1185	-	-	-	-	-	0.52	0.18	0.11	0.46	-	-	-	-	-
α-Terpineol	1197	2.60	2.06	2.00	1.77	2.62	-	-	-	-	2.39	1.97	1.84	1.63	1.82
Estragol	1213	43.00	44.60	44.70	43.20	44.60	tr	0.08	0.10	0.13	0.33	0.25	0.07	0.39	0.19

table 1. (continued).

Components	KI*	*O. basilicum*					*O. canum*				*O. gratissimum*				
		7h	10h	12h30	16h	19h	7h	10h	16h	19h	7h	10h	12h30	16h	19h
Nerol	1234	0.14	0.12	0.12	0.10	0.14	-	-	-	-	-	-	-	-	-
Neral	1246	0.42	0.33	0.36	0.31	0.45	-	-	-	-	-	-	-	-	-
Chavicol	1259	0.42	0.34	0.20	0.32	0.78	-	-	-	-	-	-	-	-	-
Geraniol	1268	0.18	0.17	0.12	0.19	0.39	-	-	-	-	-	-	-	-	-
Geranial	1275	0.33	0.33	0.28	0.24	0.27	-	-	-	-	-	-	-	-	-
Thymol	1312	-	-	-	-	-	-	-	-	-	37.70	35.10	28.30	30.70	32.60
Carvacrol	1316	-	-	-	-	-	29.00	16.10	12.00	30.80	2.10	0.14	1.13	1.16	1.37
Methyl eugenol	1409	-	-	-	-	-	0.19	0.31	0.21	0.28	-	-	-	-	-
Sesquiterpenes hydrocarbons															
α-Copaene	1376	-	-	-	-	-	0.17	0.13	0.19	0.08	-	-	-	-	-
α-Caryophyllene	1381	-	-	-	-	-	0.25	1.55	0.72	0.33	0.31	0.23	0.21	0.40	0.33
β-Caryophyllene	1424	-	-	-	-	-	0.15	11.30	9.50	3.58	3.44	2.4	2.33	5.32	4.27
trans-α-Berga motene	1436	-	-	-	-	-	0.29	0.43	0.26	0.26	-	-	-	-	-
b-Bergamotene	1444	3.53	2.14	1.79	1.29	3.61	-	-	-	-	-	-	-	-	-
ã-Elemene	1448	0.23	0.09	0.18	0.14	0.19	0.69	1.38	2.22	1.09	-	-	-	-	-
α-Humulene	1457	0.66	0.43	0.38	0.31	0.61	-	-	-	-	-	-	-	-	-
Germancrene D	1481	-	-	-	-	-	0.17	1.07	0.95	0.28	-	-	-	-	-
β-Selinene	1484	-	-	-	-	-	2.74	6.20	9.22	4.74	3.18	2.23	1.53	3.37	2.74
δ-Cadinene	1530	0.91	0.62	0.54	0.44	1.03	-	-	-	-	0.37	0.32	0.22	0.39	0.35
Oxygenated sesquiterpenes															
Caryophyllene oxide	1580	-	-	-	-	-	0.35	0.16	0.47	0.46	0.79	0.16	0.22	0.28	0.40
Cubenol	1627	0.23	0.17	0.16	0.12	0.31	-	-	-	-	-	-	-	-	-
τ-Cadinol	1652	2.41	1.80	1.71	1.31	3.58	-	-	-	-	0.49	0.39	0.19	0.58	0.54
Total identified (%)		97.03	97.18	97.07	97.51	96.79	93.38	88.24	96.18	91.72	94.68	95.74	96.54	95.66	95.15
Essential oil yield **		0.65	0.57	0.65	0.75	0.70	1.71	1.69	1.70	1.78	0.78	1.70	1.80	1.70	1.60

tr: trace (≤ 0.01 %); * KI: Kovats index determined on a CP Wax 52 CB GC column

** Essential oil yield was expressed as %w/w

In regard to essential oils of *O. canum*, the complex mixture of volatile molecules was dominated by carvacrol, *p*-cymene and 3-carene, respectively, as major constituents. A previous study on the *O. canum* essential oils showed that two chemotypes, a linalool type (32-50 %) and a terpinen-4-ol type (25-53 %), were described from different regions in Benin [18]. However, according to the present study, it can be suggested that the investigated plant may constitute a new chemotype: carvacrol and p-cymene. The other reported chemotypes of this species are camphor/longipinanol from India [27], citral from Croatia [7], 1,8-cineole from Burkina Faso [28,29] and Comoro Island [30], limonene/1,8-cineole and linalool/geraniol from Cameroon [36] and methyl chavicol/linalool from Brazil [37]. In the present investigation, the influence of the harvesting time on the composition of essential oils was also observed. Specifically, the content of carvacrol in *O. canum* leaves decreased gradually from the beginning of the day (7h) (29.0 %) to reach a minimum in the afternoon (12.0 %) before ascending to 30.8 % at 19h. On the other hand, β-selinene content increased during the day except at 19h. Considering the second major constituent (p-cymene), a diurnal variation was observed (17.5-26.2 %), while there were slight changes in other components as α-pinene (5.6-7.9 %), β-myrcene (4.4-6.1 %) and 3-carene (8.3-10.7 %).

Thymol was found as major constituent in leaf essential oil of *O. gratissimum*, followed by γ-terpinene and p-cymene. These results were in agreement with the work of Ngassoum *et al.*,[38] suggesting the chemotype thymol/γ-terpinene/p-cymene. On the other hand, Kpoviessi *et al.*,[31] found that p-cymene was predominant in *O. gratissimum*, followed by thymol and γ-terpinene. The investigated chemotype differs from those reported previously, which were the eugenol type [12,27,34], linalool type [39], methyl cinnamate type [40] and ethyl cinnamate type [41]. Variations of the percentages of chemical constituents were observed according to daytime of collection, including major constituent as thymol (28.3-37.7 %), γ-terpinene (12.5-19.3 %) and p-cymene (12.1-18 %). Being highest in the morning at 7h, the content of thymol (37.7 %) decreased progressively to 28.3 % at 12h30 before ascending to 32.6 % at 19h, while p-cymene content highly increased at 12h30. In contrast to thymol behaviour, γ-terpinene, the second major constituent in *O. gratissimum* oil, was the lowest at 7h, and then constantly reached high content from 10h to 16h before decreasing at 19h. This variation could be due to interconversion of between components, which referred to the complexity of biosynthetic reactions taking place in the plant during daytime [20]. Other major components in *O. gratissimum* oils such as α-pinene, β-pinene and 2-carene followed the similar variation than γ-terpinene and p-cymene.

Comparison of our findings to those reported in previous studies showed differences in terms of yields and chemical compositions of these oils. This could be due to a number of factors including differences in agronomic conditions [14], planting site or geographical locations [12,20], season at the time of collection [10], stage of development [20], processing of plant materials before extraction of oils [16,20] and occurrence of chemotypes (varieties) [27,36].

Antibacterial activity of essential oils

The antibacterial activity of essential oils of *O. basilicum*, *O. canum* and *O. gratissimum* against foodborne pathogenic *L. monocytogenes* and *S.* Typhimurium is presented in Tables 2 and 3. The three *Ocimum* species exhibited antimicrobial activity against both pathogens. The results from paper disc diffusion assay followed by MIC and MBC indicated that *O. basilicum* oils collected at different timeframes showed similar weak activities with diameters of inhibition zones ranging from 10.6-11.9 mm and MICs/MBCs of 6-16 µL/mL / 8-32 µL/mL for *L. monocytogenes* and 10.0 -11.5 mm with MICs/MBCs of 2-12 µL/mL / 2.50 -12 µL/mL for *S.* Typhimurium. The antimicrobial activity of *O. basilicum* var. *difforme* oil of similar composition against bacteria including *L. monocytogenes* and *L. ivanovii* using the disc diffusion method was already reported by Carovic-Stanko and co-authors [7]. We observed that the antimicrobial effect varied in function of harvesting time since the growths of both *L. monocytogenes* and *S.* Typhimurium were inhibited by

Table 2. Diameters of inhibitory zones (mm) of *O. basilicum*, *O. canum* and *O. gratissimum* essential oils and reference compounds against food pathogenic *L. monocytogenes* NCTC 11994 and *S. Typhimurium* ATCC 14028

Time of harvest	*Listeria monocytogenes*		*Salmonella typhimurium*	
	O. basilicum	**Linalool***	***O. basilicum***	**Linalool***
7h	11.7 ± 0.4[a]	ND	11.1 ± 0.2[ab]	8.8 ± 0.1[a]
10h	10.8 ± 1.5[a]	ND	10.1 ± 0.6[a]	8.9 ± 0.1[a]
12h30	11.9 ± 0.2[a]	ND	10.0 ± 1.1[a]	8.9 ± 0.2[a]
16h	11.1 ± 0.4[a]	ND	11.5 ± 0.8[b]	9.2 ± 0.0[b]
19h	10.6 ± 1.4[a]	ND	10.8 ± 0.4[ab]	8.8 ± 0.2[a]
	O. canum	**Carvacrol***	***O. canum***	**Carvacrol***
7h	31.8 ± 0.9[ab]	29.3 ± 0.7[a]	18.8 ± 0.5[ac]	20.0 ± 0.7[a]
10h	30.3 ± 0.9[b]	25.5 ± 0.7[b]	18.4 ± 0.9[a]	18.1 ± 0.4[b]
16h	24.5 ± 1.1[c]	23.3 ± 0.7[c]	13.0 ± 0.8[b]	17.0 ± 0.3[c]
19h	32.4 ± 0.9[a]	30.4 ± 0.9[a]	20.1 ± 0.6[c]	20.4 ± 0.2[a]
	O. gratissimum	**Thymol***	***O. gratissimum***	**Thymol***
7h	33.7 ± 0.3[a]	31.4 ± 0.4[a]	21.6 ± 0.7[a]	21.5 ± 0.6[a]
10h	32.0 ± 0.4[ac]	31.1 ± 0.4[a]	18.9 ± 0.8[b]	21.2 ± 0.3[a]
12h30	33.0 ± 0.5[b]	28.3 ± 0.2[b]	19.1 ± 0.5[b]	20.5 ± 0.2[b]
16h	32.3 ± 0.5[bcd]	29.1 ± 0.3[c]	19.5 ± 0.5[b]	21.0 ± 0.3[ab]
19h	32.9 ± 0.5[ad]	30.2 ± 0.3[d]	19.3 ± 0.7[b]	21.0 ± 0.2[ab]
Antibiotics**				
Ampicillin	33.7 ± 1.2	33.7 ± 1.2	28.8 ± 0.3	28.8 ± 0.3
Chloramphenicol	27.8 ± 1.0	27.8 ± 1.0	29.0 ± 0.6	29.0 ± 0.6
Streptomycin	18.9 ± 0.6	18.9 ± 0.6	16.2 ± 0.4	16.2 ± 0.4
Blank control	ND	ND	ND	ND

* Major active component of the oil of each species tested at the same concentration as the one present in the different essential oils

** Ampicillin (10 µg), chloramphenicol (30 µg) and streptomycin (10 µg) used as reference standard

Values are mean diameter of inhibition zone (mm) ± standard deviation of five determinations

Means followed by different letters in the same column for each essential oil and their main components represent significant differences ($p < 0.05$)

ND: not detected (diameter of inhibition zone < 7 mm considered as no antimicrobial activity)

O. basilicum oils collected from 7h to 16h with MICs of 6 µL/mL and 2-3 µL/mL, respectively, whereas *O. basilicum* oil from 19h appeared to be less active with MIC of 12 µL/mL. This antimicrobial activity seemed to be related to the content of linalool, as the sample collected at 19h had the lowest linalool content. Some studies also demonstrated that higher linalool containing oils of *O. basilicum* exhibited higher antimicrobial activity [7,10]. Furthermore, the major compound (methylchavicol = estragol) was previously shown to be devoid of antimicrobial activity [5]. We also observed that linalool may explain a significative part of the activity on *S.* Typhimurium even though no inhibition zone was observed on the strain of *L. monocytogenes* we used (Table 2). This compound is already known to possess antimicrobial activities [9,10,32,42,43].

On the other hand, *O. canum* and *O. gratissimum* exhibited strong antimicrobial activities. Based on the results obtained from paper disc diffusion assay, the antibacterial activity of *O. canum*

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *O. basilicum* (OB), *O. canum* (OC) and *O. gratissimum* (OG) essential oils and their main active components

Sample-time of harvest	*Listeria monocytogenes*		*Salmonella Typhimurium*	
	MIC*	MBC*	MIC*	MBC*
OB-7h	6.00 ± 2.31^{a}	8.00 ± 0.00^{a}	3.00 ± 1.15^{a}	3.00 ± 1.15^{a}
OB-10h	6.00 ± 2.31^{a}	8.00 ± 0.00^{a}	2.00 ± 0.00^{a}	2.50 ± 1.00^{a}
OB-12h30	6.00 ± 2.31^{a}	8.00 ± 0.00^{a}	2.75 ± 1.50^{a}	3.00 ± 1.15^{a}
OB-16h	6.00 ± 2.31^{a}	8.00 ± 0.00^{a}	2.00 ± 0.00^{a}	2.50 ± 1.00^{a}
OB-19h	16.00 ± 0.00^{b}	32.00 ± 0.00^{b}	12.00 ± 4.62^{b}	12.00 ± 4.62^{b}
Linalool**	1.00 ± 0.00^{c}	2.00 ± 0.00^{c}	2.00 ± 0.00^{a}	2.50 ± 1.00^{a}
OC-7h	ND	ND	ND	ND
OC-10h	1.00 ± 0.00^{a}	1.50 ± 0.58^{a}	2.00 ± 0.00^{a}	2.50 ± 1.00^{a}
OC-16h	ND	ND	ND	ND
OC-19h	0.50 ± 0.00^{b}	1.25 ± 0.00^{a}	1.00 ± 0.00^{b}	1.25 ± 0.00^{b}
Carvacrol**	0.13 ± 0.00^{c}	0.31 ± 0.13^{b}	0.13 ± 0.00^{c}	0.13 ± 0.00^{c}
OG-7h	0.38 ± 0.14^{a}	0.44 ± 0.14^{a}	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}
OG-10h	0.50 ± 0.00^{ac}	0.75 ± 0.29^{ac}	1.00 ± 0.00^{a}	1.25 ± 0.50^{ab}
OG-12h30	1.50 ± 0.58^{b}	1.50 ± 0.58^{b}	1.50 ± 0.58^{b}	1.75 ± 0.50^{b}
OG-16h	0.75 ± 0.29 ^{c}	0.88 ± 0.25^{c}	1.00 ± 0.00^{a}	1.50 ± 0.58^{ab}
OG-19h	0.50 ± 0.00^{ac}	0.63 ± 0.25^{ac}	1.00 ± 0.00^{a}	1.25 ± 0.50^{ab}
Thymol**	0.25 ± 0.00^{a}	0.44 ± 0.00^{a}	0.25 ± 0.00^{c}	0.44 ± 0.13^{c}

ND: not determined

* MIC and MBC were expressed as µL/mL

** Linalool for *O. basilicum*, carvacrol for *O. canum* and thymol for *O. gratissimum*

Values are mean ± standard deviation of four determinations for each essential oils and components

Means followed by different letters in the same column for each essential oil and their main components represent significant difference ($p < 0.05$)

decreased progressively from 7h to 16h before recovering its activity at 19h ($p < 0.05$). The *O. canum* oils collected at 7h and 19h were clearly found to be the most active against both pathogens with no significant difference. The MIC/MBC values at 19h were 0.50/1.25 µL/mL against *L. monocytogenes*, and 1.0/1.25 µL/mL against *S.* Typhimurium. Previously, *O. canum* oil was found to be effective against a wide range of bacteria including *L. monocytogenes*, *L. ivanovii*, *L. innocua* and *S. enterica* using paper disc diffusion assay [7,29]. In this study, the highest antibacterial activity corresponded to the highest content in carvacrol (30.8 %) present in oil of *O. canum* harvested at 19h. Tests realized with pure carvacrol, known to possess antimicrobial activities [42-45] showed that it can almost completely explain the antibacterial activity of the oils.

Even if all oils of *O. gratissimum* give a quite similar effect in the paper disc diffusion test against *L. monocytogenes,* the activity was found to be lower (MIC and MBC 1.50 µL/mL) at noon time ($p < 0.05$). Against *S.* Typhimurium, their activities were also similar with MIC of 1.00 µL/mL, except at 12h30 showing a higher MIC of 1.50 µL/mL. This could be related to lower thymol content (28.3 %). The activity of *O. gratissimum* oil against some bacteria and fungi were also reported in previous works [11,31,39,46]. Among previously published studies, the antibacterial activity of thymol-containing oil of *O. gratissimum* was only reported in the work of Ngassoum and co-authors [37] against some Gram-negative and Gram-positive bacteria other than *L. monocytogenes* and *S.* Typhimurium. Thymol was also previously shown to possess antimicro-

bial activities [42-45] and inhibition diameters obtained for thymol in the same concentration as in the different oils show that it is responsible for an important part of the activity, but other antimicrobial components are also present.

Interestingly, to our knowledge, it is the first time that the antimicrobial activities of these carvacrol and thymol types of *O. canum* and *O. gratissimum* against *L. monocytogenes* and *S.* Typhimurium are described.

Conclusion

Overall, *O. basilicum* oils showed weak antimicrobial activity against both pathogenic *L. monocytogenes* and *S.* Typhimurium, while *O. canum* and *O. gratissimum* oils exhibited prominent inhibitory actions. The impacts of harvesting time on the antimicrobial efficacy and the compositions of the essential oils from the three *Ocimum* species collected in Benin were also observed. This effect has to be taken into account, in addition to the effects of the chemotype, geographic localization, composition of the soil, maturity of the plant or season of collection. Our findings will help to select the best harvesting time for optimal antimicrobial activity. Further studies should be considered to evaluate their practical effectiveness in food preservation. Furthermore, the safety and toxicity of these essential oils need to be evaluated beforehand.

Acknowledgement

We thank the University Cooperation for Development (CUD, Belgium) program for providing scholarship, the International Foundation for Science (IFS) and Belgian National Fund for Scientiûc Research (FNRS) for financial support. Thank to Prof. Joëlle Quetin Leclercq for her scientific advice. Also thank to Prof. Frédéric Farnir and Mr. Luc Do Duc (Unit of Biostatistics, Bioinformatics, Economics and Animal Selection, Department of Animal Production, University of Liège) for statistical analysis of data.

References

1. **Robison, M.M. and Zhang, X. (2011).** The world medicines situation 2011. Traditional medicines: global situation, issues and challenges. WHO, Geneva.
2. **Mijovic, G. Andric, B., Terzic, D., Lopicic, M. and Dupanovic, B. (2012).** Antibiotic susceptibility of *Salmonella* spp.: a comparison of two surveys with 5 years interval. J. of IMAB, 18: 216-219.
3. **Newell, D.G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., Opsteegh M., Langelaar, M., Threfall, J. and Scheutz, F. (2010).** Food-borne diseases - The challenges of 20 years ago still persist while new ones continue to emerge, Int. J. Food Microbiol., 139: S3-S15.
4. **EFSA and ECDC. (2011).** The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. EFSA J., 9: 1-378.
5. **Bozin, B., Mimica-Dukic, N., Simin, N. and Anackov, G. (2006).** Characterization of the volatile composition of essential oils of some *Lamiaceae* spices and the antimicrobial and antioxidant activities of the entire oils. J. Agric. Food Chem., 54: 1822-1828.
6. **Nweze, E.I. and Eze, E.E. (2009).** Justification of the use of *Ocimum gratissimum* L in herbal medicine and its interaction with the disc antibiotics. MBC Complement. Altern. Med., 9: 37-42.
7. **Carovic-Stanko, K., Orlic, S., Politeo, O., Strikic, F., Kolak, I., Milos, M. and Satovic Z. (2010).** Composition and antibacterial activities of essential oils of seven *Ocimum* taxa. Food Chem., 119: 196-201.
8. **Tajkarimi, M.M., Ibrahim, S.A. and Cliver, D.O. (2010).** Antimicrobial herb and spice compounds in food. Food Control, 21: 1199-1218.
9. **Runyoro, D., Ngassapa, O., Vagionas, K., Aligiannis, N., Graikou, K. and Chinou, I. (2010).** Chemical composition and antimicrobial activity of the essential oils of four *Ocimum* species growing in Tanzania. Food Chem., 119: 311-316.

10. **Hussain, A.I., Anwar, F., Sherazi, S.T.H. and Przybylski, R. (2008).** Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chem., 108: 986-995.
11. **Nakamura, C.V., Ishida, K., Faccin, L.C., Filho, B.P.D., Cortez, D.A.G., Rozental, S., de Souza, W. and Ueda-Nakamura, T. (2004).** *In vitro* activity of essential oil from *Ocimum gratissimum* L. against four *Candida* species. Res. Microbiol., 155: 579-586.
12. **Dambolena, J.S., Zunino, M.P., López, A.G., Rubinstein, H.R., Zygadlo, J.A., Mwangi, J.W., Thoithi, G.N., Kibwage, I.O., Mwalukumbi, J.M. and Kariuki, S.T. (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., 11: 410-414.
13. **Kwee, E.M. and Niemeyer, E.D. (2011).** Variations in phenolic composition and antioxidant properties among 15 basil (*Ocimum basilicum* L.) cultivars. Food Chem., 128: 1044-1050.
14. **Klimánková, E., Holadová, K., Hajšlová, J., Èajka, T., Poustka, J. and Koudela, M. (2008).** Aroma profiles of five basil (*Ocimum basilicum* L.) cultivars grown under conventional and organic conditions. Food Chem., 107: 464-472.
15. **Zheljazkov, V.D., Cantrell, C.L., Tekwani, B. and Khan, S.I. (2008).** Content, composition, and bioactivity of the essential oils of three basil genotypes as a function of harvesting. J. Agric. Food Chem., 56: 380-385.
16. **Di Cesare, L.F., Forni, E., Viscardi, D. and Nani, R.C. (2003).** Changes in the chemical composition of basil caused by different drying procedures. J. Agric. Food Chem., 51: 3575-3581.
17. **Prasannabalaji, N., Muralitharan, G., Sivanandan, R.N., Kumaran, S. and Pugazhvendan, S.R. (2012).** Antibacterial activities of some Indian traditional plant extracts. Asian Pac. J. Trop. Dis., 2(1): S291-S295.
18. **Yayi, E., Moudachirou, M. and Chalchat, J.C. (2001).** Chemotyping of three *Ocimum* species from Benin: *O. basilicum*, *O. canum* and *O. gratissimum*. J. Essent. Oil Res., 13: 13-17.
19. **De Vasconcelos Silva, M.G., Craveiro, A.A., Abreu Matos, F.J., Machado, M.I.L. and Alencar, J.W. (1999).** Chemical variation during daytime of constituents of the essential oil of *Ocimum gratissimum* leaves. Fitoterapia, 70: 32-34.
20. **Yayi, E., Gbenou, J.D., Ahoussi, L.A., Moudachirou, M. and Chalchat, J.C. (2004).** *Ocimum gratissimum* L., siège de variations chimiques complexes au cours du développement. C.R. Chim., 7: 1013-1018.
21. **Bruneton, J. (1993).** Pharmacognosie: phytochimie, plantes médicinales. Technique et Documentation-Lavoisier, Paris, pp. 387-404.
22. **Kpoviessi, D.S.S., Gbaguidi, F.A., Kossouoh, C., Agbani, P., Yayi-Ladekan, E., Sinsin, B., Moudachirou, M., Accrombessi, G.C. and Quetin-Leclercq, J. (2011).** Chemical composition and seasonal variation of essential oil of *Sclerocarya birrea* (A. Rich.) Hochst subsp birrea leaves from Benin. J. Med. Plants Res., 5(18): 1640-4646.
23. **Adams, R.P. (2007).** Identification of essential oil components by gas chromatography and mass spectrometry. Allured Publ. Corp., Carol Stream, Illinois.
24. **Van Den Dool, H. and Kratz, P.D. (1963).** A generalisation of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr. A, 11: 463-471.
25. **Bouhdid, S., Abrini, J., Zhiri, A., Espuny, M.J. and Manresa, A. (2009).** Investigation of functional and morphological changes in *Pseudomonas aeruginosa* and *Staphylococcus aureus* cells induced by *Origanum compactum* essential oil. J. Appl. Microbiol., 106: 1558-1568.
26. **Mann, C.M. and Markham, J.L. (1998).** A new method for determining the minimum inhibitory concentration of essential oils. J. Appl. Microbiol., 84: 538-544.

27. **Verma, R.S., Padalia, R.C., Chauhan, A. and Thul, S.T. (2013).** Exploring compositional diversity in the essential oils of 34 *Ocimum* taxa from Indian flora. Ind. Crop. Prod., 45: 7-19.
28. **Djibo, A.K., Samaté, A.D. and Nacro, M. (2004).** Composition chimique de l'huile essentielle de *Ocimum americanum* Linn., syn. *O. canum* Sims du Burkina Faso. C.R. Chim., 7: 1033-1037.
29. **Bassole, I.H.N., Nebie, R., Savadogo, A., Ouattara, C.T., Barro, N. and Traore, S.A. (2005).** Composition and antimicrobial activities of the leaf and flower essential oils of *Lippia chevalieri* and *Ocimum canum* from Burkina Faso. Afr. J. Biotechnol., 4: 1156-1160.
30. **Hussane, S.O.S., Ghanmi, M., Satrani, B., Farah, A., Amarti, F., Achmet, S.M. and Chaouch A. (2011).** Composition chimique et bioactivité des huile essentielles de deux provenances d' *Ocimum canum* S. de l'île de la Grande Comore. Phytother., 9: 18-24.
31. **Kpoviessi, B.G.H.K., Ladeken, E.Y., Kpoviessi, D.S.S., Gbaguidi, F., Yehouenou, B., Quentin-Leclercq, J., Figueredo, G., Moudachirou, M. and Accombessi, G.C. (2012).** Chemical variation of essential oil constituents of *Ocimum gratissimum* L. from Benin, and impact on antimicrobial properties and toxicity against *Artemia salina* LEACH. Chem. Biodivers., 9: 139-150.
32. **Bagamboula, C.F., Uyttendaele, M. and Debevere, J. (2004).** Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. Food Microbiol., 21: 33-42.
33. **Oussalah, M., Caillet, S., Saucier, L. and Lacroix, M. (2006).** Antimicrobial effects of selected plant essential oils on the growth of a *Pseudomonas putida* strain isolated from meat. Meat Sci., 73: 236-244.
34. **Salles Trevisan, M.T., Vasconcelos Silva, M.G. Pfundstein, B., Spiegelhalder, B. and Owen, R.W. (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.
35. **Lee, S.J., Umano, K., Shibamoto, T. and Lee, K.G. (2005).** Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. Food Chem., 91: 131-137.
36. **Ngassoum, M.B., Ousmaila, H., Ngamo, L.T., Maponmetsem, P.M., Jirovetz, L. and Buchbauer, G. (2004).** Aroma compounds of essential oils of two varieties of the spice plant *Ocimum canum* Sims from northern Cameroon. J. Food Compos. Anal., 17: 197-204.
37. **Nascimento, J.C., Barbosa, L.C.A., Paula, V.F., David, J.M., Fontana, R., Silva, L.A.M. and França, R.S. (2011).** Chemical composition and antimicrobial activity of essential oils of *Ocimum canum* Sims. and *Ocimum selloi* Benth. An. Acad. Bras. Cienc., 83: 787-799.
38. **Ngassoum, M.B., Essia-Ngang, J.J., Tatsadjieu, L.N., Jirovetz, L., Buchbauer, G. and Adjoudji, O. (2003).** Antimicrobial study of essential oils of *Ocimum gratissimum* leaves and *Zanthoxylum xanthoxyloides* fruits from Cameroon. Fitoterapia, 74: 284-287.
39. **Stefan, M., Zamfirache, M., Padurariu, C., Trutã, E. and Gostin, I. (2013).** The composition and antibacterial activity of essential oils in three *Ocimum* species growing in Romania. Cent. Eur. J. Biol., 8: 600-608.
40. **Prakash, B., Shukla, R., Singh, P., Mishra, P.K., Dubey, N.K. and Kharwar, R.N. (2011).** Efficacy of chemically characterized *Ocimum gratissimum* L. essential oil as an antioxidant and a safe plant based antimicrobial against fungal and aflatoxin B1 contamination of spices. Food Res. Int., 44: 385-390.
41. **Dubey, N.K., Tiwari, T.N., Mandin, D., Andriamboavonjy, H. and Chaumont, J.P. (2000).** Antifungal properties of *Ocimum gratissimum* essential oil (ethyl cinnamate chemotype). Fitoterapia, 71: 567-569.
42. **Dorman, H.J.D. and Deans, S.G. (2000).** Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol., 88: 308-316.
43. **Burt, S.A. (2004).** Essential oils: their antibacterial properties and potential applications in foods-

a review. Int. J. Food Microbiol., 94: 223-253.

44. **Kotan, R., Cakir, A., Dadasoglu, F., Aydin, T., Cakmakci, R., Ozer, H., Kordali, S., Mete, E. and Dikbas, N. (2010).** Antibacterial activities of essential oils and extracts of Turkish *Achillea*, *Satureja* and *Thymus* species against plant pathogenic bacteria. J. Sci. Food. Agric., 90: 145-160.
45. **Castilho, P.C., Savluchinska-Feio, S., Weinhold, T.S. and Gouveia, S. (2012).** Evaluation of the antimicrobial and antioxidant activities of essential oils, extracts and their main components from oregano from Madeira Island, Portugal. Food Control, 23: 552-558.
46. **Anand, A.K., Mohan, M., Haider, S.Z. and Sharma, A. (2011).** Essential oil composition and antimicrobial activity of three *Ocimum* species from Uttarakhand (India). Int. J. Pharm. Pharm. Sci., 3(3): 223-225.