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Chemical composition, antioxidant effects and antimicrobial activities of some spices' essential oils on food pathogenic bacteria

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***Thymus vulgaris, Cinnamomum zeylanicum* and *Ocimum gratissimum* are spices widely used as aroma enhancers and food preservatives. This work assessed the chemical composition, antioxidant and antimicrobial effect of their essential oils on some food pathogenic bacteria, namely, *Staphylococcus aureus, Citrobacter freundii, Enterobacter cloacae, Morganella morganii, Escherichia coli, Salmonella typhimurium, Proteus vulgaris* and *Shigella flexineri*. After chemical analyses of the essential oils by gas chromatography and gas chromatography coupled to mass spectroscopy, the antimicrobial effects were subsequently assessed by disk and microdilution methods, while the antioxidant evaluations were performed by free radical scavenging activity. *T. vulgaris* essential oil composed of p-cymene (45.90%) and thymol (23.72%) which exhibited the highest inhibitory diameters of 20.33±0.58 and 18.00±1 mm, respectively, on the growth of *S. aureus* and *C. freundii. O. gratissimum* essential oil with thymol as major compound (47.11%) was more active to inhibit the growth of *C. freundii* and *S. flexineri* with respective inhibitory diameters of 18±1.73 and 16±2 mm. Essential oil from dry leaves of *C. zeylanicum* containing cinnamaldehyde (82.23%) and linalool (12.12%) was found to have the lowest values for minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) (≤3.53 mg/mL) considering the growth of *S. flexineri*, *C. freundii* and *E. cloacae*. Regarding the antioxidant effect, *C. zeylanicum* and *T. vulgaris* essential oil showed the most scavenging effect with half-maximal DPPH scavenging concentration (SC_{50}) of 2.5×10^{-5} and 6.5×10^{-5} mg/ml, respectively. Their antioxidant effects were higher than conventionally used antioxidants in food products, butylhydroxyltoluene (BHT) and vitamin C.**

Key words: Spices, essential oils, chemical composition, antibacterial and antioxidant effects.

INTRODUCTION

Spices are used as flavour and aroma in many processed and cooked food. Different amounts of spices are used by different populations all over the world. Chemical components mainly belonging to the essential oil (EO) fraction are responsible for the flavour and aroma of spices. Some of these compounds have medicinal properties and can exert bactericidal and bacteriostatic effects (Nwinyi et al., 2009). These antimicrobial effects

are provided by the presence of peptides, alkaloids and EO compounds, which are the major components in these plants (Okigbo and Igwe, 2007). Associated to these antimicrobial effects, spices have been attributed antioxidant properties. In fact, these activities due to compounds that, when present at low concentrations compared to that of an oxidizing substrate, markedly delay or prevent its oxidation (Cavero et al., 2005). Hence, food or supplements containing spices or their compounds may contribute to alleviate the pathologies associated with the presence of reactive oxygen species (ROS) originating from oxidative processes in human cells (Nwinyi et al., 2009). The whole plant, part of it, flowers or fruits are generally used as spices. Added to this, the different agro-ecological environment where the spices are produced can influence the composition and therefore their biological activity. Applications of spices for their antimicrobial and antioxidant properties have been proposed by some authors (Hernández-Ochoa et al., 2014; Burt, 2004; Essia Ngang et al., 2014) and these properties have been related to the composition of the spices (Hyldgaard et al., 2012). In this perspective, it is important to evaluate the effectiveness of the antimicrobial and antioxidant properties of spices typical for certain locality, because of important differences in activity that may occur as a result of the different chemical composition. This is the rational that brought us to assess the chemical composition, the antimicrobial and antioxidant properties of EOs from *Thymus vulgaris, Cinnamomum zeylanicum* and *Ocimum gratissimum*.

T. vulgaris is the most widely cultivated species of the Laminaceae family (Alonso, 2004; Al-Bayati et al., 2008). It is used as spice and traditional drug for antispasmolytic, antibacterial and antifungal illnesses (Özgüven and Tansi, 1998). Regarding *C. zeylanicum*, many species are reported throughout the world, but the most used are those from Sri-Lanka (Richard and Multon, 1992). Its EO is rich in cinnamaldehyde and eugenol and has shown antimicrobial activities on food borne pathogens (Paranagana et al., 2003; Chang et al., 2001). On the other hand, *C. zeylanicum* that belongs to the family of Leguminocaeae, has its origin in Asia (Ndoye, 2001). In many parts of Africa, the plant is used to cure upper respiratory tract infections, diarrhoea, pneumonia, teeth, and gum disorder (Nwinyi et al., 2009).

MATERIALS AND METHODS

Plant

Samples of *T. vulgaris, O. gratissimum* and *C. zeylanicum* were collected from spice sellers in Bafoussam (West region), Yaounde (Center region) and Lolodorf (South region) of Cameroon, respectively. After collection, the spices were identified by the Cameroon National Herbarium and a voucher specimen deposited with the following codes N° 25746/SRF/Cam, 5817/SRF/Cam, and 22309/SRF/Cam for *T. vulgaris, O. gratissimum*, and *C. zeylanicum*, respectively. For *C. zeylanicum*, based on the work of Richard and Multon (1992), who attributed these activities to different parts of the plant, leaves and stem barks were separated for the study.

Preparation of EOs

The EOs of the spices were extracted by hydro distillation using a Clevenger apparatus. Oils recovered were dried over anhydrous sodium sulphate and stored at +4°C until usage. The extraction yield was calculated as the ratio of oil recovered mass over the spices mass used multiplied by 100 (Ndoye, 2001).

Chemical analyses of EOs

Gas chromatography (GC)

GC analyses were performed on a Varian gas chromatograph, model CP-3380, with flame ionization detector containing two silica capillary columns: HP5 J&W Agilent (5%-Phenyl-methylpolysiloxane) capillary column (30 m × 0.25 mm i.d. × 0.25 µm film) and Supelcowax 10 (polyethylene glycol) fused capillary column (30 m × 0.25 mm i.d. × 0.25 µm film); N_2 was the carrier gas at 0.8 ml/min; injection type 0.1 µl of pure sample, split ratio 1:100; injector temperature 220°C, detector temperature 250°C; temperature program 50 to 200°C at 5°C/min, then kept at 200°C for 10 min. The linear retention indices of the components were determined relative to the retention times of a series of *n*-alkanes.

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analyses were performed using a Hewlett–Packard GC 5890 series II equipped with a HP5 (5 % Phenylmethylpolysiloxane) fused silica column (30 m × 0.25 mm; film thickness 0.25 µm) and a DB-Wax fused silica column (30 m × 0.25 mm; film thickness 0.25 µm) interfaced with a quadrupole detector (Model 5972); temperature program (50 to 200°C at 5°C/min); injector temperature, 220°C; MS transfer line temperature, 180°C; carrier gas, helium at a flow rate of 0.6 ml/min; injection type, split, 1:10 (1 µl 10:100 CH_2Cl_2 solution); ionization voltage, 70 eV; electron multiplier 1460 eV; scan range 35 to 300 amu; scan rate, 2.96 scan/s.

Qualitative analysis

The identification of the constituents was based on comparison of their relative retention times and mass spectra with either that of authentic samples or with published data in the literature (Adams, 2007).

Preparation of test microorganism

Microbial strains of *Staphylococcus aureus, Salmonella*

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tiphymurium, Proteus vulgaris, Enterobacter cloacae, Shigella flexneri, Morganella morganii, Escherichia coli, and *Citrobacter freundii* were obtained from the culture collection of the Microbiology Laboratory of the Department of Microbiology, University of Yaoundé I, Cameroon. Strains, originally stored at –80°C were sub-cultured twice in Brain Heart Infusion (BHI) at 37°C for 24 h and conserved on Muller Hinton slants at 4°C throughout the work. These strains were chosen for their implication in food borne diseases outbreaks. Prior to each test, a loop of the strain was inoculated in Mueller Hinton broth and incubated at 37°C for 24 h. Based on preliminary assessments, the overnight cultures were diluted in order to use about 10^6 cells/ml for the antimicrobial susceptibility test.

Antimicrobial activities of the EOs

The disk method was performed according to NCCL (2009) method with some adaptations. Briefly, 100 µl of an inoculum of 10^6 cells/ml were spread on sterile Mueller Hinton agar plates and let to dry at ambient temperature. 10 µl of the EO solubilised in 10% Tween 20 solution in order to obtain a concentration of 500 mg/ml were deposited on a 6 mm diameter disk and placed at the centre of the plate containing the microorganism. A disk containing 10 µl of 10% Tween 20 solution was used as control, while disk containing 10 µg of gentamicin was adopted as reference. Inhibition diameters (intended as the zone surrounding the disk with no bacterial growth) were measured after incubation at 37°C for 24 h on each of the three repetitions performed and expressed as mean ± standard deviation. The inhibition diameter was calculated as the difference between the test and the control.

For minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC), microdilution method was used according to Bajpai et al. (2008) and Yèhouenou et al. (2010) in a 96 wells plate with single well covers. EOs diluted in 10% dimethyl sulfoxide (DMSO) were added to one of the series of wells containing the strain culture at 10^6 cells/ml and 0.02 mg/L of phenol red in order to have a concentration of 452.5 mg/L. Subsequently, series of 2 fold dilutions were prepared until a final concentration of the EO of 0.022 mg/L. A well containing the culture and phenol red without EO was used as positive control, while another well without the strain was used as negative control. After 24 h of incubation at 37°C, wells where the phenol red had changed to yellow were considered as having microbial growth, hence, indicating that the EO concentration used was not active. The MIC was considered as the lowest concentration where the phenol red did not change the initial red colour. Gentamicin used as reference antimicrobial was tested with the same methodology up to a concentration of 1600 µg/L.

For the MBC, 100 µl of the wells without visible bacterial growth were inoculated in a fresh Mueller Hinton broth and incubated at 37°C for 24 h. Following this, the lowest concentration of the EO which did not permit any growth was considered as MBC. Results for the MIC and MBC were reported as mean of the three repetitions and their standard deviation.

Free radical scavenging activity determination

Stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was used for the determination of the free radical scavenging activity of the EOs according to Brand-William et al. (1995) adapted to EO as described by Agnaniet et al. (2004). Briefly, 100 µl of the EO methanolic solution was added to 1900 µl of an ethanolic solution of 0.04 mg/ml of DPPH and the optical density (OD) read after 30 min on a Jenway 6305 Spectrophotometer at 517 nm. Four successive concentrations of the methanolic solutions were prepared for each EO. The negative control consisted of 1900 µl of ethanolic solution of DPPH and 100 µl of methanol and the positive control made of 1900 µl of ethanolic solution of DPPH and 100 µl of methanolic solution of BHT (various concentrations) or vitamin C (various concentrations) were used as reference. The DPPH scavenging activity (%) was calculated as follows:

DPPH Scavenging activity (%) = [(OD control – OD sample) / OD control] ×100

SC_{50} (concentration of the tested substance that provides 50% scavenging activity) was determined graphically on the best curve fitting the experimental points representing the percentage scavenging activities as a function of the antioxidant concentration. The data was represented as mean of the three replicates and their standard deviation.

RESULTS

EO extraction and analyses

The hydro distillation of the different parts of *C. zeylanicum* plant gave different yield ranged from 1.05 to 1.10%, while those of *T. vulgaris* and *O. gratissimum* were 0.16 and 0.48%, respectively. Regarding the composition of the EOs, Table 1 shows the percentage content of the different compounds identified. The lowest percentage of compounds identified was 98.89% obtained with the EO of *O. gratissimum*, while the highest was 100% for the EO of *C. zeylanicum* fresh leaves. Concerning the composition; *T. vulgaris* was mainly composed of p-cymene (45.90%), thymol (23.72%), sabinene hydrate (5.90%), while some popular antimicrobial compounds like carvacrol and eugenol were detected in low levels of 2.02 and 0.4%, respectively. In *O. gratissimum* EO, thymol was the major compound (47.11%) followed by ɣ-terpinene and p-cymene at 16.60 and 14.06%, respectively. The percentage of carvacrol was very low, 0.62% in this EO, as compared to *T. vulgaris* EO. Regarding *C. zeylanicum*, it can be observed that the composition variability was less than that of the previous two EOs. In fact, only 9 compounds were identified. Among the different plant material, the EO of dry and fresh leaves of *C. zeylanicum* was quite similar in composition, demonstrating that the physical state did not affect the composition. On the other hand, *C. zeylanicum* stems EO demonstrated a quite different composition with respect to the leaves. As it can be observed, the EO of the leaves was mainly composed of cinnamaldehyde (82 to 84%) and linalool (11 to 12%) with traces of thymol and carvacrol (between 0.57 and 1.10%). *C. zeylanicum* stem EO was composed of 68.11% α-terpinene, about 10% of thymol and 10% of carvacrol. Moreover, it contained 8.47% of α-guaiene which was not found in *Thymus* and *Ocimum* species.

Antibacterial activities of EOs

Disk diffusion method was used to assess the inhibitory

Table 1. Chemical composition of the essential oils tested.

S/N	Compounds names	RI * (HP-5)	*T. vulgaris* (%)	*O. gratissimum* (%)	*C. zeylanicum* (%)		
					Dry leaves	Fresh leaves	Dry stems
1	α-thujene	928	1.20	-	-	-	-
2	α-pinene	937	1.56	4.10	0.80	0.86	0.68
3	camphene	954	1.79	1.22	0.42	0.46	0.45
4	β-pinene	976	0.55	0.67	0.34	0.37	0.37
5	myrcene	981	0.29	0.37	-	-	-
6	α-phellandrene	991	1.49	3.76	-	-	-
7	α-terpinene	1021	-	0.80	1.11	1.11	68.11
8	p-cymene	1031	45.90	14.06	-	-	-
9	limonene	1034	0.51	1.05	-	-	-
10	*trans*- β-*ocimene*	1038	-	0.53	-	-	-
11	*cis*- β-*ocimene*	1049	-	0.31	-	-	-
12	*Cis- Sabinene hydrate*	1064	5.90	-	-	-	-
13	*ɣ-terpinene*	1066		16.60	-	-	-
14	linalool	1094	3.96	1.56	12.12	11.26	0.46
15	borneol	1154	2.82	0.49	-	-	-
16	p-menth-1,5-dièn-8-ol	1174	1.25		-	-	-
17	terpinen-4-ol	1184	1.24	1.08	-	-	-
18	α -terpineol	1194	-	0.44	-	-	-
19	(E)-cinnamaldehyde	1278	-	-	82.23	84.16	0.30
20	thymol	1300	23.72	47.11	0.98	0.57	10.40
21	carvacrol	1307	2.02	0.62	1.10	0.88	10.44
22	eugenol	1365	0.4	-	-	-	-
23	α-copaene	1389	-	0.35	-	-	-
24	(E)-β-caryophyllene	1435	1.53	0.59	-	-	-
25	α-guaiène	1440	-	-	0.41	0.33	8.47
26	δ-cadinene	1504	0.25	0.96	-	-	-
27	α -cadinene	1514		0.34	-	-	-
28	caryophyllene oxyde	1536	0.43	0.25	-	-	-
29	eudesmol	1606	2.47	1.63	-	-	-
30	Epi- α-eudesmol	1658	0.33	-	-	-	-
% compounds identified			**99.61**	**98.89**	**99.51**	**100**	**99.68**
31	NI	1286	0.36	-	-	-	-
32	NI	1781	-	-	0.49	-	0.31

*According to Adams (2007).

effect of the EOs on the growth of selected pathogens. Table 2 shows the diameters of inhibition obtained from *T. vulgaris, O. gratissimum* and different parts of *C. zeylanicum* on tested bacterial species. The absence of inhibition zone was observed when the EO of *C. zeylanicum* dry stem bark was tested against *S. aureus*, *S. typhimurium* and *E. coli*. On the other hand, the highest sensitivity to the EOs was observed for *S. aureus* in the presence of *T. vulgaris* EO (20.33±0.58 mm), followed by *C. freundii*, 18.00±1 mm and 18.00±1.73 mm in the presence of *T. vulgaris* and *O. gratissimum* EOs, respectively. There was no statistical difference between the inhibition diameters of *C. zeylanicum* fresh and dry leaves EOs against the microorganisms tested. In general, the EOs that were tested at 500 mg/L (this means 50 µg of the EO deposited on the disks) gave lower or comparable inhibition zone compared to those obtained with gentamicin whose disk contained 10 µg of the antibiotics.

The MIC and MBC of EOs and that of gentamicin used as reference were assessed by the microdilution method. The results presented in Table 3 are expressed in mg/L for the EOs and µg/L for gentamicin. Due to the results obtained with the disk method, *C. zeylanicum* fresh leaves were not tested since it was demonstrated to have the same composition and antimicrobial potential as *C.*

Table 2. Diameters of inhibition of the essential oils of *T. vulgaris, O. gratissimum, C. zeylanicum* (dry leaves, fresh leaves and dry stems) and gentamicin on selected microorganism using the disk method.

Strain	*T. vulgaris*	*O. gratissimum*	*C. zeylanicum*			Gentamicin
			Dry leaves	Fresh leaves	Dry stems	
	Diameter (mm)					
S. aureus	20.33±0.58	13.67±2.31	12±1.73	9.33±1.53	0	22.67±0.58
S. typhimurium	12±2.65	11.33±2.52	9±1.00	10±1.00	0	18.33±0.58
S. flexineri	14.67±2.08	16±2.00	13.33±0.58	12.33±2.08	18±2	25.33±1.53
C. freundii	18±1.00	18±1.73	15±2.65	14.33±1.53	14.67±1.53	19±00
E. cloacae	11.17±2.08	15.33±3.51	10±1.00	12±3.61	14.33±3.21	15±0
E. coli	16±1.00	13±1.00	10.67±2.08	10.67±0.58	0	29.67±0.58
M. morgana	13.67±1.53	16.33±1.53	12.67±1.15	14±00	14±1.00	21.33±3.06

Table 3. Minimal inhibitory (MIC) and bactericidal concentrations (MBC) of the essential oils of *T. vulgaris, O. gratissimum, C. zeylanicum* (dry leaves and dry stems) and gentamicin on selected microorganism.

Strain	*T. vulgaris* (mg/ml)		*O. gratissimum* (mg/ml)		*C. zeylanicum* (mg/ml)				Gentamicin (µg/ml)	
					Dry leaves		Dry stems			
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	28.28	28.28	7.07	14.14	-	-	-	-	0.10	0.4
S. typhimurium	56.56	113.12	7.07	28.28	-	-	-	-	50	50
S. flexineri	14.14	56.56	14.14	28.28	1.77	1.77	226.25	452.5	100	100
C. freundii	56.56	452.50	56.56	452.5	0.88	3.53	452.5	-	100	200
E. cloacae	-	-	14.14	28.28	3.53	3.53	28.28	452.5	400	400
E. coli	14.14	28.28	7.07	14.14	-	-	-	-	100	1600
M. morgani	3.53	7.07	14.14	28.28	7.07	14.14	-	-	50	50

-No activity up to the highest concentration tested. *MIC and MBC of EO are expressed in mg/ml for the EOs and in µg/ ml for gentamicine and the standard deviations were all zero.

zeylanicum dry leaves. *T. vulgaris* and *O. gratissimum* EOs showed the most potent antimicrobial activity. In fact, it was possible to obtain a MIC and MBC with the EOs of these spices on all the microorganisms tested except on *E. Cloacae,* which were not sensitive to *T. vulgaris* up to 452.5 mg/ml. Gentamicin chosen as reference antibacterial compound was more active than the EOs. The MIC and MBC reported in Table 3 were the lowest when *S. aureus* was exposed to it (to what? gentamicin or EO; according to Table 3 it should be gentamicin). Among the Gram negative bacteria, *S. typhimurium* and *M. morgani* were the most sensitive to this antibiotic. Regarding the EOs, *C. zeylanicum* dry leaves EO was the most active to inhibit the growth of *S. flexneri*, *C. freundii* and *E. cloacae* than the other EOs tested. *C. zeylanicum* dry stem bark EO was the less active EO in general. Moreover, *C. zeylanicum* EOs, no matter the part chosen were not active on *S. typhimurium* and *E. coli* up to 452.5 mg/L.

Antioxidant properties of the EOs

The antioxidant properties of the EOs were assessed using the DPPH scavenging method. Table 4 represents the DPPH scavenging percentages obtained with EOs and the reference antioxidants BHT and vitamin C. From these data, a comparison index, namely, the SC_{50} was obtained as described in material and methods and also reported in the same table. The higher the SC_{50}, the lower the scavenging activity of the substance. It can be observed that *C. zeylanicum* EOs obtained from dry and fresh leaves had the best scavenging properties (2.5×10^{-5} mg/ml) even compared to the reference compounds. *T. vulgaris* EO also demonstrated a good antioxidant property as its SC_{50} was 6.5×10^{-5} mg/ml. These values are lower than that of BHT and vitamin C which exhibited SC_{50} of 245×10^{-5} and 11×10^{-5} mg/ml, respectively. In general, among the substances tested, BHT was the less efficient in scavenging the DPPH radicals.

DISCUSSION

Testing and proving of biological activity of EOs is one of the major aims of the study of natural substances. It is now accepted by all the scientific community that these studies should pay attention to the variability of the

Table 4. DPPH scavenging percentage (DPPH %) of the essential oils of *T. vulgaris, O. gratissimum, C. zeylanicum* (dry and fresh LEAVES), BHT and vitamin C at different concentrations (mg/ml).

	O. gratissimum		*T. vulgaris*		*C. zeylanicum*				BHT		Vitamin C	
					Dry leaves		Fresh leaves					
	Conc.	DS%	Conc.	DS%	Conc.	DS%	Conc.	DS%	Conc.	DS%	Conc.	DS%
	2.25×10^{-5}	25	2.15×10^{-4}	29	4.75×10^{-5}	75.2	4.75×10^{-5}	72	2×10^{-3}	15	1×10^{-4}	18.8
	4.5×10^{-5}	42	4.3×10^{-4}	54.5	9.5×10^{-5}	86	9.5×10^{-5}	83.5	3×10^{-3}	64	1.5×10^{-4}	82
	9×10^{-5}	60	6.45×10^{-4}	61	19×10^{-5}	91.2	19×10^{-5}	87	5×10^{-3}	86	2.5×10^{-4}	96
	13.5×10^{-5}	63.5	10.75×10^{-4}	76.5	28×10^{-5}	97.5	28×10^{-5}	99.9	10×10^{-3}	89	5×10^{-4}	100
SC_{50}	6.5×10^{-5}		47×10^{-5}		2.5×10^{-5}		2.5×10^{-5}		245×10^{-5}		11×10^{-5}	

Where, Conc. = concentration of essential oils, BHT or Vitamin C in mg/ml; DS% = %DPPH scavenging activity, and SC_{50} = the concentration necessary for the scavenging of 50% DPPH radical from solution.

biological material concerned. In fact, it is known that the composition of EOs can be different due to the species, the agro-ecological factors and the part of the plant that is being analysed (Bruneton, 1999; Brada et al., 2007). In this specific study, the EOs tested showed different composition considering the species. Within the same species, dry and fresh leaves EOs of *C. zeylanicum* had roughly the same composition, but different from that of dry stems. The compositions of the EOs of these different parts of *C. zeylanicum* were also different from that of the same plant studied in Asia and Mid Orient. Richard and Multon (1992) and Cuvelier (1997) observed that EOs of leaves of *C. zeylanicum* had eugenol and campheme as their major components whereas the barks were rich in cinnamaldehydes. Later on, Yèhonenou et al. (2010) when analysing EOs of leaves of the same plant reported similar composition to that of this work, confirming that the agro-ecological factors can be determinant to the composition of EOs of the same species. To support these findings, in some countries *C. zeylanicum* is a big tree while those harvested in Cameroon are very small plants with a stem that cannot be easily dissociated from the bark.

Regarding *T. vulgaris* EO, the composition obtained in this work indicated that the major components were p-cymene and thymol. These results are similar to those of Moghtader et al. (2012), but differ in the relative percentages of the compounds. In Moghtader et al. (2012), thymol was the major component followed by p-cymene, which is opposite to the findings in this work. Many chemo-types of *T. vulgaris* have been identified in literature. Thymol, carvacrol, thymol and even borneol were identified as chemo-types for *T. vulgaris* EOs collected from different parts of Morocco (Chebli et al., 2003). Few years ago, Imelouane et al. (2009) discovered a camphene chemo-type in Eastern Morocco. The *T. vulgaris* from Bafoussam, Cameroon, can be considered as a p-cymene chemo-type.

O. gratissimum EO analysed in this work had thymol as the major compound and also contained p-cymene and ɣ-terpinene. This is in accordance with the conclusions of Orwa et al. (2009) who observed that eugenol, thymol and carvacrol are the major compounds generally found in the EO of this plant.

The antimicrobial properties of the EOs tested in this work were assessed by disk diffusion and microdilution methods. The first method can be considered as an explorative assessment that helps in a preliminary screening. In fact, this permitted to assess that dry leaves and fresh leaves EOs of *C. zeylanicum* had the same activity and hence only the dry leaves EOs were used to assess the MIC and MBC. EO from the leaves of *C. zeylanicum* was the most active and that of the dry stem was the less active, in particular on *S. aureus*, *S. typhimurium* and *E. coli*. In fact, this EO contains 68% of α-terpinene, 10% of carvacrol and 10.4% of thymol. Among these compounds, carvacrol and thymol are mostly endowed with antimicrobial properties (Burt, 2004; Chiasson et al., 2005). The high content in cinnamaldehyde (>80%) of the leaves EO of this plant is probably the main reason of its antimicrobial activity. As a matter of fact, cinnamaldehyde antimicrobial properties according to Walsh et al. (2003) acts on bacteria by inhibiting the biosynthetic enzymes and can also, according to Oussalah et al. (2006) contribute in the reduction of bacteria intracellular pH. Hadri et al. (2014) observed that EO from *C. zeylanicum* barks with 91% cinnamaldehyde inhibited the growth of *S. aureus* and *E. coli*, while this was not the case in our study with an EO containing more than 80% of the same compound. These different results could be explained by the additional 8.5% of cinnamaldehyde acetate of the EO or by the strain variability.

T. vulgaris did not show antimicrobial activity on the growth of *E. cloacae*. The antimicrobial activity of this EO has been generally attributed to the high content in thymol and carvacrol (Sokovic et al., 2007; Khodaei Motlagh et al., 2014). In our study, thymol was present at 23% and p-cymene at 46%, while carvacrol was only 2%. The synergy between thymol and carvacrol may be the

base of the antimicrobial properties of this EO. Knowing that thymol is a more potent antimicrobial than p-cymene, the inversion of the percentage contains those of these compounds in *O. gratissimum* EO (thymol 47% and p-cymene 14%) gave way to a more important antimicrobial activity. It was observed that MIC and MBC of *O. gratissimum* EO were generally lower than those obtained with *T. vulgaris* for the same microorganism. Beside the fact that antimicrobial activities of thymol are well known, the interaction of all the molecules can have a synergic effect that may potentiate the activity of the whole EO. In this regard, a synergic activity has been reported for many compounds (Jayaprakasha et al., 2003; Matasyoh et al., 2007). The EOs activities are attributed to their disorganising effect of the molecules on the cell membrane, leading to a non control of the exchange between cytoplasm and the external medium (Bouhdid et al., 2009; Gardini et al., 2009). After entering into the microorganism's cytoplasm, other mode of action could be the acidification (Wright, 2002) and the destruction of the genetic material (Brackman et al., 2008; De Martino et al., 2009).

The antioxidant activity of the EOs was also assessed and compared to BHT, a primary antioxidant with the ability to inactivate free radicals by transferring a proton (Cuvelier, 1997) and vitamin C as a secondary antioxidant that blocks oxygen and metals that can faster the oxidation (Cuvelier, 1997). In the case of this study, *C. zeylanicum* leaves EO was the most active in scavenging the DPPH followed by the EO of *T. vulgaris*. This may be due to the presence of linalool in larger extent than that of cinnamaldehyde. Although an *in vivo* antioxidant property have been reported for cinnamaldehyde in rat kidney (Gowder and Devaroy, 2006), this aromatic aldehyde has a low proton transferring property than linalool. On the other hand, thymol's percentage is higher in *O. gratissimum* EO compared to *T. vulgaricus*. The latter gave the best scavenging properties. The presence of 4% linalool and 45% p-cymene may be the reason for this activity. De Oliveira et al. (2015) observed that p-cymene has an antioxidant potential *in vivo* as well as a neuroprotective agent in the brain. The reason behind the good scavenging performances of the EOs with respect to the reference compounds may be the interactivity of all the compounds composing the EOs. It can be observed from these results that these EOs can well replace BHT in the limitation of oxidation in food and tissues.

Conclusion

EOs of *T. vulgaris, O. gratissimum* and *C. zeylanicum* showed a high composition variability depending on the species, and within the same species of *C. zeylanicum*, between leaves and stem. This variability was also highly influenced by the agro-ecological factors as demonstrated by comparison of the results with those in the literature. Moreover, the studied antimicrobial properties were of large spectra for *T. vulgaris* and *O. gratissimum* EOs, while that of *C. zeylanicum* leaves was more active to inhibit the growth of *S. flexneri* and *C. freudii* than the other EOs. These EOs demonstrated to be good natural antioxidants that may be used in food industries and in the prevention of oxidative degenerative diseases.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Adams RP (2007). Identification of essential oil components, by Gas Chromatography/Mass Spectrometry, 4th edition, Allured Publishing Corporation Carol Stream, IL.

Agnaniet H, Makani T, Akagah A, Menut C, Bessiere JM (2004). Volatile constituents and antioxidant activity of essential oils from Lippia multiflora Mold growing in Gabon. Flavour Flagr. J. 20(1)34-38.

Al-Bayati FA (2008). Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. J. Ethnopharmacol. 166(3):403-406.

Bajpai KV, Dung NT, Kwon JO, Kang SC (2008). Analysis and the potential application of essential oil and leaf extracts of Silene Americana L. to control food spoilage and food-borne pathogens. J. Food Technol. 227:1613-1620.

Bouhdid S, Abrinin J, Zhiri A, Espuny MJ, Munresa A (2009). Investigation of functional and morphological changes in *Pseudomonas aeruginos* and *Staphylococcus aureus* cells induced by *Origanum compactum* essential oil. J. Appl. Microbiol. 106:1558-1568.

Brackman G, Defoirdt T, Miyamoto C, Bossier P, Calenbergh SV, Nelis H, Coenye T (2008). Cinnamaldehyde and cinnamaldehyde derivatives reduce virulence in *Vibrio* spp. by decreasing the DNA-binding activity of the quorum sensing response regulator LuxR. BMC Microbiol. 8:149.

Brada M, Mohamed B, Marlier M, Carlier A, Lognay G (2007). Variabilité de la composition chimique des huiles essentielles de *Mentha rotundifoglia* du Nord d'Algerie. Agron. Soc. Environ. 11(1):3-7.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. LWT Food Sci. Technol. *2*8:25-30.

Bruneton J (1999). Pharmacologie, Phytochimie, plantes medicinales. 3th Tec and Doc, Lavoisier Paris. P 915.

Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods. Int. J. Food Microbiol. 94:223-253.

Cavero S, Jame L, Martin-ĂIvarez PJ, Señoráns F.J, Reglero G, Ibañez E (2005). *In vitro* antioxidant analysis of supercritical fluid extracts from rosmary (*Rosmarinus officinalis* L). J. Eur. Food Res. Technol. 221:478-486.

Chang C, Gonzalez F, Rothermel B, Sun L, Johnston SA, Kodadek T (2001). The Gal4 activation domain binds Sug2 protein, a proteasome component, *in vivo* and *in vitro.* J. Biol. Chem. 276(33):30956-30963.

Chebli CH, Achouri M, Hassani LMI, Hmamouchi M (2003). Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against *Botrytis cinerea* Pers. Fr. J. Ethnopharmacol. 89(1):165-169.

Chiasson F, Borsa J, Lacroix M (2005). Combined effect of carvacrol and packaging conditions on radiosensitivity of *Escherichia coli* and *Salmonella typhi* in ground beef. J. Food Prot. 68:2567-2570.

Cuvelier ME (1997). Molécules antioxidantes: relation structure-activité. 16eme journée internationales des huiles essentielles.

De Martino L, de Feo V, Nazzaro F (2009). Chemical composition and *in vitro* antimicrobial and mutagenic activities of seven *Lamiaceae*

essential oils. Molecules 14:4213-4230.
De Oliveira TM, de Carvalho RB, da Costa IH, de Oliveira GA, de Souza AA, de Lima SG, de Freitas RM (2015). Evaluation of p-cymene, a natural antioxidant. Pharm Biol. 53(3):423-428.
Essia Ngang JJ, Nyegue MA, Foe Ndoye C, Tchuenchieu Kamgain AD, Sado Kamdem SL, Lanciotti R, Gardini F, Etoa FX (2014). Characterization of Mexican Coriander (*Eryngium foetidum*) Essential Oil and Its Inactivation of *Listeria monocytogenes* In Vitro and during Mild Thermal Pasteurization of Pineapple Juice. J. Food Prot. 77(3):435-443
Gardini F, Belletti N, Ndagijimana M, Guerzoni ME, Tchoumbougnang F, Amvam PH, Micci C, Lanciotti R, Sado SL (2009). Composition of four essential oils obtained from plants from Cameroon, and their bactericidal and bacteriostatic activity against *Listeria monocytogenes*, *Salmonella enteritidis* and *Staphylococcus aureus*. Afr. J. Microbiol. Res. 3:264-271.
Gowder SJ, Devaraj H (2006). Effect of the food flavour cinnamaldehyde on the antioxidant status of rat kidney. Basic Clin. Pharmacol. Toxicol. 99(5):379-382.
Hadri Z, Allem R, Marin GP (2014). Effect of essential oil of Cinnamomum zeylanicum on some pathogenic bacteria. Afr. J. Microbiol. Res. 8(10):1026-1031
Hernández-Ochoa L, Aguirre-Prieto YB, Nevárez-Moorillón GV, Gutierrez-Mendez N, Salas-Muñoz E. (2014). Use of essential oils and extracts from spices in meat protection. J. Food Sci. Technol. 51(5):957-963.
Hyldgaard M, Mygind T, Meyer RL (2012). Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front. Food Microbiol. 3(12):1-24
Imelouane B, Amhamdi H, Wathelet JP, Ankit M, Khedid K, Bachiri A (2009). Chemical composition and antimicrobial activity of essential oil of Thyme (*Thymus vulgaris*) from Eastern Morocco. Int. J. Agric. Biol. 11:205-208.
Jayaprakasha GK, Mohan J, Rao L, Sakariah KK (2003). Volatile constituants from *Cinnamomum zeylanicum* Stalks and their antioxidant activities. J. Agric. Food Chem. 51:4344-4348.
Khodaei Motlagh M, Kazemi M, Ghasemi HA, Farahani K, Hossein A, Yahyaei M, Rezaei M, De Rensis F, Taddei S (2014). Antibacterial effect of medicinal plant essence (*Thymus vulgaris*) on major bacterial mastitis pathogen in vitro. Int. J. Adv. Biol. Biomed. Res. 2:286-294.
Mahdi Khodaei M, Mahdi K, Hossein AG, Amir Hossein KF, Mohhamad Y, Mohhamad R, Fabio DR and Simone T (2014). Antibacterial effect of medicinal plant essence (Thymus vulgaris) on major bacterial mastitis pathogen in vitro. Int. J. Adv. Biol. Biomed. Res. 2(2):286-294
Matasyoh LG, Wachira FN, Kinyua MG, Muigai AWT, Mukiama TK (2007). Chemical composition and antimicrobial activity of essential oil of *Ocimum gratissimum* L. growing in Eastern Kenya. Afr. J. Biotechnol. 6:760-765.
Moghtader M (2012). Antifungal effects of the essential oil from *Thymus vulgaris* L. and comparison with synthetic thymol on *Aspergillus niger.* J. Yeast Fungal Res. 3(6):83-88.
NCCLS-National Committee for Clinical Laboratory Standard (2009). Methods for Disk Susceptibility Tests for Bacteria that Grow Aerobically. *NCCL approved standard*, 8th edition M_7-A_7, vol 3. NCCLS: Wayne, PA, USA. P 7.
Ndoye C (2001). Etude chimique et évaluation des propriétés antiradicalaires et antioxydantes des huiles essentielles d'espèces aromatiques tropicales en provenance d'Est du Cameroun. Thèse de Doctorat, Université de Monpellier II. P 319.
Nwinyi OC, Chinedu NS, Ajani OO, Ikpo CO, Ogunniran KO (2009). Antibacterial effects of extracts of *Ocimum gratissimum* and *piper guineense* on *Escherichia coli* and *Staphylococcus aureus.* Afr. J. Food Sci. 3(1):22-25.
Okigbo RN, Igwe M (2007). The antimicrobial effects of *Piper guineense* uziza and *Phyllantus amarus* ebe-benizo on *Candida albicans* and *Streptococcus faecalis*. Acta Microbiol. Immunol. Hung. 54(4):353-366.
Orwa C, Mutua A, Kindt R., Jamnadass R, Anthony S (2009). Agroforestree Database: a tree reference and selection guide version 4.0. World Agroforestry. Available at: http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp.
Oussalah M, Caillet S, Lacroix M (2006). Mechanism of action of Spanish oregano, Chinese cinnamon, and savory essential oils against cell membranes and walls of *Escherichia coli O57:H7* and *Listeria monocytogenes*. J. Food Prot. 69(5):1046-1055.
Özgüven M, Tansi S (1998). Drug yield and essential oil of *Thymus vulgaris* L as in influenced by ecological and ontogenetical variation, Turk. J. Agric. For. 22:537-542.
Richard H, Multon JL (1992). Epices et aromates. ENSIA, Collection Sciences et Techniques agroalimentaires. pp. 90-96.
Sokovic M, Marin PD, Brkić D, Van Griensven LJD (2007). Chemical composition and antibacterial activity of essential oils of ten aromatic plants against human pathogenic bacteria. Food 1(1):220-226.
Walsh SE, Maillard JY, Russell AD, Catrenich CE, Charbonneau DL, Bartolo RG (2003). Activity and mechanisms of action of selected biocidal agents on Gram-positive and Gram-negative bacteria. J. Appl. Microbiol. 94:240-247.
Wright CW (2002). Medicinal and aromatic plants industrial profiles. Vetiveria, Taylor and Francis, Artemisia. pp. 344-344.
Yèhouenou B, Noudogbèssi JP, Sessou P, Avlessi F, Sohounhloué D (2010). Etude chimique et activités antimicrobiennes d'extraits volatils des feuilles et fruits de *Xylopia aethiopica* (DUNAL) A. Rich. contre les pathogènes des denrées alimentaires. J. Soc Ouest. Afr. Chim. 29:19-27.