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Chemical composition and antimicrobial properties of different basil essential oils chemotypes from Togo

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Abstract

The aerial parts essential oils of *Ocimum basilicum* (Lamiaceae) from Togo were steam-distilled and investigated for their percentage composition (GC and GC/MS) and *in vitro* antimicrobial activities. Five oil chemotypes were identified and classified as follows in line with their principal components: estragole type; linalool/estragole type; methyleugenol type; methyleugenol/t-anethole type; t-anethole type. The *in vitro* microbiological experiments revealed that only the methyleugenol and methyleugenol/t-anethole chemotypes were active against tested fungi and bacteria. Their minimum inhibitory concentrations (MIC) ranged from 80-150 µL/L and from 200-500 µL/L respectively. Likewise, on tested bacteria the MIC varied from 200-400 µL/L and from 250-500 µL/L respectively. These findings are supportive of the potential of both basil oil chemotypes for use as active ingredients in natural antibiotic drugs.

Introduction

The genus *Ocimum* collectively called basil (in English), includes around 30 plant species from tropical and subtropical areas (Paton, 1992). *Ocimum* is widely cultivated and extensively used for food, perfumery, cosmetics, pesticides, medicine, and traditional rituals because of their natural aroma and flavor and other properties (Alburquerque, 1996; Darrah, 1974). Literature reports that *O. basilicum* leaf essential oils or leaf powder have effective insecticidal and pesticidal actvities against *Vigna unguiculata* pests *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae) (Keita et al., 2001; Ketoh et al., 2002). *O. basilicum* commonly grows semi-wild and is cultivated in Togo at small scale in vegetable gardens. Current domestic uses are only for food and folk medicine (Adjanohoun et al., 1986). Nevertheless, a large scale production of the basil essential for well-known value-added applications (Berrada et al., 1987; Archtander, 1994) is quite viable under local circumstances.

The chemical composition of *O. basilicum* essential oils has been intensively investigated throughout the world (Ekundayo et al., 1987; Sanda et al.; 1998; Yayi et al., 2001), indicating that the estragole chemotype and the linalool -estragole one are the most widely distributed.

At the same time, only very little work has been done on the chemical composition and the antimicrobial activities of basil oils from plants growing in Togo (Sanda et al.; 1998; Koumaglo et al., 1996). In addition, there are very few data available either for practical use or for basic research needs about antimicrobial properties of the essential oils of *O. basilicum* growing in Togo (Baba-Moussa et al., 1997; Chaumont et al., 2001).

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In order to help fill this deficiency, partly at least, the aim of the present work was to investigate basil oils extract of plants sampled in different localities belonging to different ecological areas of Togo. Specifically, oils percentage composition was determined along with their bacteriostatic and fungistatic activity using 14 bacterial and 14 fungal strains.

The principal finding expected from this applied research is the candidate basil oil(s) that could be produced in Togo for use as source(s) of active ingredients in natural antibiotics or cosmetics.

Materials and Methods

Plant material sampling and volatile oils isolation: Leaves and inflorescences of *O. basilicum* used in this work were harvested from plants at full flowering stage in vegetable gardens in five various locations of Togo from May to October 2007.

Plant specimen was identified by Prof. Akpagana, Departement de Botanique, Faculté des Sciences at the Université de Lomé (Togo), where voucher specimens were deposited in the herbarium.

A sample (50 g) of air-dried plant material was extracted by the hydrodistillation technique during 2 hours in a modified Clevenger-type glass apparatus (Craveiro et al., 1976). The extracted crude essential oils were stored in hermetically sealed dark glass flasks with rubber lids, covered with aluminium foil to protect the contents from light and kept under refrigeration at 4°C until use without any prior purification.

Essential oil analyses by gas chromatography: Gas chromatographic analysis was carried out on a Varian 3300 type gas chromatograph equipped with FID detector. An apolar capillary column DB-5 (30 m x 0.25 mm i.d.; film thickness 0.25 µm) and on a polar column supelcowax 10 with the same characteristics as above mentioned were used. DB-5 column operating conditions were as follows: from 50°C (5 min), 50°C to 250°C at the rate of 2°C/min and supelcowax 10 from 50 °C (5 min), 50°C to 200°C at 2°C/min. The injector and detector temperatures were respectively 250°C and 300°C. The carrier gas was helium at a flow rate of 1.5 mL/min. Samples (0.2 µL) of undiluted essential oil were injected manually.

Gas chromatography-mass spectrometry analysis: The GC/MS analysis was carried out on a Hewlett Packard 5890 series II chromatograph, coupled with a mass spectrometer of the Hewlett Packard 5971 series type operating in the EI mode at 70 eV. The capillary column type was DB5-MS (30 m x 0.25 mm i.d.; film thickness 0.25 µm). The amount of sample injected and GC/MS parameters were the same as above.

Identification of components: The components of oils samples were identified by their retention time, retention indices relative to C_8-C_{24} n-alkanes, computer matching with Wiley 275L library and as well as by comparison of their mass spectra with the authentic samples or with data already available in the literature (Kondjoyan and Berdagué, 1996; Adams, 2001).

The percentage of composition of the identified compounds was computed from the GC peak area without any correction factor and was calculated relatively.

In vitro antifungal testing: Fungal strains used are listed in Table II. They were supplied by Institut Pasteur de Paris and Hôpital Saint Jacques de Besançon, France. The fungi were cultivated on a sabouraud agar medium in which was added chloramphenicol 1%, all purchased from BioMerieux Co. (Paris, France). Pure estragole, linalool and methyleugenol commercial standards were also purchased from BioMerieux Co. (Paris, France).

The antimicrobial activities of the essential oils were assessed according to agar dilution method (Benjilali et al., 1986; Griffin et al., 2000). The tested essential oil and its pure major components from commercial origin were diluted in a minimal quantity of ethanol 95% 1/10 v/v to which was added an aqueous solution of Tween 80 (final concentration of 1% v/v) in order to obtain a homogeneous mixture. The later was incorporated as appropriate to the microbiological culture medium under solidification to obtain final concentrations of the active ingredient that ranged from 10 to 500 µL/L. The mixture was then poured into 3 cm diameter petri dishes.

After solidification fungal strains were respectively seeded as described below:

(i) dermatophytes were seeded with a disc of approximately 2 mm, from a mycelia carpet of preculture, laid in the middle area of a new petri dishes, upper side against the new culture medium;

(ii) 1 mL of a suspension of 10^5 conidia per mL of *Aspergillus fumigatus* or 10^5 blastospores per mL of yeast was poured on the surface of the culture medium. Incubation time and temperatures depended on the fungal strains: 24 hours at 37°C for *Candida albicans* and *Aspergillus*, 48 hours at 37°C for *Cryptococcus*, 14 days at 24°C for the dermatophytes and *Scopulariopsis brevicaulis*.

Antibacterial testing: Bacterial strains were supplied by Institut Pasteur Collection de Paris and Leeds University Microbiology Laboratory, United Kingdom. They were cultivated on 1.3% (m/v) nutrient broth-

Table I: Chemical composition of *O. basilicum* volatile oils from Togo

Identified compounds*	Peak area [%] of plant material sampling localities					
	RI**	Lomé	Lo-J.B***.	Adéticopé	Bassar	Sokodé
Monoterpene hydrocarbons		3.03	12.91	1.73	0.83	4.20
α-Pinene	941	0.16	0.53			0.40
Sabinene	976	0.31	0.57			
Myrcene	993	0.28	0.59			0.42
P-Cymene	1030		0.87			0.51
Limonene	1033	0.15	6.22	1.52	0.83	0.42
(Z)-β-Ocimene	1046		3.16			0.74
(E)-Cis-ocimene	1058	1.77		0.21		
γ-Terpinene	1068	0.20				0.17
Terpinolene	1100	0.16	0.97			1.54
Oxygenated monoterpenes		5.97	5.97	3.41	22.21	49.09
1,8 Cineole	1023	2.25				3.62
Linalool	1099	1.71		2.91	17.30	41.21
Camphor	1146	0.74		0.50	1.29	0.47
Terpineol-4	1171	0.52				2.48
Estragole	1198	85.50		10.02		22.17
t-Anethole	1253			32.56	74.64	
Carvacrol	1273				2.69	
Thymol	1290				0.46	
Bornyl acetate	1289	0.22			0.47	0.88
Methyleugenol	1293	0.35	74.45	42.31		
Geranyl formiate	1381	0.53				0.44
Sesquiterpene hydrocarbons		3.72	5.25	6.27	2.29	13.52
β-Elemene	1387	0.43		0.27		1.20
β-Caryophyllene	1420	0.40				0.16
(E)-α-Bergamotene	1440	1.63		3.64	0.64	7.56
α-Caryophyllene	1452			0.25		0.36
Germacrene D	1487	0,29	0.61	0.24		0.56
β-Selinene	1493			0.18		0.77
α-Zingiberene	1494			0.36		
Bicyclogermacrene	1502	0.11				0.41
α-Muurolene	1508	0.28	0.57			0.93
Germacrene A	1513	0.58		0.41		0.14
γ-Cadinene	1514	0	4.07	0.92	1.65	1.43
Oxygenated sesquiterpenes		0.53		3.73		0.38
Γ-Cadinol	1619	0.53		3.73		0.38
Total identified (%)		98.57	92.26	99.67	99.97	89.36

Table II: Antifungal activities of different *O. basilicum* essential oils chemotypes from Togo

Fungal strains	MIC* (mL/L)							
	Basil oils chemotypes					Linalool	Méthyl eugenol	Estragole
	Ob[1]*	Ob[2]*	Ob[3]*	Ob[4]*	Ob[5]*			
Dermatophytes								
Trichophyton mentagrophytes (B)	>500	400	80	250	>500	>500	60	>500
T. interdigitale (B) *	>500	450	100	300	>500	>500	150	>500
T. rubrum (B)	>500	400	150	300	>500	>500	150	>500
T. soudanense (B)	>500	500	100	200	>500	>500	300	>500
T. violaceum (B)	500	500	100	300	>500	450	300	500
Microsporum canis (CIP) *	>500	400	200	300	>500	600	150	>500
Microsporum gypseum (CIP)	>500	500	200	250	500	500	150	>500
Epidermophyton flocosum (B)	>500	500	200	300	>500	500	200	>500
Imperfect filamentous fungi								
Aspergillus fumigatus (B)	>500	>500	150	500	>500	>500	600	>500
Scopulariopsis brevicaulis (B)	>500	>500	100	>500	>500	>500	500	>500
Scytalidium dimidiatum (B)	>500	>500	150	>500	>500	>500	>500	>500
Scytalidium hyalinum (B)	>500	>500	200	>500	>500	>500	>500	>500
Pathogenic yeasts								
Candida albicans (B)	>500	>500	80	500	>500	>500	100	>500
Cryptococcus neoformans (B)	>500	>500	100	400	>500	>500	100	>500

*** MIC**: Minimum inhibition concentration; **Ob[1]** : Estragole chemotype; **Ob[2]** : Linalol/estragole chemotype ; **Ob[3]** Methyleugenol : chemotype; **Ob[4]: M**ethyleugenol/t-anethole chemotype ; **Ob[5]** : t-anethole chemotype; **B** : Hôpital Saint Jacques de Besançon, France; **CIP**: Collection Institut Pasteur de Paris

Culture media with or without ethanol 95% and Tween 80 were used as controls.

The antimicrobial activities were evaluated by the determination of the minimum inhibitory concentration (MIC). The MIC of a tested active ingredient was determined as the lowest concentration of the test antimicrobial ingredient sample that resulted in a complete inhibition of visible growth of the microorganisms. All tests were carried out in triplicate.

Results and Discussion

Basil oil extraction yields were in the range of 1.4% to 2.2%, based on plant material dry weight. Identified oil constituents and percentage and their relative percentages are listed in Table I.

Using the relative importance of the oils major constituents as classification criteria permitted to determine five chemotypes as listed below:

i) Estragole chemotype (sample from Lomé): It contained mainly estragole (85.50%), with a little amount of 1,8-cineole (2.25%). The estragole content in this sample was similar to previous findings (Guenther, 1949; Baritaux et al., 1992; Yayi et al., 2001).

ii) Linalol-estragole chemotype (sample from Sokodé): this type of basil oil contained linalool (41.21%), estragole (22.17%) and (E)-α-bergamotene (7.56%) as major constituents. This chemotype was very close to the European type previously reported by other workers in Nigeria (Ekundayo et al., 1987) and in Benin (Yayi et al., 2001).

iii) Methyleugenol chemotype (sample from Botanical garden, UL): This basil oil type contained mainly methyleugenol (74.45%), limonene (6.22%), γ-cadinene (4.07%) and (Z)-β-ocimene (3.16%).

iv) Methyleugenol and t-anethole chemotype (sample from Adéticopé, Lomé): This essential oil contained mthyleugenol (41.10%) and the t-anethole (32.56%)

Table III: Antibacterial activities of different basil essential oils chemotypes from Togo

Cutaneous bacterial strains	MIC * (mL/L)							
	Basil oils chemotypes					Linalol	Méthyl eugénol	Estragole
	O b1**	Ob2	Ob3	Ob4	Ob5			
Feet microflora								
*Staphylococcus epidermidis (*L1S2) ***	>500	>500	200	300	>500	>500	>500	>500
S. hominis (L8S2) ***	>500	>500	200	400	>500	>500	>500	>500
*S. cohnii** (L6S3)	>500	>500	150	300	>500	>500	>500	>500
Coryneform gr. B (L 16C3) ***	>500	400	100	300	>500	>500	>500	>500
Coryneform gr. C (L3C3) ***	>500	>500	150	250	>500	>500	>500	>500
Coryneform gr. D2 (L19C1) ***	>500	500	150	200	>500	>500	>500	>500
Micrococcus sedentarius (L7B5) ***	>500	400	200	300	500	>500	>500	>500
Acinetobacter sp. (LLH5DC1)	>500	500	300	400	400	>500	500	>500
Moraxella sp. (LH7SV1)	>500	500	250	500	>500	400	>500	>500
Alcaligenes sp. (LH4TV1)	>500	400	300	300	500	>500	>500	>500
Armpits microflora								
Staphylococcus xylosus (IP 8166)	>500	>500	300	400	>500	>500	>500	>500
S. haemolyticus (IP 8156) ***	>500	>500	200	300	>500	>500	>500	>500
Corynebacterium xerosis (IP5216) ***	>500	>500	150	250	>500	>500	>500	>500
*Micrococcus luteus** (L1C5)	>500	>500	400	500	>500	>500	>500	>500

* **MIC:** Minimum inhibitory concentration; ** **Ob1** : Estragole chemotype ; **Ob2** : Linalol/ estragole chemotype; **Ob3** : Methyleugénol chimiotype; **Ob4** :Methyleugenol/t-anethole chemotype; **Ob5** : t-anéthole chemotype; *** : Bacteria strains responsible of bad odours; **IP** : Bacteria strains provided by Institut Pasteur de Paris; **L, LH** : Bacteria strains provided by Dr. Marshall, Leeds University Microbiology Laboratory (United Kingdom)

agar-granulated (NB-AG) culture medium (AES Labortories), in 9 cm diameter petri dishes. After cooling and solidification, the petri dishes were automatically inoculated with the bacterial suspensions 10^5 CFU/mL with STEERS apparatus. The petri dishes were incubated at 37°C for 48 hours under aerobic conditions.

Major constituents: Others meaningful constituents were estragole (10.02%), (E)-α-bergamotene (3.64%) and linalool (2.91%).

v) t-Anethole chemotype (sample from Bassar): This sample contained mainly t-anethole (74.64%), linalool (17.30%) and carvacrol (2.69%).

To our knowledge, the last three basil oil chemotypes have not yet been reported. *O. basilicum* has been thoroughly investigated with regards to volatile oil composition but some of our findings are supportive of the idea that continuing chemical inventory on this species is still of scientific interest to support action aimed at plant biodiversity knowledge, conservation and sustainable exploitation.

The findings on the chemical composition of basil oils in Togo were very instructive with regard to the possible applications. Hence, the estragole chemotype found here resembled that described by Ekundayo et al. (1987) and Baritaux et al. (1992), which is well-known for its valuable usage in perfumery and food (Kayibou, 1992). In addition, this chemotype has been recently indicated as a possible pesticide against larva and adult of *Callosobruchus maculatus* known as a major post-harvest pest of *Vigna unguiculata* in storage (Keita et al., 2001; Ketoh et al., 2002).

The pesticidal activity of the volatile oil of *Clausena anisata,* containing mainly estragole and t-anethole (Moudachirou et al., 1997; Okunde and Olaifa, 1987). Such indications on the pesticidal properties of this essential oil containing estragole and t-anethole is certainly indicative of the biocide potential of these natural molecules which might be endowed with

antimicrobial properties (Friedman et al., 2002), our focus in this investigation.

The experimental data in Table II show that all fungal strains tested were sensitive to the metyleugenol type of basil oil, but dermatophytes and pathogenic yeasts were more particularly affected. Hence, markedly low MICs (80 µL/L) were recorded with *T. mentagrophytes var mentagrophytes*. Along with dermatophytes, imperfect filamentous fungi were also highly sensitive to the test volatile oil; MICs were in the range of 100-150 µL/L. Likewise, the methyleugenol oil chemotype appeared very toxic to pathogenic yeast strains including *Candida*, *Cryptococcus*, *Aspergillus,* and *Scopulariopsis,* MICs varying from 80 to 100 µL/L. Also noticeable was the antifungal effect (MIC: 150 µL/L and 200 µL/L) of this oil chemotype on both strains of *Scytalidium sp*. The later, which often resist conventional antibiotics, are usually reported in human dermatomycosis in tropical and subtropical countries (Alvarez et al., 2000). The African *Trichophyton soudanense,* a parasite frequent in school environment (Ouaffak et al., 2001; Vandemeulebroucke et al., 1999) was interestingly also sensitive to the tested essential oils (MICs 100 and 200 µL/L). It was also the case for *Cryptococcus neoformans*, a hazardous opportunist yeast, which is a resistant germ usually infecting humans affected by HIV/AIDS, which group of patients is known to be generally at high risk with regard to mycosis opportune affections.

The high antifungal activity of this basil oil chemotype on those pathogenic fungi like dermatophytes, filamentous fungi and yeasts confirmed the excellent fungal growth inhibition properties previously reported as a characteristic of essential oils rich in methyleugenol and/or other phenol derivatives (Chaumont and Leger, 1989). In this work there is no doubt that the antifungal activity of *O. basilicum* chemotype methyleugenol against tested fungi is a predictable consequence of its high content in methyleugenol known as one of the phenolic volatile molecules endowed with antimicrobial properties (Chaumont and Leger, 1989; Viollon and Chaumont, 1994). In comparison, commercial linalool and estragole tested as standards in this study were found non-effective unlike pure methyleugenol standard.

The methyleugenol/t-anethole chemotype showed a moderate antifungal activity only on dermatophytes with MICs ranged from 250-300 µL/L but not filamentous fungi and yeasts were not affected. The estragole type, the linalool/estragole type, and the t-anethole type were ineffective on all fungal strain tested in this work. A lesson learned from the findings mentioned above was that estragole and t-anethole, which are reported as possible natural pesticides, could not serve as natural fungicidal ingredients unlike methyleugenol against the fungal strains tested in this work. This simply indicates the broader understanding of what is generically termed to as the biological activity of essential oils.

Table III shows that all feet microflora bacterial strains tested were only sensitive to methyleugenol and methyleugenol/t-anethole oil chemotypes.

The MICs, which were in the range of 200-500 µL/L were slightly higher than those recorded on fungal strains in this study, indicating a lesser activity. The results recorded against common armpit microflora bacterial strains, were especially promising for a possible use against *Corybacterium xerosis*, the principal one of the bacteria responsible for bad odors. Its growth was interestingly inhibited with at a dose of 100 µL/L antibacterial test suspension. Note that this bacteria metabolizes human steroids produced via transpiration to yield small and bad-smelling volatile molecules (Rennie et al., 1991).

The findings in this study indicated bacteriostatic effects against feet microflora germs like *Staphylococcus epidermidis* and *Staphylococcus hominis* (MIC: 200 and 300 µL/L) partly responsible for feet bad odors (Marshall et al., 1987; Marshall et al., 1988). Methyleugenol and methyleugenol/t-anethole oil chemotypes samples were also very active against the *Coryneform* (*Corynebacterium* gr B, C and D2) (MIC: from 100-300 µL/L) which produce proteinase and lipase, both enzymes that cause feet surface skin bad odors (Marshall et al., 1987; Marshall et al., 1988). Both oil chemotypes also showed a moderate antibacterial activity against Gram-negative bacteria (*Acinetobacter* sp, *Moraxella* sp.: MIC from 250- 500 µL/L) and Gram-positve bacteria (*Micrococcus luteus*, *Staphylococcus epidermidis:* MIC: from 200-500 µL/L). The later are involved in nosocomial infections (Bergogne-Bérézin, 1995; CDC, 1999).

The proven antibacterial activities of the methyleugenol and metyleugenol/t-anethole types of basil oil found in this study are quite indicative of their potential as possible active ingredients for use in the formulation of deodorants for armpits or feet. Developing sustainable large scale consumer goods like natural drugs and cosmetics based on basil oil as well as on other essential oils bearing aromatic plants growing in Togo is of an obvious economic interest while helping preserve plant biodiversity.

In conclusion, the prospective investigation of the percentage composition of *O. basilicum* essential oils along with the evaluation of their antimicrobial properties on human normal cutaneous microflora bacterial and fungal strains is quite a typical applied research. The ultimate goal of this type of investigation is to help protect plant biodiversity and validate on a

scientific basis the potential of some valuable species whether for food or non-food applications. The present study has shown that it is feasible to use some kinds of the leaf essential oil of *O. basilicum* growing in Togo as a natural powerful antimicrobial ingredient whether in traditional or in modern medicines as well as in cosmetics. Such oils are the methyleugenol and the methyleugenol/t-anethole types. Conversely, the estragole type, the linalool/estragole type, and the t-anethole type, which were also included in the investigated samples, did show any useful antimicrobial properties.

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