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Short Communication

Antifungal Activity of the Essential Oils from *Ocimum gratissimum* L. Grown in Togo

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Abstract

The aerial parts of *Ocimum gratissimum* L. (Lamiaceae) harvested in Togo was steam-distilled and investigated for essential oil composition (GC and GC/MS) and *in vitro* antifungal activities. Thymol (31.79 %), p-cymene (15.57 %) and *γ*-terpinene (12.34 %) and were the major components of the oil. Other notable components identified in this oil were myrcene (6.94 %) and *α*-thujene (6.11 %).The *in vitro* antifungal activity was recorded with the minimum inhibitory concentrations (MICs) ranging from 80 to 150 µl.l^{-1} , 150 to 500 µl.l^{-1} and from 100 to 150 µl.l^{-1} respectively on dermatophytes, imperfect filamentous fungi and pathogenic yeasts. Likewise, on tested fungi the minimum fungicidal concentration (MFC) varied from 300 µl.l^{-1} to 500 µl.l^{-1}, 500 to 700 µl.l^{-1} and from 250 to 300 µl.l^{-1}, respectively on dermatophytes, imperfect filamentous fungi and pathogenic yeasts.

Keywords: *O.gratissimum*, Antifungal, Essential oil; Thymol.

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1. Introduction

Tree basil (*Ocimum gratissimum* L., (Lamiaceae) is a well known species and commonly used in folk medicine in Africa [1, 2]. The genus *Ocimum* collectively known as basil, includes around 30 plant species from tropical and subtropical areas [3]. *Ocimum* are widely cultivated and extensively used for food, perfumery, cosmetics, pesticides, medicine, and traditional rituals because of their natural aroma and flavour and other properties [4, 5]. *O. gratissimum* is commonly used in folk medicine to treat different diseases like upper respiratory tract infections, diarrhoea, headaches, ophthalmic, skin

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diseases, pneumonia and also a treatment of cough, fever, and conjunctivitis [6, 7]. Local populations in West Africa use the fresh leaves as mouth antiseptic [8, 9]. *O. gratissimum* herb is also used both as anthelmintic, antiparasitic [10, 11], antimalarial [11], and as condiment that impart an acceptable flavour [12]. An infusion can be used as a digestive remedy, being taken to settle a wide range of problems such colic and stomach pains and diarrhoea [1]. The plant is also used as a fumigant against pests [13, 14]. Previous studies showed that *O. gratissimum* essential oils chemotypes displayed antimicrobial activities [15-17].The chemical composition of *O. gratissimum* essential oils has been intensively investigated [18-20], indicating that the thymol chemotype is 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 with p-cymene/thymol as their major components showing moderate antimicrobial activities [19, 21]. The present study reports the chemical composition and the *in vitro* evaluation of *O. gratissimum* essential oil along with its fungistatic and fungicidal activities against seventeen fungal strains.

2. Material and Methods

2.1. *Plant material and isolation of volatile oils*

Leaves and inflorescences of *O. gratissimum* L. used in this work were harvested from plants at full flowering stage from the experimental field of the *Unité de Recherche sur les Agroressources et la Santé Environnementale* at the *Université de Lomé* in October 2007. Plant specimen was identified by Pr. Akpagana, Departement de Botanique, Faculté des Sciences at the Université de Lomé (Togo), where Voucher specimen was deposited in the Herbarium under reference 250K. A sample (50 g) of air-dried plant material was extracted by the hydro-distillation technique for 2 hours in a modified Clevenger-type glass apparatus [22]. The extracted crude essential oil was stored in hermetically sealed dark glass flask with rubber lids, covered with aluminium foil to protect the content from light and kept under refrigeration at 4°C until use without any prior purification.

2.2. *Essential oil analyses*

2.2.1. *Gas chromatography analysis*

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 250 °C and 300°C, respectively. The carrier gas was helium at a flow rate of 1.50 ml/min. Samples (0.2 µl) of non diluted essential oil were injected manually.

2.2.2. C*hromatography-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.

2.2.3. *Identification of components*

The components of oil samples was identified by their retention time, retention indices relative to C_5-C_{18} n-alkanes, computer matching with with Willet 275.L library and as well as by comparison of their mass spectra with the authentic samples or with data already available in the literature [23, 24]. The percentage of composition of the identified compounds was computed from the GC peak area without any correction factor and was calculated relatively.

2.3. *In vitro antifungal testing*

Fungal strains used are listed in Table 2. The strains were supplied by the Institut Pasteur de Paris (IP), Hôpital Saint Jacques de Besançon (France) (B) and Laboratoire de Bactériologie-Parasitologie du Centre Hospitalier Universitaire de Pointe à Pitre/Abymes, Guadeloupe (G). The fungi were cultivated on a Sabouraud Agar Medium in which was added chloramphenicol 1%, all purchased from BioMerieux Co. (Paris, France). Pure thymol, γ-terpinene, and p-cymene commercial standards were also purchased from BioMerieux Co. (Paris, France).The antimicrobial activities of the essential oil were assessed according to Agar dilution method [25]. 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 Tween80 (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 $\mu l.l^{-1}$. 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 millilitre of *Aspergillus fumigatus* or 10^5 blastospores per millilitre 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*.

The antifungal activities were evaluated by the determination of the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC). The minimum inhibitory concentrations (MICs) were determined as the lowest concentration of the test active ingredient that resulted in a complete inhibition of visible growth of the microorganisms. The minimum fungicidal concentrations (MFCs) were determined as the lowest concentration of the test active ingredient which did not allow any visible growth of the microorganisms after subculture. All tests were carried out in triplicate.

3. Results and Discussion

3.1. *Chemical analysis*

The steam distillation of the leaves and inflorescences of *O. gratissimum* produced a light-Yellow essential oil in a 1.8 % yield based on dried material. It gave off a flavour

Table 1. Chemical composition of *Ocimum gratissimum* essential oil from Togo.

Compounds	RI*	Peak Area [%]#
Monoterpene hydrocarbons		**56.21**
α-thujene	930	6.11
α-pinene	940	1.84
sabinene	976	0.56
β-pinene	990	0.74
mycene	992	6.94
α-phellandrene	1010	0.35
α-terpinene	1023	4.02
β- phellandrene	1027	0.28
para-cymene	**1030**	**15.57**
limonene	1036	0.57
Ocimene (Z) - β	1037	0.43
Ocimene (E)- β	1058	1.75
γ -terpinene	1078	**12.34**
p-cymenene	1091	2.11
terpinolene	1095	2.60
Oxygenated monoterpenes		**37.85**
thymol	**1290**	**31.79**
carvacrol	1299	1.44
cis hydrate de sabinene	1076	0.76
tr-hydrate de sabinene	1109	2.26
linalool	1113	0,47
Terpineol-4	1179	1.13
Sesquiterpene hydrocarbons		**3.80**
α-copaene	1377	0.85
β-elemene	1387	0.45
β-caryophyllene	1420	1.71
α-humulene	1454	0.30
α-selinene	1498	0.49
Total identified		**98.06**

*Retention indexon apolar DB-5 column. #Peak area percentage is based on apolar DB-5 column, and values represent average of three determinations.

reminiscent close to that of thymol. The oil constituents and its relative percentage are listed in Table 1.

Twenty six components were identified in the essential oil of *O. gratissimum* representing 98.06 % out of the total detected compounds. Thymol (31.79%) p-cymene (15.57 %) and γ-terpinene (12.34 %) and were the major components. Other notable components identified in this oil were myrcene (6.94 %) and α-thujene (6.11 %). This oil consisted of sixteen monoterpene hydrocarbons (57.06 %), six oxygenated monoterpenes (37.85 %) and four sesquiterne hydrocarbons (2.95).

This chemical composition was closer to two previously described samples of *O. gratissimum* from Rwanda by Ntezurubanza *et al*. [26] with 35.42 to 47.85 % in thymol amount and samples of *O. gratissimum* oils collected in various localities in Benin described by Yayi *et al.* [20] with thymol amount varied from 25.9 to 65.4 %.

3. 2. *Antifungal activities*

The experimental data in Table 2 show that all fungal strains tested were sensitive to the essential oil of *O. gratissimum* but dermatophytes were particularly affected.

Table 2. Antifungal activity of *O. gratissimum* essential oil from Togo.

Fungal strains	**Essential oil**		**Major components**		
	MIC (µL/L)	**MFC** (µL/L)	**MIC** (µL/L)		
	Ocimum gratissimum		Thymol	γ-terpinene	p-cymene
Dermatophytes					
Trichophyton. mentagrophytes (B)*	100	350	50	>500	>500
T. interdigitale (B)	80	300	100	>500	>500
T. interdigitale (G)	80	300	100	>500	>500
T. rubrum (B)	80	300	50	>500	>500
T. erinaceum (B)	80	300	50	>500	>500
T. soudanense (B)	80	300	40	>500	>500
T. violaceum (B)	150	300	30	>500	>500
Microsporum canis (IP) *	80	300	50	>500	>500
Microsporum canis (G) *	100	300	50	>500	>500
Microsporum gypseum (IP)	100	300	50	>500	>500
Epidermophyton flocosum (B)	150	300	50	>500	>500
Imperfect filamentous fungi					
Aspergillus fumigatus (B)	150	500	125	>500	>500
Scopulariopsis brevicaulis (B)	200	500	75	>500	>500
Scytalidium dimidiatum	500	700	150	>500	>500
Pathogenic yeasts					
Candida albicans (B)	150	500	75	>500	>500
Cryptococcus neoformans (B)	100	300	50	>500	>500
Malassezia pachydermatis (IP)	150	300	50	>500	>500

* Fungal strains origins.
(B) **:** Hôpital Saint Jacques de Besançon (France); IP : Institut Pasteur de Paris (France) ; G : Laboratoire de Bactériologie-Parasitologie du Centre Hospitalier Universitaire de Pointe à Pitre/Abymes, Guadeloupe.

Hence, markedly low MICs (80 $\mu l.l^{-1}$) were recorded with *T. interdigitale, T. rubrum, T. erinaceum, T. soudanense and Microsporum canis.* The fungicidal activity of the test essential oil sample was quite identical on all dermatophytes (MFC: 300 $\mu l.l^{-1}$) except *T. mentagrophytes v*ar *mentagrophytes* (MFC: 350 $\mu l.l^{-1)}$. Along with dermatophytes, filamentous fungi were also sensitive to the test volatile oil; MICs were in the range of 150 $\mu l.l^{-1}$ to 500 $\mu l.l^{-1}$. Likewise, the essential oil sample appeared toxic to pathogenic yeast strains like *Candida*, *Cryptococcus*, *Aspergillus,* and *Scopulariopsis* with antifungal effect (MIC: from 100 to 150 $\mu l.l^{-1}$; MFC: from 250 to 300 $\mu l.l^{-1)}$. Also quite noticeable was the antifungal effect (MIC: 500 $\mu l.l^{-1}$; MFC: 700 $\mu l.l^{-1}$) of the test oil on strain of *Scytalidium dimidiatum*. The later, which often resist conventional antibiotics, is a very frequent human parasites in Caribbean and in Subsaharan Africa regions. The African *Trichophyton soudanense,* a parasite frequent in school environment [27] is interestingly also sensitive to the tested essential oil. 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 chemotype of *O. gratissimum* essential oil 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 thymol and/or other phenol derivatives [28].

In this work it is obvious that the antifungal potential of *O. gratissimum* against tested fungi is a predictable consequence of its high content in thymol because it has been reported that the volatile oil of *O. gratissimum* contains mostly phenols, particularly thymol [29, 30] and that these are probably responsible for its reported antimicrobial properties. But commercial p-cymene and γ-terpinene tested as standards in this study were found non effective unlike pure thymol standard.

4. Conclusion

In this paper, the investigation of the percentage chemical of the essential oils of *O. gratissimum* confirmed the existence of previously discovered chemotypes containing respectively thymol as principal constituent this plant oil in Togo. The assessment of the antifungal properties of this chemotype on fungal strains responsible of various superficial mycosis is quite a typical applied research. The present study has shown that it is feasible to use *O. gratissimum* aerial part essential oil rich in phenolic derivatives as natural powerful antifungal ingredient.

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