

# Aromatic Plants of Tropical Central Africa. Part XXXII.† Chemical Composition and Antifungal Activity of Thirteen Essential Oils from Aromatic Plants of Cameroon

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**ABSTRACT:** The essential oils of *Hoslundia opposita* Vahl. (leaves), *Hyptis lanceolata* Poit. (whole plant), *Hyptis suaveolens* Poit. (leaves), *Ocimum basilicum* L. (whole plant), *Ocimum canum* Sim. (whole plant), *Ocimum gratissimum* L. (leaves), *Plectranthus glandulosus* Hook (leaves), *Thymus vulgaris* L. (whole plant), *Piper capense* L. (leaves and seeds), *Piper guineense* Schum. et Thom. (leaves and seeds) and *Bixa orellana* L. (leaves) which were obtained by hydrodistillation from plants collected in different regions of Cameroon, were analysed by GC and combined GC–MS. The oils of *Hoslundia opposita*, *Hyptis lanceolata*, *H. suaveolens*, *T. vulgaris*, *P. capense*, *P. guineense* and *B. orellana* were found to be rich in hydrocarbons (>58%). The most abundant compounds in the oils of *O. basilicum*, *O. canum* and *P. glandulosus* were alcohols and oxides (>40%), while in the oil of *O. gratissimum* the amounts of hydrocarbons and oxygen-containing components were roughly the same. It is of interest to note the presence in *O. basilicum* and *P. guineense* (leaves) of aromatic compounds in a sizeable amount (13.5% and 25.6% respectively). The antifungal activity of these essential oils against *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus flavus*, *Aureobasidium pullulans*, *Microsporum gypseum*, *Trichophyton rubrum*, and *Trichoderma viride* were also investigated. Two methods were used for these antifungal tests: the microatmosphere method and the standardized broth dilution micromethod. Three oils (from *Ocimum gratissimum*, *Thymus vulgaris* and *Ocimum basilicum*) showed strong antifungal activity. © 1998 John Wiley & Sons, Ltd.

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**KEY WORDS:** antifungal activity; essential oils; *Hoslundia opposita* Vahl.; *Hyptis lanceolata* Poit.; *Hyptis suaveolens* Poit.; *Ocimum basilicum* L.; *Ocimum canum* Sim.; *Ocimum gratissimum* L.; *Plectranthus glandulosus* Hook; *Thymus vulgaris* L.; *Piper capense* L.; *Piper guineense* Schum. et Thom.; *Bixa orellana* L.; *Candida albicans*; *Cryptococcus neoformans*; *Aspergillus flavus*; *Aureobasidium pullulans*; *Microsporum gypseum*; *Trichoderma viride*; *Trichophyton rubrum*

## Introduction

Numerous investigations have established the remarkable antifungal properties of essential oils<sup>2–5</sup> and the increasing frequency of serious fungal infections. This last fact is attributed to such factors as the increasing use of cytotoxic and immunosuppressive drugs to treat malignant and non-malignant diseases, the increasing prevalence of infection due to human immunodeficiency virus, the widespread use of newer and more powerful antifungal agents<sup>6</sup> and the high expense of marketed antifungals, particularly in African countries. All these factors are reinforced by the fact that

antifungal treatments are always too long because of fungus resistance to standard treatments.

The aim of this work is therefore to search for new antifungals of plant origin which would be effective and less expensive than the standard ones. We report here the examination of 13 essential oils extracted from some Cameroonian aromatic plants for antifungal activity.

## Experimental

### Plant Material and Isolation Procedure

The plant samples were collected between November 1992 and January 1995 in the Yaoundé region and in November 1992 and July 1993 in the Bazou and

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Bafoussam region (western province of Cameroon) (Table 1). The botanical identification of the collected material was performed at the National Herbarium of Yaoundé, where voucher specimens are conserved. Fresh leaves were cut into small pieces and fresh fruits were ground using a blender. Batches of 300–500 g of plant material were then submitted to hydrodistillation for 6 hours using a Clevenger-type apparatus. The oils obtained in each case were dried over anhydrous  $\text{Na}_2\text{SO}_4$  and subjected to instrumental analysis.

### Identification of Oil Components

GC analyses were performed on two fused-silica capillary columns (25 m  $\times$  0.32 mm, coated with OV-101 or Carbowax 20M); the oven temperature was programmed from 50°C to 200°C at 5°C/min.

GC–MS analyses were carried out on a Hewlett-Packard capillary GC–quadrupole MS system (Model 5970) fitted with a 25 m  $\times$  0.23 mm i.d. fused-silica column coated with DB-1, using the same gas-chromatographic parameters. Authentic reference compounds, as well as published mass spectra and retention indices<sup>7–9</sup> were used as the basis for the identification of the oil components.

### Screening for Antifungal Activity

Antifungal tests of the essential oils were carried out using seven strains of fungi from the Laboratory of Plant Biology and Cryptogamy of the Faculty of Pharmacy of Reims (FPR): two potentially pathogenic yeasts (*Candida albicans* FPR 006 and *Cryptococcus neoformans* FPR 008); three destructive and contaminant filamentous fungi (*Aspergillus flavus* FPR 023, *Aureobasidium pullulans* FPR 014 and *Trichoderma viride* FPR 010); and two dermatomycosis agents (*Microsporum gypseum* FPR 003 and *Trichophyton rubrum* FPR 012). These fungi were maintained on Sabouraud agar (SA), (glucose 20 g; peptone 10 g; agar 15 g; distilled water up to 1000 ml).

Two methods were used for the *in vitro* assays:

- (a) The microatmosphere method of Kellner and Kober,<sup>10</sup> as modified by Maruzella *et al.*,<sup>11</sup> Grubb,<sup>12</sup> Sarbach<sup>13</sup> and Joubert *et al.*,<sup>14</sup> allows the determination of the antifungal activity the vapour phase of the essential oils which diffuse towards the agar in an inverted Petri dish. This method was performed using five fungi: *Candida albicans*, *Aspergillus flavus*, *Aureobasidium pullulans*, *Trichoderma viride* and *Microsporum gypseum*.  
The microatmosphere method consist in pouring 2 ml of SA in Petri plates measuring 3 cm in diameter. Petri plates were then seeded with a small amount of a 7-days-old mycelium culture of the tested dermatophyte or filamentous fungus. In the case of yeasts, the SA was overlaid with 0.5 ml of a 2-days culture of yeasts diluted in Sabouraud glucose broth (SGB), (glucose 20 g; peptone 10 g; distilled water up to 1000 ml) to a concentration of  $10^5$  colony forming units per ml (CFU/ml). The Petri dishes were then inverted and the determined amount of essential oil impregnated on sterile filter paper discs (6 mm diameter) deposited on the inverted lid of a Petri dish. The inverted Petri dishes were then incubated for 7 days at 25°C for filamentous fungi and dermatophytes, and 2 days at 37°C for yeasts. MIQ (minimal inhibitory quantities) of essential oil which inhibit the total growth of fungi were noted after 2 or 7 days.
- (b) The MIC (minimal inhibitory concentrations) which inhibit the visible growth of fungi was also determined by the standardized broth dilution micromethod of Torres-Rodriguez *et al.*<sup>15</sup> using Sabouraud glucose broth. The MIC of five oils (the three more active and the two less active) selected by the microatmosphere method were determined on six fungi: *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus flavus*, *Aureobasidium pullulans*, *Microsporum gypseum* and *Trichophyton rubrum*. The yeasts inocula were a dilution of 2-days cultures

**Table 1.** Essential oils yields from 11 plants collected in three areas of Cameroon

Sample No.	Species	Date of harvest	Locality	Oil yields (wt%)
1	<i>Hoshundia opposita</i> Vahl. (fresh leaves)	February 1993	Yaoundé	0.02
2	<i>Hyptis lanceolata</i> Poit. (fresh whole plant)	July 1993	Bazou	0.05
3	<i>Hyptis suaveolens</i> Poit. (fresh leaves)	November 1992	Yaoundé	0.07
4	<i>Ocimum basilicum</i> L. (fresh whole plant)	January 1995	Yaoundé	0.04
5	<i>Ocimum canum</i> Sim. (fresh whole plant)	July 1994	Yaoundé	0.35
6	<i>Ocimum gratissimum</i> L. (fresh leaves)	July 1993	Yaoundé	0.47
7	<i>Plectranthus glandulosus</i> Hook. (fresh leaves)	November 1992	Bafoussam	0.42
8	<i>Thymus vulgaris</i> L. (fresh whole plant)	June 1993	Bafoussam	0.20
9	<i>Piper capense</i> L. (fresh leaves)	July 1993	Bafoussam	0.25
10	<i>Piper capense</i> L. (fresh fruits)	July 1993	Bafoussam	1.40
11	<i>Piper guineense</i> Schum. et Thom. (fresh leaves)	April 1993	Bazou	0.47
12	<i>Piper guineense</i> Schum. et Thom. (fresh fruits)	April 1993	Bazou	3.80
13	<i>Bixa orellana</i> L. (fresh leaves)	October 1993	Yaoundé	0.33

on yeast agar in tryptone saline solution (TSS) to a final concentration of  $10^4$  CFU/ml when 50  $\mu$ l of this suspension are introduced in a well of sterile cell 24-well flat bottomed lidded plates. Filamentous and dermatophytes inocula were obtained by suspending spores in TSS from 7-day cultures of fungi in SA. These suspensions were sifted on a filter with pores measuring 100  $\mu$ m and adjusted by counting on haematometer to a final concentration of  $2 \times 10^2$  CFU/ml when 50  $\mu$ l of each suspension were introduced in each well on a row of a plate. The first, which was used as a growth control, contained no oil. The essential oil diluted in a mixture of methanol (in such a manner as to obtain a maximum of 10% in each well) and SGB are distributed in each well to obtain a serial twofold dilution ranging in concentration from 10 000 to 39 ppm. After mixing, the plates were incubated for 7 days at 25°C. Growth was noted every day from the second to the seventh. MIC were also noted every day.

## Results and discussion

Table 2 shows the composition of the essential oils of the 11 species of plants used for this study. These results will be commented on in detail in other papers.

Nevertheless, an examination of this table reveals that the species could be readily characterized by the oil composition: *Hoslundia opposita*, *Hyptis lanceolata*, *Hyptis suaveolens*, *Thymus vulgaris*, *Piper capense*, *Piper guineense* and *Bixa orellana* (hydrocarbon-rich oils); *Ocimum basilicum*, *Plectranthus glandulosus* and *Ocimum canum* (rich in oxygen-containing components); *Ocimum gratissimum* contained equal amounts of hydrocarbon and oxygen-containing components. The essential oil of *Piper guineense* (leaves) was also characterized by a high percentage of myristicin and that of *O. basilicum* by a significant amount of eugenol. Further, it is of interest to note that among the 72 compounds identified in these extracts, nine are well-known for their antifungal activity:<sup>16-18</sup> thymol, carvacrol, linalol,  $\gamma$ -terpinene, *p*-cymene, 1,8-cineole, eugenol, fenchone and fenchol.

## Antifungal activity

The MIQs of each extract and each fungus, determined by the microatmosphere method, are shown in Table 3. These values represent the mean of three determinations. Plants are listed in decreasing order of activity. The table indicates that *Ocimum gratissimum*, *Thymus vulgaris* and *Ocimum basilicum*, with median MIQ

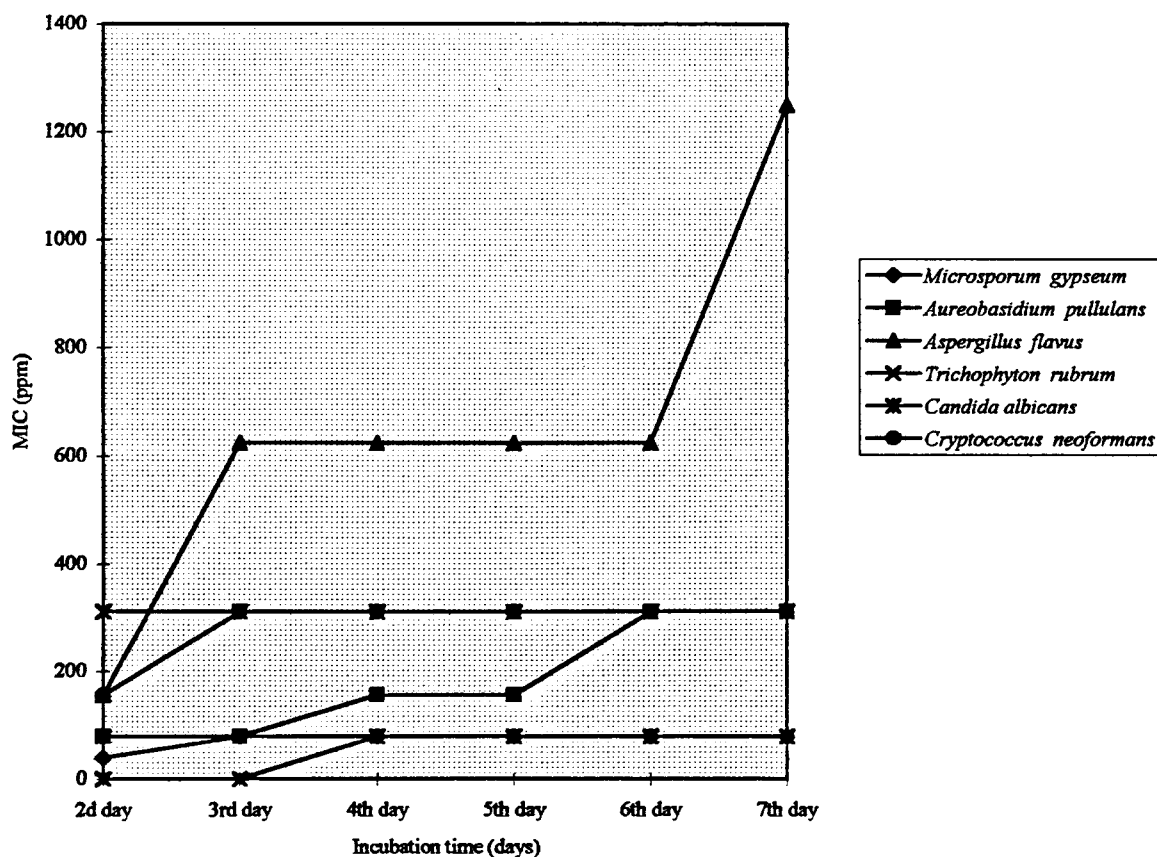


Figure 1. Evolution of MIC (ppm) of *Ocimum gratissimum* according to incubation time (days)

**Table 2.** Chemical composition of the 13 Cameroonian essential oils

RI*	Compounds	<i>Hostundia opposita</i> Vahl. (fresh leaves)	<i>Hyptis lanceolata</i> Poit. (fresh whole plant)	<i>Hyptis suaveolens</i> Poit. (fresh leaves)	<i>Ocimum basilicum</i> L. (fresh whole plant)	<i>Ocimum canum</i> Sim. (fresh whole plant)	<i>Ocimum gratissimum</i> L. (fresh leaves)	<i>Plectranthus glandulosus</i> Hook. (fresh leaves)	<i>Thymus vulgaris</i> L. (fresh whole plant)	<i>Piper capense</i> L. (fresh leaves)	<i>Piper capense</i> L. (fresh fruits)	<i>Piper guineense</i> Schum. et Thom. (fresh leaves)	<i>Piper guineense</i> Schum. et Thom. (fresh fruits)	<i>Bixa orellana</i> L. (fresh leaves)
<i>Monoterpene hydrocarbons</i>														
930	$\alpha$ -Thujene		0.3	0.5	<0.1	0.2	3.5		1.2	0.2	1.1	<0.1	<0.1	<0.1
935	$\alpha$ -Pinene	0.2	1.6	1.3	0.8	2.8	1.1	1.1	1.0	20.4	14.4	0.4	10.5	0.3
944	Camphene			0.7		<0.1	0.1	0.7	1.2	0.3	0.6	0.2	1.2	
970	Sabinene	1.2	8.4	16.4	<0.1	1.2	0.7	0.3	0.4	4.1	17.4	<0.1	3.5	
975	$\beta$ -Pinene	0.7	9.6	5.2	<0.1	5.8	0.4	0.4	0.3	66.0	46.8	1.0	27.2	0.4
985	Myrcene	0.2		1.3	2.1	1.0	3.2	1.2	1.7	3.0	1.6	0.1	1.5	0.1
1001	$\alpha$ -Phellandrene	<0.1	0.1	<0.1			0.3	0.3	0.2	<0.1		<0.1	1.3	
1007	$\delta$ -3-carene			0.3			0.2	0.5		<0.1	0.1	<0.1	2.1	
1012	$\alpha$ -Terpinene		0.5	2.1		0.3	2.8	0.4			0.1		0.3	
1018	<i>p</i> -Cymene		0.3	0.6			7.0		23.6	<0.1	0.1		0.3	
1027	Limonene	1.2	1.0	3.1	10.4		1.1		1.5	1.5	1.4	0.2		0.1
1027	$\beta$ -Phellandrene												2.1	
1030	( <i>Z</i> )- $\beta$ -Ocimene	1.2	0.4			<0.1	0.6	0.2		0.2	0.2	0.2	0.6	0.9
1042	( <i>E</i> )- $\beta$ -Ocimene	<0.1	1.9	<0.1	1.8		0.2			0.2	0.3	1.2	0.7	
1054	$\gamma$ -Terpinene		1.0	3.7	0.9	0.6	20.0		22.7	0.1	0.1	<0.1	0.3	0.3
1082	Terpinolene		0.2	8.4		0.2	0.2	5.2	<0.1	0.1	0.1	<0.1	1.2	0.1
<i>Oxygen-containing monoterpenes</i>														
1027	1,8-Cineole			0.1	3.1	78.3		8.2						
1061	( <i>E</i> )-Sabinene hydrate		<0.1			1.1	1.0			0.1	0.1			
1080	Fenchone						0.2	42.0						
1088	Linalol	2.1	1.0	0.3	50.8		0.4	0.6	5.2	0.3	0.2	4.5	3.5	<0.1
1106	Fenchol			0.3				13.5						
1134	Camphor		0.1	0.3					1.9		0.2	0.1	0.5	
1170	Terpinen-4-ol	0.3	2.0	7.8	3.5	1.0	1.0	0.2	1.3	0.1	0.3	<0.1	<0.1	
1181	$\alpha$ -Terpineol	0.6	0.5	0.5	1.2	3.2	<0.1	0.7	0.3	0.2	<0.1	<0.1	0.8	0.1
1284	Thymol	1.2	<0.1				46.2	6.0	27.2		0.4			
1294	Carvacrol						0.2		3.3					
<i>Sesquiterpene hydrocarbons</i>														
1337	$\delta$ -Elemene	2.1					<0.1					<0.1		0.9
1352	$\alpha$ -Cubebene	2.0	0.2								<0.1	<0.1	1.6	0.1
1378	$\alpha$ -Copaene	12.3	0.7	<0.1	<0.1	0.2	0.4	0.5	0.1	0.1	0.3	<0.1	6.3	0.6
1386	$\beta$ -Bourbonene		2.1					0.2						
1388	$\beta$ -Cubebene									0.1	0.1		2.3	
1391	$\beta$ -Elemene	6.5	7.2	0.6	0.7		0.2	0.6		<0.1	0.3	2.8		3.3
1411	$\alpha$ -Gurjunene	1.3											1.4	
1423	$\beta$ -Caryophyllene	10.3	10.2	20.3	2.2	0.3	2.3	1.5	3.5	1.0	4.0	2.1	4.3	3.0
1430	$\beta$ -Gurjunene	1.8							0.1	<0.1		0.1		
1440	Aromadendrene		0.4	3.5										0.2
1452	Amorphene		1.2											
1454	( <i>E</i> )- $\beta$ -Farnesene		0.3		<0.1				0.1	<0.1	0.8	20.4	4.0	
1456	$\alpha$ -Humulene	1.7	1.7	0.4	0.5	0.6	0.3	0.2	<0.1	0.1	0.5	1.1	1.0	2.1
1462	<i>allo</i> -Aromadendrene		1.0	1.3			0.2							
1473	Ishwarane													43.5
1475	Guaiene*		0.4				1.5			<0.1	0.2			2.7
1475	$\gamma$ -Muurolene		1.5					0.3		<0.1			0.4	6.2
1478	<i>ar</i> -Curcumene							0.3	0.1			3.5		
1481	Germacrene D	15.1	19.1		2.2			6.0	0.6		1.0	5.2	3.0	3.2
1490	Valencene									<0.1	0.5			3.3
1492	$\alpha$ -Selinene			1.2		0.2	0.7							
1494	$\alpha$ -Zingiberene												1.5	
1496	Bicyclgermacrene	4.3	3.1	2.5	0.8									
1500	( <i>E,E</i> )- $\alpha$ -Farnesene		0.4		<0.1			0.7	0.1			2.5	5.5	
1512	$\gamma$ -Cadinene		1.6		1.0		1.0	0.2		<0.1	<0.1			
1514	Bisabolene*				0.5					<0.1	0.4	2.1		
1518	$\delta$ -Cadinene	10.5	3.7	0.2		0.2	0.8	0.8	0.3	0.1	0.1	<0.1		3.4

Table continued on next page

Table 2. Continued.

RI*	Compounds	<i>Hostundia opposita</i> Vahl. (fresh leaves)	<i>Hyptis lanceolata</i> Poit. (fresh whole plant)	<i>Hyptis suaveolens</i> Poit. (fresh leaves)	<i>Ocimum basilicum</i> L. (fresh whole plant)	<i>Ocimum canum</i> Sim. (fresh whole plant)	<i>Ocimum gratissimum</i> L. (fresh leaves)	<i>Plectranthus glandulosus</i> Hook. (fresh leaves)	<i>Thymus vulgaris</i> L. (fresh whole plant)	<i>Piper capense</i> L. (fresh leaves)	<i>Piper capense</i> L. (fresh fruits)	<i>Piper guineense</i> Schum. et Thom. (fresh leaves)	<i>Piper guineense</i> Schum. et Thom. (fresh fruits)	<i>Bixa orellana</i> L. (fresh leaves)
1530	Cadina-1,4-diene													1.3
1558	Germacrene B	6.6								0.1	0.1		1.7	
<i>Oxygen-containing sesquiterpenes</i>														
1554	Nerolidol*		0.4							0.2	0.3	1.1		2.3
1573	Spathulenol	1.2	1.5	1.0		<0.1					<0.1			0.4
1578	Caryophyllene epoxide	1.0	2.3	0.3		0.4	0.1	0.6						0.7
1592	Guaiol													7.3
1593	Humulene epoxide	1.0	0.3	0.3		0.9								
1618	$\beta$ -Bisabolol											1.0		
1635	T-Muurolool	0.9	0.6	0.8			0.4	0.4						1.1
1636	T-Cadinol	1.6	1.4	0.5	3.4		0.1							
1640	Torreyol		1.1		<0.1				0.2					
1648	$\alpha$ -Cadinol	2.0	1.8	0.5	<0.1			0.4	0.2					1.7
1648	$\alpha$ -Eudesmol													1.3
1658	Bulnesol													2.3
1677	$\alpha$ -Bisabolol			0.2							1.1	0.5		
1681	Bergamotol*			6.2										
<i>Benzenoid compounds</i>														
1178	Safrole											0.2	3.3	
1338	Eugenol				13.5									
1532	Myristicin											25.6		

RI = Retention indices on OV-101 column.

\* Correct isomer not identified.

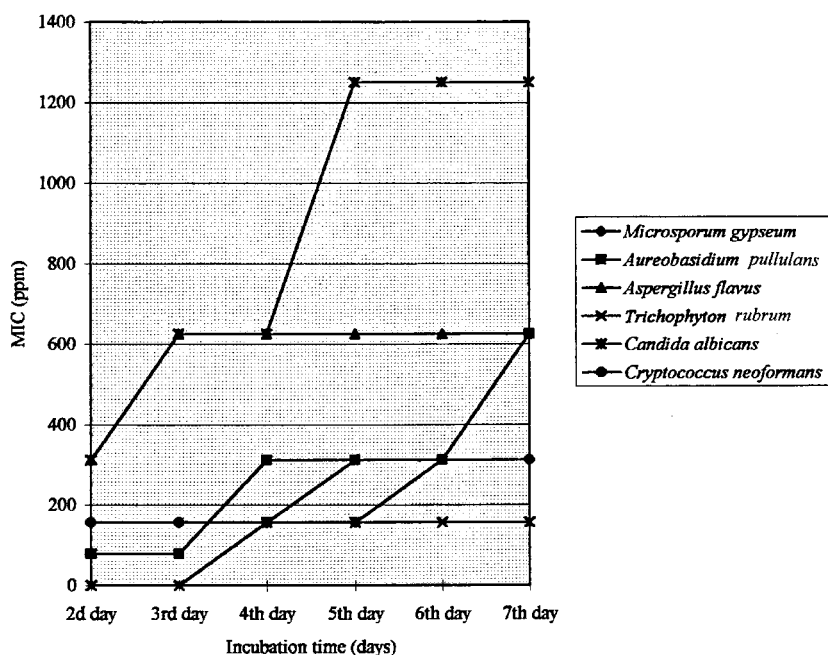


Figure 2. Evolution of MIC (ppm) of *Thymus vulgaris* according to incubation time (days)

**Table 3.** Minimal inhibitory quantity (MIQ) (in  $\mu\text{l}$ ) of 13 essential oils against five fungi in the microatmosphere assay after 7 days of incubation

Essential oils	Fungi				
	<i>Microsporium gypseum</i>	<i>Aureobasidium pullulans</i>	<i>Candida albicans</i>	<i>Aspergillus flavus</i>	<i>Trichoderma viride</i>
<i>Ocimum gratissimum</i>	5	5	5	5	5
<i>Thymus vulgaris</i>	5	5	5	5	5
<i>Ocimum basilicum</i>	5	5	5	7.5	5
<i>Plectranthus glandulosus</i>	5	7.5	12.5	15	15
<i>Piper capense</i> (fruits)	5	15	12.5	15	15
<i>Piper guineense</i> (leaves)	5	>15	>15	>15	>15
<i>Piper guineense</i> (fruits)	5	>15	>15	>15	>15
<i>Hyptis suaveolens</i>	7.5	>15	>15	>15	>15
<i>Hoslundia opposita</i>	12.5	>15	>15	>15	>15
<i>Hyptis lanceolata</i>	15	15	>15	>15	>15
<i>Ocimum canum</i>	>15	>15	>15	>15	>15
<i>Piper capense</i> (leaves)	>15	>15	>15	>15	>15
<i>Bixa orellana</i>	>15	>15	>15	>15	>15

**Table 4.** Minimal inhibitory concentrations (MIC) (in ppm) of five essential oils after 7 days of incubation

Essential oils	Fungi					
	<i>Microsporium gypseum</i>	<i>Aureobasidium pullulans</i>	<i>Aspergillus flavus</i>	<i>Trichophyton rubrum</i>	<i>Candida albicans</i>	<i>Cryptococcus neoformans</i>
<i>Ocimum gratissimum</i>	78	312	1250	78	312	312
<i>Thymus vulgaris</i>	312	625	625	156	1250	312
<i>Ocimum basilicum</i>	625	1250	5000	312	5000	1250
<i>Hyptis suaveolens</i>	625	5000	10000	156	10000	5000
<i>Hyptis lanceolata</i>	5000	10000	10000	625	10000	10000

equal to 5  $\mu\text{l}$  and 5.5  $\mu\text{l}$ , were the most active, whereas *Ocimum canum* and *Bixa orellana* were not active until 15  $\mu\text{l}$ . Table 4 indicates the MIC determined by the broth dilution micromethod after 7 days of incubation. These results report the mean of three determinations. Plants are classified in decreasing order of activity. Tables 3 and 4 lead to the conclusion that the essential oils of *Ocimum gratissimum*, *Thymus vulgaris* and *Ocimum basilicum* have an important antifungal activity on several test fungi (with several median MIC < 1000 ppm and several median MIQ = 5  $\mu\text{l}$ ).

In comparison, the MIC of nystatin and clotrimazole, determined by the agar diffusion method on *Candida albicans* by Chaumont and Bardey,<sup>5</sup> are 100 and 50 ppm respectively. In the same conditions, the MIC of *Trichophyton rubrum* was 50 and 20 ppm respectively. The MIC of carbendazim on *Aspergillus flavus* is 3000 ppm according to Singh and Upadhyay.<sup>19</sup> In liquid medium, the MIC value of isoconazole on *C. albicans* amounts to 1000 ppm according to Regli *et al.*<sup>20</sup>

Figures 1, 2 and 3 illustrate the MIC values variation for the three more active volatile oils according to the incubation time for each fungus. These figures give an idea of the nature of antifungal activity (fungistatic or fungicidal activity), from which we observe that:

1. The essential oil of *Ocimum gratissimum* leaves (Figure 1) are fungistatic on *Aureobasidium pullulans* at 78 and 156 ppm, the concentrations

for which the inhibition is partial; and fungicidal on *Microsporium gypseum* and *Trichophyton rubrum* at 78 ppm *Candida albicans* and *Cryptococcus neoformans* at 312 ppm.

2. The oil of *Thymus vulgaris* (Figure 2) shows a fungicidal activity against *Trichophyton rubrum*, *Candida albicans*, *Microsporium gypseum*, *Aureobasidium pullulans* and *Aspergillus flavus* at 156, 625, 312 and 1250 ppm respectively. This activity is not fungistatic on *A. pullulans* until 625 ppm.
3. The volatile oil of *Ocimum basilicum* (Figure 3) is fungicidal against *Trichophyton rubrum*, *Candida albicans*, *Microsporium gypseum*, *Aureobasidium pullulans* and *Aspergillus flavus* at 312, 5000, 625, 1250 and 5000 ppm respectively. This activity is not fungistatic on *Cryptococcus neoformans* until 1250 ppm.

Table 5 indicates the concentrations of the nine constituents with known antifungal activity identified in the 13 oils during the chemical analysis. The remarkable activity of *Ocimum gratissimum* essential oil was attributed to *p*-cymene, eugenol, linalol and citronellol by Tripathi *et al.*<sup>16</sup> for the Indian species. This activity may be assigned to thymol (46.2%),  $\gamma$ -terpinene (20.0%), *p*-cymene (7.0%), carvacrol (0.2%) and linalol (0.4%) for the Cameroonian species. The activity of the oil of *Thymus vulgaris*, attributed to thymol by Pellecuer *et al.*<sup>17</sup> as well as Loret and

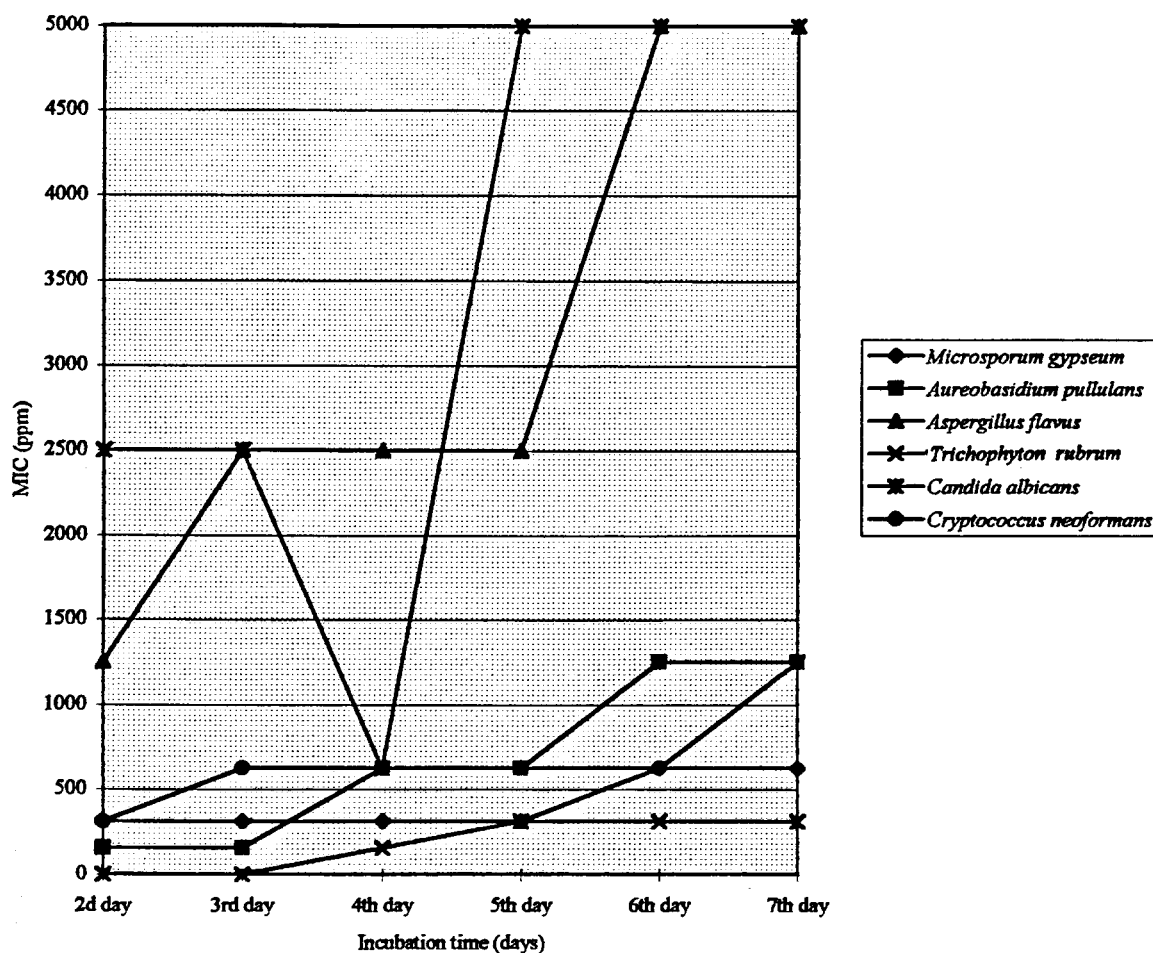


Figure 3. Evolution of MIC (ppm) of *Ocimum basilicum* according to incubation time (days)

Barrandon<sup>18</sup> may also be assigned here to thymol (27.2%), *p*-cymene (23.6%),  $\gamma$ -terpinene (22.7%) and carvacrol (3.3%). The *Ocimum basilicum* and *Plectranthus glandulosus* essential oils activities, about which no work on antifungal activities is reported, may be imputed here to linalol (50.8%), eugenol (13.5%) and 1,8-cineole (3.1%) for *O. basilicum*; fenchone (42.0%), fenchol (13.5%), 1,8-cineole (8.2%) and thymol (6.0%) for *P. glandulosus*.

The difference observed between the essential oils of fruits and leaves of *Piper capense* in their activity could be due to the presence of thymol (0.4%) in the fruits.

The *Ocimum canum* oil, for which five chemotypes are known (camphor,<sup>21</sup> methyl cinnamate,<sup>22</sup> citral,<sup>23</sup> linalol and eugenol<sup>24</sup>) shows a high level of 1,8-cineole (78.3%) but, curiously, no antifungal activity.

The essential oils of *Hoslundia opposita*, *Hyptis lanceolata*, *Hyptis suaveolens*, *Piper guineense* and *Bixa*

Table 5. Percentage of constituents with known antifungal activity in essential oils from Cameroon

Essential Oils	Constituents								
	Thymol	Carvacrol	Linalol	$\gamma$ -Terpinene	<i>p</i> -Cymene	1,8-Cineole	Eugenol	Fenchone	Fenchol
<i>Ocimum gratissimum</i>	46.2	0.2	0.4	20.0	7.0				
<i>Thymus vulgaris</i>	27.2	3.3	5.2	22.7	23.6				
<i>Ocimum basilicum</i>			50.8				13.5		
<i>Plectranthus glandulosus</i>	6.0		0.6			3.1		42.0	13.5
<i>Piper capense</i> (fruits)	0.4		0.2	0.1	0.1				
<i>Piper guineense</i> (leaves)			4.5						
<i>Piper guineense</i> (fruits)			3.5	0.3	0.3				
<i>Hyptis suaveolens</i>			0.3	3.7	0.6	0.1			0.3
<i>Hoslundia opposita</i>	1.2		2.1						
<i>Hyptis lanceolata</i>	<0.1		1.0	1.0	0.3				
<i>Ocimum canum</i> (leaves)				0.6		78.3			
<i>Piper capense</i>		0.2	0.3	0.1	<0.1				
<i>Bixa orellana</i>			<0.1	0.3					

*orellana* did not contain high contents of any well-known antifungal compounds. In agreement, the biological assays revealed weak activity on all test fungi.

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