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Antioxidant and Antifungal Activities of the Essential Oils of *Ocimum gratissimum* from Yaoundé and Dschang (Cameroon)

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Abstract: Due to the harmful effect of free radical on physiological and pathological state of our body on one hand, and the increase of the fungal infection on the other hand, drug that can reduce free radical and inhibit fungal growth is needed. This work aims to evaluate the antioxidant and antifungal activities of the *Ocimum gratissimum* L. essential oils from center and west region of Cameroon. Essential oil was analyzed by GC (gas chromatography) and GC coupled with MS (mass spectrometry). The antioxidant activities of essential oils were studied by the Diphenylpicrylhydrazyl method and the β-carotene bleaching test. The antifungal activities were assessed using micro-dilution technique for yeasts and agar dilution method for *Aspergillus*. Thymol and γ-terpinene, eugenol and thymol were the major compounds for Yaoundé and Dschang, respectively. The scavenging capacity of sample from Dschang was higher than that of Yaoundé. Also, the β-carotene bleaching tests of the sample from Dschang were better than that from Yaoundé. The antifungal activity of the sample from Yaoundé was higher than that from Dschang on yeasts and *Aspergillus* isolates, respectively. This work presents and compares the chemical composition, antioxidant and antifungal activity of *Ocimum gratissimum* essential oil from center and west region of Cameroon.

Key words: *Ocimum gratissimum* L., antioxidant, antifungal, gas chromatography, gas chromatography coupled with mass spectrometry.

1. Introduction

The metabolism of oxygen continuously produces small quantity of reactive oxygen derivatives. The physiological role of these molecules can have a function in cell signalization, phagocytosis and cell cycle [1]. The production of free radicals species grow up and induce the stress state called oxidative stress [1, 2]. Due to its capacity to damage almost all the type of molecules in the organism, the oxygen derivative species are implicated in a large number of pathologies as well as chronic or acute diseases [3]. On the other hand, these reactive oxygen derivatives are produced during fungal infection [3]. The incidence of these fungal infections has dramatically increased with the number of immunocompromized patients. The genus frequently involved is *Aspergillus*, *Candida* and *Cryptococcus* [4, 5]. They cause 90% of death on the infected persons [6]. The genus *Aspergillus* is responsible for 20% of total fungal infections and is involved in 66%-99% of death on the pulmonary *Aspergillosis*, sinuses and brain infections [7]. The genus *Candida* is responsible for 64.7% of fungal infections with about 26.4% of death cases [6]. The

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genus *Cryptococcus* accounts for about 90% in HIV positive and represents 11.7% of death among these individuals [8].

The reasons for this upsurge of infection are the aggressive therapeutic agents, transplantations, immuno-depressions [9], alcoholic cirrhosis [10], increase of the drug costs [11] and the development of fungi resistance to available drugs [12]. As the available antioxidant molecules, the antifungal drugs possess side effects. Thus, searching for alternative antioxidant and antifungal compounds from natural source has been a major concern in recent years.

Plant extracts are used as natural source of antioxidant and antifungal molecules due to their potent pharmacological activities, low side effect and economic viability [13-15]. *Ocimum gratissimum* belongs to the group of plants known as spices [16]. The plant is found throughout the tropics and subtropics, and its greatest variability occurs in tropical Africa and India [17]. In Cameroon, some studies have shown the antioxidant activity of *Ocimum gratissimum* L. essential oils from different sites in the east region [16]. The essential oils of this plant also exhibit toxic potentials when taken in certain quantity [18]. Its antibacterial activity was showed by Matasyoh et al. [19] on Kenyan's species. The biological activities of essential oils are due to its chemical composition [13, 14, 16] which depends on the site of growth of the plant [16, 17, 20]. The aim of this study is to characterize the chemical composition of the essential oils of *Ocimum gratissimum* L. from Yaoundé and Dschang (center and west region of Cameroon, respectively), and to compare their antioxidant and antifungal activities.

2. Materials and Methods

2.1 Plant Material

The leaves were collected from Nkolondom II for the Yaoundé species and Bafou for Dschang species, on August 2009. The identification of the plant was done in the Cameroon National Herbarium and voucher specimen was deposited under identification number 5817/SRF/Cam.

2.2 Fungal Strains

The fungal material was made of three mould isolates of *Aspergillus* genus (*Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger*) and three yeasts (*Candida albicans* ATCC24433, *Candida parapsilosis* ATCC22019 and *Cryptococcus neoformans* IP95026) obtained from "Centre Pasteur du Cameroun". These fungal strains and isolates were maintained on Sabouraud Dextrose Agar.

2.3 Extraction of Essential Oils

The plant samples were hydro-distilled for 5 h using a Clevenger-type apparatus. Essential oils obtained were dried over anhydrous sodium sulphate and stored at 4 ℃ until use for further experiments. The extraction yields were calculated in percentage (*w*/*w*) relatively to the starting plant material.

2.4 Chemical Analysis of the Essential Oils

The essential oils were analysed by GC (gas chromatography) and GC coupled with MS (mass spectrometry).

2.4.1 Gas Chromatography

The oil was analysed on a Varian CP-3380 GC with flame ionisation detector fitted with a fused silica capillary column (30 m × 0.25 mm coated with DB5, film thickness 0.25 μm); temperature program 50 -200 ℃ at 5 ℃/min, injector temperature 200 ℃, detector temperature 200 ℃, carrier gas N_2 at 1 mL/min.

The linear retention indices of the components were determined relatively to the retention times of a series of *n*-alkanes and the percentage compositions were obtained from electronic integration measurements without taking into account relative response factors.

2.4.2 Gas Chromatography/Mass Spectrometry

GC/MS analyses were performed using a Hewlett-Packard apparatus equipped with an HP1 fused silica column (30 m × 0.25 mm, film thickness

0.25 μm) and interfaced with a quadrupole detector (GC-quadrupole MS system, model 5970). Column temperature was programmed from 70-200 ℃ at 10 ℃/min; injector temperature was 200 ℃. Helium was used as carrier gas at a flow rate of 0.6 mL/min, the MS was operated at 70 eV.

2.4.3 Identification of Components

The identification of the constituents was assigned on the basis of comparison of their retention indices and their mass spectra with those given in Refs. [21-23] with the data bank NBS75K and with the stored laboratory mass spectral library [16, 20].

2.5 Determination of the Antioxidant Activities

2.5.1 DPPH Test

The antiradical activity was determined using DPPH (2, 2-diphenyl-1-picrylhydrazyl), which was dissolved in ethanol to give 100 μm solution. The accurate C_{DPPH} (DPPH concentration) was determined by spectrophotometric method following Eq (1):

$$A_{517} = 9832 \times C_{DPPH} \quad (1)$$

where, 9,832 is the molecular extinction coefficient of DPPH determined independently in ethanol.

2.0 mL of the ethanolic solution of DPPH was added 100 μL of a methanolic solution of a reference molecule BHT (butylated hydroxyl toluene) at different concentrations. The oil was tested using the same method. The control (without antioxidant) was represented by the DPPH ethanolic solution containing 100 μL of methanol. Decrease in absorption was measured at 517 nm after 2 h, at room temperature. The decrease in absorption induced by the test compound was calculated by subtracting that of the control. The concentration required for SC_{50} (50% scavenging concentration) was determined graphically. All the spectrophotometric measurements were performed using a SAFAS UV-mc^2 Spectrophotometer, equipped with a multi-cell/multi-kinetic measurement system and with a thermostated cell-case [13, 14].

2.5.2 β-carotene Bleaching Test

Antioxidant activity was evaluated using a β-carotene/linoleate model system. A solution of β-carotene from Fluka (7235-40-7) was prepared by dissolving 2.0 mg of β-carotene in 10 mL chloroform. 1.0 mL of this solution was pipetted into a round-bottomed flask which contained 20 μL purified linoleic acid from Avocado (60-33-3) and 200 mg Tween 40 emulsifier from Aldrich (9005-66-7). After chloroform was removed under vacuum using a rotary evaporator at 40 ℃, 50 mL aerated distilled water was added to the flask with vigorous shaking. The antioxidant activity was evaluated by measuring, at 470 nm, the kinetics of discoloration of β-carotene in the absence (control) and presence of the antioxidant solution (10 μL methanolic solutions containing different concentrations of essential oil or BHT for comparative purposes) at 50 ℃ [13, 24].

A blank was prepared under the above conditions but without β-carotene. All the kinetics were obtained according to Eq (2):

$$A = A_0 e^{-kt} + C \quad (2)$$

where, A_0 is the absorbance at time zero, C *is* the absorbance at infinite time and k *is* the degradation rate constant of β-carotene, from which Ip (inhibition percentages) were calculated using Eq (3):

$$Ip = 100\,(k_0 - k)/k_0 \quad (3)$$

where, k_0 and k are the degradation rate constants of β-carotene in the absence and presence of inhibitor. The plot of the inhibition percentage as a function of the inhibitor concentration enabled the determination of the IC_{50} of the sample.

All the spectrophotometric measurements were performed using the same apparatus as in scavenging assay.

2.6 Antifungal Activities

2.6.1 Antifungal Susceptibility Testing

Agar dilution and agar disc diffusion methods were respectively used to assess the sensitivity of moulds and yeasts to essential oil. For moulds, the experiments were designed as previously described [25]. For yeasts, the disc diffusion method was used. Briefly, 10 mL of

Sabouraud Dextrose Agar were aseptically poured on 54 mm Petri dishes and allow solidifying. After solidification, 2 mL of 2.5 $\times 10^5$ CFU/mL inoculums from each yeasts were inoculated on Petri dishes, allowed to stand for 10-15 minutes after what, 15 μL of essential oil (1 mg/mL to 0.125 mg/mL) were place aseptically on sterilized piece of filter papers and the prepared filter paper was placed at the center of the prepared Petri dishes. The Petri dishes were sealed using paraffin paper and incubated at 37 °C for 48 h. The test was done in triplicate. Nystatin (1.33 mg/mL) was used as positive control and treated as essential oils. The negative controls contain filter paper with no antifungal substance. The susceptibility of microorganisms was determined by measuring the diameter of the inhibition zone after incubation.

2.6.2 Determination of MIC (Minimal Inhibitory Concentration) and MFC (Minimal Fungicidal Concentration)

Agar dilution and broth micro-dilution methods were respectively used to assess MIC and MFC of moulds and yeasts of essential oil using the method previously described [25].

2.7 Statistical Analysis

Results are presented as means ± *SD* (standard deviation) of the three measurements. The analyses of variance followed by LSD (least significant difference) post hoc determination were performed using the statistical software Statgraphics 5.0 for Windows. Statistical significance was set at $P < 0.05$.

3. Results and Discussion

3.1 Yields of Essentials Oil's Extraction

The yield of 0.19% with yellowish color was obtained with the species of Yaoundé and 0.61% for that of Dschang.

The yield of *Ocimum gratissimum*-Yaoundé was lower than that obtained by Tchoumbougnang [20] who had a percentage yield of 0.47% and 0.42% from the samples of Yaoundé and Bazou, respectively. Rather, yield was similar to that obtained by Pessoa et al. [26] (0.2%). This variation in yield can be explained by the geographic difference, period of harvest, postharvest treatment and extraction conditions [16, 20].

3.2 Chemical Composition

After GC and GC/MS analyses, the chemical composition of our essential oils is shown in Table 1.

From Table 1, the essential oil from Yaoundé was rich in monoterpenes (91.6%) on which monoterpene hydrocarbon and oxygenated monoterpenes were 46.1% and 45.5%, respectively. Sesquiterpenes form only 6.1% of the essential oil. The major compounds were thymol (40.7%), γ-terpinene (24.5%) and p-cymene (5.9%).

As in the Yaoundé species, the essential oil from Dschang was rich in monoterpene (41.2%) on which monoterpene hydrocarbon was 18.6% and oxygenated monoterpene was 22.6%, with thymol as the major component; on the other hand, the oil was characterized by a high content of eugenol (46.2%). Sesquiterpenes form only 10.6% of the whole essential oil, of which sesquiterpene hydrocarbons represent 9.1% and oxygenated sesquiterpenes only 1.5%.

The identifiable constituents of our essential oils on the qualitative point were generally the same for the two species. Nevertheless, there was a slight difference, with the presence of oxygenated sesquiterpene in the essential oil from Dschang (0.2%), while there was none in the Yaoundé species. The presence of some chemical compounds is dependent on the geographical situation of the plant. These results were similar to those previously obtained by Ndoye [16] for the essential oils from six localities of east Cameroon. This author obtained a variation in the presence of oxygenated sesquiterpene (0%-2.5%) among the six species. Matasyoh et al. [19] obtained 0% oxygenated sesquiterpene on the Kenyan's species.

Based on the majority of compounds, the essential oil from Yaoundé showed similar results with that previously obtained by Ndoye [16], Tchoumbougnang [20] and Nguefack [27] for plants collected in East,

Table 1 Chemical composition of essential oils.

KI	Compounds	Percentages	
		O. gratissimum (Dschang)	*O. gratissimum* (Yaoundé)
Monoterpenes		41.2	91.6
Monoterpene hydrocarbons		18.6	46.1
928	α-thujene	0.6	3.8
938	α-pinene	0.2	1.0
954	camphene	-	0.1
970	sabinene	0.1	-
977	β-pinene	0.1	0.7
991	myrcene	1	3.8
1,002	α-phellandrene	tr	0.3
1,010	Δ-3-Carene	tr	0.2
1,014	α-terpinene	0.7	3.0
1,018	p-cymene	5.9	5.9
1,027	limonene	1.7	1.6
1,039	(E)-β-ocimene	0.1	0.2
1,057	γ-terpinene	7.6	24.5
1,081	p-cymenene	0.6	0.8
1,085	terpinolene	tr	0.2
Oxygenated monoterpenes		22.6	45.5
1,074	sabinene hydrate	-	0.2
1,093	linalool	0.7	3.0
1,107	thujone	tr	0.2
1,127	limonene oxide	-	tr
1,135	t-pinocarveol	0.2	0.5
1,159	isoborneol	tr	-
1,163	borneol	-	0.2
1,175	terpinen-4-ol	0.9	1.3
1,197	α-terpineol	0.2	tr
1,230	neral	-	tr
1,280	thymol methyl-ether	tr	-
1,288	thymol	20.6	40.70
Sesquiterpenes		10.6	6.1
Sesquiterpene hydrocarbons		9.1	5.9
1,388	β-elemene	0.7	0.3
1,397	isocaryophyllene	-	0.1
1,398	longifolene	0.1	-
1,434	β-caryophyllene	3.0	2.7
1,450	α-humulene	-	0.9
1,453	(E)-β-farnesene	0.4	0.2
1,456	(Z)-β-farnesene	tr	-
1,468	α-humulene	0.3	0.2
1,480	germacrene D	0.1	0.5
1,494	α-selimene	0.1	0.4
1,497	α-curcumene	tr	-
1,500	(E,E)-α-farnesene	3.0	0.6
1,511	β-selimene	0.8	-
1,530	Δ-cadinene	0.4	-
1,535	epi-α-selinene	0.2	-
Oxygenated sesquiterpenes		1.5	0.2
1,593	humulene oxide	0.1	0.2
1,597	caryophyllene oxide	1.3	-
1,633	t-cadinol	0.1	-
Aromatic compound		46.2	0.6
1,354	eugenol	46.2	0.6

tr = percentage lower than 0.1%; - = not present; KI = Kovats index.

Littoral and Center region of Cameroon, respectively. These essential oils from the Center and East Regions of Cameroon can be classified as thymol/γ-terpinene chemotype. Meanwhile, that of Dschang can be classified as eugenol/thymol chemotype and its chemical composition does not correspond to anyone in literature.

3.3 Antioxidant Activity

3.3.1 DPPH Test

3.3.1.1 BHT Activity

Fig. 1 represents the dose response curve of DPPH radical scavenging activity of BHT.

This graph enabled us to determine the scavenging concentration of BHT. An SC_{50} value of 0.07 ± 0.01 mg/mL was obtained.

3.3.1.2 Essentials Oils Activity

The curves of Fig. 2 show the scavenging capacity of the different essential oils.

From the curve, it shows that scavenging capacity of the DPPH radical increases as concentration increases. The SC_{50} values were extrapolated and are shown in Table 2.

From these SC_{50} values, the effective concentration, that is, the concentration of essential oil necessary to capture 50% of DPPH radical (EC_{50}) and the antiradical capacity was calculated and shown in Table 2.

It was found that *O. gratissimum* from Dschang $(5.88 \pm 0.99) \times 10^{-4}$ and BHT $(1.22 \pm 0.00) \times 10^{-3}$ had the highest antiradical activities than *O. gratissimum* of Yaoundé $(3.37 \pm 0.19) \times 10^{-5}$. There was a statistically significant difference ($P < 0.05$) among the activities of the essential oils, but there was no statistical significance difference ($P < 0.05$) among the activities of *O. gratissimum*-Dschang and BHT.

Our essentials oils are rich in phenolic compounds (41.3% for essential oil from Yaoundé and 66.8% for essential oil from Dschang). Both oils showed antiradical activity, but the relative high activity of essential oil of Dschang can be attributed to its high phenolic content. This activity could be explained by the capacity of phenolic compounds to stabilize DPPH by hydrogen donation and formed DPPH-H as previously reported by Brand-Williams et al. [28].

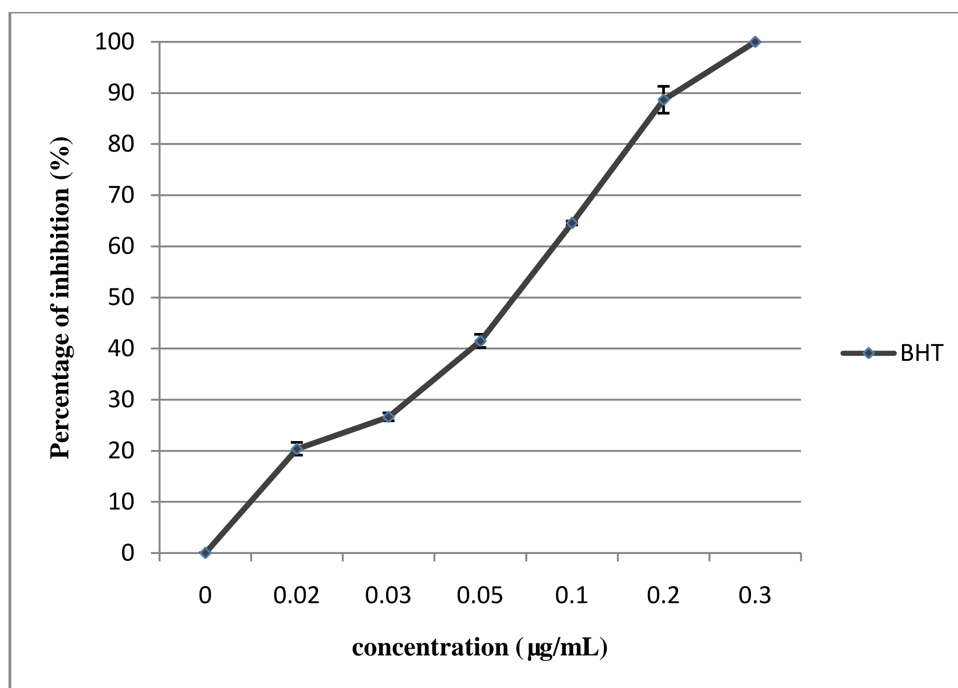

Fig. 1 DPPH scavenging activities of BHT.

BHT = butylated hydroxytoluen.

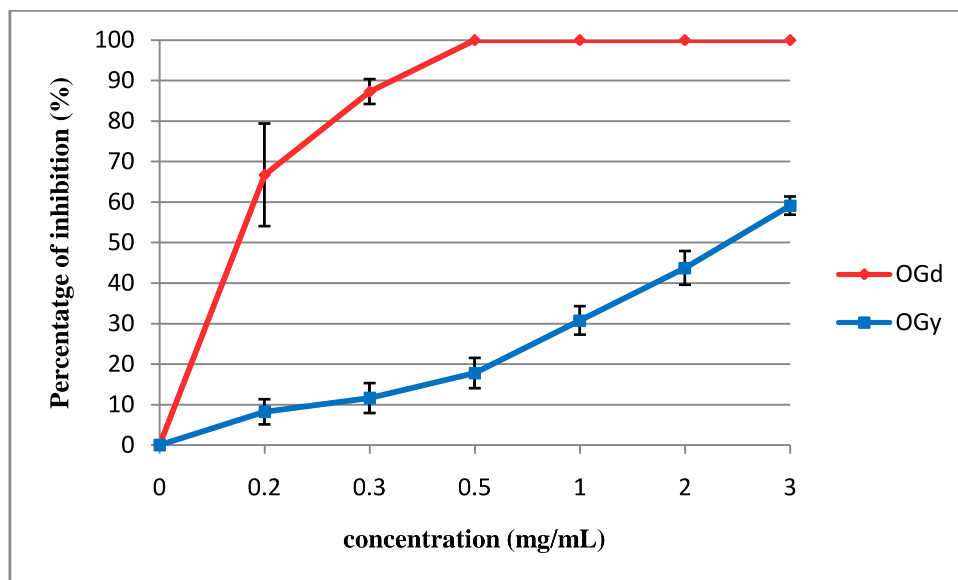

Fig. 2 DPPH scavenging activities of essentials oils.

OGd = *Ocimum gratissimum* from Dschang, OGy = *Ocimum gratissimum* from Yaoundé.

Table 2 SC_{50}, EC_{50} and AC values of the essential oils and BHT.

	$SC_{50} \pm SD$ (%)	$CE_{50} \pm SD$ (%)	$AC \pm SD$ (%)
O. gratissimum-YDE (mg/mL)	2.39 ± 0.13^{a}	$(2.97 \pm 0.16) \times 10^{4c}$	$(3.37 \pm 0.19) \times 10^{-5e}$
O. gratissimum-DSC (mg/mL)	0.15 ± 0.00^{b}	$(1.70 \pm 0.40) \times 10^{3d}$	$(5.88 \pm 0.99) \times 10^{-4f}$
BHT (mg/mL)	0.07 ± 0.01^{b}	$(8.23 \pm 0.00) \times 10^{2d}$	$(1.22 \pm 0.00) \times 10^{-3f}$

O.gratissimum-YDE = *Ocimum gratissimum* from Yaoundé; *O. gratissimum*-DSC = *Ocimum gratissimum* from Dschang; SC_{50} = scavenging concentration 50; EC_{50} = efficient concentration 50; AC = anti oxydant capacity; SD = standard deviation; a, b, c, d, e and f = statisticaly significantl difference.

3.3.2 B-carotene Bleaching Test

3.3.2.1 BHT Activity

The antioxidant activity of BHT (used as the reference antioxidant) was analyzed and the results are plotted as shown in Fig. 3.

The inhibitory concentration of the BHT to the oxidation of linoleic acid was determined. An IC_{50} value of 0.10 ± 0.00 was obtained.

3.3.2.2 Essential Oils Activity

The antioxidant activity of the respective essential oils was analyzed and the results are plotted as shown in Fig. 4.

From the curves of Fig. 4, the inhibitory concentration of the essential oils was determined. For *O. gratissimum*-Dschang, the IC_{50} value of $(0.30 \pm 0.03) \times 10^{-3}$ mg/mL was obtained, while an IC_{50} value of $(3.28 \pm 0.10) \times 10^{-3}$ mg/mL was obtained from *O. gratissimum*-Yaoundé. The values obtained showed that the sample of Dschang was a better antioxidant than that of Yaoundé with a statistical difference ($P < 0.05$). With respect to the reference, there was no statistical difference ($P < 0.05$) between *O. gratissimum*-Dschang and BHT. The activities observed can be explained by the higher content of phenolic compounds.

The antioxidant activity can be attributed to the phenol content of the essential oil [13, 14, 16], but other compounds in the essential oil can also increase these activities as previously reported [29, 30]. Our essentials oils are rich in other compounds such asγ-terpinene, α-terpinene and terpinolene for both Yaoundé and Dschang samples. Kim et al. [30] reported that the essential oil from tea tree had higher antioxidant than BHT. This oil was rich in α-terpinene, γ-terpinene and terpinolene. Therefore, we can suggest that the antioxidant activities of our essential oil could

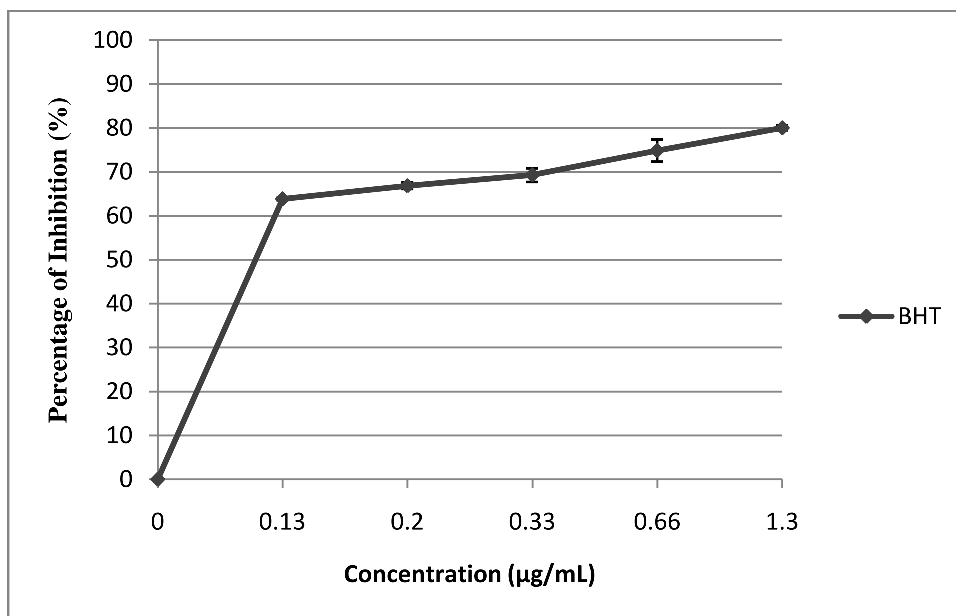

Fig. 3 Antioxidant activity of BHT.

BHT = butylated hydroxytoluen.

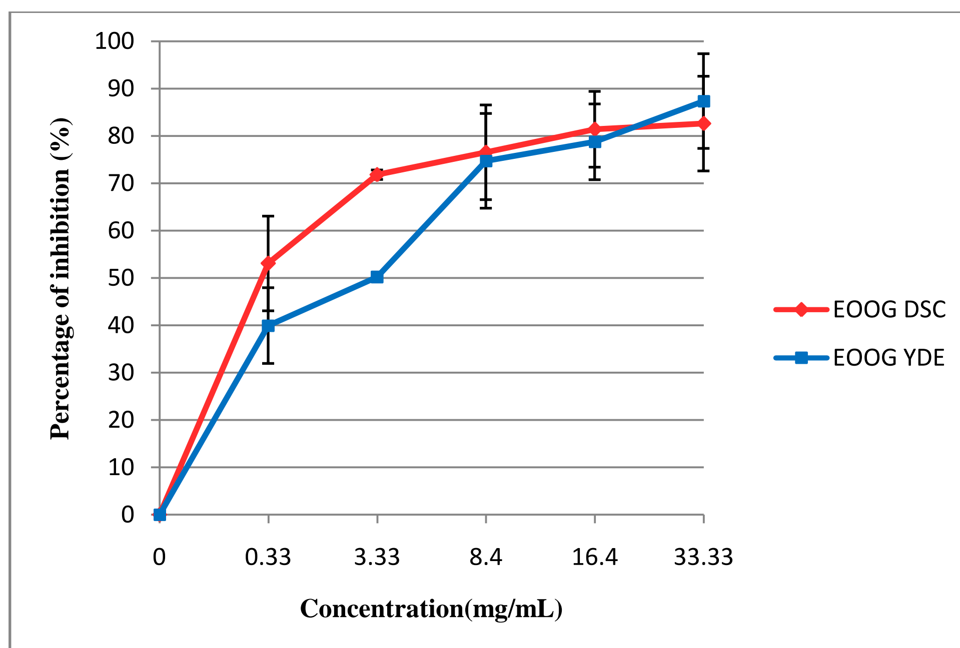

Fig. 4 Antioxidant activities of essentials oils.

EOOG DSC = *Ocimum gratissimum* from Dschang, EOOG YDE = *Ocimum gratissimum* from Yaoundé.

be due to the synergistic action between terpinene (in his various configurations), terpinolene and phenol compounds.

3.4 Antifungal Activity

The disc diffusion method is actively used to evaluate the biological activities of natural substances and plant extracts. But in the case of the solutions with a weak activity, high quantity of extracts are needed and the countenance of disc is very poor [3], in addition the essential oils contain high amount of hydrophobic compound and the interaction between disc surface and

these molecules is very weak. Therefore, this method was used in this study just to find any active concentration as previously used by Mohammedi [31].

3.4.1 Results of MIC and MFC on Moulds

The results from Table 3 show the inhibitory effect of essentials oils on three moulds tested.

All these essential oils revealed statistically the same activity ($P < 0.05$) and all the *Aspergillus* were resistant to the *Amphotericine B* (*Fungizone*) and *Griseofulvin*. In fact, the MIC is higher than 2 mg/mL.

The MIC of *Aspergillus flavus* obtained in this study was smaller than that obtained by Tchoumbougnang [32]. In fact, this author, using *Ocimum gratissimum* harvested in Yaoundé and dried before essential oil extraction, obtained the MIC of 1.25 μL/mL on *Aspergillus flavus* FPR023. Using microathmosphere technique [33] has enable us to obtained the MIC of 80×10^{-3} mg/disc on *Aspergillus niger*. The MFC/MIC ratio revealed that the essential oils tested have fungicidal activity on tested strains.

3.4.2 Results of MIC and MFC on Yeasts

Table 4 gives the results.

In general, these two essential oils revealed good anti-yeast activity as compared to the reference drug found in Cameroon markets: Nystatin. From Table 4, we observed that the essential oil from Yaoundé revealed the high activity on the tested yeasts strains with 0.32 mg/mL ≤ MIC ≤ 2.57 mg/mL. The most sensitive strain was *Candida albicans* with MIC of 0.32 mg/mL and 2.50 mg/mL, respectively. The most resistant was *Cryptoccocus neoformans* with MIC of 2.57 mg/mL and 4.68 mg/mL for essential oils from Yaoundé and Dschang, respectively. Instead, the activities observed here on another *Candida albicans* strain were weaker than that obtained by Lemos et al. [34]. In fact, these authors, using the same method, obtain MIC of 0.25 mg/mL.

Table 3 Different parameters of the anti *Aspergillus* activities of essential oils and reference molecules.

	IC50±SD(mg/mL)×10-3		MIC ±SD(mg/mL)×10-3				MFC		MFC/MIC	
Strains	OGd	OGy	OGd	OGy	*AmB*	*Gris*				
A.Fl	0.27b±0.00	0.15b±0.02	0.34ce±0.01	0.28de±0.06	>2	>2	0.47[a]	0.43[a]	1.43	1.76
A.Fu	0.27b±0.01	0.17b±0.01	0.34ce ±0.01	0.31ce±0.01	>2	>2	0.45[a]	0.40[a]	1.41	1.47
A.Ng	0.37bc±0.01	0.28b±0.02	0.42ce±0.01	0.33ce±0.01	>2	>2	0.45[a]	0.40[a]	1.14	1.40

IC_{50} = inhibitory concentration 50; MIC = minimal inhibitrory concentration; *A.Fl = Aspergillus flavus*; *A.Fu = Aspergillus fumigatus; A.Ng = Aspergillus niger*; OGd = *Ocimumgratissimum* harvested in Dschang; OGy = *Ocimumgratissimum* harvested in Yaoundé; *AmB = Amphotericine B* (*fungizone*); *Gris = griseofulvine*; a, b, c, d and e = no significant differences.

Table 4 Anti-yeast parameters.

Methods	Parameters (mg/mL)	*Candida albicans ATCC24433*			*Candida parapsilosis ATCC22019*			*Cryptoccocusneoformans IP95026*		
		OGy	OGd	N	OGy	OGd	N	OGy	OGd	N
Micro dilution	MAC	ND	ND	ND	ND	ND	ND	ND	ND	ND
	IC_{50}	ND	ND	ND	ND	ND	ND	ND	ND	ND
	MIC	0.32	2.50	25.90	0.64	2.50	≤3.24	2.57	4.68	25.90
	MFC	ND	ND	ND	ND	ND	ND	ND	ND	ND
Absorbance (625nm)	MAC	0.08	1.12	8.00	0.25	0.24	8.00	1.12	2.99	8
	IC_{50}	2.80	33.60	152.00	0.83	0.24	12.00	10.39	8.55	12
	MIC	≥80	≥80	584.00	11.97	5.06	72.00	≥80	≥150	72
	MFC	ND	ND	ND	ND	ND	ND	ND	ND	ND
Subculture and colony count	MAC	ND	ND	ND	ND	ND	ND	ND	ND	ND
	IC_{50}	ND	ND	ND	ND	ND	ND	ND	ND	ND
	MIC	ND	ND	ND	ND	ND	ND	ND	ND	ND
	MFC	0.32	10	25.9	5.15	10	25.90	2.57	4.68	51.00

MAC = minimal antifungal concentration; IC_{50} = inhibitory concentration 50; MIC = minimal inhibitory concentration; MFC = minimal fungicidal contration.

Table 5 MFC/MIC ratios.

Parameters	MFC/MIC		
	Candida albicans	*Candida parapsilosis*	*Cryptoccocus neoformans*
OGy	1	8	1
OGd	4	4	1
Nystatin	1	>4	1.96

OGY = *Ocimum gratissimum* from Yaoundé; OGD = *Ocimumgratissimum* from Dschang.

The change of coloration in this method is due to the presence of acetic acid. These compounds are produced during the fungal growth by hydrolysis of glucose contained in the medium [35]. This acetic acid is a weak acid and to change the coloration of the medium, the presence of high amount is needed. Therefore, it is possible that we consider that the inhibition of yeasts is complete when there are a small number of yeast cells.

Using these three methods, the anti-yeast parameters obtained here are high than those revealed by the Nystatin as previously shown by Nakamura et al. [36]. In fact, these authors showed that *Ocimum gratissimum* L. from Brazil is more active on yeast than Nystatin. The variation observed in the result of spectrophotometric method can be explained by the effect of the oils on yeast. In fact, this method is based on the evaluation of the turbidity of the solution but the subculture revealed that the yeasts observed in the solution are not viable. These observations confirm the previous description of the effect of the eugenol rich essential oils of *Ocimum gratissimum* L. on some *Candida* species [37]. Using electronic transmission microscopy on yeasts previously treated with sub-MIC concentration, these authors observed that the deleterious effect of the essential oil on the cell wall of the yeast may be the main reason for the decrease in the rate of yeast growing, because the integrity of the cell wall is necessary for cell division.

In the aim to assess the fungicidal degree of essential oil, we calculate the MFC/MIC ratio and the results are in Table 5

Table 5 shows that the essential oils and Nystatin are fungicidal for *Cryptococcus neoformans* with MFC/MIC lower than 4, and fungistatic for *Candida parapsilopsis* with MFC/MIC ratio greater than 4. The essential oil from Yaoundé and Nystatin are fungicidal on *Candida albicans*, instead, the essential oil from Dschang is fungistatic on this same strain.

Many authors have shown that phenolic compounds have an important antifungal activity [27, 31]. The antifungal properties of essential oils were good. But the essential oil from *Ocimum gratissimum* harvested in Yaoundé revealed statistically the same antifungal activities than that harvested in Dschang on moulds ($P < 0.05$). Except on *Candida albicans*, these two essential oils revealed the same activities on yeasts. According to their chemical composition, essential oils from Yaoundé contain less phenolic compound than that of Dschang. This observation reveals that the inhibitory effects on yeast observed are not only due to the presence of the phenolic compounds. These activities can also be caused by the hydrocarbon monoterpene as previously shown. Our essential oils contained monoterpen hydrocarbons in the range of 46.1% in the essential oil from *Ocimum gratissimum* L. harvested in Yaoundé and 18.6% for the one harvested in Dschang. Bouzouita et al. [37], starting with essential oil containing α-pinene (59.1%), obtained the MIC of 7.5 μg/mL.

4. Conclusions

The results of this study show that the essential oils of *Ocimum gratissimum* from Yaoundé and Dschang belongs to two different chemotypes and has antioxidant as well as antifungal activities. In general, *Ocimum gratissimum* from Dschang has a better antioxidant activity compared to that of Yaoundé but the contrary was observed with regard to the antifungal activities. The best antioxidant potential and the considerable antifungal activity exhibited by *Ocimum*

gratissimum show that this essential oil is a source of compounds which could be used for the prevention of oxidative stress related diseases. The antioxidant properties of this extract probably explain partly, the use of these plants in traditional medicine for the treatment of infectious diseases and inflammations. However, if this essential oil is to be used for preservation or medicinal purposes, issues of safety and toxicity need to be addressed.

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