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Antifungal activity of *Ocimum basilicum* and *Ocimum gratissimum* essential oils and their nanoemulsions against selected *Trichophyton* species

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Abstract: The outcome of antifungal treatments is hindered by various conditions, such as resistance and tolerance of certain fungal pathogens. Mycoses of nails and skin, which are primarily caused by dermatophytes, are the most common fungal infections, with *Trichophyton rubrum* being the most common dermatophytic pathogen, followed by *Trichophyton interdigitale.* Thus, the search for effective treatments against dermatophyte infections is valuable. This study is sought to investigate the antidermatophytic and antioxidant activities of essential oils and nanoemulsions of the leaves and stems from *Ocimum gratissimum* and *Ocimum basilicum*. The plants' essential oils, which were obtained by hydrodistillation using a Clevenger-type apparatus, were further analyzed by gas chromatography and gas chromatography coupled with spectrometry mass (GC/MS). The nanoemulsions were obtained by spontaneous emulsification using Tween 80 and their stabilities were evaluated using distilled water and methylene blue dye. The antidermatophytic effects of the essential oils were evaluated using an agar diffusion method and by determination of minimum inhibitory concentrations (MICs). The antioxidant activity was carried out by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) colorimetric method. As a result, the GC/MS analysis of essential oil from *O. gratissimum* revealed the presence of γ-terpinene (33.73%), thymol (26.44%) and 1.8 cineol (16.65%), whereas the *O. basilicum*'s essential oil was dominated by linalool (55.32%), eucalyptol (16.78%) and eugenol (7.45%). Essential oils and nanoemulsions from *O. gratissimum* (MICs : 1000 and 2000 ppm, respectively) and *O. basilicum* (MICs : 5750 and 6750 ppm, respectively) revealed fungicidal activity against *Trichophyton rubrum*, whereas only *O. gratissimum* showed moderate activity against *T. interdigitale*. Moreover, essential oils and nanoemulsions from *O. gratissimum* and *O. basilicum* scavenged the free radicals of DPPH, thus revealing antioxidant activity. This novel contribution demonstrates the antidermatophytic activity of essential oils and nanoemulsions from *O. gratissimum* and *O. basilicum*, thus supporting the traditional use of these plants in ethnomedicine.

Keywords: dermatophyte infections; *Trichophyton* species; *Ocimum gratissimum*; *Ocimum basilicum*; essential oils; nanoemulsions

1. Introduction

Dermatophytoses are mycotic infections caused by a group of fungi that usually remain localized to the superficial layers of the skin, hair or nails [1,2]. Despite their propensity to infect the exterior aspects of the host, dermatophytic fungi prefer a warm, moist environment for growth, and as a consequence, infections are common in tropical regions [1]. These fungi are classified in the anamorphic genera *Epidermophyton*, *Microsporum* and *Trichophyton* [3]. Recent estimates proposed an annual incidence of 6·5 million invasive fungal infections and 3·8 million deaths, of which about 68% (2·5 million) were directly attributable [4]. Superficial mycoses are the most prevalent forms in humans, thus affecting about 20-25% of the world's population [4]. In Africa, dermatophyte infection is widespread depending on geographic location, with a prevalence of 14-86% in children [1]. Mycoses of nails and skin, which are primarily caused by dermatophytes, are the most common fungal infections, with *Trichophyton rubrum*, being the most common dermatophytic pathogen, followed by *Trichophyton interdigitale* [5]. The reduced size of their genome with the expansion of particular gene families indicate that these fungi are highly specialized and have the ability to degrade hard keratin and escape the immune system during infection [6]. Dermatophytes establish infection following successful adherence of arthroconidia to the surface of keratinized tissues. The proteolytic enzymes released during adherence and invasion not only ascertain their survival but also allow the persistence of infection in the host [7]. The treatment of dermatophytosis involves the use of a number of drugs, such as griseofulvin, terbinafine, ketoconazole and itraconazole, among others. Although these treatments are effective against dermatophytes, their use is often limited due to the number of adverse effects they cause, their high cost, the length of treatment, and the development of resistance [8].

To address their healthcare needs, many people have turned to traditional healers, herbal medicines, ancient medicinal knowledge and home remedies [9]. In addition, there is a growing interest of researchers around the world in medicinal plants and their products, as a result of antifungal drug resistance [10,11]. This is due to their medicinal properties against infectious diseases. Interestingly, medicinal plant extracts and essential oils are factories of valuable natural bioactive compounds, which exhibit antibacterial and antifungal properties, thereby combating microbial resistance [12]. Examples of such plants include *Ocimum basilicum* and *Ocimum gratissimum*, which are widely used by local populations in Africa to cure various diseases, including fungal infections. For example, the infusion of *Ocimum gratissimum* is used to overcome headache, giddiness, cold and cough, whereas *O. gratissimum*-based formulations are recommended for the treatment of ear infections and dermatoses in Côte d'Ivoire [13]. In Nigeria and Togo, *O. gratissimum* is used to relieve fever, diarrhoea and dysentery [14]. The decoction of *O. gratissimum* stems is used to cure hepatitis, candidoses and wound infections [14].

On the other hand, *O. basilicum* or Sweet basil is an important essential oil crop, medicinal plant, and culinary herb that belongs to the Lamiaceae family [15,16]. In Africa, people have been using *O. basilicum* to treat malaria, typhoid, coughs and colds, yellow fever, candidiasis, influenza, genito-urinary infections, sore eyes and ear infections, among others [17]. *O. basilicum* and *O. gratissimum* are used in different places of Cameroon as spices and for the traditional treatment of of headaches, respiratory problems, influenza, stomach pain, inflammations of the throat, diarrhea, worms, and kidney malfunction [18].

Modern research has unveiled the antibacterial activity of *O. basilicum* [11,19–21] and *O. gratissimum* [22–29] against human and plant pathogenic bacteria. Antifungal activity of *O. basilicum* and *O. gratissimum* extracts was also revealed against fungal pathogens in plants (*Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Fusarium oxysporum* ; [30,31]) ; and humans (*Scopulariopsis breicaulis* and *Cryptococcus neoformans* [32] ; *Candida* species [33–35]).

However, very little is known about the antifungal activity of botanicals from *O. basilicum* and *O. gratissimum* against the *Trichophyton* species, which are responsible for fungal infections of the scalp, groin (jock itch), body, feet (athlete's foot), fingernails, and toenails.

Thus, the present study aims to investigate the antifungal activity of essential oils and nanoemulsions from *O. basilicum* and *O. gratissimum* against *Trichophyton rubrum* and *Trichophyton interdigitale*. Moreover, the antioxidant activity of essential oils and their nanoemulsions is also evaluated.

2. Results and Discussion

2.1. Results

2.1.1. Yields of Extraction

The essential oils were obtained as bright and dark yellow substances for *Ocimum gratissimum* and *Ocimum basilicum*, respectively. The yields of extraction were found to be 1.12% and 0.62% for *O. gratissimum* and *O. basilicum*, respectively (Table 1).

Table 1. : Yield of extraction and physical characteristics of the essential oils from *Ocimum gratissimum* and *Ocimum basilicum.*

Plant species	Organs	Yield of extraction	Color	Density
Ocimum gratissimum	**Leaves and twigs**	1.12±0.02	Bright yellow	0.89
Ocimum basilicum	**Leaves and twigs**	0.62±0.01	Dark yellow	0.88

To determine the phytochemical composition of the as-prepared essential oils from *Ocimum gratissimum* and *Ocimum basilicum*, the oil samples were subjected to gas chromatography and gas chromatography tandem mass spectrometry.

2.1.2. Chemical Composition of the Essential Oils

The chromatograms of the essential oils from *Ocimum gratissimum* and *Ocimum basilicum* are illustrated in Figures 1 and 2. The Kovats indices were calculated following the analysis of the chromatograms to afford a tentative phytochemical analysis of the oils.

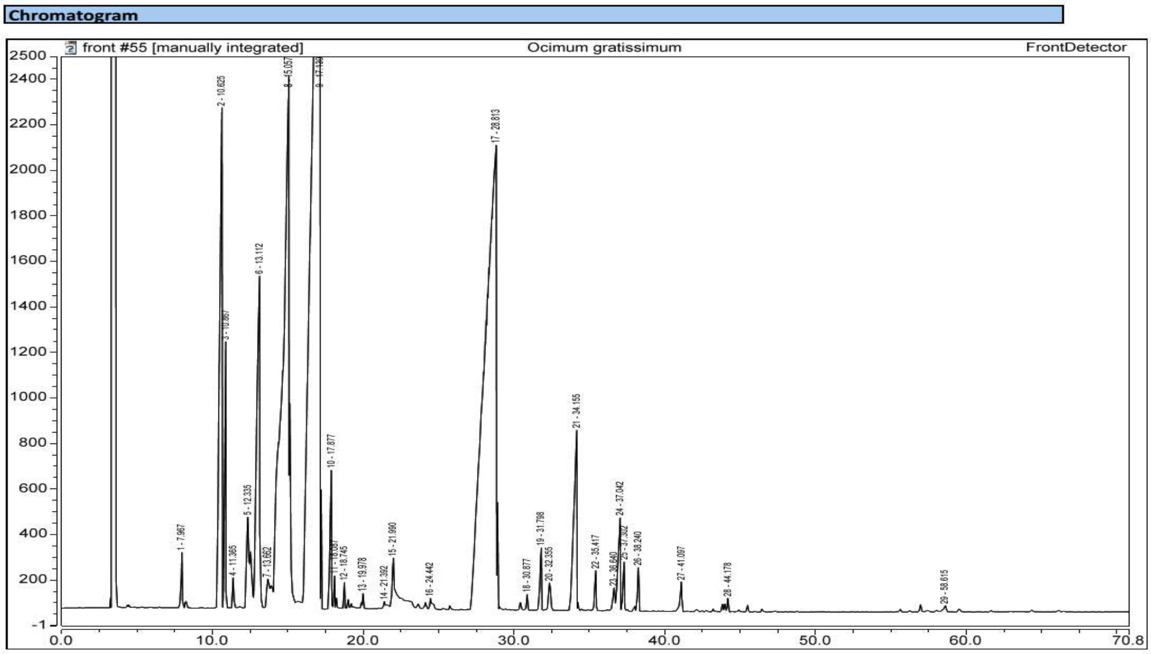

Figure 1. Chromatogram of the essential oil from *Ocimum gratissimum.*

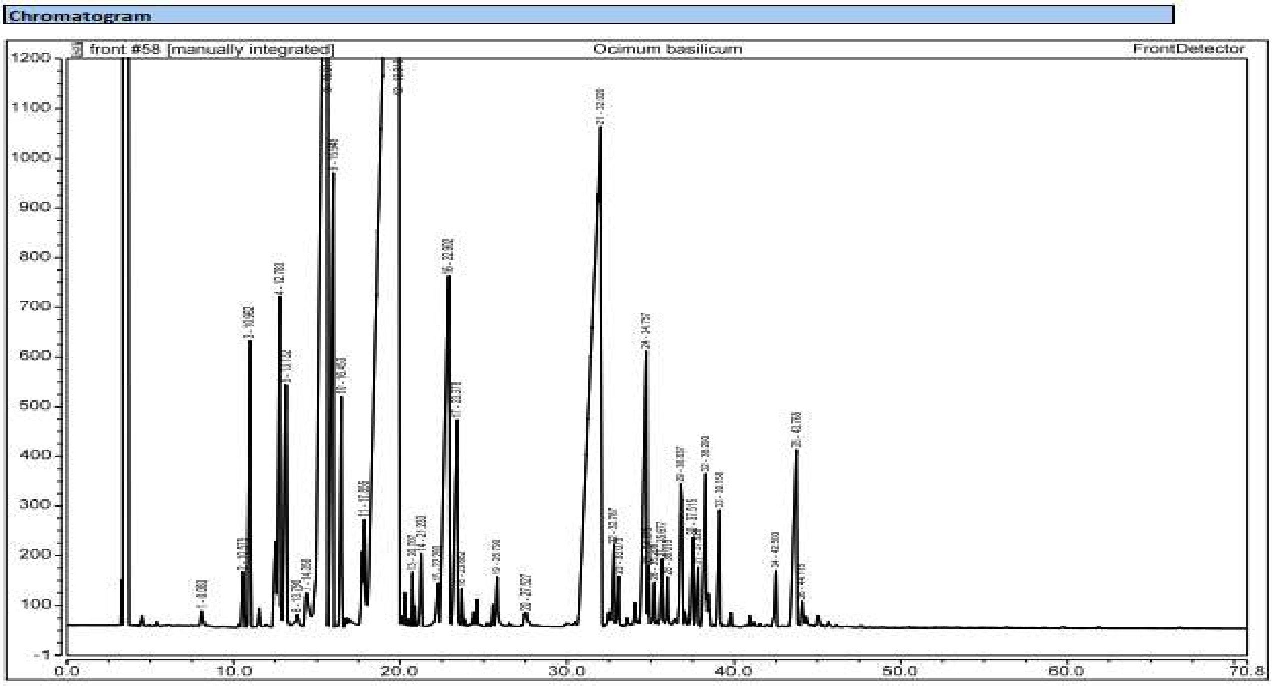

Figure 2. : Chromatogram of the essential oil of *Ocimum basilicum.*

The GC/MS analysis aided in the complete characterization of the essential oils as shown in table 2. According to table 2, the essential oil from *Ocimum gratissimum* was dominated by hydrocarbonated monoterpenes, such as γ-terpinène (33.73%), α-thujène (6.05%), oxygenated monoterpenes (thymol, 26.44% ; and 1,8-cinéole, 16.65%), hydrocarbonated (β-caryophyllene, 2.64%), and oxygenated (β-6 elemene, 0.25%), sesquiterpenes. Moreover, the essential oil from *Ocimum basilicum* was found to be rich in oxygenated monoterpenes, such as eucalyptol (16.78%), linalol (55.32%), eugenol (7.45%), hydrocarbonated ((-)-endo-α-bergamotène, 2.75%), and oxygenated (tau-cadinol, 2.20%) (Table 2).

Table 2. : Chemical composition of essential oils obtained from *Ocimum gratissimum* and *Ocimum basilicum.*

	Kovats' indices	Name of the compound	*Ocimum gratissimum*	*Ocimum basilicum*
	Monoterpenes		**93.87**	**92.51**
	Hydrocarbonated monoterpenes		**48.22**	**7.93**
1	812	α-pinene	1.55	1.20
2	825	β-pinene	-	2.30
3	828	α-myrcene	4.73	1.34
4	848	α-ocimene	-	1.96
5	926	**α-thujene**	**6.05**	-
6	945	Camphene	0.18	-
7	969	Sabinene	1.43	-
8	1002	α-terpinene	0.42	-
9	1076	**γ-terpinene**	**33.73**	1.13
10	1097	Terpinolene	0.13	-
Oxygenated monoterpenes			**45.65**	**84.58**
11	843	**Eucalyptol**	-	**16.78**
12	865	Fenchone	-	1.09
13	1032	**1,8-cineole**	**16.65**	-
14	1092	**Linalol**	0.97	**55.32**
15	1110	Trans-thujone	0.11	-
16	1135	Isocitral exo	0.09	-
17	1164	Borneol	0.08	-
18	1176	Terpinen-4-ol	1.08	3.94

19	1226	Thymol methyléther	0.14	-
20	1314	**Thymol**	**26.44**	-
21	1360	**Eugenol**	0.09	**7.45**
Sesquiterpenes			**5.8**	**7.46**
Hydrocarbonated sesquiterpenes			**5.37**	**5.26**
20	1015	(-)-endo-α-bergamotene	-	2.75
21	1036	Bicyclogermacrene	-	0.67
22	1042	Υ muurolene	-	0.97
23	1380	β-cubebene	0.47	-
24	1393	β-elemene	0.27	-
25	1432	β-caryophyllene	2.64	-
26	1460	α-humulene	0.26	-
27	1487	Germacreme D	0.13	0.87
28	1496	α-curcumene	0.88	-
29	1502	α-selimene	0.36	-
30	1524	d-cadinene	0.36	-
Oxygenated sesquiterpenes			**0.43**	**2.20**
31	1080	Tau-cardinol	-	2.20
32	1591	β-6-elemene	0.25	-
33	1665	Hydroxycaryophyllene	0.18	-
TOTAL			**99.67**	**99.97**

Overall, the essential oil from *O. gratissimum* was dominated by γ-terpinene (33.73%), thymol (26.44%), 1,8-cineole (16.65%) and thujene (6.05%), whereas *O. basilicum* essential oil comprised mostly eugenol (16.78%) and linalool (55.32%).

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2.1.3. Characterization of the Nanoemulsions

a. Physical characteristics

The nanoemulsions were obtained by mixing separately the essential oils from *O. basilicum* and *O. gratissimum* with Tween 80 and distilled water in the respective proportions of 5/5/90 (w/w). Nanoemulsions from essential oils of *O. basilicum* and *O. gratissimum* were obtained as white, homogenous and milky substances.

b. Stability of emulsion

To evaluate the stability of the as-prepared nanoemulsions, a water soluble dye (methylene blue) was used for the centrifugation. After adding a few drops of the methylene blue solution in each nanoemulsion, the mixtures were centrifuged for 30 minutes at 1000, 2000 and 3000 rpm. Since the preparation was homogenous at different speeds of centrifugation, the nanoemulsions were considered to be stable.

c. Dilution test

This experiment was used to assess the type of emulsion, whether it was water/oil or oil/water emulsion for each nanoemulsion. The addition of water to each nanoemulsion did not modify the appearance of the emulsion, to conclude that the as-prepared nanoemulsions were water/oil emulsions.

d. Measure of pH

It is essential to measure the pH of a nanoformulated drug, which is intended to be used for potential application on the skin. In this study, the pH of the nanoemulsions from the essential oils of *Ocimum basilicum* and *Ocimum gratissimum* were obtained as 6.0 and 6.2, respectively. Accumulated evidence has shown that skin with pH values below 5.0 is in a better condition than skin with pH values above 5.0, as shown by measuring the biophysical parameters of barrier function, moisturization and scaling [36,37]. Other reports demonstrated that the mean pH value of the skin ranges between 5.4 and 7.0 [38,39].

2.1.4. In Vitro Antifungal Activity

The antifungal activity of essential oils and nanoformulations from *O. basilicum* and *O. gratissimum* were assayed against *Trichophyton rubrum* and *Trichophyton interdigitale*. Figure 3 and 4 illustrates, respectively, the inhibition zones of *Trichophyton rubrum and Trichophyton interdigitale* by *O. gratissimum*'s essential oil at various concentrations *viz.* 250, 350, 500, 750 and 1000 ppm. As the concentrations of the essential oils increase, there is a disappearance of the fungal growth. The highest antifungal activity was observed at 1000 ppm.

Negative control **250 ppm** **350 ppm**

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500 ppm **750 ppm** **1000 ppm**

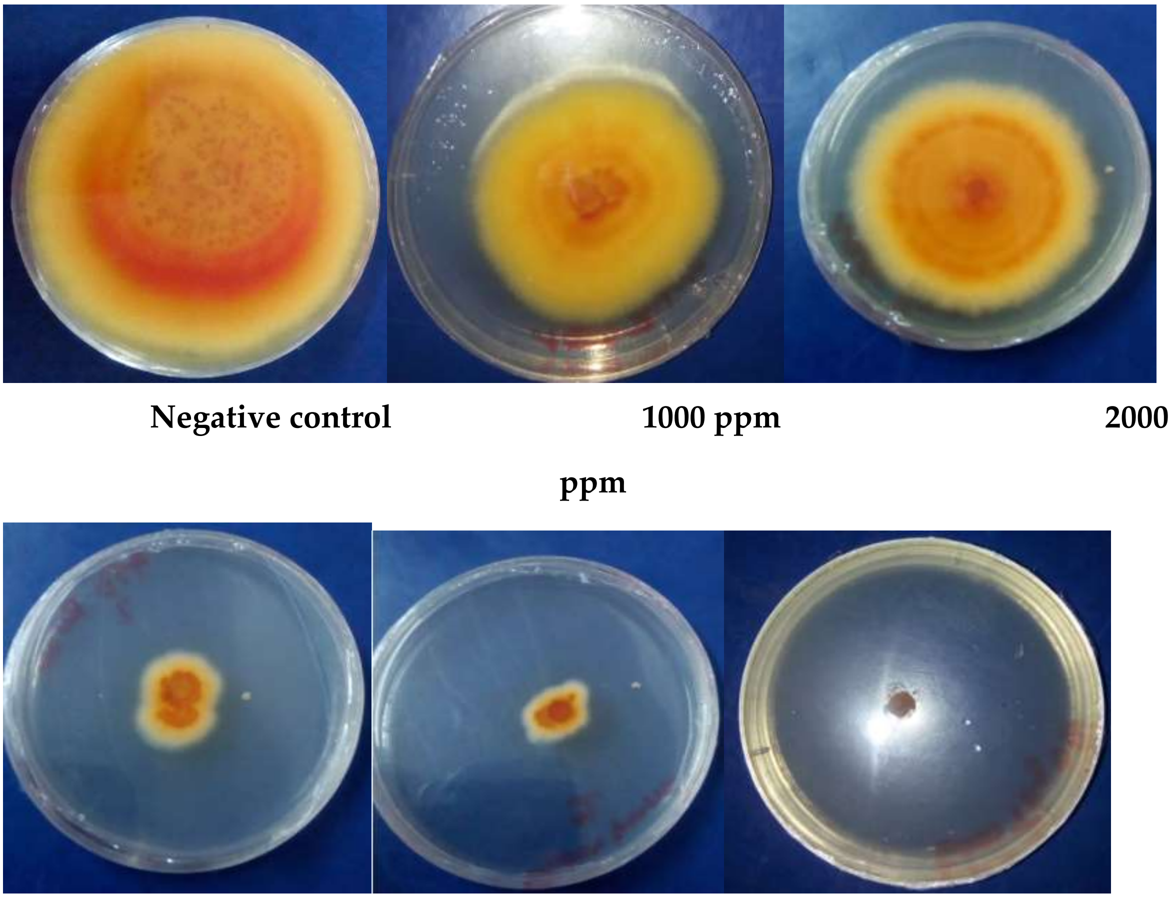
Figure 3 : Inhibition of *Trichophyton rubrum* upon treatment with various concentrations of *Ocimum gratissimum*

Negative control **250 ppm** **350 ppm**

500 ppm **750 ppm** **1000 ppm**

Figure 4. Inhibition of *Trichophyton interdigitale* upon treatment with different concentrations of *Ocimum gratissimum*.

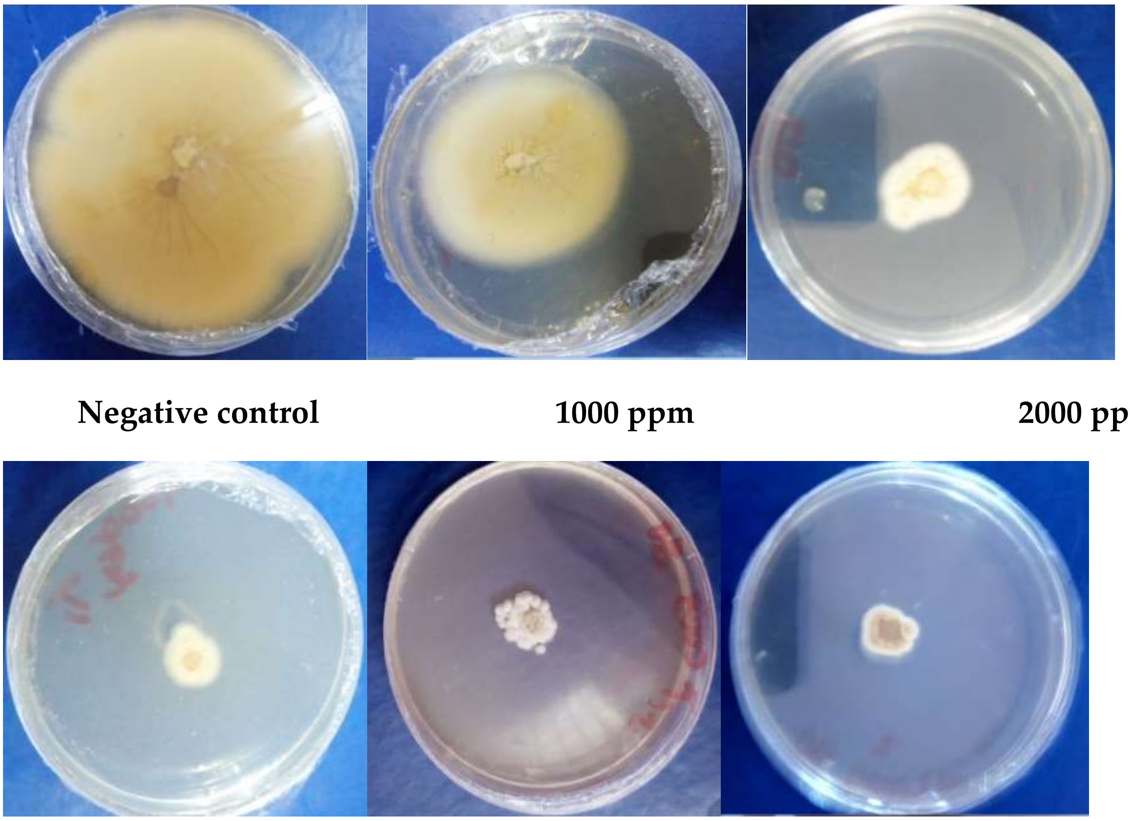
Figure 5 and 6 illustrates, respectively, the inhibition zones of *Trichophyton rubrum and Trichophyton interdigitale* by *O. basilicum*'s essential oil at various concentrations *viz.* 250, 350, 500, 750 and 1000 ppm. As the concentrations of the essential oils increase, there is a disappearance of the fungal growth. The highest antifungal activity was observed at 1000 ppm.



Negative control 1000 ppm 2000 ppm

3000 ppm 4000 ppm 5000 ppm

Figure 5. Inhibition of *Trichophyton rubrum* by treatment with various concentrations of *Ocimum basilicum*.

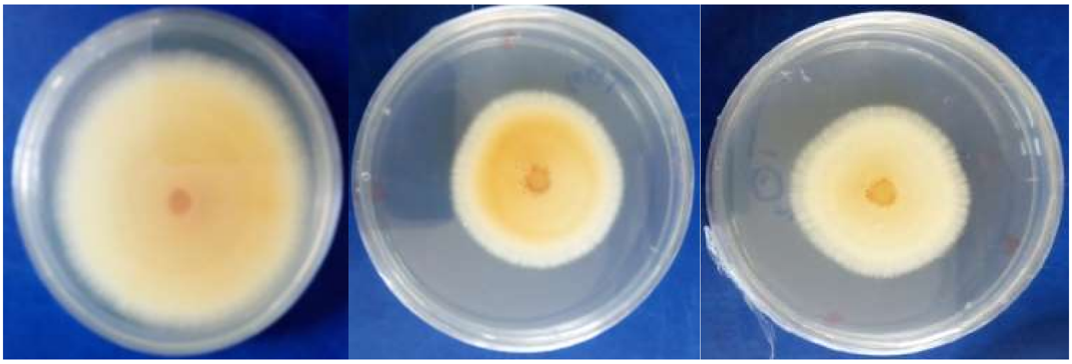


Negative control 1000 ppm 2000 ppm

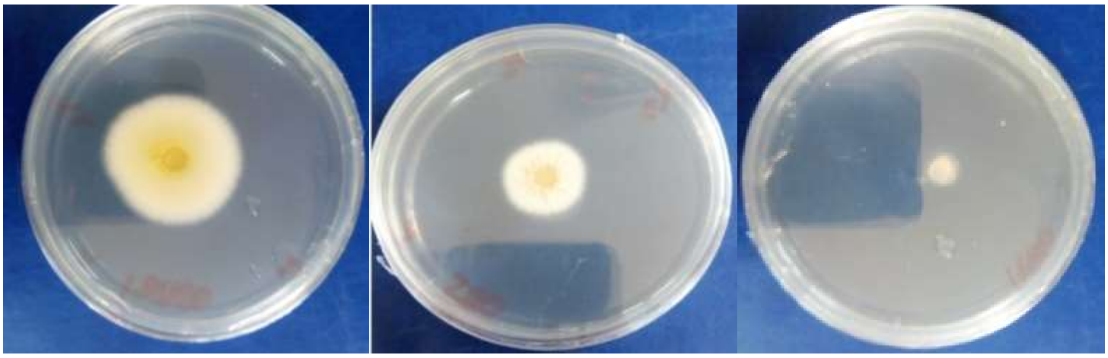
3000 ppm 4000 ppm 5000 ppm

Figure 6. Inhibitory effects of various concentrations of *Ocimum basilicum* on *Trichophyton interdigitale*.

Figure 7 and 8 shows the inhibition of *Trichophyton rubrum and Trichophyton interdigitale* by the nanoemulsion prepared from *O. gratissimum*'s essential oil at various concentrations *viz.* 1000, 1250, 1500, 1750 and 2000 ppm. The reduction of the fungal growth is proportional to the increase in the concentration of the nanoemulsion. The highest antifungal activity was observed at 2000 ppm.



Negative control **1000 ppm** **1250 ppm**



1500 ppm **1750 ppm** **2000 ppm**

Figure 7. Inhibitory effects of various concentrations of nanoemulsion from *Ocimum gratissimum* vis-à-vis *Trichophyton rubrum*.

Negative control **1000 ppm** **1250 ppm**

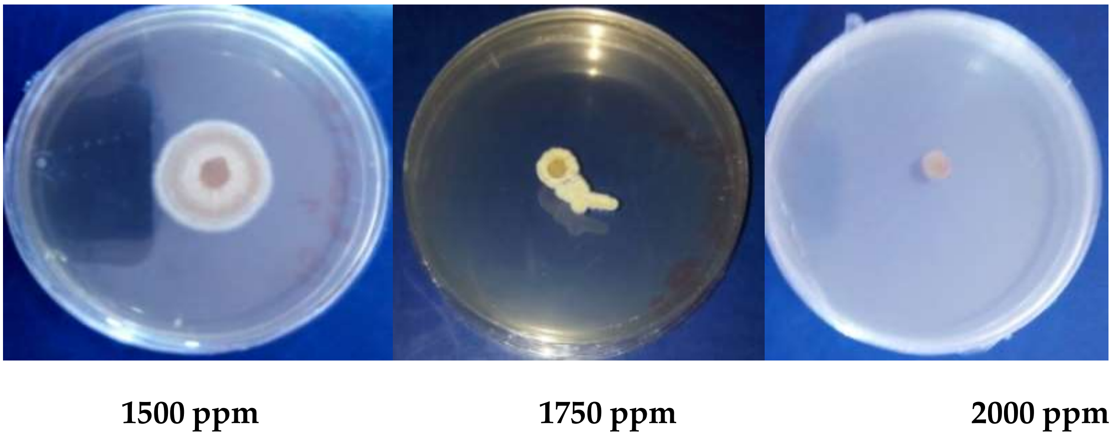


Figure 8. Inhibitory activity of various concentrations of nanoemulsion from *Ocimum gratissimum* vis-à-vis *Trichophyton interdigitale*.

Figure 9 and 10 depicts the inhibitory effects of nanoemulsion from *O. basilicum*'s essential oil at various concentrations *viz.* 5000, 6000, 6250, 6500 and 6750 ppm against *Trichophyton rubrum and Trichophyton interdigitale*, respectively. The reduction of the dermatophyte's growth is proportional to the increase in the concentration of the nanoemulsion. The highest antifungal activity was observed at 2000 ppm.

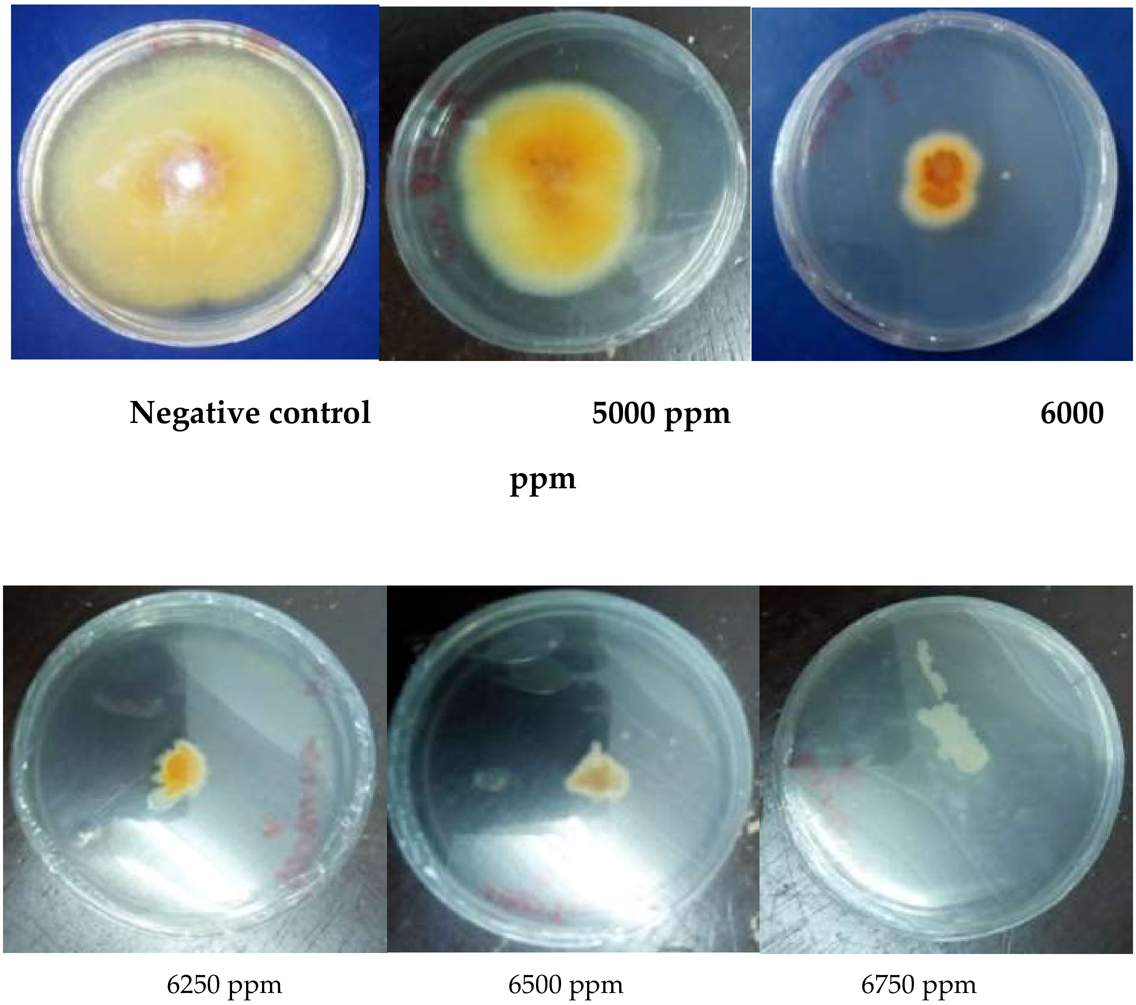
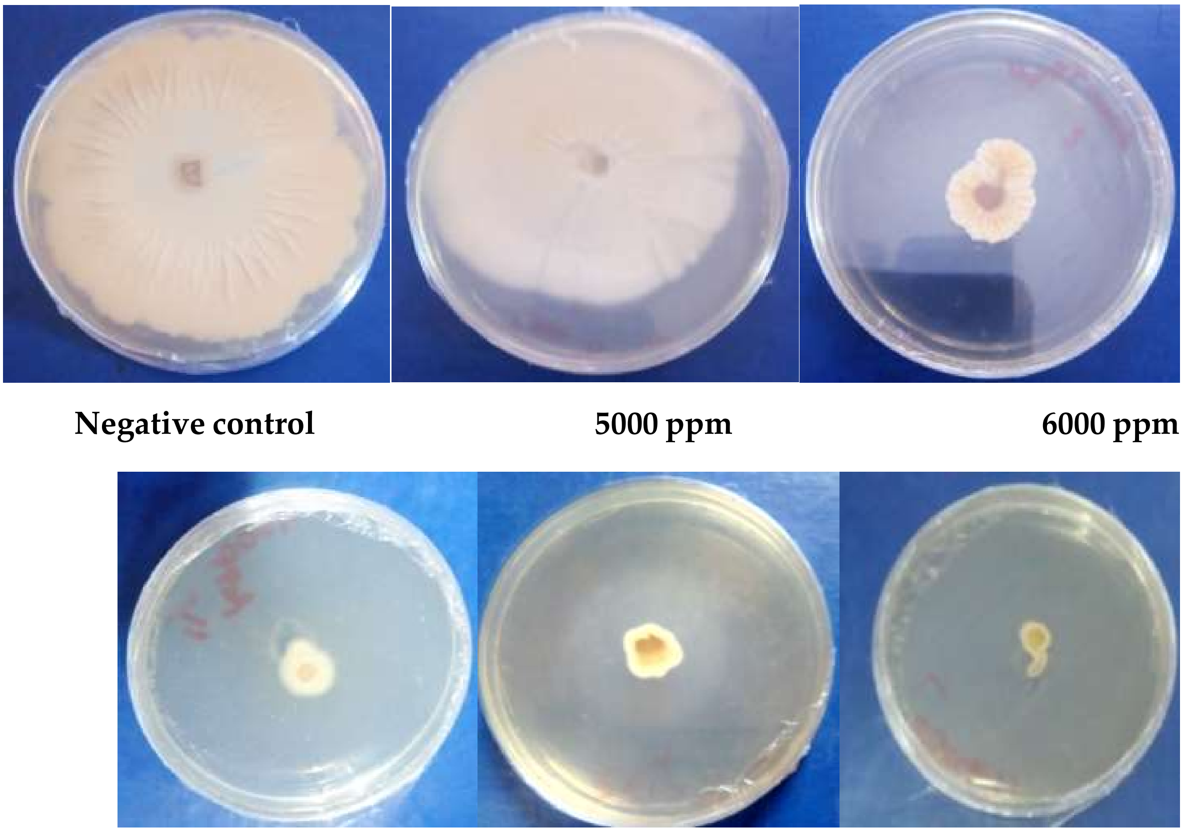


Figure 9. Inhibitory effects of various concentrations of nanoemulsion from *Ocimum basilicum* vis-à-vis *Trichophyton rubrum*.

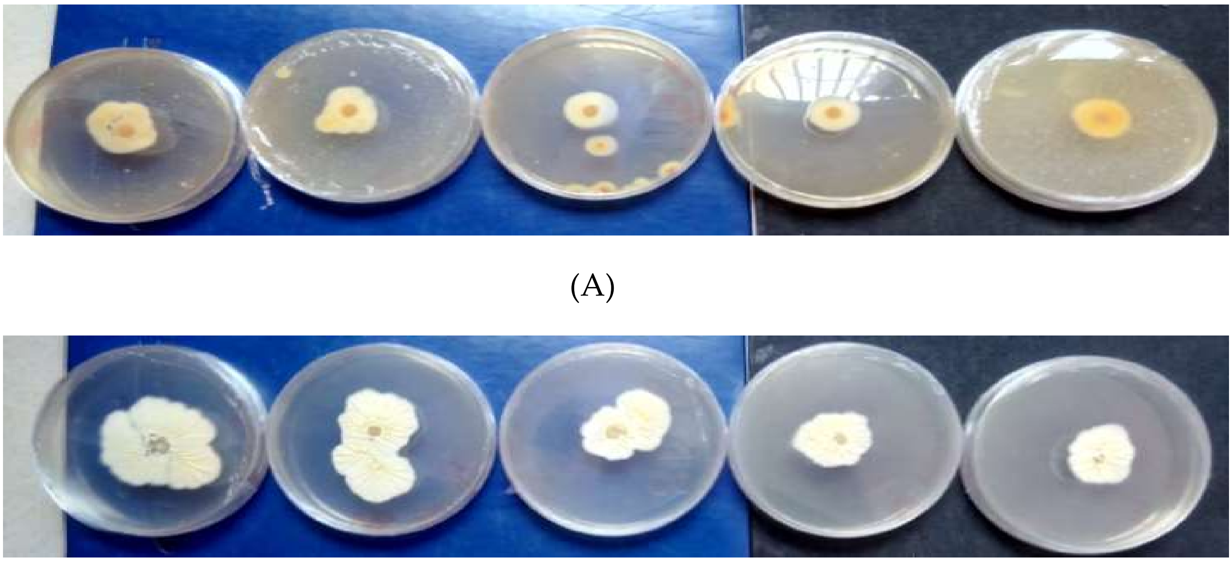


Negative control **5000 ppm** **6000 ppm**

6250 ppm **6500 ppm** **6750 ppm**

Figure 10. Inhibitory activity of various concentrations of nanoemulsion from *Ocimum basilicum* vis-à-vis *Trichophyton interdigitale*.

In antifungal assays, griseofulvin was used as a positive control. Figure 11 illustrates the inhibitory effects of griseofulvin on the growth of *Trichophyton rubrum* and *Trichophyton interdigitale*. As shown in Figure 11, as high as 5000 ppm of griseofulvin did not completely eradicate the growth of *Trichophyton rubrum* and *Trichophyton interdigitale.*



(A)

(B)

1000 ppm 2000 ppm 3000 ppm 4000 ppm 5000 ppm

Figure 11. Inhibitory effects of various concentrations of griseofulvin (positive control) against *Trichophyton rubrum* (A) and *Trichophyton interdigitale* (B).

These results demonstrate that antifungal activity of the essential oils and nanoemulsion from *Ocimum gratissimum* is greater than that obtained with the reference antifungal compound griseofulvin. Indeed, 21 days' incubation of essential oils or nanoemulsion from *Ocimum gratissimum* at lower concentrations (1000-5000 ppm) showed more pronounced inhibition of the mycelial growth compared with the activity of griseofulvin. However, the essential oils and nanoemulsion from *Ocimum basilicum* showed moderate antifungal activity at higher concentrations (5000-6750 ppm).

-Determination of the minimum inhibitory (MICs) and minimum fungicidal (MFCs) concentrations

The concentration where no radial growth of germs was visible after 21 days of incubation of fungi with essential oils and nanoformulations, corresponded to the minimum inhibitory concentrations (MICs) (Table 3). Against *Trichophyton rubrum*, the essential oil and nanoemulsion of *Ocimum gratissimum* showed minimum inhibitory (MICs) and minimum fungicidal (MFCs) concentrations of 1000 and 2000 ppm, respectively. Against *Trichophyton interdigitale, Ocimum gratissimum* afforded common minimum inhibitory and minimum fungicidal concentration (2000 ppm). The incubation of various concentrations of the essential oil and nanoemulsion of *Ocimum basilicum* with *Trichophyton rubrum* afforded a common MIC and MFC value of 5750 ppm. Against *Trichophyton interdigitale, Ocimum basilicum'* essential oil and nanoemulsion yielded a common MIC and MFC value of 6750 ppm. Irrespective of the fungal strain tested, the standard antifungal agent griseofulvin yielded MIC value of more than 5000 ppm (Table 3).

Table 3. Minimum inhibitory (MICs) and minimum fungicidal (MFCs) concentrations.

Sample (OG)	Essential oil		Nanoemulsion		Griseofulvin	
	TR	**TI**	**TR**	**TI**	**TR**	**TI**
MIC (ppm)	**1000**	**2000**	**2000**	**2000**	>5000	
MFC (ppm)	**1000**	**2000**	**2000**	**2000**		
Sample (OB)	**Essential oil**		**Nanoemulsion**		**Griseofulvin**	
	TR	**TI**	**TR**	**TI**	**TR**	**TI**
MIC (ppm)	**5750**	/	**6750**	/	>5000	
MFC (ppm)	**5750**	/	**6750**	/		

TR : Trichophyton rubrum and TI : Trichophyton interdigitale ; OB : Ocimum basilicum ; OG : Ocimum gratissimum.

3.1.5. Antioxidant Activity

In this study, the antioxidant activity of the essential oils and nanoemulsion from *Ocimum gratissimum* and *Ocimum basilicum* was evaluated using DPPH assay. As a result, the essential oils of *Ocimum basilicum* and *Ocimum gratissimum* scavenged the free radicals of DPPH with median scavenging concentrations (SC_{50}S) of 100 and 500 ppm, respectively, vs butylated hydroxytoluene (SC_{50} : 200 ppm) (Figure 12).

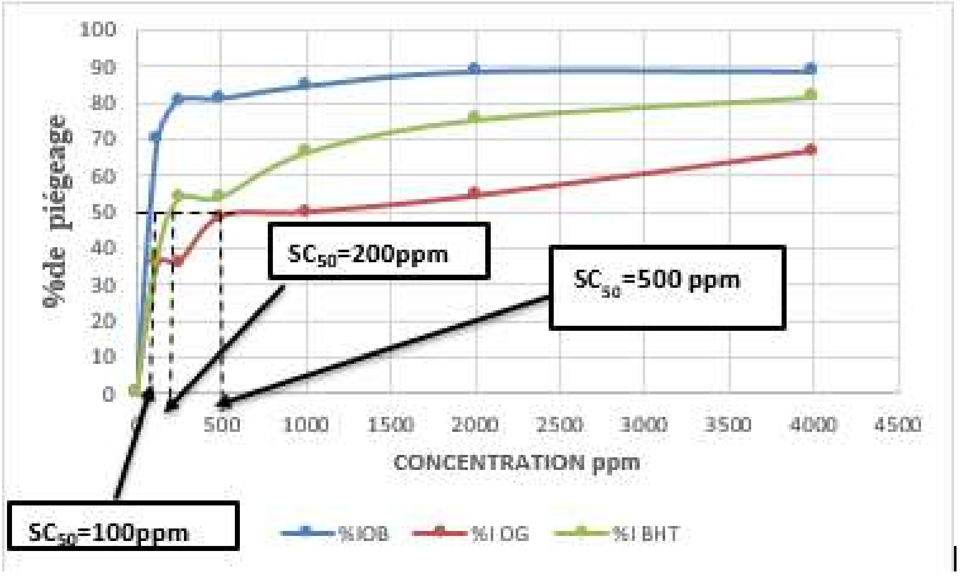


Figure 12. : Percentages of DPPH radical scavenging as a function of concentrations of essential oils of *O. gratissimum* and *O. basilicum* and the butylated hydroxytoluene (BHT).

The nanoemulsions, which were prepared from *O. gratissimum* and *O. basilicum* essential oils, scavenged the free radicals of DPPH with a common median radical scavenging concentration of 100 and 4800 ppm, respectively, vs butylated hydroxytoluene (SC_{50} : 200 ppm) (Figure 13).

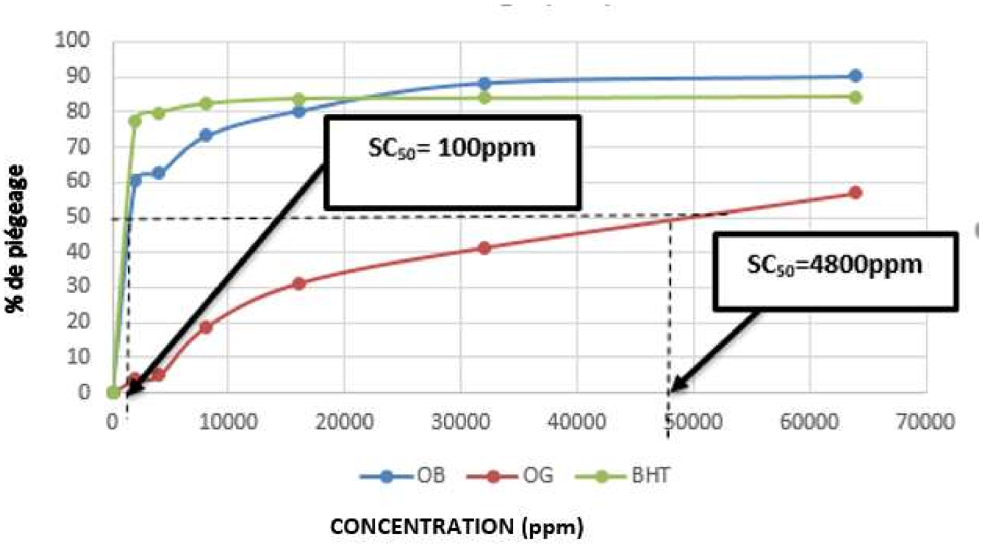


Figure 13. : Percentages of DPPH radical scavenging as a function of concentrations of nanoformulations obtained from the essential oils of *O. gratissimum* and *O. basilicum* and the butylated hydroxytoluene (BHT).

The median efficient concentrations ($CE_{50,}$ g/mol) and the antiradical potential (AP, mol/g) of essential oils (5000 g/mol and 0.0002 mol/g) and nanoformulations (4800 g/mol and 0.00002 mol/g) were also calculated for *O. gratissimum*. Moreover, essential oils (1000 g/mol and 0.001 mol/g) and nanoformulations (1000 g/mol and 0.001 mol/g) obtained from *O. basilicum* showed more pronounced antioxidant activity, as evidenced by their median efficient concentrations and antiradical potential.

2.2. Discussion

Fungal pathogens have become a major threat to human health, infecting billions of people with approximately 1.5 million deaths annually [40,43].

There are several classes of azole antifungal drugs, including (i) imidazoles, such as clotrimazole, econazole, and miconazole, which are commonly used to treat skin and nail infections, (ii) triazoles, such as fluconazole, itraconazole, and voriconazole, which are used to treat systemic fungal infections and (iii) allylamines, such as terbinafine, which is used to treat dermatophyte infections [43,44]. However, most of these drugs have become resistant to the majority of fungal pathogens, thus reducing their effectiveness against re-emerging fungal strains. In addition to drug resistance, a number of undesirable side effects have been documented on these antifungals. Medicinal plants, which have been traditionally used for the treatment of superficial skin infections, can afford a number of botanicals with antifungal properties [45,46]. Such plants include *Ocimum gratissimum* and *Ocimum basilicum*, which are well known for their antimicrobial properties [47]. *Ocimum gratissimum* and *Ocimum basilicum* are two medicinal plants that have been extensively used to treat skin infections [48]. Thus, the scientific validation of *Ocimum gratissimum* and *Ocimum basilicum* in the treatment of certain dermatophytes, is valuable. The present study evaluated the antidermatophytic effects of essential oils from *Ocimum gratissimum* and *Ocimum basilicum* and their nanoformulations against *Trichophyton rubrum* and *Trichophyton interdigitale.* Essential oils from *Ocimum basilicum* (yield of extraction : 1.12%) and *Ocimum gratissimum* (yield of extraction : 0.62 %) were obtained as light yellowish and dark yellow colour, respectively. Accumulated evidence has shown that essential oils from *Ocimum basilicum* and *Ocimum gratissimum* collected in Benin and Nigeria, exhibited yields of extractions ranging from 0.63%-1.25%, and from 1.07%-1.25% [10,49,50]. The slight difference in the values of yields of extraction might be attributed to the difference in the plant harvesting period ; plants' collection in dry season might afford higher yields of essential's oil extraction than that from plants collected during wet season [51]. The GC-MS analysis of the essential oils from *O. basilicum* and *O. gratissimum* revealed that these oils were mainly dominated by volatile compounds, such as monoterpenes and sesquiterpenes. In fact, the essential oil from *O. gratissimum* was dominated by γ-terpinene (33.73%), thymol (26.44%), 1,8-cineole (16.65%) and thujene (6.05%), whereas *O. basilicum* essential oil comprised mostly eugenol (16.78%) and linalool (55.32%). Although Fokou et al. [34] (linalool (55.32%), eucalyptol (16.78%) and eugenol (7.45%)) reported almost similar composition of the essential oil from *O. basilicum*, another study by Kpodekon et al. [10] (estragole (31.3%), linalool (24.0%) and eugenol (21.7%)) revealed different composition for the essential oil of *O. basilicum* collected in a different country. This observation suggests that the phytochemical composition of a plant's essential oil varies based on the place of plant's collection. *O. gratissimum's* essential oil, which was isolated by Tchoumbougnang et al. [52] from a plant collected in Douala (Cameroon), was dominated by monoterpenes, such as β-cymene (32.1%) and thymol (24.3%). The essentail oils prepared from two varieties of *O. gratissimum*, which were collected from Gabon by Agnaniet et al. [53] revealed 33.3% p-cymene and 31.5% thymol as thee main constituants for the first variety and 75.4% eugenol as the major compound of the second plant species. On the other hand, Kpodekon et al. [10] identified thymol (43.41%), as the main compound of *O. gratissimum* along with p-cymene (12.3%) and γ-terpinene (12.1%). The chemical variability of essential oils from each plant could be linked to the ecology and composition of the soil on which the plant grew up [54]. Since nanoemulsion technology has emerged as the most promising delivery channel for lipophilic components such as nutraceuticals, flavors, antioxidants and antimicrobial agents, nanoemulsions from essential oils of *O. basilicum* and *O. gratissimum* were prepared and evaluated for antifungal activity against selected fungi. The essential oil from *O. gratissimum* and its nanoformulation demonstrated antifungal activity against *Trichophyton rubrum* and *Trichophyton interdigitale* with minimum inhibitory (MICs) and minimum fungicidal (MFCs) concentrations varying from 1000 to 2000 ppm. Moreover, only *Trichophyton rubrum* was inhibited by the essential oil of *O. basilicum* and its nanoformulation, thus yielding common MIC and MFC values of 5750 and 6750 ppm, respectively. According to the MIC/MFC ratio values, which are found to be less than 2, it can be speculated that *O. gratissimum* and *O. basilicum* essential oils and their nanoformulation have a fungicidal orientation. Noteworthy, *O. basilicum* was found to be less active than *O. gratissimum* ; however, both the *Trichophyton* strains were found to be resistant to griseofulvin (MICs and MFCs >5000 ppm), the reference antifungal drug used. Growing evidence has shown the antifungal activity of 1,8-cineole [55,56], a compound that was identified among the major constituents of the essential oil of *O. gratissimum.* Major constituents of *O. basilicum,* such as eugenol (MIC value : 256 µg/ml [57]) and linalool (MIC value : ≤ 512µg/ml [58]) were reported to exhibit antifungal activity against *Trichophyton rubrum*. Thus, it is not unreasonable to speculate that 1,8-cineole might be the phytochemical responsible for the antifungal activity of *O. gratissimum*, whereas eugenol and linalool could have compounded the antifungal effects of *O. basilicum*. A recent paper published by Aliabasi et al. [59] revealed that eugenol anti-*Trichophyton* effects via inhibition of the ergosterol synthesis, or by affecting the keratinase activity, and SUB3 gene expression [59]. It has also been reported that linalool exert fungicidal effects by partially interacting with 1,3-β-glucan synthase [60]. On the other hand, 1,8-cineole induce ROS-dependent lethality and ROS-independent virulence inhibition in certain fungi [61]. Because of their nanosize, nanoemulsions are thought to interact with the cell wall biosynthesis in many fungi and induce several morphological disruption effects [62]. Donsi et al. [63] also demonstrated that nanosize particles below 200 nm activates passive transport mechanisms to easily cross the cell membrane of microorganisms.

Furthermore the antioxidant effects of essential oils of *O. basilicum* and *O. gratissimum* and their nanoformulations were also demonstrated through the DPPH assay. The major constituent of *O. basilicum* that is 1,8-cineole is well known for its antioxidant properties, which is mediated, in part, by activating the Nrf2/Keap1 system, a critical regulator of cellular protective responses to oxidative stress and inflammation [55]. The antioxidant activity of eugenol and linalool (major botanicals of *O. gratissimum*'s essential oil) is generally attributed to the inhibition of lipid peroxidation [64].

Overall, this study demonstrates the antifungal activity of the essential oils of *O. basilicum* and *O. gratissimum*, as well as their nanoformulations against *Trichophyton rubrum* and *Trichophyton interdigitale*, thus validating the traditional use of these plants in the treatment of certain dermatophytes. The antioxidant activity of *O. basilicum* and *O. gratissimum* is also unveiled. Nevertheless, additional studies, including toxicity and *in vivo* antidermatophytic tests, as well as antifungal mechanisms of action are desired to support the safe use of *O. basilicum* and *O. gratissimum* in ethnomedicine.

3. Conclusions

The present study sought to investigate anti-*Trichophyton* and antioxidant activities of essential oils from *O. basilicum* and *O. gratissimum*, and their nanoformulations. As a result, essential oils, which were obtained respectively as yellowish and dark yellow oily substances from *O. basilicum* and *O. gratissimum* by hydrodistillation using a clevenger apparatus, were further subjected to the fabrication of nanoemulsions. These nanoemulsions were obtained as whitist and milky substances. The GC-MS analysis of *O. gratissimum*'s essential oil yielded γ-terpinene (33.73%), thymol (26.44%), 1,8-cineole (16.65%) and thujene (6.05%) as its major constituents, whereas *O. basilicum* essential oil was dominated by eugenol (16.78%) and linalool (55.32%). The characterization of stability of the as-prepared nanoemulsions by centrifugation, dilution test and pH measurements revealed that the nanopreparations were stable under the experimental conditions. The incubation of *Trichophyton rubrum* and *Trichophyton interdigitale* with the essential oils of *O. basilicum* and *O. gratissimum* and their nanoformulations showed growth inhibition of these pathogenic fungi with MIC and MFC ranging from 1000 to 5750 ppm and 2000 to 6750 ppm, respectively. The antifungal activity was characterized as fungicidal since the MFC/MIC ratio was found to be less than 2. The antioxidant activity of essential oils and their nanoformulations was also unveiled with median scavenging concentrations varying respectively from 100 to 500 ppm and from 100 to 4800 ppm. The antiradical potential of *O. basilicum* and *O. gratissimum* were found to be greater than that of BHT, a standard antioxidant compound.

Overall, the present study demonstrates the antidermatophytic effects of essential oils of *O. basilicum* and *O. gratissimum* and their nanoformulations, thus substantiating the traditional use of these plants in the treatment of dermatophytic fungi. However, a detailed characterization of the fabricated nanoemulsions from essential oils of *O. basilicum* and *O. gratissimum* is warranted. Additional studies, including toxicity, *in vivo* antidermatophytic effects, and pharmacokinetics of *O. basilicum* and *O. gratissimum* botanicals are expected for the successful utilization of these plants in traditional healing practices.

3. Materials and Methods

3.1. Material

3.1.1. Plant Material

The leaves of *Ocimum gratissimum* and *Ocimum basilicum* were collected from Douala (Littoral Region of Cameroon). The identification of the plant was done at the National Herbarium of Cameroon (NHC) in Yaounde, where a voucher specimen was deposited under number 5817/SRF/Cam.

3.1.2. Fungal Strains

The fungal strains used in this study included *Trichophyton rubrum* and *Trichophyton interdigitale*, which were supplied by the Douala Obstetrics and Paediatrics Hospital, Douala, Cameroon. These fungal strains and isolates were maintained on Sabouraud Dextrose Agar.

3.1.3. Material for Fungal Cell Culture

In this study, Mueller Hinton Agar was used for the development of the bacterial strains, whereas Mueller Hinton Broth was employed for the determination of the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). These media were obtained from Liofilchem® S.r.L (Scozia, Zona Industriale 64026, Roseto degli Abruzzi, Italy). Other reagents included McFarland standard 0.5, sterile distilled water, physiological water (normal saline), tween 80 and anhydrous sodium sulfate (Sigma-Aldrich, Darmstadt, Germany).

3.2. Methods

3.2.1. Extraction of the Essential Oils

The essential oils were extracted from fresh leaves of *O. gratissimum* and *O. basilicum* separately using a Clevenger-type apparatus. Briefly, the collected plant materials were washed and then chopped. Next, the plant material was introduced to a round bottom flask, with 1 kg for every 500 mL of water. Each mixture was then brought to a boil for a period of 4 to 5 h. During this process, the vapor underwent condensation and was divided into 2 phases, with the superior phase consisting of the EO of each plant, which was collected. The water contained in the essential oils was then dried using anhydrous sodium sulfate. The oils were further weighed and bottled in a tinted glass 60 mL bottle and refrigerated at 4 °C. Then, the yields of the EOs were expressed as percentages that were calculated using the following formula :

$Y = (Me/Mp) \times 100$
Where
Y = yield of essential oil in percentage
Me = mass of essential oil in grams
Mp = mass of plant biomass in grams

3.2.2. GC-MS Analysis of Essential Oil of Ocimum Gratissimum and Ocimum basilicum

The essential oils were analyzed by gas chromatography (GC) on a Varian CP-3380 GC with a flame ionization detector fitted with a fused silica capillary column (30 m × 0.25 mm coated with DB5, film thickness 0.25 μm), with a temperature program of 50–200 °C at 5 °C/min, with common injection and and detection temperature of 200 °C, and N2 as the carrier gas (flow rate : 1 mL/min).

Afterwards, gas chromatography coupled with mass spectrometry (GC-MS) was conducted using a Hewlett-Packard apparatus (model 5970) equipped with an HP1 fused silica column (30 m × 0.25 mm, film thickness 0.25 μm), interfaced with a quadrupole detector (GC-quadrupole MS system). For GC-MS, the column temperature was programmed from 70° to 200 °C at 10 °C/min, whereas the injector temperature was set at 220 °C. Helium was used as the carrier gas at a flow rate of 0.6 mL/min, and the mass spectrometer was operated at 70 eV [65]. For each run, 0.1 μL of essential oil diluted in 10% hexane was injected. The linear retention indices of the compounds were relatively determined by the retention times of a series of n-alkanes, and the percentage compositions were obtained from electronic integration measurements, without taking into account the relative response factors [65,66]. After analysis by GC/GC-MS, the identification of different constituents of the oils was confirmed by comparison of retention times and mass spectra with known values reported in the literature [65,67]. For each compound identified, the retention index (Kovats retention index) was determined according to the following formula :

$$\mathbf{IK} = \mathbf{100}\left(\mathbf{n} + \frac{\mathbf{Tr(X) - Tr(Cn)}}{\mathbf{Tr(Cn+1) - Tr(Cn)}}\right)$$

IK = Kovats retention index
Tr (Cn) = retention time of alkane at n atoms of carbons
Tr (Cn + 1) = retention time of alkane at (n + 1) atoms of carbons
Tr (x) = retention time for compound x

3.2.3. Preparation of the Nanoemulsions

The nanoemulsions were prepared according to a previously described protocol [68]. In this experiment, 5% tween 80 was used as a surfactant, corresponding to an hydrophile-lipophile balance (HLB) of 15. In fact, the nanoemulsion was prepared by mixing Tween 80 (5%), essential oil (5%) and distilled water (90%). Briefly, 0.5g of Tween 80 were mixed with 0.5g of each essential oil under mechanical agitation at 400 rotations per minute (rpm) for 30 min. Next, 9 mL of distilled water was added to the mixture at 2 mL/min while stirring at 400 rpm. After water's addition, the preparation was kept under agitation for an additional two hours. Afterwards, the as-prepared nanoformulations were further characterized.

3.2.4. Characterization of the Nanoemulsions

a-Macroscopic examination

The characteristics of the as-prepared nanoemulsions were done by macroscopic examination using naked eyes.

b-Stability assessment by centrifugation

The stability was assessed by centrifugation of the nanoemulsion upon addition of a few drops of methylene blue at different velocities *viz.* 1000, 2000 and 3000 rpm for 10 minutes.

b-Direction of the emulsion

It is always necessary to determine the direction of the emulsion after its preparation. To this end, the as-prepared nanoemulsion was washed with water followed by a visual examination. On the other hand, the methylene blue dye was also used to assess the direction of the emulsion. In brief, a few drops of methylene blue were added to the nanoemulsion, followed by a visual examination.

c-Determination of pH

The pH was determined using a pH meter at room temperature.

3.2.5. Antidermatophytic Assay

a. Preparation of microbial inocula

The suspensions of selected fungi were prepared from 48 hours old fungal cultures, which were under incubation at 37°C on Sabouraud Dextrose Agar medium. Thus, two to three colonies of each microorganism were collected under sterile conditions (with a bec bunsen flamme) using a platinum loop and added to 10 mL of normal saline (NaCl 0.9%) and then homogenized to obtain a turbidity equivalent to 0.5 Mc Farland ($1.5x10^8$ CFU/mL) as recommended by the « Comité de l'antibiogramme de la société française de microbiologie » [69]. The microbial suspension was further diluted 20 times using Sabouraud Dextrose Broth (SDB) to adjust the number of fungal colonies to approximately $1.5x10^4$ CFU/ml.

b. Preparation of solutions

b.1. Preparation of essential oils

The as-prepared essential oils were added to dimethylsulfoxide (DMSO) solution (1:9; v/v) to achieve a final concentration of 103000 ppm. This solution was further diluted using the SDB medium to yield test concentrations of 12800 ppm and 3200 ppm, the latter being considered as the concentration in the first well of the microplate [69].

b.2. Preparation of the sterility control

To verify whether the as-prepared essential oils are free of germs, the sterility control was assessed by inoculating a few microliters of stock solutions of essential oils onto the SDA (agar), followed by an incubation at 37°C for 24-48 hrs.

c. Inhibition test

The antidermatophytic activity of the essential oils was evaluated by incorporation in agar medium as previously described by Lahlou et al. [70]. Different concentrations of the SDB diluted essential oils were distributed into a 90 mm petri dish. After solidification of the culture medium, a 3 mm mycelial disc taken from a 4 days pre-culture was placed at the center of the Petri dishes. The preparation was incubated at room temperature and the mycelial growth was followed by measuring the diameter of the mycelial disc daily for 14 days. Each experiment was done in triplicate.

The percentages of inhibition were calculated using the following formula :

$$\%\mathbf{I} = \left(\frac{\mathbf{Dt - De}}{\mathbf{Dt}}\right)\boldsymbol{x}\mathbf{100}$$

Dt = diameter of the fungal growth in the negative control Petri dish ; **De** = diameter of the fungal growth in the test Petri dish. Griseofulvin was used as positive control.

d. Determination of the minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC)

The MIC of the essential oil were determined as the smallest concentration of essential oil which totally inhibited the visible growth of the dermatophyte until the end of the experiment. The MFC was determined by transferring the mycelial discs from the Petri dishes where the growth inhibition was complete to new dishes containing the SDA medium not supplemented with essential oil. The petri dishes were incubated for 14 days. Thus, the essential oil was considered to be fungicidal when there was no dermatophyte growth, or fungistatic when dermatophyte resume growth.

3.3. Statistical Analysis of Data

All tests were performed in triplicate. The results were expressed as a mean plus or minus standard deviation. Analysis of variance and testing of multiple ranges were performed to highlight significant differences between the means. Pearson's correlations were performed to test the significance link between percentages and concentration of essential oils and positive controls. The analyzes were all carried out using StatGraphics Centurium software 17.1.8 version, and the significance level was set at 0.05.

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