



(RESEARCH ARTICLE)



ANTIFUNGAL ACTIVITY OF THREE ESSENTIAL OIL EXTRACTS AGAINST RESISTANT STRAINS OF PATHOGENIC FUNGI

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World Journal of Advanced Research and Reviews, 2026, 32(01), 288–294

Publication history: Received on 26 August 2026; revised on 02 October 2026; accepted on 05 October 2026

Article DOI: <https://doi.org/10.30574/wjarr.2026.32.1.2535>

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ABSTRACT

Background: This study is part of the ongoing effort to combat antifungal resistance. The aim of this work is to determine the antifungal activity of essential oil extracts from three plants (*Cymbopogon citratus* [CC], *Ocimum gratissimum* [OG], and *Cymbopogon nardus* [CN])—compared to two reference compounds (fluconazole and amphotericin B)—against resistant strains (*Candida albicans* NR-29449 and *Aspergillus fumigatus* NR-45108).

Methods: Oil extracts were produced using the Clevenger-type hydrodistillation technique. Hygienic quality was assessed via agar plating. Finally, the antifungal activity of the extracts was evaluated using a microdilution plate assay with RPMI medium containing 2% glucose, followed by analysis using a spectrophotometer coupled with an automated Minimum Inhibitory Concentration (MIC) reader.

Results: The essential oil extracts obtained showed relatively low yields 0.46, 0.38, and 0.32, respectively for *Cymbopogon citratus* (CC), *Ocimum gratissimum* (OG), and *Cymbopogon nardus* (CN). No contaminating microorganisms such as aerobic mesophilic bacteria, *Enterobacteriaceae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, or yeasts and molds were detected, demonstrating the good hygienic quality of our extracts. Antifungal activity was determined based on the Minimum Inhibitory Concentrations (MICs) of the oil extracts. The following MICs were determined: MIC (CC) = 0.17 µL/µL, MIC (OG) = 0.14 µL/µL, MIC (CN) = 0.25 µL/µL, and MIC (FCA) = 0.25 µL/µL for *Candida albicans* NR-29449. Similarly, for *Aspergillus fumigatus* NR-45108: MIC (CC) = 0.17 µL/µL, MIC (OG) = 0.15 µL/µL, MIC (CN) = 0.05 µL/µL, and MIC (AMPH B) = 0.25 µL/µL.

Conclusion: It appears that the Minimum Inhibitory Concentrations of the extracts are all lower than those of the reference compounds. This suggests that these essential oil extracts contain molecules possessing promising antifungal properties.

Keywords: Antifungal activity, Essential oil extract, Resistant strains, Pathogenic fungi

1 INTRODUCTION

Antifungal resistance poses a serious threat to modern healthcare systems. Indeed, managing *Candida* infections present significant challenges, including the ineffectiveness of certain agents, the toxicity of available drugs, resistance to common antifungals, and the high cost of antifungal medication [1]. Studies have also demonstrated the renal and hematological toxicity associated with amphotericin B [2]. Furthermore, the intensive use of fungicides particularly azoles to boost agricultural yields fosters the emergence of resistant fungal strains [3,4] Azole resistance in *Aspergillus fumigatus* has been documented in numerous regions [5] Consequently, the high prevalence of this pathogen has led the [6] to classify the microorganism as a public health threat.

Isolates carrying the TR34/L98H mutation are resistant to several azoles notably itraconazole (a clinically used azole), to which they exhibit high tolerance, and voriconazole (the first-line treatment for aspergillosis) [7]. These isolates, which drive antifungal resistance, also pose a public health threat [8]. Given these challenges, there is a pressing need to identify new, more suitable, accessible, and cost-effective alternative compounds. This has sparked interest in aromatic plants such as *Cymbopogon citratus*, *Ocimum gratissimum*, and *Cymbopogon nardus* and their essential oil extracts. These plants, known for their medicinal properties, are potential sources of bioactive substances that can be identified and utilized to develop new natural antifungal agents capable of combating fungi resistant to conventional antifungals.

2 MATERIAL

The plant material consists of leaves from *Cymbopogon citratus*, *Ocimum gratissimum*, and *Cymbopogon nardus*, from which the oil is to be extracted. These plants were harvested in Korhogo in May 2026. The technical equipment comprises the standard apparatus found in biochemistry and microbiology laboratories. The microbial material was obtained from the biobank of the Institute Pasteur de Côte d'Ivoire in Adiopodoumé and consists of two reference strains: *Aspergillus fumigatus* (NR 45108) and *Candida albicans* (NR 29449).

3 METHODS

3.1 Essential oil extraction

Essential oils from the leaves of *Cymbopogon citratus*, *Ocimum gratissimum*, and *Cymbopogon nardus* were extracted using Clevenger-type apparatus. The leaves were hydrodistilled for 5 hours. The collected oil was dried over a column of anhydrous sodium sulfate (Na_2SO_4), weighed in a tared vial, and stored at approximately 4°C, protected from light. The extraction yield was calculated using the formula:

$$\text{Yield (\%)} = \text{Mass of essential oil} / \text{Mass of plant material} \times 100$$

3.2 Microbiological quality analysis of oil extracts

3.2.1 Enumeration of total aerobic mesophilic flora (TAMF)

TAMF enumeration was performed using the pour-plate method on Plate Count Agar (PCA). After pouring and solidification, the plates were incubated at 30°C for 72 hours.

3.2.2 Detection and enumeration of *Enterobacteriaceae*

Detection of *Enterobacteriaceae* was carried out by plating on Drigalski agar, incubated at 37°C for 24 to 48 hours.

3.2.3 Detection of *Staphylococcus aureus*

Detection of *Staphylococcus aureus* was conducted by plating on Chapman agar (Mannitol Salt Agar), a selective medium used to identify halotolerant staphylococci. The plates were incubated at 37°C for 24 to 48 hours.

3.2.4 Detection of *Pseudomonas aeruginosa*

Detection of *Pseudomonas aeruginosa* was performed by plating on Cetrimide agar, a selective medium that inhibits most other bacteria. The plates were incubated at 37°C for 24 to 48 hours.

3.3 Enumeration of yeasts and molds

Yeast and mold counts were determined by surface plating on Sabouraud agar supplemented with chloramphenicol (0.1 g/L) to inhibit bacterial growth. The plates were incubated at 25°C for 5 to 7 days.

3.4 Antifungal activity of essential oil extracts

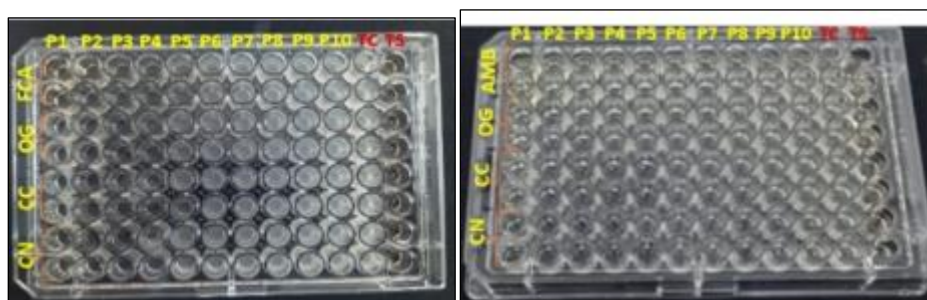
Antifungal activity was evaluated using the broth microdilution method. This method involves determining inhibitory concentrations and comparing them to those of Fluconazole (FCA) and Amphotericin B, which serve as reference compounds. Minimum Inhibitory Concentrations (MICs) were read using an automated system coupled with a spectrophotometer. The extract's MIC was calculated as the means of the MIC values obtained for each extract concentration tested.

3.5 Preparation of the fungal inoculum

A single colony was picked from a fresh 24-hour *Candida albicans* culture and suspended in distilled water containing Tween. The mixture was incubated at 37°C for 48 hours, after which the concentration was adjusted to a 0.5 McFarland standard using a densitometer.

3.6 Preparation of extract solutions

Ten tubes are used. The tubes are numbered 1 through 8, with one tube labeled TC (growth control) and another labeled TS (sterility control). Each numbered tube contains 1 mL of Sabouraud chloramphenicol agar, except for the TS tube. 1000 μ L of the extract is drawn and transferred into tube number 1, resulting in a concentration (C1) of 1000 μ L/mL. Half of this concentration is transferred to the next tube, yielding a concentration (C2) of 500 μ L/mL, and so on, down to a concentration (C8) of 7.81 μ L/mL. If no growth is observed at this final concentration, a subculture is performed on an agar Petri dish. If the subculture reveals no growth, a dilution series is then prepared starting from concentration C8 (7.81 μ L/mL) down to concentration C10 (0.02 μ L/mL).



A- Test with NR-29449 *Candida albicans* B- Test with NR-45108 *Aspergillus fumigatus*

Figure 1: Microdilution culture of the strains under study in RPMI with 2% glucose

The microdilution test is performed using the wells of the plates, along with two resistant reference strains: *Candida albicans* (NR-29449) and *Aspergillus fumigatus* (NR-45108). Two rows of wells are used for each extract. In the first row, 100 μ L of extract is added to 100 μ L of RPMI medium containing 2% glucose in each well. In the second row, 100 μ L of the reference compound is added to the RPMI medium containing 2% glucose. The reference compounds are fluconazole for *Candida albicans* and amphotericin B for *Aspergillus fumigatus*. The plates are then incubated at 37°C for 48 hours and read using a spectrophotometer at 530 nm.

3.7 Statistical analysis

The data collected were entered into and organized using Microsoft Excel 2016 software. Statistical analysis was performed via ANOVA using Fisher's LSD test at a 5% probability level ($p < 0.05$), using STATGRAPHICS Plus 5.0 software for Windows.

4 RESULTS

The various essential oil extractions from the plants studied yielded generally low results, as shown in Table 1:

Table 1: Yield of obtained oil extracts

Yield (%)	Extracts from various plants		
	Cympogon citratus	Ocimum gratissimum	Cymbopogon nardus
	46 %	38 %	32 %

Microbiological quality parameters showed 0 CFU/mL for each extract studied and for all parameters indicated (Table 2).

Table 2: Microbiological quality parameters of the produced oil extracts

Targeted microorganisms (UFC / mL)	Extracts from various plants		
	<i>Cympogon citratus</i>	<i>Ocimum gratissimum</i>	<i>Cymbopogon nardus</i>
Germes aérobies mésophiles	0	0	0
Entérobactérie	0	0	0
<i>Staphylococcus aureus</i>	0	0	0
<i>Pseudomonas aeruginosa</i>	0	0	0
Levures	0	0	0
Moisissures	0	0	0

Compliance criterion: 0 CFU/MI

4.1 Antifungal parameters of essential oil extracts

For the *Candida albicans* strain, the extracts yielded mean inhibitory concentrations ranging from 0.05 ± 0.02 to 0.17 ± 0.09 $\mu\text{L}/\mu\text{L}$, compared to 0.25 ± 0.00 $\mu\text{L}/\mu\text{L}$ for the reference molecule (Table 2).

Table 3: MIC for *Candida albicans*

Strain	NR-29449 <i>Candida albicans</i>					
Tested solutions	CMI test1	CMI test2	CMI test3	CMI test4	CMI Average	S.deviation Average
FCA	25.10^{-2}	25.10^{-2}	25.10^{-2}	25.10^{-2}	25.10^{-2}	10^{-2}
Ocimum gratissimum (OG)	24.10^{-2}	6.10^{-2}	12.10^{-2}	12.10^{-2}	14.10^{-2}	8.10^{-2}
Cymbopogon citratus (CC)	12.10^{-2}	24.10^{-2}	6.10^{-2}	24.10^{-2}	17.10^{-2}	9.10^{-2}
Cymbopogon nardus (CN)	6.10^{-2}	6.10^{-2}	3.10^{-2}	6.10^{-2}	5.10^{-2}	2.10^{-2}

Table 3 presents the various minimum inhibitory concentrations of the extracts, which range from 0.05 ± 0.02 to 0.17 ± 0.09 $\mu\text{L}/\mu\text{L}$, whereas that of the reference molecule is 0.25 ± 0.18 .

Table 3: MIC for *Aspergillus fumigatus*

Strain	NR-45108 <i>Aspergillus fumigatus</i>					
Tested solutions	CMI test1	CMI test2	CMI test3	CMI test4	CMI Average	S.deviation Average
Amphotericine B	12.10^{-2}	12.10^{-2}	50.10^{-2}	25.10^{-2}	25.10^{-2}	18.10^{-2}
<i>Ocimum gratissimum</i> (OG)	24.10^{-2}	12.10^{-2}	12.10^{-2}	12.10^{-2}	15.10^{-2}	6.10^{-2}
<i>Cymbopogon citratus</i> (CC)	24.10^{-2}	12.10^{-2}	24.10^{-2}	6.10^{-2}	17.10^{-2}	$0,09.10^{-2}$
<i>Cymbopogon nardus</i> (CN)	3.10^{-2}	6.10^{-2}	6.10^{-2}	6.10^{-2}	5.10^{-2}	2.10^{-2}

Figures 1 and 2 compare the minimum inhibitory concentrations of the essential oil extracts with those of the two reference molecules. The results show that the concentrations of all the extracts are lower than those of the reference molecules, with the *Cymbopogon nardus* essential oil extract exhibiting the lowest value.

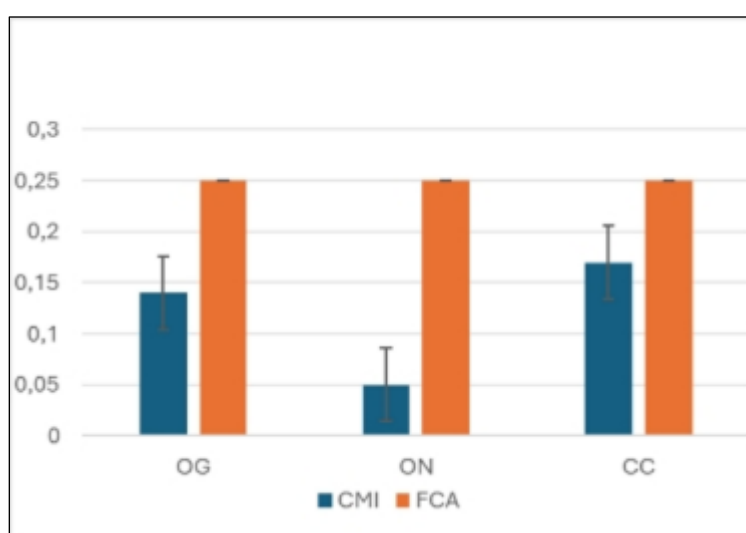


Figure 1: Diagrams comparing the various minimum inhibitory concentrations of the essential oil extract with the reference molecule fluconazole (FCA).

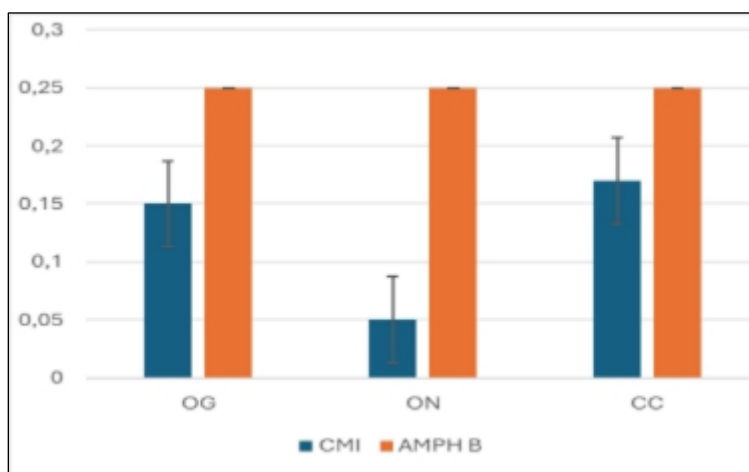


Figure 2: Diagrams comparing the various minimum inhibitory concentrations of the essential oil extract with the reference molecule Amphotericin B (AMPH B).

5 DISCUSSION

The yield of *Cymbopogon citratus* essential oil obtained in this study is very close to that obtained by [9], using the same extraction method (hydrodistillation), those researchers obtained a yield of 0.40%. However, this result differs from those obtained by [10], who reported yields of 0.30% and 0.35%, respectively. Nevertheless, it is close to the oil yields obtained from *Ocimum gratissimum* and *Cymbopogon nardus*. This difference in yield could likely be attributed to the timing of the leaf harvest, but above all to the composition or texture of the soil where the various plants were grown.

The obtained essential oil extracts showed no presence of infectious microorganisms specifically *aerobic mesophilic bacteria*, *Enterobacteriaceae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, yeasts, and molds. This indicates that the essential oils produced were not contaminated during production and are therefore of good hygienic quality.

Regarding antifungal activity, the essential oil extracts all demonstrated favorable minimum inhibitory concentrations against both *Candida albicans* and *Aspergillus fumigatus*. The minimum inhibitory concentrations obtained for the various essential oil extracts ranged from 0.05 to 0.18 $\mu\text{L}/\mu\text{L}$. These values are significantly lower than those of the reference compounds (Amphotericin B and Fluconazole), demonstrating the efficacy of these essential oils in combating infections caused by these pathogens compared to the reference drugs available in pharmacies. This result suggests that the essential oil extracts of *Cymbopogon citratus*, *Ocimum gratissimum*, and *Cymbopogon nardus* possess antifungal properties comparable to or better than the reference compounds studied.

Furthermore, research by [10] in Chad and by [11] has revealed that the essential oils of *Cymbopogon citratus* and *Ocimum gratissimum* are composed primarily of oxygenated monoterpene phytochemicals. Given this high monoterpene content, the antifungal activity observed could be attributed to these phytochemicals. Similar studies such as that of [9] have demonstrated that *Cymbopogon citratus* essential oil extract inhibits the growth of *Aspergillus fumigatus*, *Aspergillus flavus*, and *Candida albicans*.

6 CONCLUSION

The essential oils produced from *Cymbopogon citratus*, *Ocimum gratissimum*, and *Cymbopogon nardus* yielded generally low concentrations, but all were free from contamination. These essential oil extracts showed good minimum inhibitory concentrations (MICs), demonstrating their inhibitory efficacy against the NR-45108 *Aspergillus fumigatus* and NR-29449 *Candida albicans* strains. Furthermore, the MICs obtained from the various essential oil extracts of *Cymbopogon citratus*, *Ocimum gratissimum*, and *Cymbopogon nardus* are lower than those of the two reference molecules studied (fluconazole and amphotericin B). Therefore, more in-depth tests on many strains could be conducted with these extracts to better assess their antifungal activity and develop an improved traditional medicine.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Acknowledgments

We extend our thanks to the Faculty of Biological Sciences and the Department of Biochemistry and Genetics at Peleforo Gon Coulibaly University in Korhogo, and particularly to the institution's Microbiology Unit. We also offer our sincere thanks to the Institute Pasteur in Côte d'Ivoire for providing laboratory equipment and biological resources.

Disclosure of conflict of interest

The authors declare no conflicts of interest.

Author contributions

Traore designed the study, contributed to data analysis alongside Aka Kouadio, and drafted the manuscript. Abiba Ouattara collected the plant material and participated in oil extraction in collaboration with Dramane Soro. Dramane was specifically responsible for the botanical description of the plants studied. Abou and Guessend contributed to the interpretation of the results. All authors read and approved the manuscript.

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