

In Vitro Antifungal Activity of Essential Oils from *Cymbopogon citratus*, *C. giganteus*, *Eucalyptus globulus*, and *Syzygium aromaticum* on Strains of *Candida albicans* and *Microsporium* Spp. in the Kisangani Region (DRC)

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Abstract

This study focused on demonstrating the inhibitory activity of essential oils from four aromatic plants, *Cymbopogon citratus*, *C. giganteus*, *Eucalyptus globulus*, and *Syzygium aromaticum*, on strains of *Candida albicans* and *Microsporium* spp. in the Kisangani region. The essential oils were extracted by hydrodistillation and the antifungal activity was evaluated by aromagram. The results revealed that, on the *C. albicans* strain, the average inhibition diameter was 3 mm for *C. citratus* essential oil, 1 mm for *C. giganteus* and *E. globulus* essential oils, and 24 mm for *S. aromaticum* essential oil. Furthermore, on the *Microsporium* spp. strain, the average inhibition diameter was 16 mm for *C. citratus* essential oil, 15.5 mm for *C. giganteus* essential oil, 8 mm for *E. globulus* essential oil, and 12 mm for *S. aromaticum* essential oil. This study shows that the inhibition diameters of the essential oils of the plant species studied have different activities on the two fungal strains (*C. albicans* and *Microsporium* spp.) tested. However, using Student's t-test, the probability obtained is p-value = 0.01, indicating a very significant difference in sensitivity between the two strains tested.

Keywords: Antifungal activity, essential oil, *Candida albicans*, *Microsporium* spp., Kisangani.**Copyright © 2026 The Author(s):** This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC BY-NC 4.0) which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited.

I. INTRODUCTION

The history of aromatic and medicinal plants is linked to the evolution of civilizations. Today, aromatic plants have considerable value thanks to the gradual discovery of applications for their essential oils (EO) in healthcare and their uses in other areas of economic interest. Their many uses mean that they are increasingly in demand on international markets [1].

EOs have many biological activities. In phytotherapy, they are used in pharmaceutical preparations. Phenolic terpenes, particularly thymol and carvacrol, are often used as antiseptics, antibacterials, and antifungals. In phytosanitary fields, for example, essential oils or their active compounds could also be used as protective agents against bacteria, fungi, and other microorganisms. The antimicrobial activities of essential oils have been reported in several studies [2]. In most cases, these activities are attributed to oxygenated

monoterpenes [3]. The discovery of new molecules would, by default, eliminate pathogenic factors and improve the health of patients. The plant kingdom, the basis of traditional medicine, is likely to provide a large number of molecules with antifungal properties [4].

Human fungal infections currently represent a real public health problem. They are one of the leading causes of death, especially among immunocompromised individuals worldwide [5]. Although modern antifungal drugs are now available, the treatment of fungal infections remains difficult, partly due to the limited number of truly effective active ingredients and their very high cost, and partly due to the emergence of strains resistant to certain commonly used antimicrobials [6].

As part of this research, we studied *in vitro* the antifungal activity of essential oils from *Cymbopogon citratus*, *C. giganteus*, *Eucalyptus globulus*, and

Syzygium aromaticum on strains of *Candida albicans* and *Microsporium* spp. with a view to proposing effective means of combating these zoopathogenic germs in the Kisangani region.

II. MATERIALS AND METHODS

II.1. Study environment

This study was conducted in the Kisangani region, capital of Tshopo province in the Democratic Republic of Congo. The city of Kisangani is located in the eastern part of the Congolese central basin at 0°, 31 North and 25°, 11 East, at an altitude of 396 m and covers an area of approximately 1,910 km² [7].

II.2. MATERIALS

II.2.1. Aromatic plants

Four aromatic plants were used, namely *C. citratus*, *C. giganteus*, *E. globulus*, and *S. aromaticum*. The plants were harvested in the morning before 7 hours 30 minutes in areas with little human traffic. Sampling was performed on healthy parts of the plant [8].

The analyses were carried out at the Microbiology and Phytopathology Laboratory of the Faculty of Natural Sciences and Biotechnology at the University of Kisangani.

II.2.2. Fungal strains

C. albicans and *Microsporium* spp. are the two zoopathogenic fungal strains used in this study.

II.3. Methods

II.3.1. Treatment of the plants studied

The leaves of *C. citratus*, *C. giganteus*, and *E. globulus* were crushed after drying at room temperature (between 25 and 30°C) in the laboratory for two weeks, and the seeds of *S. aromaticum* were purchased from vendors at the central market in Kisangani for extraction.

II.3.2. Extraction of essential oils

The essential oils were extracted using the hydrodistillation method. The mixture of 800 grams of powder and seven liters of water was boiled for four hours. The essential oil collected was then placed in a hermetically sealed glass bottle and stored in the refrigerator [9].

II.3.3. Extraction yield

This was determined using the following formula:

$$\text{Yield} = M'/M * 100$$

Were

- ✓ Yield: Essential oil yield as a percentage;
- ✓ M': Mass of essential oil in grams;
- ✓ M: Mass of plant material used in grams.

II.3.4. Organoleptic and physicochemical characterization of essential oils

The appearance, color, and odor of the EOs were considered for the organoleptic characterizations. However, density, pH, miscibility in water and 70% ethanol, and refractive index were taken into account for the physicochemical qualities.

II.3.5. Preculture of strains to be tested

The strains to be tested were transferred to tubes, each containing 3 ml of peptone water for revivification, which were then incubated for 24 hours at 37°C for the *C. albicans* strain and for 48 hours at 27°C for the *Microsporium* spp. strain.

II.3.6. Impregnation of discs

The 7 mm diameter discs were impregnated with a few drops of essential oil, then placed in Petri dishes where they were dried for at least 5 days.

II.3.7. Obtaining strains

The zoopathogenic fungal strain of *C. albicans* was kindly provided by the Provincial Public Health Laboratory in Kisangani. However, for *Microsporium* spp. at the periphery of the lesions, samples were taken by scraping the scales with a swab. Broken hairs were pulled out and collected in test tubes containing physiological saline. Sabouraud culture medium supplemented with chloramphenicol was used. The scales and hairs were placed in tubes containing slanted agar using a swab previously dipped in sterile physiological saline and pressed lightly into the medium. The cultures were incubated in an oven set at 27°C [10, 11].

II.3.8. Evaluation of antifungal activity

The method chosen to determine the antifungal activity of essential oils on our two strains is the aromatogram method [12]. The principle of this method is based on the migration of essential oils by diffusion in agar, which is inspired by the antibiogram and has been generalized to essential oils. Filter paper discs with a diameter of 7 mm impregnated with essential oils are placed on the surface of an agar medium (Mueller Hinton for *C. albicans* and Sabouraud for *Microsporium* spp.), previously seeded with fungal suspensions aged 24 hours at 37°C and 48 hours at 27°C for *C. albicans* and *Microsporium* spp. strains, respectively [10].

II.3.9. Expression of results

❖ Interpretation of sensitivity to essential oils

Inhibition halos of varying sizes will form around the discs depending on the fungicidal power of the essential oil, that's to say a clear, transparent, circular halo with a sharp edge. The inhibition diameter of the essential oils tested was determined by measuring the diameter of the halos in mm (excluding the disc). And, if the diameters of the inhibition zones are [14].

- ✓ Ø < 8: Resistant strain;

- ✓ $9 < \emptyset < 14$: Sensitive strain;
- ✓ $15 < \emptyset < 20$: Very sensitive strain;
- ✓ $\emptyset > 21$: Extremely sensitive strain.

II.3.10. Statistical analyses

The inhibition diameters encoded in Excel 2016 were processed using R software (version 4.4.0). Student's t-test (at 0.05) was used to compare the sensitivities of the two strains studied.

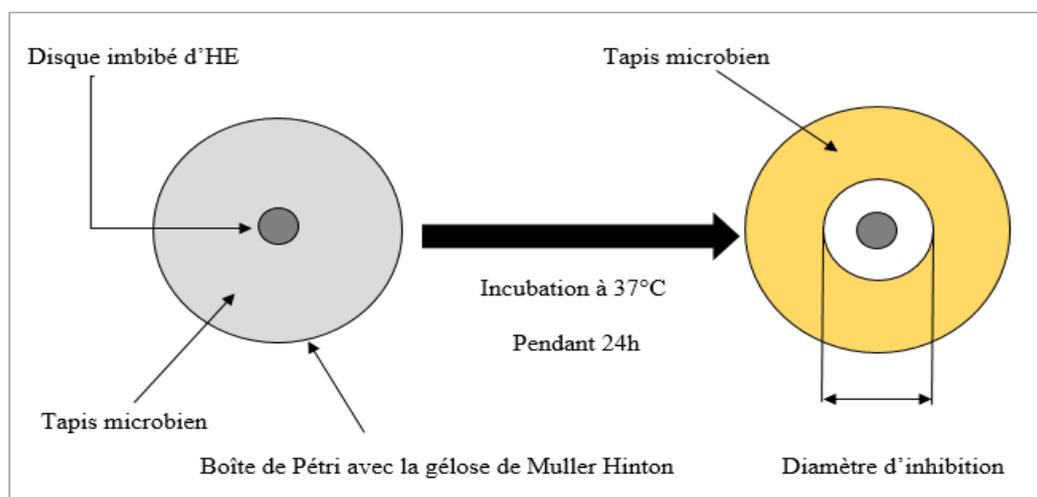


Figure 1: Aromatogram technique [13]

III. RESULTS AND DISCUSSION

III.1. Essential oil extraction yield

It was determined that *S. aromaticum* has a high yield compared to other species, at 0.85%, followed by *E. globulus* at 0.48%, then *C. citratus* at 0.31%, and finally *C. giganteus* at 0.19%. This high yield could be explained by its natural richness in volatile compounds.

Our results are lower than those obtained by [15], on *C. citratus*, which was 0.36%. After comparing our results with those of [16], this author found a yield identical to ours, either 0.19% in *C. giganteus* essential oil. When comparing the results obtained in this research

with those of [17], on *E. globulus* essential oil, which obtained 0.07%, our results are higher. Furthermore, this value is also lower than those of [18], who found a value of 1.57 after extraction of *E. globulus* essential oil. Thus, [19], found that the essential oil yield of *S. aromaticum* is 3.67%, a value well above our results. These differences in essential oil yield are probably due to several factors, including soil, climate, environment, extraction method, and harvest period [20, 16].

III.2. Organoleptic qualities

The results for organoleptic qualities are illustrated in Figure 2 below:



Figure 2: Essential oil of *S. aromaticum* (A), *C. giganteus* (B), *C. citratus* (C), and *E. globulus* (D)

Essential oils are liquid substances of varying thickness with a very distinctive odor. They are extremely volatile, which distinguishes them from fixed oils, and quickly lose their properties [21].

The organoleptic qualities showed similarities between the four essential oils of the plant species studied, with characteristic liquid appearances and odors. However, only the color marks the organoleptic difference between these aromatic plant extracts, with dark yellow for *C. citratus*, pale yellow for *C. giganteus* and *S. aromaticum*, and reddish for *E. globulus*. This variability is thought to be due to the abundance of pigments in the plants studied [22].

According to [16], the essential oil of *C. citratus* was yellow in color, while that of *C. giganteus* obtained from [20], was greenish-yellow. Furthermore, [23], found that *E. globulus* essential oil was yellowish in color, while *S. aromaticum* essential oil was light yellow according to [19]. These color differences could be explained not only by the organs used for extraction but also by the shelf life of these essential oils [9].

III.3. Physicochemical characteristics

III.3.1. Density at 25°C

It should be noted that *S. aromaticum* has a high density compared to other species, at 1.00, followed by *E. globulus* at 0.84, then *C. giganteus* at 0.83, and finally *C. citratus* at 0.77.

The density value of *C. citratus* differs from that generally reported in the literature, where the density of essential oils is usually between 0.85 and 1.10 [24]. This variation is attributed to the specific chemical composition of each essential oil, in particular the proportion of major compounds such as sesquiterpenes and monoterpenes, as well as the possible presence of residues or impurities.

Compared to the experimental values of [17], on the density of *E. globulus* essential oil (0.96) and [16], on *C. citratus* essential oil (0.64), these values are lower. The density found in this study is higher than that found by [25], for *C. giganteus* essential oil, which is 0.66. However, this value is close to those found by [26], who found a density of 1.06 for *S. aromaticum* essential oil.

III.3.2. pH

It should be noted that the highest pH value was observed in *S. aromaticum* essential oil, at 4.69, followed by *E. globulus* at 3.98, then *C. citratus* at 3.26, and finally *C. giganteus* at 3.17.

All essential oils from the plants studied had acidic pH values. The relative acidity observed is thought to be due to the presence of certain volatile acidic

compounds such as short-chain carboxylic acids or certain phenols in the essential oils studied [27].

Comparing the pH values of *C. citratus* essential oil with those found by [28], which were 4.11, we note that the values are quite similar. Nevertheless, the pH results for *C. giganteus* EO obtained in this study are very close to those obtained by [29], either 3.20. Other authors have recorded a significantly higher pH ; in fact, in his study, [19] obtained a pH of 6.79 for *S. aromaticum* EO. Similarly, [30], found a pH of 3.34 for *E. globulus* essential oil. These differences in pH values are thought to be due to the nature and molecular abundance of the oils studied [31].

III.3.3. Miscibility of essential oils in water and 70% ethanol

The essential oils of *C. citratus* and *C. giganteus* are slightly miscible in water, while the other two are immiscible, including *E. globulus* and *S. aromaticum*. Furthermore, all the essential oils studied are miscible in 70% ethanol.

These results are consistent with those reported in several previous studies, which describe the insolubility of essential oils in water and their solubility in polar organic solvents such as ethanol and ether [15].

III.3.4. Refractive index

There is a diversity of refractive index values among the four essential oils of the plant species studied. The highest refractive index value was observed in *S. aromaticum*, at 1.532, followed by *E. globulus* at 1.504, then *C. giganteus* at 1.491, and finally *C. citratus* at 1.483.

The refractive index depends on the chemical composition, which increases with the length of the acid chains, their degree of substitution, and temperature. It varies mainly with the content of monoterpenes and oxygenated derivatives. A high monoterpene content will result in a high index [32].

According to French standardization norms (AFSN), the refractive index of essential oils generally ranges between 1.495 and 1.513 at 25°C. A value of 1.495 indicates a high-quality oil, while 1.513 suggests a lower-quality oil. The refractive index values obtained in this research are close to the range mentioned by AFSN standards.

III.4. Sensitivity of strains to essential oils

III.4.1. Sensitivity of *C. albicans* and *Microsporum* spp. strains to essential oils

The results of the sensitivity of *C. albicans* and *Microsporum* spp. strains are illustrated in Figures 3 and 4.

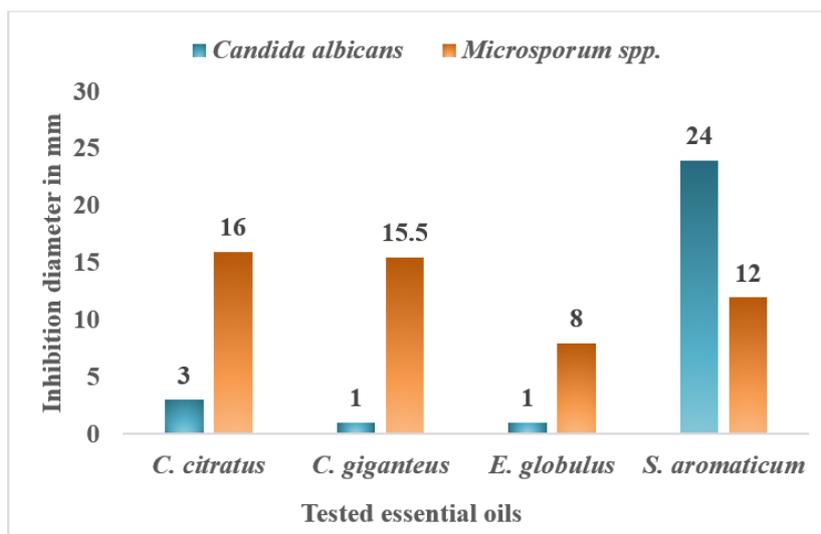


Figure 3: Inhibition diameters of essential oils on strains of *C. albicans* and *Microsporium spp.*

This figure shows that, for the *C. albicans* strain, *S. aromaticum* EO had the highest inhibition zone diameter, at 24 mm, while *C. giganteus* and *E. globulus* EOs had the lowest inhibition zone diameters, at 1 mm after 24 hours of incubation.

As for the *Microsporium spp.* strain, *C. citratus* essential oil showed significant activity, with a diameter of 16 mm. *E. globulus* essential oil had the lowest inhibition diameter, at 8 mm after 48 hours of incubation.

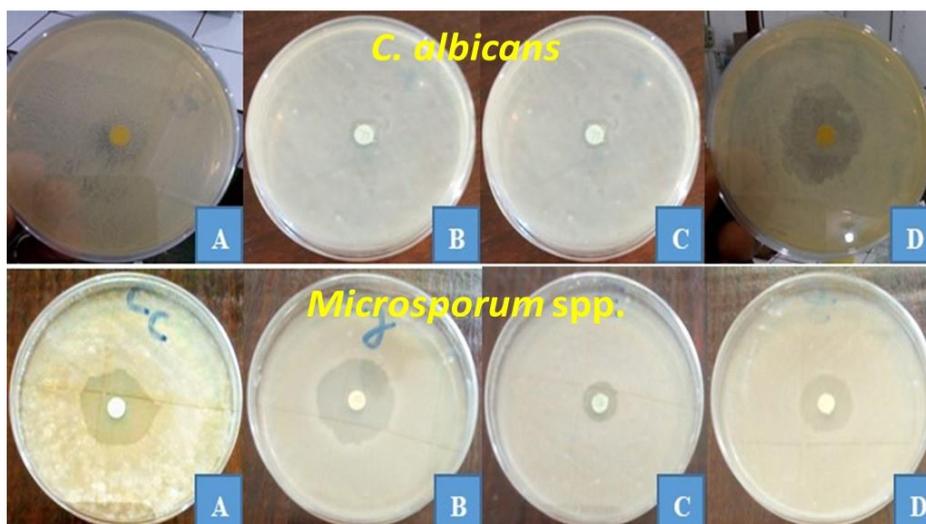


Figure 4: Inhibition zones of essential oils on strains of *C. albicans* and *Microsporium spp.*: *C. citratus* (A), *C. giganteus* (B), *E. globulus* (C), and *S. aromaticum* (D). After 48 hours of incubation

All of the essential oils tested had antifungal activity on the strains studied, but with different inhibition diameters. This variability is thought to be due to the antifungal properties of these aromatic plant essences.

The *C. albicans* strain appears to be the most resistant to the essential oils of *C. citratus*, *C. giganteus*, and *E. globulus*, as these essential oils have the smallest inhibition zone diameters on this strain. Only the essential oil of *S. aromaticum* showed the largest inhibition zone diameter.

However, [33], working on *G. glabra* essential oil, found an inhibition zone diameter of 13 mm on the *C. albicans* strain. Our results are identical to those found by [25], who conducted a study on *C. giganteus* essential oil, tested on the *C. albicans* strain, and measured an inhibition zone diameter of 1 mm. Furthermore, [34], were able to obtain a small inhibition of *C. albicans* growth with *Mentha piperita* essential oil.

The same is true of the results found by [35], who report that *C. citratus* essential oil exhibited the strongest anti-candidal activity among the 18 Thai essential oils tested. Compared to *E. globulus* essential

oil, the work of [36], which obtained an average diameter of 13 mm across a range of tested fungi, shows results superior to ours. These differences in activity are thought to be due to the chemical composition of each essential oil.

Our results corroborate those found by [19], on *S. aromaticum* essential oil, which found inhibition zone diameters of 23 mm and 25 mm on the two *Klebsiella* strains tested, respectively. These results would explain the effectiveness of this aromatic essence against microorganisms.

The values obtained in this study are contrary to those of [17], who found a diameter of 18 mm for *E. globulus* essential oil on the *S. aureus* strain. The difference in the species of the strains tested may be the reason for this.

[37], found that *C. giganteus* essential oil was active on the LNCaP prostate cancer cell line. Our results are consistent with those of this author. Compared to the results of [19], who obtained a diameter of approximately 15 mm for *S. aromaticum* essential oil on the *Klebsiella* strain. These results would be justified by the effectiveness of this essential oil.

Table 1 provides information on the evaluation of the antifungal activity of essential oils

Strain/EO	<i>C. citratus</i>	<i>C. giganteus</i>	<i>E. globulus</i>	<i>S. aromaticum</i>
<i>C. albicans</i>	R	R	R	ES
<i>Microsporium spp.</i>	VS	VS	R	S

Legend: R: Resistant, S: Sensitive, VS: Very sensitive, and ES: Extremely sensitive

Based on inhibition diameters as a criterion, this table shows that the *C. albicans* strain is completely resistant to *C. citratus*, *C. giganteus*, and *E. globulus* essential oils. However, it is extremely sensitive to *S. aromaticum* essential oil. In addition, the *Microsporium* spp. strain is resistant to *E. globulus* essential oil and sensitive to *S. aromaticum* essential oil, except for *C. citratus* and *C. giganteus* essential oils, to which it is very sensitive.

Our observations partially agree with those of [42], who found resistance of the *S. typhi* strain to *E. globulus* essential oil. With regard to the work of [36], this author found a range of bacteria to be sensitive to *E. globulus* essential oil. This result differs from ours, and this difference in activity could be explained by the nature of the strains tested.

However, these results are consistent with those found by [43], for the *Microsporium* spp. strain, which found two strains of *E. coli* to be highly sensitive to *C. Citratus* essential oil. This could be explained by the effectiveness of this essential oil against microorganisms due to its specific chemical composition.

Our results for *C. citratus* essential oil on the *Microsporium* spp. strain confirm the theory that this essential oil is anti-infectious. Its main chemical component is citral [38]. It has antifungal properties that can be used to treat certain fungal infections radically [39].

Several studies have been conducted around the world to evaluate the biological activities of *C. citratus* essential oil and its main compounds [40]. From a microbiological point of view, this oil has been classified as one of the most powerful antimicrobials [41]. In addition, it has proven properties, but the amount needed to inhibit these germs varies (from 1 to 100mg/ml) depending on the microbial strains [41].

III.4.2. Classification of the sensitivity of *C. albicans* and *Microsporium* spp. strains to essential oils

The antifungal activity of essential oils from four species (*C. citratus*, *C. giganteus*, *E. globulus*, and *S. aromaticum*), tested on strains of *C. albicans* and *Microsporium* spp., is evaluated according to the diameters of the inhibition zones. The strains are assessed according to the criteria.

According to this study, the results are consistent with those of other researchers, since the *S. aromaticum* essential oil studied has antifungal activity against the fungal pathogens tested. [44], on *Penicillium* sp., *Aspergillus flavus*, *Aspergillus niger*, and *Trichoderma* sp. are good examples of this.

IV. CONCLUSION AND RECOMMENDATIONS

The aim of this research was to evaluate, *in vitro*, the inhibitory activity of essential oils from the leaves of *Cymbopogon citratus*, *C. giganteus*, *Eucalyptus globulus*, and the seeds of *Syzygium aromaticum* on strains of *Candida albicans* and *Microsporium* spp.

After evaluation by aromagram, the essential oils of these four plants showed, on the *C. albicans* strain, average inhibition diameters of 3 mm for *C. citratus* essential oil, 1 mm for *C. giganteus* and *E. globulus* essential oils, and 24 mm for *S. aromaticum* essential oil, while on the *Microsporium* spp. strain, an average inhibition diameter of 16 mm for *C. citratus* essential oil, 15.5 mm for *C. giganteus* essential oil, 8 mm for *E. globulus* essential oil, and 12 mm for *S. aromaticum* essential oil.

Thus, the inhibition diameters of the essential oils of the plant species studied have different activities on the two fungal strains (*C. albicans* and *Microsporum* spp.) tested. Applying Student's t-test, the probability obtained is p-value = 0.01, showing that there is a very significant difference between the two strains tested.

We recommend that future researchers continue studying the antifungal activity of essential oils from other plants on strains of *C. albicans* and *Microsporum* spp., verify the synergistic effects of the essential oils from the plants used, and finally analyze the chemical composition of the essential oils to identify the active molecules responsible for the antifungal activity.

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