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# Antifungal activity of the essential oil of *Micromeria barbata* an endemic Lebanese *Micromeria* species collected at North Lebanon

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## Abstract

The spreading of the resistance to antifungal drugs is a significant issue to the public health and requires to find out a new and innovative alternative for the treatment of resistant fungi and yeasts strains. *Micromeria barbata* a *Lamiaceae* perennial aromatic herb naturally grown in the rocky areas of the Mediterranean region, is represented by 14 species and 22 taxa. *M. barbata* and *M. libanotica* are endemic Lebanese species. The aim of this study is to assess the antifungal activity of *M. barbata* essential oil towards 20 strains of filamentous fungi (dermatophytes and molds) and 20 yeast. The inhibition ratio has been calculated for each fungi for all the essential oil dilutions used. The obtained results for filamentous fungi showed a high antifungal activity of the essential oil even at high dilution. A similar results was observed event towards strains presenting antifungal drug resistant. The method used in this work to assess the antimicrobial effect of essential oil was smooth, rapid and accurate. *M. barbata* essential oil used in this study may be useful as an alternative antimicrobial agent for the treatment of many fungal infections and nosocomial infections.

*Keywords*: *Micromeria barbata*, Essential oil, antifungal activity.

## Introduction

Essential oils have a long history of use as antimicrobial agents. They have been used in a number of pharmaceutical, food, and cosmetic products because these oils effectively inhibit the growth of a wide range of microorganisms, and they cause fewer side effects than synthetic antimicrobial agents in humans [1]. Advances in medical treatment have led to improved survival in the general population, but these advances have also led to a large numbers of individuals being at risk for fungal infection such as those who have indwelling catheters, those who are in intensive care, those who have received various immunosuppressive therapies, and those who are undergoing organ or stem cell transplantation. Fungi have emerged as a major cause of human disease especially among the immune compromised and those hospitalized with serious underlying disease [2-5]. The incidence of invasive opportunistic mycoses has increased because of the expanding population of immunocompromised patients, including solid-organ transplant and hematopoietic stem cell transplant recipients, patients with cancer, patients with the acquired immune deficiency syndrome, premature neonates, elderly patients, and patients recovering from major surgery [6, 7].

The list of opportunistic fungi causing serious, life-threatening infection increases every year [4, 8-12]. In addition to *Candida* spp. *Aspergillus* spp. and *Cryptococcus* spp. the opportunistic fungi include yeasts other than *Candida* species, non- dematiaceous or hyaline molds, and the pigmented or dematiaceous fungi [5, 9, 11, 12]. *Candida. albicans* is the predominant cause of invasive fungal infections [13] and represents a serious public health challenge with increasing medical and economic importance due to the high mortality rates and increased costs of care and duration of hospitalization [14, 15]. It is the third- or the fourth-most-common isolate in

nosocomial bloodstream infections in the USA. In addition, candidosis is the most common invasive fungal infection in critically ill non neutropenic patients [16], it is followed by *C. glabrata* and other non-*C. albicans*. This change in epidemiology could be associated with severe immunosuppression or illness, prematurity, exposure to broad-spectrum antibiotics [13].
Dermatophytes are involved in serious human pathogenic infections that have increased during the last decades, particularly among high risk patients [17, 18]. These infections are a major cause of morbidity-associated superficial mycoses, with frequent relapses and often refractory to therapy [18]. Various plant materials are believed to have antifungal activity and many essential oils have been reported to have antifungal activities with no side effects on humans and animals [19]. Previous *in vitro* and *in vivo* investigations suggested that the essential oils could be used as an effective antifungal agents [20]. The selection of plants for evaluation was based on traditional usage for treatment of infectious diseases [21-23]. Essential oils and plant extracts have long been known and used throughout the world for the treatment of many conditions, including skin conditions, and have less deleterious side effects than corresponding synthetic drugs [24].
During the last years, a number of studies have been carried out concerning the application of essential oils as antimicrobial agents [25]. *Micromeria* a *Lamiaceae* perennial aromatic herb naturally grown in the rocky areas of the Mediterranean region, is represented by 14 species and 22 taxa [26]. *M. barbata* and *M. libanotica* are endemic in Lebanon [27]. *M. barbata* is a perennial, aromatic and glandular plant. It flowers in summer – autumn until December and January. *M. barbata* is a medicinal plant traditionally recognized by the villagers in Lebanon for its stimulating effects of the digestive system as well as for respiratory illnesses. The aim of this study is to evaluate the antifungal activity of the essential oil of *M. barbata* against yeast molds and dermatophytes. This study was carried out on different clinical, as well as on environmental isolates and on reference strains.

## 2. Materials and methods

### 2.1. Essential Oil Extraction:

The samples of *M. barbata* collected in summer 2014 in the mountain of the Deniyeh region, north Lebanon, were cool dried. The essential oil was extracted by Hydro-distillation (3 hours) using a Clevenger–type apparatus yielding yellowish oil. The composition (percent) was calculated on moisture free basis to be 2%. The oil was collected in dark glass and stored at temperature between 4 to 8°C till analysis and use.

### 2.2. Fungal strains

The strains used in this study were kindly provided by Professor P. Bouchara (Groupe d'Etude des Interactions Hôte-Pathogène - University of Angers –France).
The tested molds were: *Aspergillus nidulans, A. flavus, A. fumigatus, A. versicolor, A. ochraceus, Fusarium* spp. *Scedosporium aurantiacum, S. dehoogii, S. boydii, Absidia corymbifera*.
The tested dermatophytes were: *Microsporum canis, M. audouini, Trichophyton mentagrophyte, T. schoenleinii, T. rubrum, T. verrucosum, T. tonsurans, T. violaceum and Epidermophyton floccosum.*

### 2.3. Yeasts

In this study ATCC strains, environmental and clinical isolates were used. The clinical isolates were collected from Nini Hospital at Tripoli North Lebanon. The studied strains were conserved in the: Collection Microbiologique de l'Université Libansaise (CMUL). They were identified by the Auxacolor® gallery (Biorad–France) and the antifungal susceptibility profile for all these strains was determined by the Fungitest® gallery (Biorad – France). Some of the yeast strains studied show resistance to antifungal drugs (Table 1). The following yeast strains have been selected and used in our study: *Geotrichum* spp. CMUL 88, *Geotrichum candidum* CMUL 043 resistant to miconazol and fluconazole, *Saccharomyces cerevisiae* CMUL 058, *S. cerevisiae* CMUL 98, *Candida glabrata* CMUL 121, *C. dubliniensis* CMUL 133, *C. albicans* CMUL 136 resistant to itraconazole, ketoconazole and fluconazole. *C. albicans* CMUL 137 resistant to ketoconazol, *C. tropicalis* CMUL 141, *C. inconspicua* CMUL 143, *C. krusei* CMUL 146 resistant to fluconazol, *C. parapsilosis* CMUL 154, *Rhodotorula rubra* CMUL 157 resistant to fluconazole, *Cryptococcus neoformans* CMUL 158, *Rhodotorula* spp. CMUL 051 resistant to itraconazole and fluconazole, *C. krusei* ATCC 34135, *C. albicans* ATCC 34135, *C. glabrata* ATCC 1512, *C. tropicalis* ATCC 13803, *C. guilliermondii* ATCC 626.

Table 1: The collection of the studied yeast strains, with the antifungal resistance profile carried out in the present study (R: resistant; S: sensitive, I = Intermediate)

| Yeast name | Antifungal µg/ml | | | | | | Origin |
|---|---|---|---|---|---|---|---|
| | 5-fluorocytosine 2-32 | Amphotericin B 2-8 | Fluconazole 8-64 | Itraconazole 0.5-4 | Ketoconazole 0.5-4 | Miconazole 0.5-4 | |
| *Geotrichum* spp. CMUL 88 | S | S | S | S | I | I | Clinical |
| *G. candidum* CMUL 043 | I | S | R | I | I | R | Clinical |
| *S. cerevisiae* CMUL 058 | S | S | S | S | I | I | Clinical |
| *C. aglabrata* CMUL 121 | S | S | I | I | I | S | Clinical |
| *C. dubliniensis* CMUL 133 | S | S | S | S | S | S | Clinical |
| *C. albicans* CMUL 136 | S | S | R | R | R | I | Clinical |
| *C. albicans* CMUL 137 | S | S | I | I | R | I | Clinical |
| *C. tropicalis* CMUL 141 | S | S | S | I | I | I | Clinical |
| *C. inconspicua* CMUL 143 | I | S | I | I | I | I | Clinical |
| *C. krusei* CMUL 146 | I | S | R | I | I | I | Clinical |
| *C. parapsilosis* CMUL 154 | S | I | S | I | S | I | Clinical |
| *R. rubra* CMUL 157 | S | S | R | I | I | I | Environment |
| *C. neoformans* CMUL 158 | S | S | S | S | I | S | Clinical |
| *Rhodotorula* spp. CMUL 051 | S | S | R | R | I | R | Environment |
| *S. cerevisiae* CMUL 98 | S | S | S | I | I | S | Clinical |
| *C. krusei* ATCC 34135 | I | I | I | I | I | I | Clinical |
| *C. albicans* ATCC 10231 | S | S | S | I | S | S | Clinical |
| *C. glabrata* ATCC 15126 | S | S | S | I | I | S | Clinical |
| *C. tropicalis* ATCC 13803 | S | S | S | S | S | I | Clinical |
| *C. guilliermondii* | S | S | S | I | I | I | Clinical |

## 2.4. Study of the antifungal activity of *M. barbata* essential oil

Because of the immiscibility of the essential oils to water and thus to the culture medium an emulsification was performed using a 0.2% agar [28]. This solution allows a homogeneous distribution of essential oils and maximizes the germ/compound contact. In this agar solution, the following dilutions were prepared as $1/10^{th}$, $1/25^{th}$, $1/50^{th}$, $1/100^{th}$, $1/200^{th}$, $1/300^{th}$ and $1/500^{th}$.
Test tubes containing each 9 ml of Sabouraud medium supplemented with chloramphenicol (CONDA®) for fungi were sterilized for 20 min at 121°C and cooled to 50°C, then 1 ml of each dilution was added aseptically in order to obtain a final concentrations of $1/100^{th}$, $1/250^{th}$, $1/500^{th}$, $1/1000^{th}$, $1/2000^{th}$, $1/3000^{th}$ and $1/5000^{th}$. The tubes were shacked to disperse the essential oil in the culture medium before pouring into Petri dishes. Positive controls containing only the culture medium and a 0.2 % agar solution were also prepared. Inoculation was performed by depositing fragments of 0.5 cm in diameter, taken from the periphery of a mycelial mat, originating from a 7 days old culture on Sabouraud agar. The Incubation was performed at 30°C during 7 days [29, 30, 31].

## 2.5. Expression of results

The inhibition percentage of the fungal growth is calculated in relation to the control without essential oil (Sabouraud + 2 % agar) using the following formula [29, 30, 31]:
Inhibition percent (%) = dt − de / dt × 100.
dt (mm) = diameter of fungal growth in the control plate.
de (mm) = diameter of fungal growth in the test plate

## 2.6. Study of the effects on yeasts

### 2.6.1. Preparation of the inoculum

From a pure and fresh yeast culture (18h old), one colony has been transferred in a sterile test tube contains Sabouroud broth and stirring for 1 to 3 minutes to be homogenized to reach around 0.5 McFarland turbidity. A sample of 10 µl of each yeast suspension was placed/spotted on the surface of the plates (12/12 cm) containing the same concentrations used in the case of filamentous fungi. This method allows to test 10 different strains in each plate. To verify the viability of the tested yeasts, 10 µl for each strain were inoculated in the same way on the surface of an oil-free Sabouraud agar. The dishes were then incubated at 35°C during 48 hours.

### 2.6.2. Reading and Interpretation

After checking the culture viability of the strains on the control plates, the strains were considered inhibited, if no growth was observed on the plate with diluted oil. All the experiment were carried out in triplicate.

## 3. Results

**Antimicrobial effect of *M. barbata* essential oil against molds and dermatophytes strains**

Table 2 and 3 and figure 1 and 2 show the results of the antifungal activity of the essential oil on molds and dermatophytes. The results showed that *M. barbata* essential oil totally inhibits the fungi growth whatever the tested dermatophytes strains for the dilution 1/100 and 1/250. At a dilution of 1/500 almost all the tested strains were totally inhibited except: *M. canis*, *T. schonleinii*. However, the antifungal activity of the essential oil decreased with the increase of the dilution. At a dilution of 1/1000 a total inhibition was observed only for *T. tonsurans*, *T. violaceum* and *E. flocosum*. The growth inhibition percentage decreased to 20 % at the dilution of 1/500 for *T. schonleinii,* while at the dilution 1/1000 no antifungal activity was measured for the essential oil against *T. tonsurans*, *T. violaceum* and *E. flocosum*.

**Figure 1:** Effect of *M. barbata* essential oil on the growth of *Fusaruim* spp. The growth of *Fusaruim* spp. was carried out on Sabouraud agar supplemented by different dilution of the essential oil

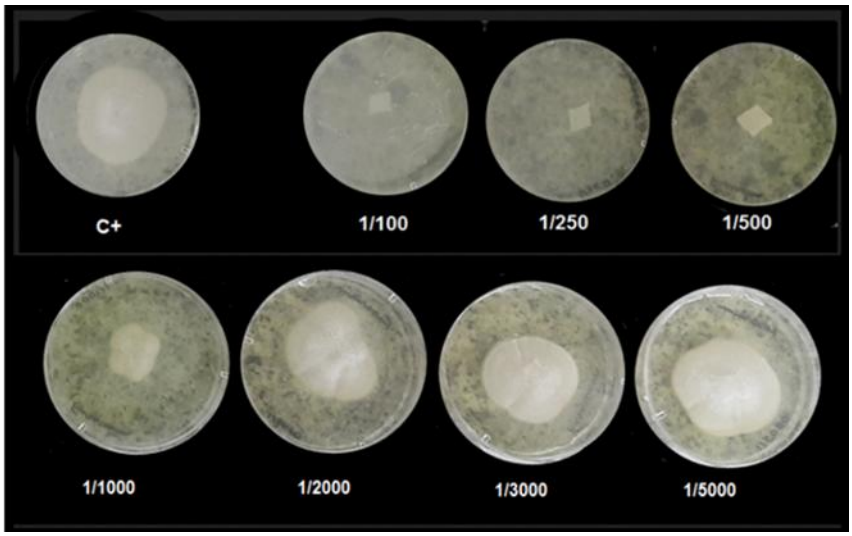
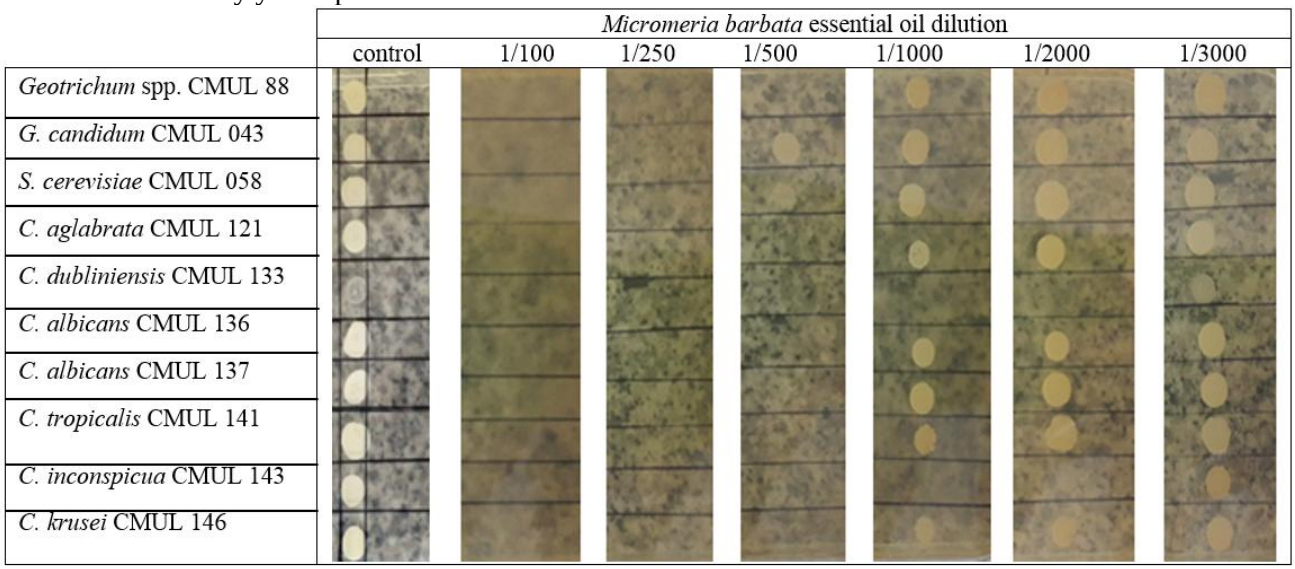



**Figure 2:** Effect of *M. barbata* essential oil on the growth of different yeast species. The growth was performed on Sabouraud agar supplemented by different dilution of the essential oil. This method allows the assessment of the resistance of many yeast species under the same condition of the added essential oil.




The results showed also that *M. barbata* essential oil inhibit totally (100%) the growth of all the mould tested strains until the dilution of 1/500 (Table 3). However, the growth inhibition activity of the essential oil decreased with the increase of the dilution. At 1/1000 a 30% inhibition of the growth was measured for the following fungi: *A. flavus, A. fumigatus, A. versicolor* and *S. aurantiacum* (Table 3). The percentage of growth inhibition exceeded 40% for *S. boydii, Fusarium* spp. and *A. ochraceus,* while in case of A. *fumigates* and *S. dehoogii* the percentage was more than 60% at a dilution of 1/1000 of *M. barbata* essential oil.

**Table 2:** Effect of *M. barbata* essential oil on the studied dermatophytes species, the results represent the inhibition percentage of the growth of the different strains.

| | *Inhibition % | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *M. canis* | *M. audouinii* | *T. mentagrophytes* | *T. schoenleinii* | *T. rubrum* | *T. verrucosum* | *T. tonsurans* | *T. violaceum* | *E. floccosum* |
| Control + | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 1\100 | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| 1\250 | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| 1\500 | 82.1% | 100% | 100% | 20% | 100% | 100% | 100% | 100% | 100% |
| 1\1000 | 10.2% | 40.5% | 5.2% | 8.0% | 65.2% | 46.7% | 100% | 100% | 100% |
| 1\2000 | 8.9% | 28.6% | 0.0% | 0.0% | 52.2% | 26.7% | 56.5% | 23.1% | 20.0% |
| 1\3000 | 2.6% | 9.5% | 0.0% | 0.0% | 26.1% | 26.7% | 26.1% | 8.7% | 0.0% |
| 1\5000 | 0.0% | 4.8% | 0.0% | 0.0% | 8.7% | 13.3% | 8.7% | 0.0% | 0.0% |

**Table 3:** Effect of *M. barbata* essential oil on the studied molds species, the results represent the inhibition percentage of the growth of the different strains.

| | **Inhibition % | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *A.nidulans* | *A. flavus* | *A. fumigatus* | *A. versicolor* | *A. ochraceus* | *Fusarium* spp. | *S. aurantiacum* | *S. dehoogi* | *S. boydii* | *A. coryntbifera* |
| Control + | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 1\100 | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| 1\250 | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| 1\500 | 100 % | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |
| 1\1000 | 35.4% | 32.0% | 62.8% | 33.3% | 44.0% | 41.4% | 37.5% | 66.6% | 45.2% | 43.2% |
| 1\2000 | 2.4% | 10.0% | 37.1% | 26.7% | 24.0% | 24.1% | 10.0% | 15.1% | 11.9% | 24.3% |
| 1\3000 | 2.4% | 4.0% | 2.8% | 0.0% | 24.0% | 6.9% | 5.0% | 9.1% | 9.5% | 10.8% |
| 1\5000 | 2.4% | 4.0% | 0.0 % | 0.0% | 24.0% | 0.0% | 0.0% | 9.1% | 4.8% | 8.1% |

***Antimicrobial effect of M. barbata essential oil against yeast strains***

The Table 4 shows that *M. barbata* essential, at a dilution of 1/250, was able to inhibit completely (100%) the growth of the tested yeast strains. The results show also that the growth inhibition activity of the *M. barbata* essential oil decreased with the increase of the dilution. The 1/500 dilution of the essential oil was able to inhibit only the growth of *C. krusei* CMUL 146 resistant to fluconazole, *C. albicans* CMUL 136 resistant to fluconazole itraconazole and ketoconazole, *C. albicans* CMUL 137 resistant to ketoconazole and *Rhodotorula* spp. CMUL 051 resistant to itraconazole , fluconazole and miconazole. *C. dubliniensis* CMUL 133, *C. albicans* CMUL 136 resistant to itraconazole, ketoconazole and fluconazole, *G. candidum* CMUL 043 resistant to miconazole and fluconazole, *S. cervisiae* CMUL 058, *C. parapsilosis* CMUL 154, *C. krusei* CMUL 146 resistant to fluconazole, *C. glabrata* ATCC 15126, *C. tropicalis* ATCC 13803, *C. guilliermondii* ATCC 62600, *R. rubra* CMUL 157 resistant to fluconazole, *Geotrichum* spp. CMUL 88 and *C. neoformans* CMUL158.

At a dilution of 1/1000 *M. barbata* essential oil completely inhibit the growth of *Rhodotorula* spp CMUL 051, *S. cervisiae* CMUL 058, *R. rubra* CMUL 157 and *Geotrichum* spp. CMUL 88. However, at a dilution of 1/2000 a growth inhibition was measured only for *Rhodotorula* spp. CMUL 051 *R. rubra* CMUL 157, and *Geotrichum* spp. CMUL 88. In addition, at a dilution of 1/3000 and 1/5000 the growth inhibition was measured only for *R. rubra* CMUL 157.

## 4. Discussion

These results showed that the essential oils of *M. barbata* have a high fungistatic activity on all the tested fungal species. Numerous studies carried out on the assessment of the antimicrobial activity (bacteria and fungi) of certain species of *Micromeria* have reported that the oils of these species had a significant antimicrobial activity [16, 26, 32-37].

To our knowledge there is few publications on the antifungal activity of the endemic Lebanese *Micromeria* species, and there are only two publication available which reported a significant antimicrobial activity on *C. albicans* [38, 39]. The results obtained in our study clearly indicate the effectiveness of *M. barbata* essential oil even at high dilution (1/1000) towards molds, dermatophytes fungi and yeasts.

The strains used within this study might be involved in opportunistic infections such as molds or might be involved in human parasitical infections such as dermatophytes group which require a long time treatment, in addition of the issues of resistant strains.

Most of the tested yeast strains in this study were isolated from patients and some of them had a resistance profile to many antifungal drugs. The significant effectiveness of *M. barbata* essential oil on the resistant strains even at high dilution of the essential oil shows that this oil may be an alternative treatment for these infections. In addition, the method used in this study is of importance since it's allowed to assess the antimicrobial effect of the essential oil against a high number of strains using the same Petri dish, in addition of the smoothness, rapidness and accuracy of the method. The observed antifungal activity can be attributed to the presence of some components such as carvacrol, α-terpinly acetate, cymene, thymol, pinene, linalool which are already known to exhibit antimicrobial activity [40-42]. A number of scientific investigations have highlighted the importance and the contribution of many plant families i.e. *Asteraceae*, *Liliaceae*, *Apocynaceae*, *Solanaceae*, *Caesalpinaceae*, *Rutaceae*, *Piperaceae*, and *Sapotaceae* used as medicinal plants [43].

Essential oils are becoming increasingly popular to be used for a wide variety of purposes, including aroma therapeutics and alternative natural medicines. During the last few years, due to the increasing development of drug resistance to antifungal agents in human dermatophytic fungi, demand for searching novel antifungal agents is being increased. The plant essential oils as natural substances could represent a potential source of new antifungal agent. Indeed, a number of studies carried out showed the potential application of essential oils as antimicrobial agents [25].

Several *in vitro* studies have been published confirming the effect of essential oil and their major compounds on plant and human pathogenic fungi. A screening assay of 72 essential oil against *Trichophyton mentagrophyte*s, using vapor phase test, was carried out by Inouye *et al.* [44]. The most active oils were *Origanum vulgare*, *Thymus serpyllum*, *Eugenia caryophyllata*, *Cymbopogon nardus*, *Pelargonium roseum*, *Lindera umbellata*, *Aniba rosaeodora*, *Thymus vulgaris*, *Lavandula latifolia*, *L. angustifolia* and *Melaleucaalterni folia*. The essential oils and their components have been used broadly against molds. The essentials oils extracts from many plants such as basil, citrus, fennel, lemon grass, oregano, rosemary and thyme have shown their considerable antifungal activity against the wide range of fungal pathogens [45]. Essential oils have been known to possess antimicrobial activity by their action through the disruption of the cell membrane [46, 47].

**Table 4:** Effect of *M. barbata* essential oil on the studied yeast species, the results represent the inhibition (−) or not (+) of the growth of the different strains.

| **Yeast name** | **Control** | **1/100** | **1/250** | **1/500** | **1/1000** | **1/2000** | **1/3000** | **1\5000** |
|---|---|---|---|---|---|---|---|---|
| *C. tropicalis* CMUL 141 | + | - | - | + | + | + | + | + |
| *C. inconspicua* CMUL 143 | + | - | - | + | + | + | + | + |
| *C. krusei* CMUL 146 | + | - | - | - | + | + | + | + |
| *C. albicans* CMUL 137 | + | - | - | - | + | + | + | + |
| *Rhodotorula* spp. CMUL 051 | + | - | - | - | - | - | + | + |
| *C. dubliniensis* CMUL 133 | + | - | - | - | + | + | + | + |
| *C. albicans* CMUL 136 | + | - | - | - | + | + | + | + |
| *G. candidum* CMUL 043 | + | - | - | - | + | + | + | + |
| *S. cervisiae* CMUL 058 | + | - | - | - | - | - | + | + |
| *C. parapsilosis* CMUL 154 | + | - | - | - | + | + | + | + |
| *C. krusei* ATCC 34135 | + | - | - | - | + | + | + | + |
| *C. albicans* ATCC 10231 | + | - | - | - | + | + | + | + |
| *C. glabrata* ATCC 15126 | + | - | - | - | + | + | + | + |
| *C. tropicalis* ATCC 13803 | + | - | - | - | + | + | + | + |
| *C. guilliermondii* ATCC 6260 | + | - | - | - | + | + | + | + |
| *R. rubra* CMUL 157 | + | - | - | - | - | - | - | - |
| *Geotrichum* spp. | + | - | - | - | - | - | + | + |
| *C. neoformans* | + | - | - | - | + | + | + | + |
| *S. cerevisiae* | + | - | - | - | + | + | + | + |
| *C. glabrata* | + | - | - | - | + | + | + | + |

Regarding the few studies on this matter, *Candida* spp. and *Aspergillus* spp. have been the species mostly used [48, 49] and, therefore, very little information is available on dermatophytes. Pinto *et al.* evaluated the ergosterol content of *T. rubrum* and showed that 0.08 µL/mL of *Thymus pulegioides* oil was able to reduce ergosterol content around 70% [50].

The essential oils have the ability to penetrate and disrupt the fungal cell wall and cytoplasmic membranes, permeablise them and finally damage mitochondrial membranes. The changes in electron flow through the electron transport system inside the mitochondria damage the lipids, proteins and nucleic acid contents [51] of the fungal cells. The essential oils could also hassle the depolarization of the mitochondrial membranes and decreasing the membrane potential, affect $Ca^{2+}$ and other ion channels, reduce the pH and also affect the proton pump and ATP pool. The changes in membranes fluidity resulted into the leakage of radicals, cytochrome C, calcium ions and proteins. Thus, permeabilization of outer and inner mitochondrial membranes leads to cell death by apoptosis and necrosis [52].

y phenolic terpenes such as carvacrol and thymol. These compounds proved to be able to attack cell walls and membranes, affecting the permeability and release of intracellular constituents, as well as several invasive targets, allowing all together inhibition of fungal infection [53]. Many essential oil have also shown fungicidal activity against dermatophyte strains [54-59]. Overall it seems that the antifungal activity of essential oil is not due to a single mechanism of action but may result from the effect of different compounds on several cell targets. Moreover, many essential oils such as those of Clove, Geranium, Lemon balm and Citronella, showed strong effect on the cell wall and membrane of *C. albicans* [60].

## Conclusion

The results obtained in this study provided evidence that the essential oil of *M. barbata* is potentially a rich source of antifungal agents against yeast and filamentous fungi. Hence, the oil used in this study may be useful as an alternative of antifungal agent for the treatment of many fungal and nosocomial infections. More pharmacological investigations are necessary. The method used within this study is of importance since it's

allowed to assess the antimicrobial effect of the essential oil against a high number of strains with the same Petri dish in addition of the smoothness, rapidness and accuracy of this method.

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