EFFECT OF *OCIMUM* ESSENTIAL OIL ON MOLD ISOLATED FROM VIETNAMESE SWIMMERS

Duc-Huy Pham[1], Bac V.G Nguyen[1]

ABSTRACT

Introduction: Mold contamination poses a significant threat to various sectors, including food safety, infrastructure, and human health. The increasing resistance of fungi to synthetic antifungal agents necessitates the exploration of natural alternatives. *Ocimum gratissimum* (L.) essential oil has shown promising antifungal activity. **Objective:** This study investigated the antifungal properties of *Ocimum gratissimum* (L.) essential oil on various mold strains. **Subjects and methods**: The antifungal effectiveness of *Ocimum gratissimum* (L.) essential oil was evaluated against pathogenic mold strains collected from clinical samples, utilizing a broth dilution technique. **Results:** *Ocimum gratissimum* essential oil displayed excellent *in vitro* antifungal efficacy against the tested pathogenic mold strains. A concentration of 0.6 µL/mL of essential oil completely inhibited 100% fungal isolates. Furthermore, a two-fold dilution (0.3 µL/mL) of *Ocimum gratissimum* oil was still inhibit Phaeohyphomycetes group. **Conclusion:** In conclusion, *O. gratissimum* essential oil demonstrates significant potential for development into novel formulations for the treatment of mold-related diseases.

Keywords: Ocimum gratissimum (L.) *essential oil, mold*

I. INTRODUCTION

Mold contamination is a widespread problem affecting various sectors, including agriculture, food storage, healthcare, and indoor environments. Fungi such as *Aspergillus*, *Penicillium*, and *Fusarium* are known for causing food spoilage, structural damage, and adverse health effects, including allergic reactions and respiratory diseases. Additionally, some mold species produce mycotoxins, which can lead to severe toxicological consequences, including immunosuppression and carcinogenesis [1]. Traditional antifungal agents such as synthetic fungicides and chemical preservatives have been commonly used for mold control. However, concerns regarding fungal resistance, environmental toxicity, and health hazards have led to an increased demand for natural and eco-friendly alternatives [2].

Ocimum essential oil, extracted from plants of the *Ocimum* genus (e.g., *Ocimum basilicum*, *Ocimum sanctum*, and *Ocimum gratissimum*), has gained attention due to its strong antifungal properties. Rich in biologically active compounds such as eugenol, linalool, and methyl chavicol, this essential oil has demonstrated inhibitory effects against a wide range of mold species [3]. These bioactive constituents exhibit multiple antifungal mechanisms, including cell membrane disruption, inhibition of ergosterol biosynthesis, oxidative stress induction, and prevention of biofilm formation. Given these properties, Ocimum essential oil presents a promising natural alternative for mold prevention and control in various applications.

[1] *Faculty of Pharmacy, University of Medicine and Pharmacy at Hochiminh City*
Responsible person: Bac V.G Nguyen
Email: nguyenvugiangbac@ump.edu.vn
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One of the primary antifungal mechanisms of Ocimum essential oil is the disruption of fungal cell membranes. Eugenol and linalool, the major constituents, interact with membrane lipids, increasing permeability and leading to the leakage of vital intracellular contents such as potassium ions, ATP, and proteins [4]. This membrane disruption severely compromises the structural integrity and viability of mold cells. Research on *Aspergillus flavus* has shown that exposure to Ocimum oil leads to significant morphological alterations, including membrane rupture, cytoplasmic disintegration, and cell death [5].

Another crucial mode of action is the inhibition of ergosterol biosynthesis, a key sterol responsible for maintaining fungal cell membrane integrity. Ergosterol plays a vital role in fungal growth, and its inhibition results in structural instability and impaired cellular function [6]. Compounds such as methyl chavicol present in Ocimum essential oil interfere with ergosterol synthesis pathways, making fungal membranes more susceptible to osmotic stress and external antifungal agents [7]. This effect is particularly useful in controlling molds that exhibit resistance to conventional antifungal drugs.

In addition to disrupting cell membranes, Ocimum essential oil also induces oxidative stress in mold cells. The production of reactive oxygen species (ROS) leads to lipid peroxidation, protein oxidation, and mitochondrial dysfunction, ultimately triggering programmed cell death (apoptosis) [8].Study on *Alternaria tenuissima* has demonstrated that exposure to essential oil significantly increases ROS levels, resulting in cellular damage and fungal inhibition [9].. This oxidative stress mechanism enhances the overall antifungal efficacy of Ocimum essential oil.

Beyond direct fungicidal effects, Ocimum essential oil also plays a crucial role in preventing mold spore germination and biofilm formation. Mold spores are responsible for fungal colonization and proliferation, while biofilms provide protection against antifungal agents, making eradication more difficult. Research has indicated that Ocimum essential oil effectively reduces spore germination rates and inhibits biofilm formation in pathogenic mold species such as *Aspergillus niger* and *Penicillium spp*. [3]. This preventive action is particularly useful in food preservation and medical applications where fungal contamination needs to be controlled before it spreads.

Due to its strong antifungal properties, Ocimum essential oil has potential applications across multiple industries. In food preservation, Ocimum oil has been tested as a natural food preservative to prevent fungal contamination in grains, fruits, and dairy products [10]. In agriculture, it serves as a biopesticide against fungal pathogens like *Fusarium* and *Alternaria*, reducing crop losses and minimizing the use of synthetic fungicides [4]. Additionally, in pharmaceutical and medical applications, Ocimum oil is being explored as a natural antifungal agent for treating skin infections and respiratory diseases caused by mold exposure [11]. Furthermore, it can be used in environmental settings as an air purifier or natural disinfectant to reduce mold spores in humid indoor environments.

In conclusion, Ocimum essential oil is a promising natural antifungal agent with multiple mechanisms of action against mold, including cell membrane disruption, ergosterol biosynthesis inhibition, oxidative stress induction, and biofilm prevention. Its broad-spectrum antifungal activity, low toxicity, and eco-friendly nature make it a viable alternative to synthetic antifungal agents. Future research should focus on optimizing formulation stability, exploring synergistic effects with other natural compounds, and conducting clinical trials to enhance its commercial application in mold prevention and control.

II. MATERIAL AND METHODS

The *Ocimum gratissimum* essential oil, with an eugenol content of 75.52% (w/w), was procured from Sarona company.

339 fungal strains utilized in this study were isolated from 109 swimmers diagnosed with otomycosis.

Quantification of Fungal Spores with a Neubauer Hemocytometer [12]:

To quantify fungal spores, we first activated mold cultures on PDA and incubated them at room temperature for five days. Following incubation, a suspension of the fungal spores was created in a physiological saline solution (0.85% NaCl, 0.05% Tween 80) and vortexed briefly. Next, a Neubauer hemocytometer was used to determine spore density. Specifically, a 10 µL aliquot of the suspension was loaded onto the chamber. After a 1-2 minute settling period, the spores were counted in five large squares (four corners and the center), with only those spores touching the top and left borders of these squares included in the count. Finally, the spore concentration was adjusted to be within the range of 1 to 5 x 10^6 CFU/mL.

Peparing stock solution:

We prepared stock solutions of *Ocimum gratissimum* essential oil at a 10% (w/w) concentration in dimethyl sulfoxide (DMSO). We then serially diluted these stock solutions in a suitable solvent to achieve the desired test concentrations for subsequent assays.

Determination of Minimum Inhibitory Concentration (MIC) by Agar Dilution Method

We determined the minimum inhibitory concentration (MIC) using the agar dilution method, adapted from Karaca [13]. First, we prepared two-fold serial dilutions of the acetic acid and *Ocimum gratissimum* essential oil stock solutions in 1.5 mL eppendorf tubes, creating a series of five decreasing concentrations. These dilutions were then further diluted 1:10 in molten potato dextrose agar (PDA) to achieve the final test concentrations, with the highest concentrations being 1.2 µL/mL for *Ocimum gratissimum* essential oil. The PDA containing each test concentration was poured into sterile Petri dishes. After the agar solidified and the surface dried for 15 minutes, we spotted 2 µL of each fungal suspension (prepared as described previously) onto the agar surface. The plates were incubated at room temperature for 3-5 days, and the MIC was defined as the lowest concentration of the test substance that visibly inhibited fungal growth. Each experiment was performed in triplicate.

III. RESULT

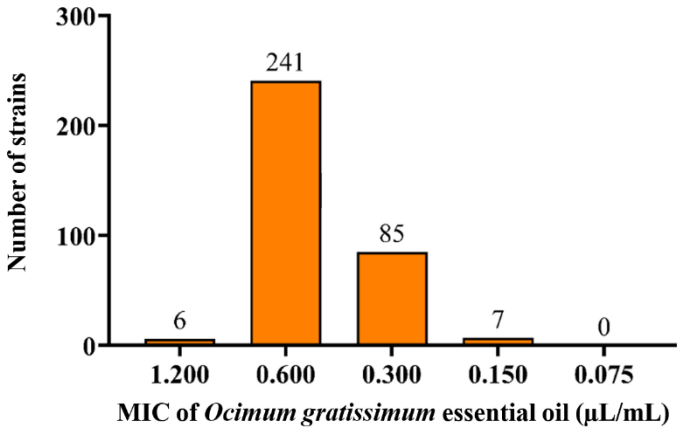

Figure 11. **Minimum inhibitory concentration of *Ocimum gratissimum* essential oil against isolated fungal strains**

Figure 1 illustrates the *in vitro* antifungal activity of *Ocimum gratissimum* essential oil against mold strains isolated from clinical specimens. The essential oil exhibited a minimum inhibitory concentration (MIC) of 0.6 µL/mL against the majority of isolates (n = 241, representing approximately 71% of the 339 total isolates). A substantial number of isolates (n = 85) were also inhibited at an MIC of 0.3 µL/mL. Fewer isolates showed sensitivity at MICs of 1.2 µL/mL (n = 6) and 0.15 µL/mL (n = 7). No inhibition was observed at the lowest tested concentration of 0.075 µL/mL.

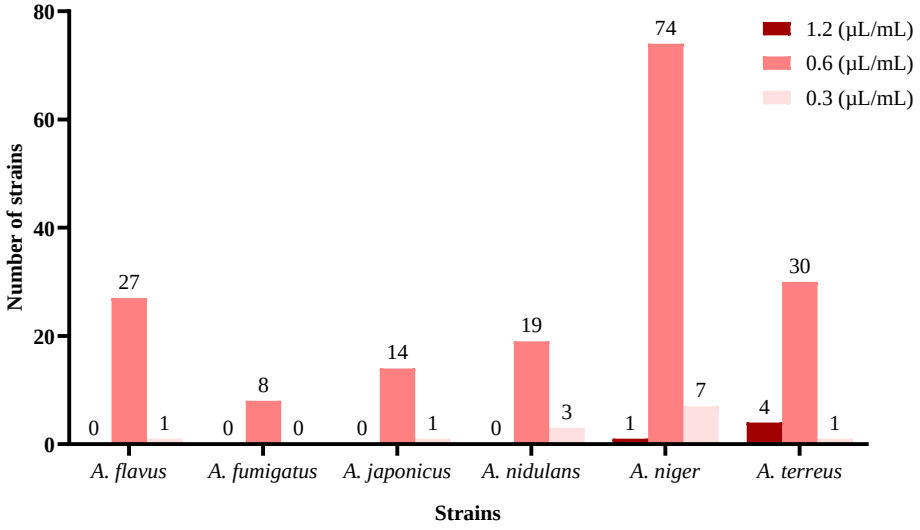

Figure 12. **Antifungal activity of *Ocimum gratissimum* essential oil on *Aspergillus* sp.**

Figure 2 presents the distribution of minimun inhibitory concentrations (MIC) of *Ocimum gratissimum* essential oil on six *Aspergillus* species (*A. flavus*, *A. fumigatus*, *A. japonicus*, *A. nidulans*, *A. niger*, and *A. terreus*), as indicated by the distribution of minimum inhibitory concentrations (MICs). The results indicate that a concentration of 0.6 µL/mL effectively inhibits the growth of all isolated *Aspergillus* species. However, four strains of *A. terreus* exhibited a MIC of 1.2 µL/mL.

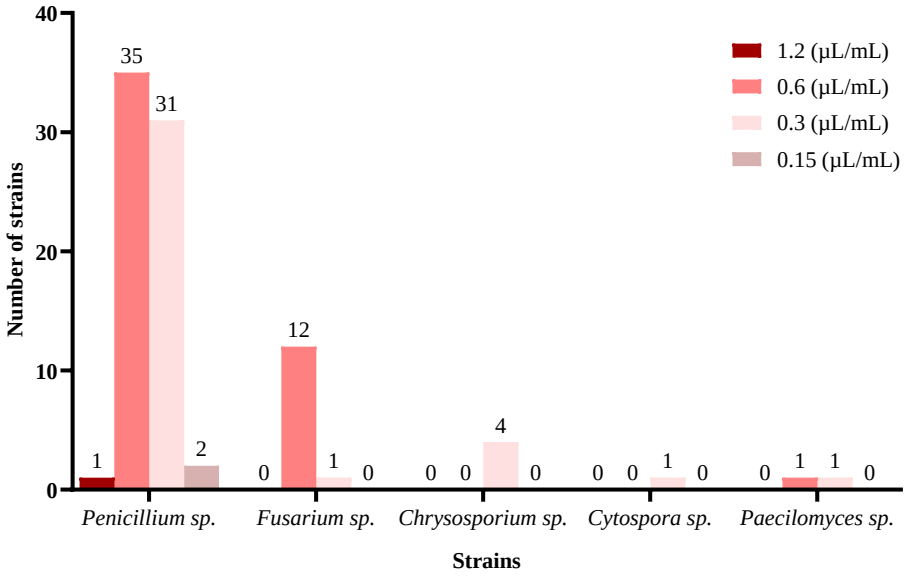

Figure 13. **Antifungal activity of *Ocimum gratissimum* essential oil on *Hyalohyphomycetes* group**

Figure 3 illustrates the antifungal activity of *Ocimum gratissimum* essential oil on the other *Hyalohyphomycetes* group. Penicillium sp. was the most prevalent genus isolated (n = 69). Penicillium sp. was the most frequently isolated genus (n = 69), with the majority of strains exhibiting MICs of either 0.6 µL/mL (n = 35, 50.72%) or 0.3 µL/mL (n = 31, 44.93%). All Fusarium sp. isolates (n = 13) demonstrated an MIC of 0.6 µL/mL. Additionally, a concentration of 0.3 µL/mL of Ocimum gratissimum essential oil represented the MIC for all isolated strains of Chrysosporium sp. (n = 4), Paecilomyces sp. (n = 1), and Cytospora sp. (n = 1).

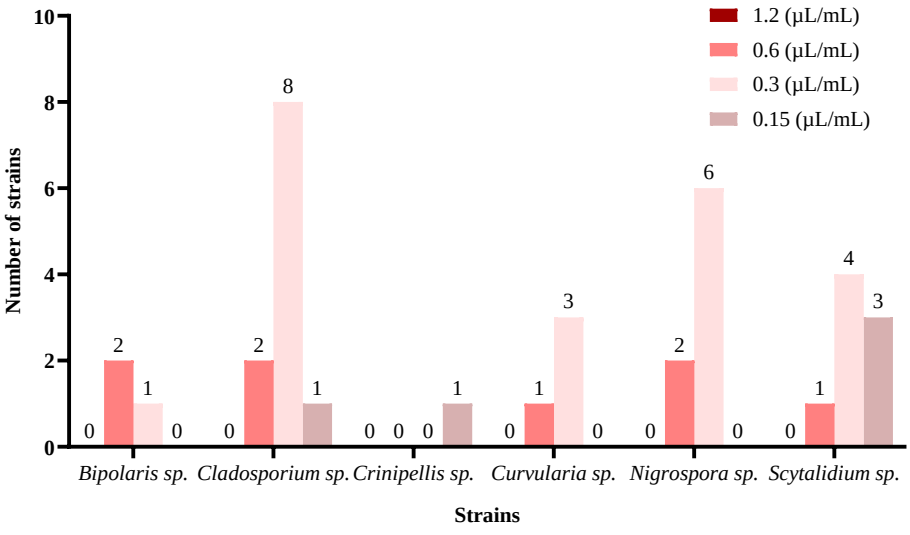

Figure 14. **Antifungal activity of *Ocimum gratissimum* essential oil on *Phaeohyphomycetes* group**

The analysis of the minimum inhibitory concentration (MIC) of *Ocimum gratissimum* essential oil against the *Phaeohyphomycetes* fungal group, as shown in Figure 4, reveals that strains belonging to this group exhibit lower MIC values compared to the *Hyalohyphomycetes* group (Figure 2 and Figure 3). Specifically, the isolated strains demonstrated MIC values ranging from 0.3 µL/mL to 0.15 µL/mL.

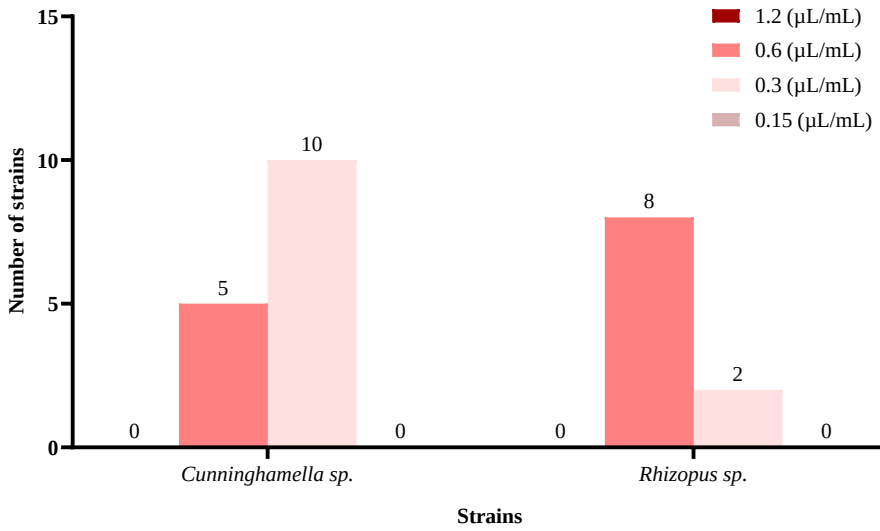

Figure 15. **Antifungal activity of *Ocimum gratissimum* essential oil on *Zygomycetes* group**

Figure 5 illustrates the antifungal activity of *Ocimum gratissimum* essential oil against isolated Zygomycetes. The results indicate that *Rhizopus* sp. (n = 8) exhibited a minimum inhibitory concentration (MIC) of 0.6 µL/mL, whereas *Cunninghamella* sp. (n = 10) were inhibited at a lower concentration of 0.3 µL/mL. These findings demonstrate the potent activity of *Ocimum gratissimum* essential oil against this fungal group.

IV. DISCUSSION

A concentration of 0.6 µL/mL exhibited activity against all fungal strains isolated from external auditory canal wet samples. The growth of isolates belonging to the genera *Aspergillus* and *Penicillium* was inhibited at this concentration (0.6 µL/mL). Notably, *A. niger* and *A. terreus*, species implicated as etiological agents of otomycosis, were susceptible at this concentration (0.6 µL/mL). Furthermore, essential oil of *Ocimum gratissimum* also demonstrated potent activity against strains belonging to the *Phaeohyphomycetes* and *Zygomycetes* groups. These finding align with previous research on the antifungal properties of *Ocimum gratissimum* essential oil. Bhanu Prakash (2011) reported a similar minimum inhibitory concentration (MIC) of 0.7 µL/mL [14]. Adjou et al. (2013) demonstrated the efficacy of *Ocimum gratissimum* essential oil against peanut-contaminating fungi, with *A. flavus* and *A. parasiticus* inhibited at 7.5 µL/mL, and *A. ochraceus* and *F. oxysporium* at 5.5 µL/mL [15]. Beyond antifungal activity, *Ocimum gratissimum* essential oil also possesses antibacterial properties; Adebolu (2005) observed complete inhibition of

Staphylococcus aureus growth at a concentration of 0.1% (w/w) [16]. The primary bioactive component of *Ocimum gratissimum* essential oil, eugenol, contributes to its suitability for topical application in the external ear. Eugenol's higher density than water and non-volatile nature at room temperature allow for sustained contact with the affected area, potentially enhancing its therapeutic efficacy [17].

V. CONCLUSION

In conclusion, *Ocimum gratissimum* essential oil holds significant promise for the development of natural product-based formulations for the treatment of otomycosis, owing to its potent antifungal activity.

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