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Original Research Article

Harnessing Herbal Strength: Exploring the Synergy between Origanum Vulgare Essential Oil and Fluconazole in Combating Candida Glabrata

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Abstract: ***Introduction*:** Fungal infections caused by Candida glabrata have significantly increased in recent years, particularly in hospital settings, posing a serious public health issue due to their growing resistance to conventional antifungals, especially azoles. In response, combining conventional antifungal agents with natural compounds like essential oils represents an innovative and promising therapeutic strategy. ***Materials and Methods*:** This study aimed to evaluate the antifungal activity of fluconazole and Origanum vulgare essential oil, both individually and in combination, against a clinical strain of Candida glabrata isolated from a urinary sample at the Hassan II Regional Hospital in Agadir. The study employed two complementary approaches: the disk diffusion method and liquid microdilution, allowing for the determination of inhibition zones and minimum inhibitory concentrations (MIC) of each agent. ***Results*:** The results from the diffusion method indicated that fluconazole exhibited moderate activity with an inhibition zone of 29.5 ± 0.5 mm and an MIC of 8 µg/mL, while O. vulgare essential oil demonstrated marked antifungal activity, with an inhibition zone of 62 ± 0.5 mm and an MIC of 0.0435 mg/mL. The combination of both agents resulted in an increased inhibition zone and a significant reduction in their respective MICs, with a Fractional Inhibitory Concentration Index (FICI) of 0.5, indicating a synergistic effect between fluconazole and the essential oil. This combination resulted in a fourfold enhancement of fluconazole's antifungal activity. ***Conclusion*:** In conclusion, these findings suggest that Origanum vulgare essential oil could enhance the effectiveness of fluconazole against Candida glabrata, opening interesting perspectives for the development of new combined antifungal approaches, particularly in the context of resistance to conventional treatments.

Keywords: Aromatogram, Antifungal, Candida Glabrata, Fluconazole, Essential Oil, Origanum Vulgare, Synergy.

INTRODUCTION

The incidence of systemic fungal infections has significantly increased in recent years, affecting millions of people worldwide (Soulaimani *et al*., 2021). These infections, often opportunistic, primarily impact immunocompromised patients, those hospitalized in intensive care, or individuals with invasive devices (Méan *et al*., 2008). Among the causative agents, Candida glabrata—recently renamed Nakaseomyces glabrata—has raised growing concerns (Takashima & Sugita, 2022). This yeast is now one of the most frequently implicated fungal pathogens in candidemia, with particularly high mortality rates, especially during septicemia (Vahedi *et al*., 2016).

One of the main challenges in managing these infections is the increasing resistance of C. glabrata to conventional antifungals (De la Cruz-Claure *et al*., 2019). This species frequently exhibits acquired resistance to azoles, such as fluconazole, as well as mutations leading to resistance against echinocandins (Vahedi *et al*., 2016). Furthermore, C. glabrata is often classified as having dose-dependent susceptibility (DDS), meaning its inhibition requires higher concentrations of antifungals than normal (Sarah *et al*.,

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2021). This concept is utilized in clinical practice, where dosing is adjusted to higher levels to enhance treatment efficacy. However, increasing the dose is limited by the risk of adverse effects, particularly hepatotoxicity, which restricts its use in certain patients (Spernovasilis & Kofteridis, 2018).

In this context, the search for natural therapeutic alternatives has gained increasing interest. Among these, essential oils (EOs) of plant origin stand out for their natural antimicrobial properties. Several studies have demonstrated their antifungal activity against species of the genus Candida (D'agostino *et al*., 2019). These EOs contain bioactive compounds such as monoterpenes and phenols (carvacrol, thymol, eugenol, cinnamaldehyde), which can disrupt fungal cell membranes or inhibit certain resistance mechanisms (Nazzaro *et al*., 2017). Extracts from plants such as thyme, oregano (Origanum vulgare), citronella (Ezzariga *et al*., 2025), and cinnamon (Tran *et al*., 2020) have shown promising in vitro results.

Moreover, several studies suggest that these essential oils can act synergistically with conventional antifungals, potentially reducing the necessary doses of drugs like fluconazole while maintaining therapeutic efficacy (Di Vito *et al*., 2023). This synergistic potential paves the way for less toxic and potentially more effective combinatorial approaches against resistant strains or DDS (Cid-Chevecich *et al*., 2022).

Our study explores the antifungal activity of Origanum vulgare essential oil on a clinical strain of Candida glabrata. Specifically, this research revolves around the following specific objectives:

- Determine the fluconazole susceptibility profile of our Candida glabrata strain.
- Study the sensitivity of Candida glabrata to Origanum vulgare essential oil.
- Investigate the potential effect of the essential oil/fluconazole combination on the growth of C. glabrata to explore possible antifungal synergy.

MATERIALS AND METHODS

Host Laboratory

This study is a prospective experimental investigation conducted at the Parasitology and Mycology Service of the Medical Laboratory at the Hassan II Regional Hospital in Agadir. The practical work took place from March 24, 2025, to May 27, 2025. This setting was chosen due to its advanced facilities and expertise in mycological research, which are essential for accurately assessing the antifungal activities being studied.

Tested Essential Oil

For our study, we utilized Origanum vulgare essential oil, specifically chemotyped and obtained through steam distillation. This essential oil is 100% pure and natural, extracted from the leaves of the plant with a concentration of 892 mg/mL. It was sourced from the Zaity association, known for its high-quality essential oils, and was stored at 4 °C to maintain its stability and potency.

Table 1: Main active compounds present in Origanum vulgare essential oil as determined by gas chromatography (GC) analysis conducted by an external laboratory from the Zaity association

Main Active Compounds	Concentration (%)
Carvacrol	68.60
Thymol	3.58
p-Cymene	6.10
γ-Terpinene	1.50
α-Terpinene	0.50
Eugenol	0.20
Cinnamaldehyde	0.10
Other Compounds	19.52

Culture Media

The culture media used in this study, presented in the table, were prepared according to the manufacturer's recommended protocol.

Table 2: Culture Media and Their Uses

Culture Media	Usage
Sabouraud Agar with Chloramphenicol	Revivification and determination of MFC
Mueller Hinton Agar supplemented with 2% glucose	Aromatogram and antifungal susceptibility testing
Mueller Hinton Broth supplemented with 2% glucose and 0.2% agar	Microdilution tests

Isolation and Preservation of the Strain

A strain of *Candida glabrata* was isolated from urine samples and preserved in Sabouraud agar with chloramphenicol under sterile conditions.

Revivification and Confirmation of the Strain

The strain was revived by subculturing on Sabouraud agar and incubated at 37 °C for 48 hours. Confirmation of the strain was performed using the API 20C AUX system, which identifies strains based on their fermentation and assimilation profiles.

Characterization of Origanum vulgare Essential Oil by FTIR

Fourier Transform Infrared Spectroscopy (FTIR) was used to identify functional groups in the essential oil. The oil was characterized using an Agilent Technologies device, with spectra compared to reference data.

Evaluation of Antifungal Activity

The antifungal activity of fluconazole and Origanum vulgare essential oil, both alone and in combination, was assessed using agar diffusion methods. Fluconazole discs were prepared and tested on Mueller Hinton agar, while essential oil was tested using aromatogram and micro-atmosphere methods.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the essential oil and fluconazole was determined using liquid microdilution methods, with concentrations tested in 96-well plates. The lowest concentration inhibiting fungal growth was recorded.

Determination of Minimum Fungicidal Concentration (MFC)

The MFC was established by culturing samples from wells without visible growth on Sabouraud agar, identifying the lowest concentration that kills 99.9% of the initial inoculum.

Synergy Testing

The synergy between Origanum vulgare essential oil and fluconazole was evaluated using a checkerboard microdilution method. The Fractional Inhibitory Concentration Index (FICI) was calculated to assess the interaction between the two agents.

Characterization of Origanum vulgare Essential Oil by FTIR

FTIR analysis of Origanum vulgare essential oil identified key functional groups, including hydroxyl (OH) groups of phenols, aromatic and aliphatic CH groups, C=C bonds of aromatic cycles, and C-O bonds of alcohols and ethers. The presence of major compounds like carvacrol and thymol was confirmed, aligning with the known chemical composition of the oil.

Evaluation of Antifungal Activity by Agar Diffusion Method

- **Fluconazole:** The antifungal activity of fluconazole showed an inhibition zone of 29.5 ± 0.5 mm, classifying the *Candida glabrata* strain as "sensitive dose-dependent" (S-DD) according to CLSI criteria. This indicates the need for higher doses of fluconazole to inhibit this strain, which may lead to potential side effects.
- **Origanum vulgare Essential Oil**: The essential oil exhibited a significant inhibition zone of 62 ± 0.5 mm, demonstrating strong antifungal activity against *Candida glabrata*. Complete inhibition was also observed using the micro-atmosphere method, attributed to its high phenolic content, particularly carvacrol (68.60%) and thymol (3.58%).
- **Synergy Between Essential Oil and Fluconazole**: The combination of fluconazole and Origanum vulgare essential oil resulted in a significantly larger inhibition zone compared to each agent alone, suggesting a synergistic effect against *Candida glabrata*.

Evaluation of Antifungal Activity by Microdilution: MIC

The MIC for fluconazole was determined to be 8 µg/mL, while the MIC for Origanum vulgare essential oil was 0.0435 mg/mL, indicating that the essential oil is more effective against this strain. The MFC for fluconazole was 32 µg/mL, and for the essential oil, it was 0.174 mg/mL, showing fungicidal effects for both agents.

Synergy Testing:

The checkerboard microdilution method revealed that the combination of fluconazole and essential oil significantly reduced the MICs of both agents, with FICI calculated at 0.5, indicating a synergistic interaction.

DISCUSSION

The results of our study clearly demonstrate the antifungal activity of Origanum vulgare essential oil against Candida glabrata, as well as its synergistic effect with fluconazole. The essential oil exhibited superior activity compared to fluconazole, with a minimum inhibitory concentration (MIC) of 0.0435 mg/mL versus 8 µg/mL for fluconazole. This strong activity can be attributed to the richness of the oil in phenolic compounds, particularly carvacrol (68.60%) and thymol (3.58%), which are known for their antifungal properties. Several studies have already shown the antifungal

activity of essential oils rich in carvacrol against various species of Candida (D'agostino *et al*., 2019; Nazzaro *et al*., 2017).

The synergistic effect observed between Origanum vulgare essential oil and fluconazole is particularly interesting. The FICI index of 0.5 indicates a clear synergy between the two agents, allowing for a fourfold reduction in the MIC of fluconazole. This synergy could be explained by several mechanisms. The phenolic compounds in the essential oil, such as carvacrol, may disrupt the fungal cell membrane, increasing its permeability and thereby facilitating the entry of fluconazole into the cell (Nazzaro *et al*., 2017). Additionally, carvacrol may inhibit efflux pumps, which are one of the main resistance mechanisms of Candida glabrata to fluconazole (Lee *et al*., 2021). By inhibiting these pumps, carvacrol could allow fluconazole to reach higher intracellular concentrations, thereby enhancing its efficacy.

Our strain of Candida glabrata has been classified as "dose-dependent susceptible" (S-DD) to fluconazole, which is characteristic of this species. This reduced sensitivity to azoles poses a significant clinical problem, as it necessitates the use of higher doses of fluconazole, thereby increasing the risk of side effects such as hepatotoxicity (Spernovasilis & Kofteridis, 2018). The combined use of fluconazole and Origanum vulgare essential oil could help reduce the dose of fluconazole needed to inhibit the growth of Candida glabrata, thus minimizing side effects while maintaining therapeutic efficacy.

Several studies have already demonstrated the synergistic effect between essential oils and conventional antifungals against various species of Candida. For example, Di Vito *et al*., (2023) showed that Thymus vulgaris essential oil acts synergistically with fluconazole against Candida albicans. Similarly, Cid-Chevecich *et al*., (2022) reported a synergistic effect between Cinnamomum zeylanicum essential oil and fluconazole against Candida tropicalis. Our study confirms these findings and extends them to Candida glabrata, a species particularly resistant to conventional antifungals.

The results of our study open interesting perspectives for the development of new therapeutic approaches against Candida glabrata infections. The combined use of conventional antifungals and essential oils could help overcome antifungal resistance, reduce necessary doses, and minimize side effects. However, further studies are needed to evaluate the toxicity of these combinations on human cells and to determine their in vivo efficacy.

CONCLUSION

Our study demonstrated the antifungal activity of Origanum vulgare essential oil against Candida glabrata, as well as its synergistic effect with fluconazole. The essential oil showed superior activity compared to fluconazole, with a MIC of 0.0435 mg/mL versus 8 µg/mL for fluconazole. The combination of the two agents reduced the MIC of fluconazole by a factor of four, with an FICI index of 0.5 indicating a clear synergistic effect.

These results suggest that Origanum vulgare essential oil could enhance the efficacy of fluconazole against Candida glabrata and open interesting perspectives for the development of new combined antifungal approaches, particularly in the context of resistance to conventional treatments. The combined use of conventional antifungals and essential oils could help overcome antifungal resistance, reduce necessary doses, and minimize side effects. However, further studies are needed to evaluate the toxicity of these combinations on human cells and to determine their in vivo efficacy. Additionally, in-depth mechanistic studies would be necessary to precisely elucidate the mechanisms responsible for the observed synergistic effect.

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