**Original Article**

# *In vitro* activity of econazole in comparison with three common antifungal agents against clinical *Candida* strains isolated from superficial infections

Abastabar M[1], Shokohi T[1]*, Rouhi Kord R[2], Badali H[1], Hashemi SJ[3], Ghasemi Z[3], Ghojoghi A[4], Baghi N[2], Abdollahi M[2,5], Hosseinpoor S[2], Rahimi N[2], Seifi Z[2], Gholami S[1], Haghani I[1], Jabari MR[2], Pagheh A[2,4]

[1] Invasive Fungi Research Center (IFRC), Department of Medical Mycology and Parasitology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[2] Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran
[3] Department of Medical Parasitology and Mycology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[4] Toxoplasmosis Research Center, Mazandaran University of Medical Sciences, Sari, Iran
[5] Department of Microbiology and Immunology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

***Corresponding author: Tahereh Shokohi,** Invasive Fungi Research Centere (IFRC)/ Department of Medical Mycology and Parasitology, School of Medicine, Km 18 Khazarabad Road, Iran. Email: shokohi.tahereh@gmail.com

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## Abstract

**Background and Purpose:** *Candida* species are the most common organisms involved in superficial fungal infections, worldwide. Although econazole is among the most frequently used topical formulations for the treatment of candidiasis, no information is available regarding the susceptibility profiles of *Candida* species in Iran.

**Materials and Methods:** *In vitro* susceptibility of 100 clinical *Candida* isolates belonging to 6 species from superficial candidiasis of Iran towards to econazole was compared with three other common antifungal agents including itraconazole, fluconazole, and miconazole. Minimum inhibitory concentrations (MICs) values were analyzed according to the Clinical and Laboratory Standards Institute (CLSI) M38-A3 document. All isolates were previously identified to the species level, using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) on ITS region.

**Results:** The MIC of econazole, itraconazole, miconazole, and fluconazole were within the range of 0.016-16, 0.032-16, 0.016-16, and 0.25-64 µg/ml, respectively. In general, econazole and miconazole were more active against *Candida* isolates, compared to the other two agents.

**Conclusion:** The present study demonstrated that for *Candida albicans* isolates, miconazole and econazole had the best effect, but in non-albicans Candida species, itraconazole and miconazole displayed more activity than other antifungal agents.

**Keywords**: Antifungal, Econazole, Candidiasis

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## Introduction

Superficial mycoses are among the most prevalent fungal infections, worldwide. These infections are caused by various fungi including *Candida* species, dermatophytes, and rarely other pathogens [1]. *Candida albicans* is currently the main causative agent in the majority of superficial candidiasis, followed by non-*Candida albicans* species, *C. tropicalis, C. glabrata, C. parapsilosis,* and *C. krusei* [1].

Over the past decades, there has been a remarkable increase in the number of infections caused by *Candida* species mainly due to the rising number of immunocompromised hosts such as transplant recipients, diabetic patients, and HIV-infected individuals [1, 2]. Oral and/or topical formulations of fluconazole, itraconazole, miconazole, clotrimazole, amphotericin B, and nystatin are usually the treatment of choice for different types of superficial candidiasis.

Azole-based therapy is the preferred treatment option, despite recent reports on the resistance of *Candida* species to these agents as the most prevalent type of antifungal resistance [3, 4]. In fact, widespread use of these antifungal agents for prophylaxis and treatment of *Candida* infections has resulted in the

emergence of resistant *Candida* species [5].

Acquired resistance to fluconazole has been reported in *C. albicans* isolates from patients with advanced AIDS, receiving prolonged azole treatment [6, 7]. As evidences from different recent studies suggest, there has been a shift towards fluconazole resistance in other *Candida* species, particularly *C. glabrata* [8-10]. Generally, the rate of azole resistance among *Candida* species is vague and variable in Iran, with the reported rates ranging from 10% to 85% for fluconazole and 12% to 62% for itraconazole [11-16].

Although a large number of azole-based antifungal agents have been used by this time for the treatment of candidiasis, further researches are required regarding the increasing resistance to these agents in Iran. Econazole is among the most frequently used topical formulations for the treatment of candidiasis [17]. However, econazole has not been used in Iran for the treatment of patients and the susceptibility profiles of different fungi against these agents have not been yet identified.

In view of the aforementioned background, in this study, we aimed to compare the *in vitro* activity of econazole with three common antifungal agents including fluconazole (FLC), itraconazole (ITC), and miconazole (MIC) against 100 clinical *Candida* strains belonging to six different species (i.e., *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. krusei, and C. guilliermondii)* isolated from patients with superficial candidiasis in Iran.

## Material and Methods

### *Fungal isolates*

Clinical strains were isolated from different specimens obtained from patients with superficial candidiasis, referred to the medical mycology laboratory of Razi Hospital in Tehran, Iran during 2014-2015. All clinical isolates were previously identified, using phenotypic criteria and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of internal transcribed spacer (ITS) region of rDNA [18].

In total, 100 *Candida* isolates belonging to six species including *C. albicans* (n=67), *C. glabrata* (n=4), *C. parapsilosis* (n=15), *C. tropicalis* (n=10), *C. krusei* (n=2), and *C. guilliermondii* (n=2) were identified. To ensure purity and viability, the isolates were cultured on Sabouraud dextrose agar (Difco Laboratories, Detroit, MI, USA) and incubated at $35^0$C until use.

### *In vitro susceptibility* testing

The MIC of antifungal agents was determined for *Candida* isolates, using the reference procedure described by the Clinical and Laboratory Standards Institute (CLSI) in accordance with guideline M38-A3 [19]. *C. krusei* (ATCC 6258) and *C. parapsilosis* (ATCC 22019) strains were used as the controls.

The reference powders of fluconazole (Pfizer), itraconazole, econazole, and miconazole (Sigma, St. Louis, MO, USA) were obtained from respective manufacturers. Fluconazole was dissolved in sterile distilled water, while other agents were prepared in 100% dimethyl sulfoxide. All agents were diluted in RPMI 1640 medium (Sigma Chemical Co.), supplemented with L-glutamine, without sodium bicarbonate buffered at pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) (Sigma, Aldrich Chemie) to provide the following concentrations: 0.016–16 mg/ml for econazole, itraconazole, and miconazole and 0.064–64 for fluconazole.

### *Inoculum preparation*

The inoculum of all *Candida* species was prepared from 24 h cultures, grown on Sabouraud dextrose agar (SDA, Difco Laboratories, Detroit, MI, USA) at 35°C. At first, several colonies were picked and suspended in 5 mL of sterile saline. The obtained suspension was vortexed for few seconds by a mixer. The cell densities were measured by a spectrophotometer at a wavelength of 530 nm, and the transmission was adjusted to 75–77%.

The suspension was diluted in RPMI 1640 medium to yield a final inoculum concentration ranged from 0.5-2.5 $\times 10^3$ CFU/ml. MIC was determined after incubation of the 96-well microplates at 35 $^0$C for 24-48 hours as the 80% or more of reduction of growth for all agents compared to the growth rate of the drug-free control well.

### *Data analysis*

MIC values were measured for antifungal agents and presented as geometric mean (GM), MIC range, $MIC_{50}$, and $MIC_{90}$. All the tests were performed in duplicate.

## Results

Table 1 summarizes the *in vitro* susceptibility of 100 isolates from six *Candida* species to fluconazole, itraconazole, econazole, and miconazole. In the present study, $MIC_{50}$, $MIC_{90}$, GM, and MIC range of all *C. albicans* isolates were determined. Since the number of *C. tropicalis, C. parapsilosis, C. glabrata, C. krusei,* and *C. guilliermondii* isolates was insufficient, $MIC_{50}$ and $MIC_{90}$ values were not measured for these species.

The MICs of econazole, itraconazole, miconazole, and fluconazole were within the range of 0.016-16, 0.032-16, 0.016-16, and 0.25-64 μg/ml, respectively. The rates of resistance to fluconazole (MIC≥ 8 μg/ml), itraconazole (MIC≥ 1 μg/ml), and miconazole (MIC≥1 μg/ml) were 57% (n=57), 69% (n=69), and 54% (n=54), respectively. However, the rate of resistance to econazole could not be calculated, given the absence of published interpretive criteria for this agent.

Overall, among *C. albicans* isolates, miconazole and econazole were more active than the two other used agents. As presented in Table 1, $MIC_{50}$ values of miconazole and econazole against *C. albicans* isolates were 4 and 8 μg/ml, while $MIC_{50}$ values of itraconazole and fluconazole were 16 and 64 μg/ml, respectively. Also, GM values of miconazole and econazole against *C. albicans* isolates were 0.75 and 1.16 μg/ml, while GM values for itraconazole and fluconazole were 5.34 and 13.45 μg/ml, respectively.

Based on the findings, miconazole, followed by econazole showed the best activity against *C. albicans* isolates (n=67). However, for all non-*Candida albicans* species (n=33), itraconazole and miconazole displayed better activity, compared to econazole and fluconazole. In addition, itraconazole was more active against *C. tropicalis and C. parapsilosis* isolates, compared to the other three agents.

Considering the MIC values of all agents, *C. albicans* isolates were more resistant than other species (Table 1). Among all isolates, *C. krusei* species was fully resistant to all used azoles, while *C. tropicalis* was the most sensitive species.

## Discussion

Despite the fact that more than 100 fungal species have been identified as important clinical pathogens causing superficial to life-threatening mycoses, infections due to *Aspergillus* and *Candida* species are the most common. Among these infections, superficial candidiasis is generally community-acquired and is considered responsible for remarkable morbidity. These infections are frequently caused by *Candida albicans* and non-*Candida albicans* species such as *C. tropicalis, C. glabrata, C. parapsilosis,* and *C. krusei* [1, 2].

The incidence of infections caused by azole-resistant Candida and non-*Candida albicans* species has increased over the past decades owing to the excessive use of azoles, especially triazoles such as fluconazole [20-22]. Considering the scarcity of available data on the susceptibility profiles of *Candida* species to econazole in Iran, in the present study, *in vitro* activity of 100 clinical *Candida* isolates from six species from patients with superficial candidiasis towards econazole was compared with three common antifungal agents including fluconazole, itraconazole, and miconazole.

In a previous study by Salehei et al. in Iran, the susceptibility patterns of vaginal *Candida* isolates to eight antifungal drugs including clotrimazole, miconazole, itraconazole, fluconazole, ketoconazole, econazole, nystatin, and terbinafine were determined, using disk diffusion method. They found that the highest sensitivity of *C. albicans* to antifungal drugs was observed against miconazole, whereas 43 (81%) isolates were resistant to fluconazole and econazole antifungals [16]. Similarly, Al-Mamari [23] using disk diffusion reported that the highest sensitivity of *C. albicans* was seen against miconazole (95%) whereas 73 isolates (78%) were resistant to fluconazole and econazole antifungals.

**Table 1.** *In vitro* antifungal susceptibilities of 100 clinical *Candida* isolates against four antifungal agents

| Isolate | Antifungal Agent | MIC (µg / ml) | | | | | | | | | | | | | MIC range | MIC 50 | MIC 90 | G mean | Resistant (Number) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.016 | 0.032 | 0.0625 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | | | | | |
| *Candida albicans* (n=67) | ECO | 0 | 1 | 1 | 1 | 2 | 3 | 10 | 10 | 1 | 15 | 23 | | | 0.016-16 | 8 | 16 | 1.16 | - |
| | ITR | 0 | 0 | 0 | 3 | 3 | 2 | 5 | 2 | 3 | 3 | 46 | | | 0.125-16 | 16 | 16 | 5.34 | 57 |
| | MIC | 2 | 0 | 0 | 4 | 1 | 3 | 5 | 6 | 5 | 11 | 30 | | | 0.016-16 | 4 | 16 | 0.75 | 43 |
| | FLU | | | 0 | 0 | 3 | 2 | 4 | 5 | 9 | 1 | 0 | 5 | 38 | 0.25-64 | 64 | 64 | 13.45 | 46 |
| *Candida parapsilosis* (n=15) | ECO | 0 | 0 | 0 | 1 | 1 | 1 | 2 | 5 | 2 | 1 | 2 | | | 0.0625-16 | 2 | - | 1.56 | - |
| | ITR | 0 | 1 | 2 | 0 | 4 | 3 | 1 | 2 | 0 | 0 | 2 | | | 0.032-16 | 0.5 | | 0.45 | 3 |
| | MIC | 1 | 0 | 0 | 1 | 0 | 5 | 3 | 2 | 1 | 1 | 2 | | | 0.016-16 | 1 | | 0.7 | 2 |
| | FLU | | | 0 | 0 | 1 | 2 | 5 | 2 | 2 | 0 | 1 | 1 | 2 | 0.25-64 | 1 | | 1.81 | 2 |
| *Candida tropicalis* (n=10) | ECO | 0 | 0 | 0 | 0 | 0 | 2 | 5 | 0 | 1 | 0 | 2 | | | 0.5-16 | 1 | - | 1.7 | - |
| | ITR | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 1 | 0 | 0 | 1 | | | 0.125-16 | 0. 5 | | 0.46 | 3 |
| | MIC | 0 | 0 | 0 | 0 | 0 | 2 | 4 | 1 | 4 | 0 | 1 | | | 0.5-16 | 1 | | 1.62 | 3 |
| | FLU | | | 0 | 0 | 0 | 0 | 5 | 1 | 1 | 2 | 0 | 0 | 1 | 1-64 | 1 | | 2.82 | 3 |
| *Candida glabrata* (n=4) | ECO | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 1 | | | 2-16 | - | - | 4.75 | - |
| | ITR | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | | | 0.25-16 | - | | 2.37 | 3 |
| | MIC | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 1 | | | 1-16 | - | | 4 | 3 |
| | FLU | | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 4-64 | - | | 26.90 | 2 |
| *C. guilliermondi* (n=2) | ECO | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | | | 2-16 | - | - | 5.65 | - |
| | ITR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | | | 0.32-16 | - | | 5.65 | 2 |
| | MIC | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | | | 0.016-16 | - | | 4 | 1 |
| | FLU | | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0.25-64 | - | | 45.25 | 2 |
| *C. krusei* (n=2) | ECO | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | | | 16 | - | - | 16 | - |
| | ITR | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | | | 2-4 | - | | 2.82 | 2 |
| | MIC | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | | | 16 | - | | 16 | 2 |
| | FLU | | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 32-64 | - | | 45.25 | 2 |
| *All Canddia spp.* (n=100) | ECO | 0 | 1 | 1 | 2 | 3 | 6 | 17 | 18 | 4 | 17 | 31 | | | 0.016-16 | - | - | 3.11 | - |
| | ITR | 0 | 1 | 2 | 3 | 9 | 7 | 8 | 7 | 4 | 4 | 55 | | | 0.032-16 | - | | 2.7 | 69 |
| | MIC | 1 | 0 | 0 | 5 | 1 | 10 | 14 | 9 | 12 | 12 | 36 | | | 0.016-16 | - | | 2.4 | 54 |
| | FLU | 0 | 0 | 0 | 0 | 4 | 4 | 14 | 8 | 13 | 3 | 1 | 9 | 44 | 0.25-64 | - | | 10.43 | 57 |

In the current study, based on the microdilution method, miconazole showed the lowest MIC, while fluconazole exhibited the highest MIC value. Previous studies in Iran, comparing the efficacy of fluconazole and other agents against *Candida* species of superficial infections, have reported inconsistent results [24-26]. In studies by Katiraee et al. [24], Pakshir et al. [25], Badiee et al. [26], and Shokohi et al. [27], resistance of *C. albicans* isolates to fluconazole was estimated at 25.7%, 26%, 10%, and 2.6%, respectively.

On the other hand, in a study by Gross et al. [28], 96.5% and 98% of *C. albicans* isolates were susceptible to fluconazole and itraconazole, respectively; however, we observed a higher rate of fluconazole resistance (46%). In the current study, all *C. krusei* isolates were resistant to the evaluated azoles, whereas in the study by Güzel et al., which evaluated vaginitis isolates [29], resistance rates of 57.1% and 32.1% against fluconazole and itraconazole were reported, respectively; also, all isolates were sensitive to miconazole.

Based on studies by Badiee et al. [26] and El Feky et al. [30], 17.4% and 40% of *C. krusei* isolates were resistant to fluconazole, respectively; conversely, these isolates were susceptible to other azoles. Similar to a study by Shokohi et al. on oropharyngeal lesions isolates [27], we reported *Candida* isolates including *C. albicans* (n=36), *C. glabrata* (n=2), *C. krusei*

(n=2), *C. guilliermondii* (n=1), *C. tropicalis* (n=1), and *C. parapsilosis* (n=1), which were fully resistant to four antifungals.

In agreement with studies by Badiee et al. [26] and El Feky et al., *C. tropicalis* was the most susceptible species to all antifungal agents in the present study. In total, azole resistance was more prevalent in non-*C. albicans* species, particularly *C. glabrata, C. krusei*, and *C. guilliermondii* as compared to *C. albicans*.

In contrast with a study by Salehei et al. in Iran [16], in the present study, two azoles, i.e., econazole and miconazole, typically showed better activity against all the isolates, compared to the other azoles. In fact, 42% of the isolates were susceptible to miconazole, while susceptibility to fluconazole and itraconazole was estimated at 40% and 28%, respectively.

Although econazole is among the most commonly used topical formulations for the treatment of candidiasis and dermatophytosis, this agent has not been routinely used in Iran. Based on our findings, econazole, similar to miconazole, showed suitable activity with a MIC range of 0.016-16 µg/ml. In summary, this study revealed that econazole is more potent than fluconazole and itraconazole against all *Candida* species.

## Conclusion

Based on the findings, it can be concluded that econazole is a suitable alternative choice for treatment of Iranian isolates of candida isolated from superficial infections.

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## Authors' Contributions

M.A. and T.S. designed and supervised the research, M.A., T.S., H.B. and SJ.H. edited the final manuscript and R.R., Z.G., I.H., S.P., A.G., N.B., M.A., S.H., N.R., MR.J., S.G. and Z.S. performed the tests.

## Conflicts of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

## Financial Disclosure

The authors declare no financial interests related to the materials of the study.

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