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Hinokitiol plus fluconazole: potent inhibitory efficacy on *Candida* species resistant to azole

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Abstract

Candida species represent the most prevalent types of yeasts affecting humans and animals. The increase in antifungal resistance has raised the issue of using these medications in tandem to improve treatment outcomes. Guided by the CLSI M27-A3 guideline, this investigation quantified the synergistic potential of hinokitiol and fluconazole against *Candida albicans*, *C. tropicalis*, *C. glabrata*, and *C. krusei*. The primary metrics for this assessment were the minimum inhibitory and fungicidal concentrations (MICs and MFCs), which were established using a broth microdilution methodology. Using data from a checkerboard microdilution test, we calculated the fractional inhibitory concentration indices (FICIs) to evaluate the interaction between hinokitiol and fluconazole. A strong synergistic interaction between hinokitiol and fluconazole was evident in every tested isolate (100%) of fluconazole-resistant *C. albicans* and *C. glabrata*. The corresponding FICI values, falling within ranges of 0.304–0.498 and 0.290–0.492, respectively, confirm this robust synergistic activity. This activity was noted against a background of universal fluconazole resistance, with hinokitiol alone showing MIC values from 0.81 µg/ml (*C. glabrata*) to 6.9 µg/ml (*C. krusei*). FICI scores spanning 0.500 to 0.750 for every *C. krusei* strain (100%) confirm a strictly additive pharmacological interaction between hinokitiol and fluconazole. Additionally, the FICI values for the combination of hinokitiol and fluconazole for *C. tropicalis* strains varied from 0.245 to 0.730, demonstrating a synergistic effect in 13 (86.7%) strains and an additive effect in 2 (13.3%) strains. This study suggests that hinokitiol may help reduce fluconazole dosages and inhibit the development of fluconazole-resistant *Candida* species

Introduction

Candida is identified as a fungus that can take advantage of certain conditions to cause disease. It can lead to fungal infections like candidiasis, which are linked to infections acquired in healthcare settings (Barros *et al*., 2013). Candidiasis is the most common fungal infection affecting people worldwide, particularly those with weakened immune systems (Biswas *et al*., 2007; Zhang *et al*., 2009).

Triazoles are employed to combat several serious fungal infections (Desnos-Ollivier *et al*., 2008). Fluconazole acts as a fungistatic agent, meaning it does not exterminate the fungi. Consequently, this can result in the emergence of resistant strains. Specifically, during prolonged or repeated treatments for ongoing cases of vulvovaginal or oropharyngeal candidiasis in patients with AIDS, fluconazole-resistant strains of *Candida* have frequently been noted (Makarova *et al*., 2003). The successive appearance of resistance mutations in genes, such as MRR1, ERG11, UPC2, and TAC1, in drug-sensitive *Candida* strains indicates that various resistance mechanisms interact and combine in effect (Tobudic *et al*., 2012). This explains why different resistant strains are selected during lengthy fluconazole therapy for those suffering from candidiasis. To tackle these challenges, it is crucial to identify and develop new effective antifungal agents.

Plants synthesize a diverse array of secondary metabolites with significant pharmacological potential. Among these is the tropolone compound hinokitiol (β-thujaplicin; $C_{10}H_{12}O_2$), isolated from the heartwood of Cupressaceous trees. Its broad bioactivity stems from its function as an iron chelator, a mechanism that can induce apoptosis via caspase-3 activation. This foundational property underpins a spectrum of documented effects, ranging from antibacterial and antifungal actions to anti-inflammatory, antioxidant, antitumor, and cell-differentiation-promoting activities (Jayakumar *et al*., 2013; Domon *et al*., 2019). Capitalizing on the established strategy of synergizing natural compounds with conventional antifungals (Carson *et al*., 2002; Shin and Lim, 2004), this study was designed to quantitatively evaluate the efficacy of hinokitiol against both standard and clinical isolates of four *Candida* species: *C. albicans*, *C. glabrata*, *C. tropicalis*, and *C. krusei*. Furthermore, we systematically investigated the *in vitro* interaction between hinokitiol and the azole drug fluconazole to assess its potential for combination therapy.

Materials and Methods

Candida strains and inoculum preparation

The reference *Candida* species (*C. albicans* ATCC 10231, *C. glabrata* ATCC 90030, *C. tropicalis* ATCC 9968, and *C. krusei* ATCC 6258) were obtained from the American Type Culture Collection located in Rockville, Maryland. Clinical samples of *Candida* were collected from the Mycology Research Center at the Faculty of Veterinary Medicine at the University of Tehran in Iran. The yeasts were isolated from the oral and vaginal areas as well as from the urine of patients diagnosed with candidiasis (see Table 1). They were then cultured on sabouraud dextrose agar (SDA) that included chloramphenicol (0.02%, Merck Co., Darmstadt, Germany) and incubated at 37°C for a duration of 2 to 4 days. Certain colonies from these cultures were spread onto a selective differential medium known as CHROMagar Candida (CHROMagarTM Microbiology, Paris, France) in Petri dishes. These cultures were also maintained at 37°C for 48 hours. The identification of yeasts was performed using the RAPID yeast plus system (Remel Inc., Lenexa, Kansas, USA) and confirmed by polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis. For the preparation of the inoculum, yeast cells from a minimum of five colonies, each measuring 1 mm, were diluted in 5 ml of sterile 0.85% saline solution. This yeast suspension was vortexed for 15 seconds. The final suspension was adjusted to yield a yeast concentration of 1×10^6 cells/ml, using a hemacytometer for accurate counting.

Antifungal agents

Hinokitiol and the antifungal agent fluconazole, which was synthesized, were sourced from Sigma-Aldrich located in St. Louis, USA, for the laboratory tests. Hinokitiol was mixed with a 5% solution of dimethyl sulfoxide (DMSO) from Samchun based in Pyeongtaek, South Korea.

Broth microdilution assay

The antifungal efficacy of hinokitiol and fluconazole against *Candida* isolates was quantified via a broth microdilution assay, performed in strict adherence to the CLSI M27-A3 standard (CLSI, 2008). The assay utilized RPMI-1640 medium (Merck, Germany), buffered to pH 7.0 with MOPS and supplemented with glucose and glutamine. Test compounds were prepared in a sterile 96-well plate as follows: each well received a 100 µl aliquot of a fluconazole dilution series (1–1024 µg/ml), a 100 µl aliquot of hinokitiol (0.15–20 µg/ml), and a 100 µl inoculum of the yeast suspension, standardized to 1×10^6

cells/ml. Appropriate positive (drug-free) and negative (yeast-free) controls were included for each strain.

Table 1. Fluconazole-resistant *Candida* isolates used in this study and their sources.

Candida glabrata	Source	*Candida tropicalis*	source	*Candida krusei*	source	*Candida albicans*	source
1	Vagina	1	Oral	1	Oral	1	Oral
2	Vagina	2	Oral	2	Oral	2	Oral
3	Vagina	3	Oral	3	Oral	3	Oral
4	Vagina	4	Oral	4	Oral	4	Oral
5	Vagina	5	Oral	5	Oral	5	Oral
6	Vagina	6	Vagina	6	Oral	6	Vagina
7	Vagina	7	Vagina	7	Vagina	7	Vagina
8	Oral	8	Vagina	8	Vagina	8	Vagina
9	Oral	9	Urine	9	Vagina	9	Vagina
10	Urine	10	Urine	10	Vagina	10	Vagina
11	Urine	11	Urine	11	Vagina	11	Vagina
12	Urine	12	Urine	12	Urine	12	Vagina
13	Urine	13	Urine	13	Urine	13	Urine
14	Urine	14	Urine	14	Urine	14	Urine
ATCC 90030		ATCC 9968		ATCC 6258		ATCC 10231	

All experiments were conducted in duplicate. Following a 48-hour incubation period at 35°C, the minimum inhibitory concentration (MIC) was determined spectrophotometrically using an ELISA reader. To establish the minimum fungicidal concentration (MFC), a 100 µl subculture from each well demonstrating no visible growth was transferred onto SDA. After a further 48-hour incubation at 35°C, the MFC was defined as the lowest compound concentration resulting in no colony formation. Fluconazole susceptibility (susceptible: MIC ≤ 8 µg/ml; dose-dependent: 16–32 µg/ml; resistant: MIC ≥ 64 µg/ml) was interpreted per CLSI (2008) breakpoints. The mode of action for hinokitiol-fungistatic or fungicidal was classified based on the MFC/MIC ratio, applying the established criterion where a ratio ≥ 4 denotes fungistatic and a ratio < 4 denotes fungicidal activity (Sharifzadeh *et al*., 2017).

Checkerboard assay for drug interaction analysis

Thepharmacodynamic interaction between hinokitiol and fluconazole was characterized using a checkerboard titration assay, following the principles outlined by De Castro *et al*(De Castro *et al*., 2015). Briefly, a two-dimensional dilution series of both agents was prepared in a 96-well microtiter plate. Each well was inoculated with 100 µl of a standardized *Candida* suspension (0.5–2.5 × 10^3 CFU/ml) and incubated at 35°C for 48 hours. The MIC for each drug combination was determined visually. The resulting data were quantified by calculating the fractional inhibitory concentration (FIC) for each agent. The FIC is defined as the MIC of the drug in combination divided by its MIC alone. The FIC index (FICI), representing the sum of the individual FICs, was then computed for each combination. The interaction was classified according to the following schema: synergy (FICI ≤ 0.5), additivity (0.5 < FICI ≤ 1.0), indifference (1.0 < FICI ≤ 4.0), or antagonism (FICI > 4.0). The entire experiment was performed in two independent replicates to ensure reproducibility.

Statistics

Findings were illustrated as geometric mean. The values for the various *Candida* groups concerning the examined agents were evaluated using a T-test (Sigma Stat, version 3.5). A p value under 0.05 was considered statistically significant.

Results and discussion

Candida species are a type of fungal pathogens that take advantage of opportunities to infect humans, particularly those with weakened immune systems or who are in the hospital. The growing resistance to treatments for candidiasis poses a significant problem. Thus, finding new antifungal agents or safer alternatives is crucial for improving treatment. This study examined the antifungal properties of hinokitiol against clinical strains of *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. krusei*, and investigated its combined effect with the man-made antifungal fluconazole.

As quantified in Table 2, all clinical isolates in this study were confirmed as resistant to fluconazole, with MICs uniformly exceeding the CLSI breakpoint for resistance (≥64 µg/ml). The resistance profiles, however, revealed significant interspecies variation. *C. albicans* and *C. tropicalis* displayed high-level resistance (MIC range: 64–256 µg/ml), with the latter showing a higher mean MIC (116.7 µg/ml vs. 97 µg/ml). *C. glabrata* exhibited a similarly elevated mean MIC (116.7 µg/ml) but across a broader range (64–512 µg/ml). Notably, *C. krusei* demonstrated the most profound resistance, with MIC values (256–1024 µg/ml) and a mean (370.5 µg/ml) that were statistically significantly higher than those of all other species evaluated ($p < 0.05$). The differences in how various *Candida* isolates respond to azoles might be due to both natural and developed antifungal resistance (Whaley *et al*., 2017). Previous studies showed that fluconazole MIC values varied from 0.125 to 64 µg/ml for various *Candida* species, such as *C. albicans*, *C. tropicalis*, *C. glabrata*, and clinical isolates of *C. krusei*, with every strain of *C. krusei* identified as resistant (Sharifzadeh *et al*., 2018; Shokri *et al*., 2021). Fluconazole functions by hindering the growth of fungi without completely eradicating them, which can allow for resistance to emerge. The propensity for fluconazole-resistant *Candida* strains to emerge is especially pronounced following extended or repeated antimicrobial regimens used to manage persistent mucosal candidiasis. Experiments involving the gradual introduction of resistance mutations in genes like ERG11, MRR1, TAC1, and UPC2 into drug-sensitive *Candida* strains show that different mechanisms of resistance can combine and reinforce each other. This helps explain why strains with multiple resistance traits tend to thrive during long-term fluconazole treatment for candidiasis (Sasse *et al*., 2012). The rise of fluconazole-resistant recurrent candidiasis poses serious concerns, especially since there are few alternative treatments available. As detailed in Table 2, hinokitiol exhibited a marked spectrum of *in vitro* efficacy against the panel of *Candida* species. The susceptibility profile revealed a clear hierarchy, with *C. glabrata* identified as the most sensitive (mean MIC: 0.81 µg/ml; range 0.64–1.20 µg/ml), followed by *C. albicans* (mean MIC: 1.34 µg/ml). In contrast, the compound's activity was substantially lower against *C. tropicalis* and *C. krusei*, which required mean inhibitory concentrations of 4.35 µg/ml and 6.9 µg/ml, respectively-a finding that underscores a notable species-dependent variation in susceptibility.

While literature on the antifungal properties of hinokitiol against *Candida* species remains limited, existing studies consistently report its efficacy. For instance, Jin *et al.* (2021) demonstrated that hinokitiol was active against a panel of species, including *C. albicans* (MIC: 2 µg/ml), *C. tropicalis* (MICs: 1-2 µg/ml), *C. glabrata* (MIC: 0.5 µg/ml), and *C. krusei* (MICs: 1-2 µg/ml). The anti-candidal activity of hinokitiol demonstrates considerable promise, particularly given its retained efficacy against fluconazole-resistant strains. This finding is corroborated by Kim *et al.* (2017) who reported potent activity against resistant isolates of *C. albicans*, *C. glabrata*, and *C. tropicalis*, with MICs of 1.6, 0.78, and 3.1 µg/ml, respectively. The MIC value for *C. albicans* observed in the present work is also consistent with the earlier report by Komaki *et al.* (2008) of 5 µg/ml. The modest divergence in the absolute numerical values likely reflects expected experimental variance arising from differences in methodological execution or the distinct provenance of the clinical isolates used in each study. Beyond *Candida*, the activity of hinokitiol against filamentous fungi is less explored but shows a broad spectrum of efficacy. For instance, it demonstrates potent activity against *Aspergillus fumigatus* (Ma *et al*., 2022), while higher MICs have been reported for other molds, including *Saprolegnia* and *Aphanomyces* species (Mori *et al*., 2002), *Aspergillus niger*,

Penicillium citrinum (Hu *et al*., 2013), and *Botrytis cinerea* (Wang *et al*., 2020), with values ranging from 12.5 to 40 µg/ml. Studies on mechanisms have shown that hinokitiol interacts with iron in the fungal cells, resulting in reduced respiratory function in fungal cells. It also suppressed the functions of mitochondrial respiratory chain complexes I and II, leading to a decreased mitochondrial membrane potential, which subsequently lowered the intracellular ATP levels and increased harmful intracellular reductive stress (Sun *et al*., 2017). According to Nakamura *et al* .(2013), a brief 30-minute treatment with 0.25 mM hinokitiol inhibited the attachment of *Candida* species to oral epithelial cells by 30-70% and reduced biofilm formation by approximately 11%. Another investigation by Kim *et al.* (2017) found that hinokitiol effectively prevented *Candida* biofilm formation at concentrations between 3.1 to 12.5 µg/ml and decreased mature biofilms at levels from 12.5 to 400 µg/ml in both fluconazole-sensitive and fluconazole-resistant *Candida* species. Hinokitiol also suppressed the expression of genes associated with adhesion, specifically HWP1 and ALS3. Additionally, it lowered the transcript levels of UME6 and HGC1, which are crucial for maintaining hyphal extension over extended periods. Furthermore, hinokitiol diminished the expression of CYR1, which is part of the signaling pathway for hyphal formation involving cAMP-PKA. It also inhibited RAS1, the upstream regulator. These findings suggest that hinokitiol could interfere with the ability of *Candida* species to adhere and develop biofilms, thereby reducing the occurrence of oral and vaginal infections triggered by *Candida*.

Table 2. The sensitivity of *Candida* species to fluconazole and hinokitiol based on CLSI standard instructions using broth microdilution method.

Candida species	Broth microdilution											
	Hinokitiol (µg/ml)								Fluconazole (µg/ml)			
	MIC				MFC				MIC			
	C.alb	C. kru	C.tro	C. gla	C.alb	C. kru	C.tro	C. gla	C.alb	C. kru	C.tro	C. gla
1	1.2	10	5	1.2	5	40	10	5	64	512	128	256
2	1.2	10	5	0.62	2.5	20	20	2.5	64	256	64	128
3	1.2	10	5	0.62	5	40	10	2.5	64	256	64	128
4	0.62	5	5	0.62	2.5	20	20	2.5	64	256	128	64
5	1.2	5	5	0.62	5	20	20	1.2	64	256	256	64
6	2.5	5	2.5	0.62	5	20	10	2.5	128	256	256	64
7	2.5	10	2.5	0.62	10	40	10	1.2	128	1024	128	512
8	1.2	5	5	1.2	5	10	10	5	128	256	64	64
9	1.2	5	2.5	1.2	2.5	20	10	2.5	128	256	128	64
10	1.2	10	5	1.2	5	20	10	5	64	512	64	64
11	2.5	5	10	0.62	10	10	20	2.5	64	1024	256	256
12	0.62	10	2.5	1.2	2.5	40	5	5	128	512	128	128
13	1.2	10	5	0.62	5	20	10	2.5	128	512	128	128
14	1.2	5	5	0.62	5	20	10	2.5	256	256	128	128
ATCC	2.5	5	5	1.2	5	20	20	2.5	128	256	64	128
GEO mean	1.34	6.9	4.35	0.81	4.8	21.9	12.03	2.73	97	370.5	116.7	116.7

C. alb: *Candida albicans*, C. kru: *Candida krusei*, C. tro: *Candida tropicalis*, C. gla: *Candida glabrata*, GEO mean: Geometric mean

Table 2 showed that the mean MFC values for hinokitiol were 4.8 µg/ml for *C. albicans*, 12.03 µg/ml for *C. tropicalis*, 2.73 µg/ml for *C. glabrata*, and 21.9 µg/ml for *C. krusei* isolates. The fungicidal efficacy of hinokitiol was confirmed by MFC/MIC ratios below 4 for all clinical *Candida*

isolates, specifically 3.58 for *C. albicans*, 2.76 for *C. tropicalis*, 3.37 for *C. glabrata*, and 3.17 for *C. krusei* (Fig. 1). This aligns with the work of Kim *et al*. (2017) who also documented the potent fungicidal action of hinokitiol against planktonic cells of various *Candida* species, including fluconazole-resistant strains.

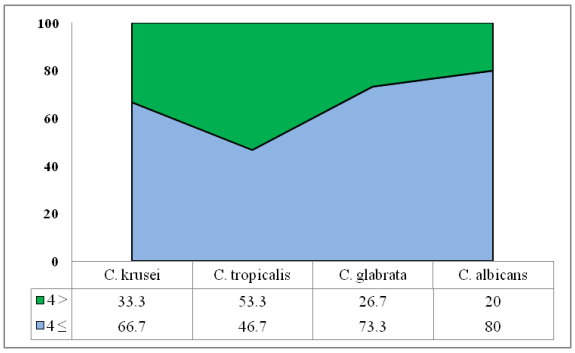

Fig. 1. Fungicidal versus fungistatic activity of hinokitiol against *C. albicans*, *C. tropicalis*, *C. glabrata,* and *C. krusei* isolates.

Methods to combat the rising fungal resistance have been urgently required because of the illness and death associated with invasive fungal infections in immunocompromised individuals. Combining fluconazole with different natural substances is one method to address treatment failures. The findings from the checkerboard microtitre assay in this study showed substantial synergistic effects between hinokitiol and fluconazole on isolates of *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. krusei* ($p < 0.05$) (Fig. 2). FICI values for the combination of hinokitiol and fluconazole varied from 0.304 to 0.498 for *C. albicans* isolates, 0.245 to 0.730 for *C. tropicalis* isolates, 0.290 to 0.492 for *C. glabrata* isolates, and 0.500 to 0.750 for *C. krusei* isolates (Tables 3 and 4). No notable difference was detected among the tested *Candida* isolates. The interaction between hinokitiol and fluconazole was found to be synergistic for all 30 tested isolates of *C. albicans* and *C. glabrata* (100%). The interaction between hinokitiol and fluconazole was predominantly synergistic or additive across the tested isolates. An additive effect was observed in all (100%) *C. krusei* isolates. For *C. tropicalis*, the combination was highly effective, demonstrating synergy in 86.7% (13 of 15) of isolates and an additive effect in the remaining 13.3% (2 isolates). No differences were observed in the effectiveness of hinokitiol combined with fluconazole against clinical isolates of *C. albicans*, *C. tropicalis*, *C. glabrata*, and *C. krusei*. In this research, no antagonism was detected for this combination. As far as we know, there are no documented findings on the combined effectiveness of fluconazole and hinokitiol against *Candida* species. Components of the essential oils can interact to produce synergistic, additive, indifferent, or antagonistic effects. The concept of synergizing fluconazole with natural compounds represents a validated strategy to enhance antifungal efficacy. A body of literature substantiates this approach, documenting significant synergistic interactions with phenolic compounds, such as eugenol, thymol, and honokiol, as well as with terpenes like linalool and citral (Jin *et al*., 2010; Pemmaraju *et al*., 2013; Sharifzadeh *et al*., 2018; Shokri *et al*., 2021). Earlier research suggested that natural antifungal agents used together could enhance the effectiveness of each medication, allowing for efficacy with reduced dosages of the individual drugs (Sharifzadeh and Shokri, 2021). In this research, the MICs of hinokitiol and fluconazole when tested singly against *Candida* species were considerably higher than the MICs observed in their combinations. Hinokitiol lowered the levels of fluconazole needed to inhibit the growth of *Candida* species. Overall, the primary mechanisms of these synergistic effects seem to involve enhancing membrane permeability, decreasing the efflux of antifungal medications, disrupting intracellular ion balance, obstructing the function of proteins and enzymes essential for fungal survival, and hindering biofilm development.

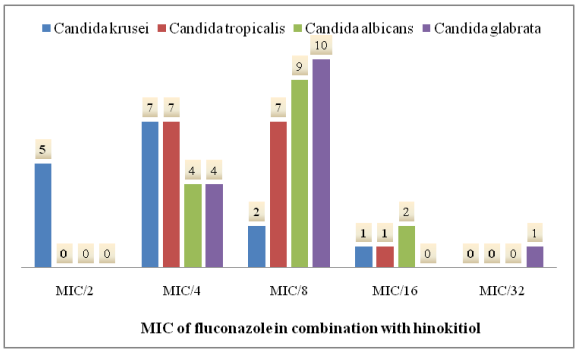

Fig. 2. MIC of fluconazole in combination with hinokitiol in comparison with fluconazole alone.

This investigation established hinokitiol as a potent antifungal agent with demonstrable *in vitro* efficacy against clinically relevant, fluconazole-resistant strains of *Candida* species. A significant finding was the marked enhancement of antifungal activity observed when hinokitiol was

combined with fluconazole, which substantially reduced the minimum effective dosage of both agents. These results underscore the potential of hinokitiol-fluconazole combination therapy as a viable strategy to overcome antifungal resistance in cases of refractory candidiasis. To translate these promising *in vitro* findings into clinical practice, subsequent validation through *in vivo* models and controlled clinical trials is essential.

Table 3. The combined effect of hinokitiol and fluconazole against fluconazole-resistant *Candida krusei* and *Candida glabrata* isolates.

Candida strains	MIC combination (µg/ml) (Hinokitiol - Fluconazole)	FIC index		FICI index	Interpretation
		Hinokitiol	Fluconazole	Hinokitiol + Fluconazole	
C. krusei 1	5 – 32	0.50	0.06	0.563	Additive
C. krusei 2	2.5 – 64	0.25	0.25	0.500	Additive
C. krusei 3	5 – 32	0.50	0.13	0.625	Additive
C. krusei 4	2.5 – 64	0.50	0.25	0.750	Additive
C. krusei 5	1.2 – 128	0.24	0.50	0.740	Additive
C. krusei 6	0.64 – 128	0.13	0.50	0.628	Additive
C. krusei 7	2.5 – 256	0.25	0.25	0.500	Additive
C. krusei 8	1.2 – 128	0.24	0.50	0.740	Additive
C. krusei 9	0.62 – 128	0.12	0.50	0.624	Additive
C. krusei 10	2.5 – 128	0.25	0.25	0.500	Additive
C. krusei 11	2.5 – 256	0.50	0.25	0.750	Additive
C. krusei 12	2.5 – 128	0.25	0.25	0.500	Additive
C. krusei 13	2.5 – 256	0.25	0.50	0.750	Additive
C. krusei 14	2.5 – 64	0.50	0.25	0.750	Additive
ATCC 6258	2.5 – 32	0.50	0.13	0.625	Additive
C. glabrata 1	0.31 – 32	0.26	0.13	0.383	Synergism
C. glabrata 2	0.15 – 32	0.24	0.25	0.492	Synergism
C. glabrata 3	0.15 – 16	0.24	0.13	0.367	Synergism
C. glabrata 4	0.15 – 16	0.24	0.25	0.492	Synergism
C. glabrata 5	0.15 – 8	0.24	0.13	0.367	Synergism
C. glabrata 6	0.15 – 8	0.24	0.13	0.367	Synergism
C. glabrata 7	0.15 – 64	0.24	0.13	0.367	Synergism
C. glabrata 8	0.31 – 8	0.26	0.13	0.383	Synergism
C. glabrata 9	0.31 – 8	0.26	0.13	0.383	Synergism
C. glabrata 10	0.15 – 16	0.13	0.25	0.375	Synergism
C. glabrata 11	0.15 – 64	0.24	0.25	0.492	Synergism
C. glabrata 12	0.31 – 16	0.26	0.13	0.383	Synergism
C. glabrata 13	0.15 – 16	0.24	0.13	0.367	Synergism
C. glabrata 14	0.15 – 16	0.24	0.13	0.367	Synergism
ATCC 90030	0.31 – 4	0.26	0.03	0.290	Synergism

Table 4. The combined effect of hinokitiol and fluconazole against fluconazole-resistant *Candida albicans* and *Candida tropicalis* isolates.

Candida strains	MIC combination (µg/mL) (Hinokitiol - Fluconazole)	FIC index		FICI index	Interpretation
		Hinokitiol	Fluconazole	Hinokitiol+ Fluconazole	
C. albicans 1	0.31 - 8	0.26	0.13	0.383	Synergism
C. albicans 2	0.31 - 8	0.26	0.13	0.383	Synergism
C. albicans 3	0.31 - 8	0.26	0.13	0.383	Synergism
C. albicans 4	0.15 - 4	0.24	0.06	0.304	Synergism
C. albicans 5	0.15 - 16	0.13	0.25	0.375	Synergism
C. albicans 6	0.31 - 32	0.12	0.25	0.374	Synergism
C. albicans 7	0.62 - 16	0.25	0.13	0.373	Synergism
C. albicans 8	0.15 -32	0.13	0.25	0.375	Synergism
C. albicans 9	0.31 - 8	0.26	0.06	0.321	Synergism
C. albicans 10	0.31 - 8	0.26	0.13	0.383	Synergism
C. albicans 11	0.62 -8	0.25	0.13	0.373	Synergism
C. albicans 12	0.15 - 16	0.24	0.13	0.367	Synergism
C. albicans 13	0.31 - 16	0.26	0.13	0.383	Synergism
C. albicans 14	0.31 - 32	0.26	0.13	0.383	Synergism
ATCC 10231	0.62 - 32	0.25	0.25	0.498	Synergism
C. tropicalis 1	1.2 - 32	0.24	0.25	0.490	Synergism

C. tropicalis 2	1.2 - 8	0.24	0.13	0.365	Synergism
C. tropicalis 3	0.62 - 16	0.12	0.25	0.374	Synergism
C. tropicalis 4	1.2 - 16	0.24	0.13	0.365	Synergism
C. tropicalis 5	0.62 - 64	0.12	0.25	0.374	Synergism
C. tropicalis 6	0.31 - 32	0.12	0.13	0.249	Synergism
C. tropicalis 7	1.2 - 32	0.48	0.25	0.730	Additive
C. tropicalis 8	1.2 - 4	0.24	0.06	0.303	Synergism
C. tropicalis 9	1.2 - 16	0.48	0.13	0.605	Additive
C. tropicalis 10	0.62 - 16	0.12	0.25	0.374	Synergism
C. tropicalis 11	1.2 - 32	0.12	0.13	0.245	Synergism
C. tropicalis 12	0.31 - 16	0.12	0.13	0.249	Synergism
C. tropicalis 13	0.62 -32	0.12	0.25	0.374	Synergism
C. tropicalis 14	1.2 -16	0.24	0.13	0.365	Synergism
ATCC 9968	0.62 - 16	0.12	0.25	0.374	Synergism

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Conflict of Interest

The authors declare no conflict of interest.

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