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ORIGINAL ARTICLE

Antifungal activity of polyphenolic compounds against fluconazole-susceptible and -resistant *Candida* species

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ABSTRACT

Background and Objectives: The rapid emergence of resistant fungi is occurring worldwide, and this crisis has been attributed to the lack of new antifungal drug development. This issue emphasizes the need for innovation in finding novel antifungals. There is an increasing interest in using the natural products of plants with high biological activity as alternatives to synthetic drugs. This study aimed to evaluate the possible applicability of polyphenols as alternative antifungal drugs to treat resistant *Candida* infections.

Materials and Methods: A panel of fluconazole-resistant (n=14) and fluconazole-susceptible (n=26) clinical *Candida* isolates was obtained from the reference culture collection. The determination of the minimum inhibitory concentrations (MICs) of fluconazole, tannic acid, rosmarinic acid, gallic acid, chlorogenic acid, caffeic, ferulic, and p-coumaric was carried out following the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: The MIC values of 40 *Candida* species isolates ranged from 0.25 to >64 µg/mL for polyphenolic compounds. The highest inhibitory effect against *Candida* species was observed with tannic acid, followed by fluconazole. Non-*albicans Candida* groups were more sensitive to tannic acid compared to *C. albicans* isolates. Significant differences were observed in the MICs of fluconazole and tannic acid against non-*albicans Candida* isolates.

Conclusion: The increasing antifungal resistance highlights the importance of evaluating new drugs that are more robust against resistance. This study suggests that tannic acid could be considered a novel antifungal agent for managing fungal infections, including multidrug-resistant non-*albicans Candida* infections.

Keywords: Tannic acid; Polyphenolic compounds; *Candida* species; Fluconazole resistance; Antifungal activity

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INTRODUCTION

The worldwide emergence of antifungal resistance is a significant concern, particularly in immunosuppressed individuals, as delays in initiating adequate empiric therapy can lead to severe and life-threatening fungal infections (1, 2). Numerous reports have highlighted the growing problems of drug resistance in fungal pathogens (3-6). Recent studies indicate that various fungi contribute to drug resistance, with limited therapeutic options available for these infections (3, 7). Alarmingly, there has been a lack of new antifungals to combat the threat of drug-resistant fungal pathogens (8). The rise in antifungal resistance is a key factor in increased morbidity and mortality rates globally. Drug-resistant fungal infections can delay recovery, lead to higher medical costs, and pose significant treatment challenges. Drug-resistant *Candida* species have been implicated in over 34,000 cases and 1,700 deaths annually in the United States (9). The number of patients with invasive *Candida* infections caused by multidrug-resistant *C. auris* has increased dramatically, increasing from 329 cases in 2018 to 1,012 in 2021, according to emerging data (3, 9). Consequently, there is a critical need to develop novel therapeutic approaches, as the growing prevalence of drug-resistant fungi is contributing to millions of deaths worldwide. The rapid global emergence of resistant fungal has been attributed to the overuse of existing antifungals and lack of new antifungal development (10-12). This issue strongly highlights the critical need for innovation to discover novel chemical classes of antifungals to prevent cross-resistance and improve treatment outcomes. Phenolic compounds consist of an aromatic ring with one or more hydroxyl groups and can be simple phenolic molecules or polymerized compounds. There is an increasing interest in using natural products from plants with high biological activities as alternatives to synthetic drugs. Among these natural compounds, some polyphenolics ones are considered as the most health-related beneficial groups (13, 14). Phenolic compounds exhibit various activities, such as antioxidant, antidiabetic, anti-inflammatory, antiallergic, cardioprotective, antihypertensive, antithrombotic, anticancer, osteoprotective, neuroprotective, anti-aging, antibacterial, antitoxin, antiviral, and antifungal properties (13-18). Moreover, the majority of previous studies have highlighted the antifungal activity of whole plant extracts (15, 17). In this area of knowledge, reports on the efficacy of phenolic compounds as antifungal agents remain limited. This study aimed to consider the potential applicability of polyphenols as alternative antifungal drugs for treating resistant *Candida* infections. Therefore, we examined the in vitro activity of tannic acid, gallic acid, rosmarinic acid, chlorogenic acid, caffeic, ferulic, and p-coumaric against a collection (n=40) of fluconazole-susceptible and -resistant *Candida* isolates.

MATERIALS AND METHODS

Polyphenolic standards. High-purity standards (more than 95%) were purchased from Sigma-Aldrich including tannic acid, gallic acid, rosmarinic acid, chlorogenic acid, caffeic, ferulic, and p-coumaric. stock solutions of polyphenolic compounds were prepared in dimethyl sulfoxide (DMSO) with the final concentration of DMSO >1%.

Antifungal susceptibility testing. A panel of fluconazole-resistant (n=14) and -susceptible (n=26) clinical *Candida* isolates, including *C. albicans* (n=13), *C. glabrata* (n=8), *C. tropicalis* (n=2), *C. parapsilosis* (n=8), *C. krusei* (n=3), *C. kefyr* (n=3), and *C. auris* (n=3), was obtained from the reference culture collection (19-24). All isolates were previously identified using both conventional and molecular methods. The *Candida* isolates were identified at the species level through polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and partial DNA sequencing of the ITS rDNA region with specific primers (23). Antifungal susceptibility testing was conducted according to the CLSI guidelines (25). The agents were dispensed into microdilution trays, with final concentration ranges of 0.063-64 µg/mL for both fluconazole (Pfizer, Groton, CT, USA) and the polyphenolic compound. The MIC endpoints were defined as a 50% reduction in growth compared to the agent-free growth control for both fluconazole and polyphenolics. All antifungal stock solutions were dissolved in DMSO, then diluted with RPMI 1640 medium (Sigma Chemical Co.) and dispensed into 96-well microdilution trays. Homogeneous suspensions were measured spectrophotometrically at 530 nm to determine percent transmission in the range 75-77. The final inoculum densities of the tested isolates were within the range of 0.5-2.5×10^3 CFU/mL, as determined by quantitative colony counts on Sa-

bouraud glucose agar. *Candida parapsilosis* (ATCC 22019) and *C. krusei* (ATCC 6258) reference strains were included as quality controls. Plates were incubated at 35°C for 24 hours, and MIC values were visually determined. All tests were repeated twice for each isolate. Statistical analysis was performed using SPSS version 18.0 (IBM, New York, NJ, USA), with a P-value less than 0.05 considered statistically significant.

RESULTS

Table 1 summarises the MIC range, geometric mean (GM) MIC, MIC_{50} and MIC_{90} for phenolic compounds against 40 fluconazole-resistant and -susceptible clinical *Candida* isolates. The MIC values of 40 *Candida* species isolates ranged from 0.25 to >64 µg/mL for polyphenolic compounds. Among the tested phenolic compounds, p-coumaric demonstrated the highest MICs (MIC range, 32->64 µg/mL; MIC_{90}, 64 µg/mL), followed by caffeic acid (MIC range, 32->64µg/mL; MIC_{90}, 32 µg/mL) and ferulic acid (MIC range, 16->64 µg/mL; MIC_{90}, 16 µg/mL). However, tannic acid exhibited the potent activity (MIC range, 0.25->64 µg/mL; MIC_{90}, 16 µg/mL) against all *Candida* species isolates, in comparison to fluconazole (MIC range, 0.5->64 µg/mL; MIC_{90}, 32 µg/mL). Non-*albicans Candida* groups were more sensitive to tannic acid (MIC range, 0.25->64 µg/mL; GM MIC, 3.17 µg/mL) than *C. albicans* isolates (MIC range, 1-16 µg/mL; GM MIC, 6.46 µg/mL). Significant differences were observed in the MICs of fluconazole and tannic acid against non-albicans *Candida* isolates ($P < 0.05$).

Table 1. In vitro activities of polyphenolic compounds against clinical *Candida* species

	MICs (µg/ml)								
Strains and polyphenolic compounds	**Range**	**MIC_{50}/MIC_{90}**	**G mean**	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**
Candida species (40)									
Fluconazole	0.5- >64	4/16	4.28			4	8	7	4
Tannic acid	0.25- >64	4/16	4.07		1	3	2	5	11
Gallic acid	1- >64	16/19.2	10.88				1		
Rosmarinic acid	2- >64	8/25.6	9.18					1	
Chlorogenic acid	2- >64	8/20.8	8					1	2
Caffeic	32- >64	32/32	32						
Ferulic	16- >64	16/16	16						
P-coumaric	32- >64	32/64	44.47						
Candida albicans (13)									
Fluconazole	0.5- >32	2/8	2			3	2	2	1
Tannic acid	1- 16	8/16	6.46			1	4	5	
Gallic acid	1- >64	64/64	27.26			1			
Rosmarinic acid	2- >64	64/64	28.76					1	
Chlorogenic acid	2- >64	64/64	27.26					1	2
Caffeic	32- >64	32/32	33.75						
Ferulic	32- >64	32/64	37.55						
P-coumaric	32- >64	32/64	44.06						
Non-*Candida albicans* (27)									
Fluconazole	0.5- >64	4/16	3.44			3	4	4	3
Tannic acid	0.25- >64	4/16	3.17		1	3	1	5	3
Gallic acid	16- >64	16/16	19.02						
Rosmarinic acid	32- >64	32/32	32						
Chlorogenic acid	8- >64	12/27.2	13.45						
Caffeic	32	32/32	32						
Ferulic	16- >64	16/16	16						
P-coumaric	32- >64	32/32	32						

DISCUSSION

The development of antifungal resistance must be considered a serious public health problem, as it can significantly impact global health (8). Multidrug resistance and pandrug resistance in fungal pathogens, such as *C. auris*, azole-resistant *Aspergillus fumigatus*, and terbinafine- and azole-resistant dermatophytes are associated with poor health outcomes (7). These pathogens are often resistant to multiple or entire classes of available antifungal agents. The limited treatment options for managing fungal infections with drug-resistant phenotypes contribute to higher morbidity and mortality rates (8). Therefore, it is essential to focus on the design and development of new classes of antifungals and innovative therapeutic strategies. Among the phenolic compounds, tannic acid exhibited significant antifungal activity against *Candida* isolates. The MIC_{50} and MIC_{90} values for tannic acid and fluconazole against all *Candida* isolates were 4 μg/mL and 16 μg/mL, respectively. The results also showed that tannic acid demonstrated strong in vitro antifungal activity against non-*albicans Candida* isolates, as indicated by its GM MIC, which was significantly lower than the one for fluconazole. The reason for the difference in antifungal activities between tannic acid and the other polyphenolic compounds remains to be elucidated. However, the high number of hydroxyl groups in the structure of tannic acid may contribute to these differences (26). Therefore, the polyphenolic compounds and their interaction with the cell membrane can significantly affect and inhibit microbial functions (26). The antifungal activity of phenolic compounds has been documented in previously published studies, with significant variability in the MIC values. Therefore, the assessment of the results is challenging, as different methods have been applied for determining the antifungal activity of polyphenolics by researchers (27). The exact mechanisms of polyphenolic compounds against *Candida* species remain unknown. However, some researchers have suggested that the action of polyphenolics on *Candida* species may be due to their ability to penetrate the cell membrane and alter cell surface charge and hydrophobicity (28). Additionally, polyphenolics may interfere with 1,3-β-glucan synthase (29), significantly inhibit ergosterol biosynthesis (30) and induce the production of reactive oxygen species (ROS) (8). Furthermore, several factors influence the mechanisms by which polyphenolics affect fungi, including inducing the induction of apoptotic mechanisms in *Candida* (31, 32) and the inhibition of efflux transporters (33). The progress of research in the upcoming years will be crucial. The compounds analysed in this study are just the beginning, however, they represent a significant step forward. To gain a more thorough understanding of the antifungal activity of tannic acid, in vitro testing should be expanded to include a wider range of drug-resistant *Candida* isolates. This would offer a more precise evaluation of its antifungal potential and help define the spectrum of activity of tannic acid against drug-resistant *Candida* species.

CONCLUSION

The increasing of antifungal resistance shows the importance of evaluating new drugs that are more robust to resistance mechanisms. Such studies should be of particular interest due to the increasing significance of resistance in both established and emerging fungi. This study indicated that tannic acid exhibits strong antifungal activity and could be considered a novel class of antifungal agents for treating fungal infections, including multidrug-resistant non-*albicans Candida* infections.

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