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Original Article

Evaluation of synergistic anticandidal and apoptotic effects of ferulic acid and caspofungin against *Candida albicans*

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ABSTRACT

This study aimed to investigate the synergy between anticandidal and apoptotic effects of ferulic acid and caspofungin against *Candida albicans* and *Candida glabrata*, with the help of a quantitative checkerboard microdilution assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as a viability dye. Apoptotic effects of caspofungin and ferulic acid concentrations (alone and combined) were analyzed for *C. albicans* and *C. glabrata* based on annexin V–propidium iodide binding capacities using flow cytometric analysis. *C. albicans* showed a synergistic effect, represented by a fractional inhibitory concentration index of < 0.5, but *C. glabrata* showed no synergistic effect (fractional inhibitory concentration index > 0.5). Early and late apoptotic effects of caspofungin and ferulic acid concentrations (1 μg/mL and 1000 μg/mL) were calculated as 55.7% and 18.3%, respectively, while their necrotic effects were determined as 5.8% and 51.6%, respectively, using flow cytometric analyses. The apoptotic effects of the combination of caspofungin and ferulic acid at concentrations of 1 μg/mL and 1000 μg/mL on *C. albicans* and *C. glabrata* were 73.0% and 48.7%, respectively. Ferulic acid also demonstrated a synergistic effect in combination with caspofungin against *C. albicans*. Another possibility is to combine the existing anticandidal drug with phytochemicals to enhance the efficacy of anticandidal drug.

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1. Introduction

A considerable increase in the incidence of deep fungal infections has been observed in the last two decades in hospital environments due to the increase in organ transplantations, rise in the incidence of AIDS, and use of invasive devices (such as catheters, artificial joints, and valves), and also in immunocompromised patients [1,2].

Candida albicans and *Candida glabrata* have been highly associated with several opportunistic fungal infections [3]. *Candida* species are the basis for the development of new antifungal drugs. However, increasing levels of *Candida* species resistant to the current antifungal drugs have been

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observed, making these agents ineffective [4]. Therefore, other therapies, which are more effective and safer than the current ones, are being explored; namely the use of plant extracts enriched in phenolic compounds [5,6].

Although the advances in medical and chemical fields have increased life expectancy, an increasing resistance of pathogenic microorganisms to conventional drugs has been observed due to the random use of chemicals and drugs, leading to the development of other complications [7].

Ferulic acid is a phenolic acid widely distributed in the plant kingdom [8]. It is one of the most abundant phenolic acids. It may be found in high concentrations in foods such as navy bean, corn bran, wheat bran, eggplant, artichokes, and beets [9]. It presents a wide variety of potential therapeutic effects helpful in the treatment of cancer, diabetes, and lung and cardiovascular diseases; hepatoprotective, neuroprotective, and photoprotective effects; and antimicrobial and anti-inflammatory properties [10,11].

Caspofungin and other agents in the echinocandin class of antifungals have assumed an increasingly important role in the therapy of invasive candidiasis [12]. These agents are nontoxic and show potent fungicidal activity against *C. albicans* and other *Candida* species [13]. Although the mechanism of action of echinocandins is known, the physiological mechanisms by which they cause cell death are not defined [14].

The minimum inhibitory concentration (MIC) values of caspofungin and ferulic acid using the microbroth dilution assay, fractional inhibitory concentration (FIC) index using the checkerboard microdilution assay, and mechanisms of *C. albicans* and *C. glabrata* cell death caused by caspofungin and ferulic acid were determined in this study. The flow cytometric analysis showed that caspofungin caused both apoptosis and necrosis of *C. albicans* and *C. glabrata* cells.

2. Materials and Methods

2.1. Fungal strains and chemicals

C. albicans (ATCC 90028) and *C. glabrata* (ATCC 90030) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Caspofungin diacetate (SML0425) and ferulic acid (1270311) were purchased from Sigma (St. Louis, MO, USA).

2.2. Antimicrobial assay

MIC is defined as the lowest concentration of the antimicrobial agent that inhibits the bacterial growth, as detected by the lack of visual turbidity. The microbiological assay was performed according to the Clinical and Laboratory Standards Institute M7-A7 broth microdilution method [15].

2.3. Checkerboard microdilution assay for ferulic acid and caspofungin

Synergy was tested by the checkerboard method; a two-dimensional array of serial concentrations of test compounds, which has been used most frequently to assess antimicrobial combinations *in vitro*. The tested dilutions were based on the MIC of the two compounds. The checkerboard test was used as the basis to calculate the FIC index [16]. The effects of the combination of caspofungin with ferulic acid were investigated by the checkerboard broth microdilution method. Drug interaction was classified as synergistic, additive, or less-than-additive based on the FIC index, which is the sum of FIC indexes for each drug. The FIC index of each drug was calculated as the MIC of that drug in combined treatment divided by the MIC of the drug used alone. Drug–drug interactions were considered synergistic if the FIC index was < 0.5; additive if the FIC index was $= 1.0$; and less-than-additive if the FIC index was > 0.5.

2.4. Analysis of apoptosis caused by Candida species using flow cytometry

C. albicans and *C. glabrata* cells (2×10^6/mL) were incubated in Sabouraud Dextrose Broth with 1 μg/mL caspofungin and 1000 μg/mL ferulic acid for 24 hours at 30°C. *C. albicans* and *C. glabrata* cells were harvested by centrifugation and washed in 0.1M potassium phosphate buffer. Annexin V/propidium iodide (PI) assays were performed according to the staining kit protocol, using 5 μg annexin V and 5 μg PI at 37°C for 20 minutes. The cells were analyzed using a BD Accuri C6 flow cytometer (Becton–Dickinson, Mansfield, MA, USA) [17].

3. Results

The primary aim of this study was to determine the MIC and FIC index of caspofungin and ferulic acid, which induced both apoptosis and necrosis, using flow cytometry. The checkerboard microdilution assay showed that ferulic acid and caspofungin exhibited antifungal activity against *C. albicans* with an MIC value of 40 μg/mL and 2 μg/mL, respectively, and against *C. glabrata* with an MIC value of 20 μg/mL and 4 μg/mL, respectively. The FIC index of ferulic acid and caspofungin was 0.0375 (Table 1). The apoptotic effects of caspofungin and ferulic acid concentrations (alone and combined) after the 24-hour incubation period, which were analyzed for *C. albicans* and *C. glabrata* based on annexin V–PI binding capacities using flow cytometry, are depicted in Figure 1. Flow cytometric analyses revealed early and late apoptotic effects of 1 μg/mL caspofungin on *C. albicans* as 2.1% and 53.6%, respectively, while their necrotic effects were determined as 5.8%. Especially late apoptotic effects on *C. albicans* were found to be increased (Figure 1). The late apoptotic effects of the combination of caspofungin and ferulic acid (1 μg/mL and 1000 μg/mL) on *C. albicans* and *C. glabrata* were 63.4% and 44.9%, respectively (Table 2 and Figure 1). These findings indicated that the anticandidal effect of the combination of caspofungin and ferulic acid on *C. albicans* than on *C. glabrata* increased depending on the concentration and prolonged incubation period. The results of flow cytometric analysis were used to determine the apoptotic effects of caspofungin and ferulic acid alone and combined against *C. albicans* and *C. glabrata*. Caspofungin exerted apoptotic activity against *C. albicans* by directly killing the cells (resulting in necrosis) and causing others to undergo programmed cell death (apoptosis).

Table 1 – Results of ferulic acid and caspofungin combination treatment on *Candida albicans* and *Candida glabrata*.

	Ferulic acid (μg/mL) (A)	Caspofungin (μg/mL) (B)	Combination (μg/mL)		FIC > 0.5–< 0.5
			(A + B)	(B + A)	
C. albicans	40	2	10	0.25	0.0375
C. glabrata	20	4	2.5	0.25	0.1875

C. albicans showed an synergistic effect (FIC < 0.5). *C. glabrata* showed a nonsynergistic effect (FIC > 0.5). FIC = fractional inhibitory concentration.

The synergistic activity of the combination of caspofungin and ferulic acid caused a pronounced reduction in the MIC of *C. albicans* and *C. glabrata*.

4. Discussion

Only a few classes of antifungals such as polyenes, azoles, echinocandins, allylamines, and flucytosine are available for the treatment of *Candida* infections [18]. However, studies on their mechanism of action alone or in synergism with known antifungals are still scarce [19]. This study aimed to explore the potential use, proposed mechanisms of action, and limitations of the phenolic acids in anticandidal therapy [20]. No study to date has evaluated the synergistic effect of caspofungin and ferulic acid on *Candida* species.

However, caspofungin has been reported to mediate apoptosis in *C. albicans* when used alone. Hao and colleagues discovered that caspofungin induced apoptosis in *C. albicans* [13]. The results of their study correlated with the present findings: 0.5 μg caspofungin induced apoptosis in *C. albicans* (early and late apoptosis rate 25%). Another study on the resistance of *C. albicans* to fluconazole using a combination of fluconazole and *Rubus chingii* extract reported a significant effect of the combination on the resistance [21]. Also, a synergistic effect of caffeic acid and fluconazole was found on the resistance of *C. albicans* to fluconazole [22]. A study on the effects of fluconazole and nystatine on *C. albicans* reported a 64-fold decrease in the MIC value [23]. Stringaro et al [24] reported the antifungal activity of the essential oil of *Mentha suaveolens*, alone or in combination with other antifungal drugs, against *C. albicans*.

Regarding the studies on the antifungal activities of ferulic acid, the most significant effect was found against *C. albicans*. In spite of various biological activities of ferulic acid, no real clinical usage of this compound has been reported. Clinical trials exploring the effect of ferulic acid on different ailments and diseases, namely, colon and pancreatic cancers, multiple myeloma, myelodysplastic syndromes, Alzheimer's disease, and psoriasis, are still in progress [25].

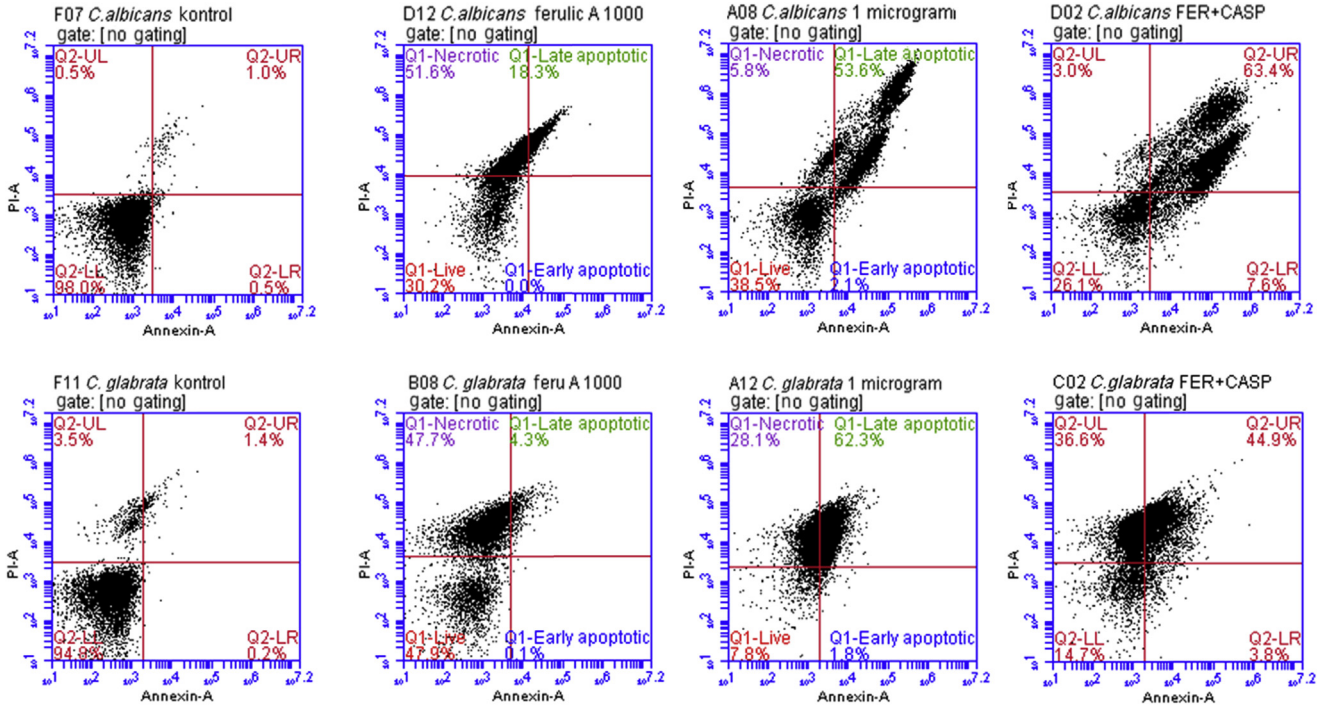

Figure 1 – *Candida albicans* and *Candida glabrata* apoptosis assay stained with annexin V-propidium iodide using flow cytometry.

Table 2 – Comparison of percentages of viable, early apoptotic, late apoptotic, and necrotic cells on *Candida albicans* and *Candida glabrata* stained with annexin V–propidium iodide using flow cytometry

Microorganism	*C. albicans*	*C. glabrata*
Control		
Viable	98.0	94.8
Early apoptotic	0.5	0.2
Late apoptotic	1.0	1.4
Necrotic	0.5	3.5
Caspofungin (1 μg/mL)		
Viable	38.5	7.8
Early apoptotic	2.1	1.8
Late apoptotic	53.6	62.3
Necrotic	5.8	28.1
Ferulic acid (1000 μg/mL)		
Viable	30.2	47.9
Early apoptotic	0.0	0.1
Late apoptotic	18.3	4.3
Necrotic	51.6	47.7
Caspofungin (1 μg/mL) + ferulic acid (1000 μg/mL)		
Viable	26.1	14.7
Early apoptotic	7.6	3.8
Late apoptotic	63.4	44.9
Necrotic	3.0	36.6

Results are shown as percentages.

Therefore, more comprehensive researches are required for a definite conclusion on the suitability of phenolic acids as successful antifungal agents in the future.

The present study highlighted the low toxic potential of phytochemicals as antifungal compounds and suggested the possible use of synergistic drug–herb combinations for the treatment of fungal contaminations. This study also highlighted various systemic interaction patterns. The findings may help identify new drug combinations with a novel mechanism of action.

In conclusion, this study demonstrated the inhibitory effect of different concentrations of ferulic acid and caspofungin on *C. albicans* and *C. glabrata*. It can be hypothesized that these effects are probably not produced by a single compound, but are a synergistic effect of different compounds. Substantial evidence is available regarding the synergistic effect of ferulic acid and existing antifungal agents, which may become a promising anticandidal treatment. However, more comprehensive research is needed to validate these fungal treatment strategies. This study evaluated the synergetic effect of two compounds on *Candida* species. It provided a different perspective in managing opportunistic fungal infections, especially against *Candida* species. The combination of ferulic acid and caspofungin exhibited a stronger fungicidal activity compared with ferulic acid or caspofungin alone.

Conflicts of Interest

The author has no conflict of interest to declare.

REFERENCES

[1] Abi-Said D, Anaissie E, Uzun O, Raad I, Pinzcowski H, Vativarian S. The epidemiology of hematogenous candidiasis caused by different *Candida* species. Clin Infect Dis 1997;4:1122–8.

[2] Eggimann P, Garbino J, Pittet D. Epidemiology of *Candida* species infections in critically ill non-immunosuppressed patients. Lancet Infect Dis 2003;3:685–702.

[3] Kim J, Sudbery P. Candida albicans, a major human fungal pathogen. J Microbiol 2011;49:171–7.

[4] Mayer FL, Wilson D, Hube B. *Candida albicans* pathogenicity mechanisms. Virulence 2013;4:119–28.

[5] Agarwal V, Lal P, Pruthi V. Effect of plant oils on *Candida albicans*. J Microbiol Immunol Infect 2010;43:447–51.

[6] Alves-Silva JM, Santos SMD, Pintado ME, Pérez-Álvarez JA, Fernández-López J, Viuda-Martos M. Chemical composition and *in vitro* antimicrobial, antifungal and antioxidant properties of essential oils obtained from some herbs widely used in Portugal. J Food Control 2012;32:371–8.

[7] Kanafani ZA, Perfect JR. Resistance to antifungal agents: mechanisms and clinical impact. Clin Infect Dis Antimicrob Resist 2008;46:120–8.

[8] Sher A. Antimicrobial activity of natural products from medicinal plants. Gomal J Med Sci 2009;7:72–8.

[9] Ou S, Kwok KC. Ferulic acid: pharmaceutical functions, preparation and applications in foods. J Sci Food Agric 2004;11:1261–9.

[10] D'archivio M, Filesi C, DI Benedetto R, Gargiulo R, Giovannini C, Masella R. Polyphenols, dietary sources and bioavailability. Ann Ist Super Sanita 2007;43:348–61.

[11] Kroon PA, Faulds CB, Ryden P, Robertson JA, Williamson G. Release of covalently bound ferulic acid from fiber in the human colon. J Agric Food Chem 1997;45:661–7.

[12] Georgopapadakou NH, Tkacz JS. The fungal cell wall as a drug target. Trends Microbiol 1995;3:98–104.

[13] Hao B, Cheng S, Clancy CJ, Nguyen MH. Caspofungin kills *Candida albicans* by causing both cellular apoptosis and necrosis. Antimic Agents Chemother 2012;57:326–32.

[14] Ramsdale M. Programmed cell death in pathogenic fungi. Biochim Biophys Acta 2008;1783:1369–80.

[15] Chaturvedi V, Ramani R, Pfaller MA. Collaborative study of the NCCLS and flow cytometry methods for antifungals susceptibility testing of *Candida albicans*. J Clin Microbiol 2004;42:2249–51.

[16] Meletiadis JI, Pournaras S, Roilides E, Walsh TJ. Defining fractional inhibitory concentration index cutoffs for additive interactions based on self-drug additive combinations, Monte Carlo simulation analysis, and *in vitro–in vivo* correlation data for antifungal drug combinations against *Aspergillus fumigatus*. Antimicrob Agents Chemother 2010;54:602–9.

[17] Bjerknes R. Flow cytometric assay for combined measurement of phagocytosis and intracellular killing of *Candida albicans*. J Immunol Methods 1984;72:229–41.

[18] Sanglard D. Resistance of human fungal pathogens to antifungal drugs. Curr Opin Microbiol 2002;5:379–85.

[19] Chandrasekar P. Management of invasive fungal infections: a role for polyenes. J Antimicrob Chemother 2011;66:457–65.

[20] Teodoro GR, Brighenti FL, Delbem AC, Delbem AC, Khouri S, Gontijo AV. Antifungal activity of extracts and isolated compounds from *Buchenavia tomentosa* on *Candida albicans* and non-*albicans*. Future Microbiol 2015;10:917–27.

[21] Han B, Chen J, Yu Y, Cao Y, Jiang Y. Antifungal activity of *Rubus chingii* extract combined with fluconazole against fluconazole-resistant *Candida albicans*. Microbiol Immunol 2016;60:82–92.

[22] Dai L, Zang C, Tian S, Liu W, Tan S, Cai Z. Design, synthesis, and evaluation of caffeic acid amides as synergists to sensitize fluconazole-resistant *Candida albicans* to fluconazole. Bioorg Med Chem Lett 2015;25:34–7.
[23] Sardi JC, Gullo FP, Freires IA, Pitangui NS, Segalla MP, Fusco-Almeida AM, Rosalen PL, Regasini LO, Mendes-Giannini MJ. Synthesis, antifungal activity of caffeic acid derivative esters, and their synergism with fluconazole and nystatin against *Candida spp*. Diagn Microbiol Infect Dis 2016;86:387–91.
[24] Stringaro A, Vavala E, Colone M. Effects of *Mentha suaveolens* essential oil alone or in combination with other drugs in *Candida albicans*. Evid Based Complement Alternat Med 2016. http://dx.doi.org/10.1155/2014/125904.
[25] Soobrattee MA, Neergheen VS, Luximon-ramma A, Aruoma OI, Bahorun T. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. Mutat Res 2005;579:200–13.