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RESEARCH

Synergistic activity between conventional antifungals and chalcone-derived compound against dermatophyte fungi and *Candida* spp.

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Abstract
The scarce antifungal arsenal, changes in the susceptibility profile of fungal agents, and lack of adherence to treatment have contributed to the increase of cases of dermatomycoses. In this context, new antimicrobial substances have gained importance. Chalcones are precursors of the flavonoid family that have multiple biological activities, have high tolerability by humans, and easy synthesis. In this study, we evaluated the in vitro antifungal activity, alone and in combination with conventional antifungal drugs, of the VS02–4′ethyl chalcone-derived compound against dermatophytes and *Candida* spp. Susceptibility testing was carried out by broth microdilution. Experiments for determination of the target of the compound on the fungal cell, time-kill kinetics, and toxicity tests in *Galleria mellonella* model were also performed. Combinatory effects were evaluated by the checkerboard method. Results showed high activity of the compound VS02–4′ethyl against dermatophytes (MIC of 7.81–31.25 µg/ml). The compound targeted the cell membrane, and the time-kill test showed the compound continues to exert gradual activity after 5 days on dermatophytes, but no significant activity on *Candida*. Low toxicity was observed at 250 mg/kg. Excellent results were observed in the combinatory test, where VS02–4′ethyl showed synergistic interactions with itraconazole, fluconazole, terbinafine, and griseofulvin, against all isolates tested. Although further investigation is needed, these results revealed the great potential of chalcone-derived compounds against fungal infections for which treatments are long and laborious.

Keywords Chalcones · Antifungal activity · Synergistic interaction · Dermatomycoses · Dermatophytes · *Candida*

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Introduction

Dermatomycoses affect 20–25% of the world population (Baghi et al. 2016; Zhan and Liu 2017; Appelt et al. 2021). They are caused by fungi that can invade the stratum corneum the skin, the intrafollicular keratinized portion of the hair, or nail plate (Corralo et al. 2014). The main etiological agents are dermatophyte fungi, especially *Trichophyton rubrum*, *T. mentagrophytes*, *Epidermophyton floccosum*, and *Microsporum canis*; and *Candida* spp. (Monod and Méhul 2019; Sylla et al. 2019). The scarce antifungal arsenal and change in the susceptibility profile of the etiological agents have contributed to the perpetuation of these mycoses in the population. Furthermore, patients often fail to adhere to long-term treatments (Gupta and Stec 2019; Lindsø Andersen et al. 2020; Gupta et al. 2021).

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In the search of new antimicrobial molecules, plant metabolites are being increasingly studied due to their beneficial biological properties (Dos Santos Ramos et al. 2016). In this context, chalcones, precursors of the flavonoid family, have great potential for exploration. They can interact with multiple biological targets, have high tolerability by humans, and the synthesis of its derivatives is simple (León-González et al. 2015). It has been reported many chemotherapeutic activities, including anticancer (Letafat et al. 2013; Ketabforoosh et al. 2014), antibacterial (Nowakowska 2007; Mahapatra et al. 2015; Marques et al. 2020), antiviral (Duran et al. 2021; Mothana et al. 2022; Valipour 2022; Nematollahi et al. 2023), antiparasitic (González et al. 2020; Nematollahi et al. 2023), and antifungal (Wang et al. 2016; Marques et al. 2020; Mirzaei et al. 2020; Gładkowski et al. 2023).

Currently, the main challenge in treating infectious diseases is the development of drug-resistant microorganisms. This resistance can emerge due to mutations, genetic changes, and phenotypic modifications. As a result, treatments that were once effective may no longer work, leading to unsuccessful disease management, a higher risk of transmission, and increased mortality rates. Therefore, developing new antimicrobial drugs is essential (Dhingra et al. 2020). Researchers are increasingly focusing on chalcones due to their proven antimicrobial properties against viruses, bacteria, fungi, and protozoa (Nematollahi et al. 2023).

Combining natural compounds, particularly chalcones, with antimicrobial drugs holds significant promise for improving the treatment of infectious diseases. By combining chalcones with existing antimicrobial drugs, it is possible to enhance the efficacy of treatments, especially in cases of resistance (Pereira et al. 2022; Chai et al. 2023). This synergy between traditional medicines and natural compounds, such as chalcones, carvacrol, gallic acid, epigallocatechin gallate, and essential oils, has already been reported in studies, reinforcing not only the expansion of the spectrum of antimicrobial activity but also opening the door to more effective therapeutic strategies for combating infectious diseases (Ayaz et al. 2019; Hellewell and Bhakta 2020; Brescini et al. 2021; Dos Santos et al. 2023; Sun et al. 2023).

In this study, we report the activity of a chalcone-derived compound, including determination of the target on the fungal cell, time-kill and toxicity analyses, and synergistic activity with conventional antifungals, against dermatophytes and *Candida* spp.

Material and methods

Fungal isolates

The list of clinical isolates and reference strains used in this study is in Table 1. In accordance with the Brazilian regulation, the isolates are registered in the SisGen (National System for the Management of Genetic Heritage and Associated Traditional Knowledge) – protocol number A2B1006.

Synthesis of the compound

The chalcone-derived compound was provided by the Laboratory of Antibiotics and Chemotherapy (LAQ) at the São Paulo State University (Unesp), Campus São José do Rio

Table 1 Dermatophytes and *Candida* isolates included in this study, the identification code, culture collection, and origin of isolation

Isolate code	Species	Culture collection	Origin
TRCBS	*T. rubrum*	CBS2266	-
TR7984	*T. rubrum*	FAMERP	Foot skin
TR6284	*T. rubrum*	FAMERP	Toenail
TMATCC	*T. mentagrophytes*	ATCC 18748	Mild dermatophytosis
TM6007	*T. mentagrophytes*	FAMERP	Foot skin
TM6085	*T. mentagrophytes*	FAMERP	Toenail
MCATCC	*M. canis*	ATCC 11621	Clinical dermatophytosis
MC8168	*M. canis*	FAMERP	Scalp
MC8295	*M. canis*	FAMERP	Scalp
EF6050	*E. floccosum*	FAMERP	Toenail
EF6069	*E. floccosum*	FAMERP	Toenail
EF8233	*E. floccosum*	FAMERP	Toenail
CGATCC	*C. glabrata*	ATCC 2001	Feces
CG6930	*C. glabrata*	FAMERP	Urine
CAUCBS	*C. auris*	CBS	-
CAU491	*C. auris*	FAMERP	-

ATCC American Type Culture Collection, *CBS* Centraalbureau voor Schimmelcultures, *FAMERP* Faculdade de Medicina de São José do Rio Preto; (-), unknown

Preto, Brazil. It was synthesized by Claisen–Schmidt aldol condensation reaction (Dos Santos et al. 2017, 2019). The components (aminoacetophenones and aldehydes) were dissolved in ethanol, and sodium hydroxide in ethanol was added as catalyst solution. The reaction product was kept under stirring for 2–6 h at RT and then poured into ice and filtered or extracted with ethyl acetate. The organic phases were combined and washed with aqueous NaHSO solution and dried over $MgSO_4$. Filtration occurred under reduced pressure. After chromatographic separation and purification, the chalcone compounds were obtained in a yield of around 40%. Characterization of compounds included determination of melting points, structure, and spectral analyses. The compound used in this study was the VS02–4′ethyl, where an ethyl group was inserted at the fourth carbon position of the first aromatic ring (Fig. 1). For the in vitro assays, the compound was solubilized in 10% DMSO (LabSynth®).

Antifungal susceptibility testing

The susceptibility profiles of the fungal isolates against the VS02–4′ethyl chalcone compound and conventional antifungal drugs (itraconazole, fluconazole, terbinafine, griseofulvin—[Sigma-Aldrich®]) were determined by broth microdilution, according to protocols M38-3rd ed (CLSI 2017) and M27-3rd ed (CLSI 2008) of the Clinical Laboratory Standard Institute.

In 96-wells plates, the VS02–4′ethyl was serially diluted in RPMI–1640 medium (Rosen Park Media Institute—Sigma-Aldrich®). For dermatophytes, final testing concentrations ranged from 250 to 0.48 µg/ml. For yeast, the concentrations ranged from 1000 to 1.95 µg/ml. Similarly, the antifungals were diluted to reach the following concentrations: 256–0.5 µg/ml, for fluconazole; 8–0.015 µg/ml, for terbinafine; and 16–0.03 µg/ml, for itraconazole and griseofulvin. Griseofulvin was only tested against dermatophytes.

Dermatophytes were cultured in Potato-dextrose agar (Oxoid ®) for 7 days, at 35 °C, and yeast in Sabouraud-dextrose agar (SDA, Oxoid ®) for 24 h, at 37 °C. The inocula were prepared in 0.9% sterile saline solution to reach final concentrations of 0.4 to 5×10^4 CFU/ml, for dermatophytes, and 0.5 to 2.5×10^3 CFU/ml, for yeast.

After inoculation, the plates were incubated at 35 °C and visual readings were carried out after 120 h, for dermatophytes, and 48 h, for yeast. Minimum inhibitory concentrations (MIC) were determined as the lowest concentration capable of inhibiting 80% of fungal growth, for fluconazole and itraconazole, and 100% of fungal growth, for griseofulvin, terbinafine, and the VS02–4′ethyl compound.

Minimum fungicidal concentrations (MFC) were determined by subculturing an aliquot from each well into agar plates, and it was considered as the lowest concentration capable of inhibiting fungal growth in the culture medium.

Sterility, growth, and solvent controls were added to each test. Tests were performed in triplicate.

Sorbitol and ergosterol assays

In addition to the compound susceptibility testing, experiments were performed with two different supplemented RPMI–1640 to determine where the VS02–4′ethyl will target on the fungal cell. The first supplement to the RPMI–1640 medium was 0.8 M sorbitol (Sigma-Aldrich®) (De Castro et al. 2015). Sorbitol acts as an osmoprotector that allows cells to grow in the presence of an inhibitor of a fungal cell wall synthesis (Svetaz et al. 2007). Consequently, increases in MIC values indicates that the compound targets the cell wall. The other supplement was the ergosterol (Sigma-Aldrich®) at 400 µg/ml (De Castro et al. 2015). If the compound activity is due to ergosterol binding, providing exogenous ergosterol would prevent binding to ergosterol of the fungal membrane, what would increase MIC values (Escalante et al. 2008).

The fungal isolates TRCBS, TMATCC, MCATCC, EF6069, CGATCC, and CAUCBS were selected for these assays. Values of MIC were compared with and without sorbitol and ergosterol supplementation.

As controls, these isolates were tested against caspofungin (Sigma-Aldrich®) and amphotericin B (Sigma-Aldrich®) (at final concentration of 16 to 0.03 µg/ml) with RPMI–1640 without and with supplementation with sorbitol and ergosterol, respectively. Sterility, growth, and solvent controls were added to each test. Tests were performed in triplicate.

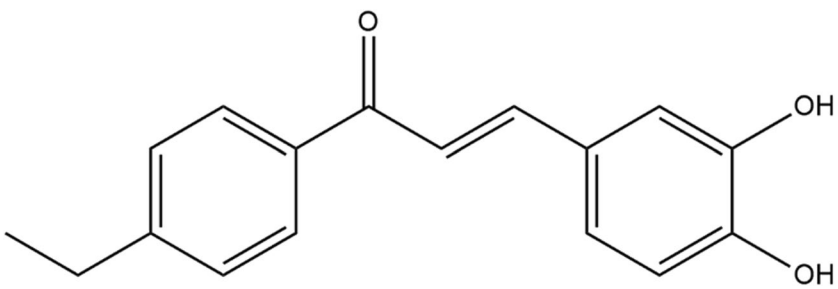

Fig. 1 Chemical structure of the chalcone-derived compound VS02–4′ethyl

Time-kill analysis

Time-kill analysis were carried out according to Klepser et al. (1998). The isolates used in this experiment were TRCBS, TMATCC, MCATCC, EF6069, CGATCC, and CAUCBS. Fungal inocula were prepared as previously described and diluted 1:1 with VS02–4′ethyl. For dermatophytes, two concentrations of the compound were tested, 500 µg/ml and 62.5 µg/ml. For *Candida* spp., the concentration of the compound was 4000 µg/ml. Controls included 10% DMSO solution, without the compound.

At predetermined times (initial moment—0 h, 8 h, 24 h, 48 h, and 120 h), an aliquot of 30 µl was inoculated on SDA plates with a Drigalski spatula. After 120 h, for dermatophytes, and 48 h, for yeasts, at 35 °C, the colonies were counted, and the results adjusted to Log_{10} CFU/ml.

Toxicity analysis

The toxicity of the VS02–4′ethyl compound was determined by an experimental model of *Galleria mellonella* larvae, according to Ignasiak and Maxwell (2017). The *G. mellonella* in vitro model has the advantage of the fact that this insect immune system is functionally and structurally similar to the innate immune system of mammals, along with the low cost of the technique and the fast of insect reproduction (Browne et al. 2013; Ignasiak and Maxwell 2017). The compound was tested at the following concentrations: 125 µg/ml, 250 µg/ml, 500 µg/ml, 1000 µg/ml, and 2000 µg/ml). For each experiment, five groups of five larvae at the sixth stage of development (250 ± 25 mg) were inoculated with compound. Five microliters were injected into the last right proleg of the larvae using a 10 µl Hamilton model 7000.5KH micro syringe. As controls, it was used untouched larvae (naïve), larvae inoculated with 99.9% ethanol (mortality control), sterile water (negative toxicity control), and 60% DMSO.

The larvae were incubated at 37 °C, deprived of food and direct lighting. Larvae survival assessments were carried out every 24 h for 5 days and pre-pupal formations were removed daily to delay their metamorphosis. Survival analysis was performed using the log-rank (Mantel-Cox) test. GraphPad Prism® software version 9.3.0 for Windows (San Diego, California, USA) was used for statistical analysis.

Checkerboard assay

The combinatorial effect between the chalcone-derived compound and conventional antifungal drugs was evaluated using the checkerboard method (CLSI 2008; Lemes et al. 2023).

In 96-wells plates, the VS02–4′ethyl was combined with each antifungal. In the assays against dermatophytes, the chalcone-derived compound concentration ranged from 125 to 0.12 µg/ml, and, for *Candida* spp., ranged from 1000 to 0.97 µg/ml. The antifungal drugs varied from 128 to 0.5 µg/ml, for fluconazole; 16 to 0.03, for itraconazole; 64 to 0.007 µg/ml, for terbinafine; and 4 to 0.06 µg/ml, for griseofulvin. Griseofulvin was only tested against dermatophytes.

The isolates TRCBS, TR7984, CGATCC, and CAUCBS were used in this assay. The inocula were prepared as previously described. Inoculation, incubation, and readings were also performed as previously described.

To evaluate the interaction between the compound and the drugs, the fractional inhibitory concentration (FIC) index was calculated, following the classification by Kumar et al. (2012), where $FIC \leq 0.5$ means synergistic interaction; $0.5 < FIC \leq 1$ means additive action; $1 < FIC \leq 2$ means indifferent interaction; and $FIC > 2$ means antagonistic interaction.

Results

Antifungal activity of VS02–4′ethyl

The chalcone-derived compound showed high activity against dermatophytes, with MIC ranging from 7.81 to 31.25 µg/ml, and geometric mean (GM) of 14.74 µg/ml. For *Candida* spp., MIC values ranged from 500 to 1000 µg/ml and GM of 840.90 µg/ml. Regarding MFC, values varied between 31.25 and > 250 µg/ml, for dermatophytes, and 1000 and > 1000 µg/ml, for *Candida* spp. (Table 2).

Regarding the antifungal drugs, terbinafine showed the most potent activity against dermatophytes (Table 2), followed by itraconazole and griseofulvin. Fluconazole showed the least activity. Against *Candida* isolates, only itraconazole exhibited high activity. Detailed results are shown in Table 2.

Sorbitol and ergosterol assays

Comparing of the experiments with and without supplementation, it was observed that the presence of sorbitol did not alter the MIC values. However, the presence of exogenous ergosterol in the tests caused the MIC values to increase up to 8 times (from 31.25 to 250 µg/ml) (Table 3). That indicates that the VS02–4′ethyl targets the cell membrane of fungi.

Time-kill analysis

Data obtained in the time-kill experiments can be seen on Fig. 2. For dermatophytes, results show that the compound caused a mean of 1.12-log reduction of viability, at 500 µg/ml, and 0.92-log reduction, at 62.5 µg/ml, after 5 days. The

Table 2 Minimum inhibitory concentrations (MIC), minimum fungicidal concentrations (MFC), and geometric mean MIC values of the chalcone-derived compound; and MIC values of fluconazole, itraconazole, terbinafine, and griseofulvin, against the isolates of *T. rubrum*, *T. mentagrophytes*, *M. canis*, *E. floccosum*, *C. glabrata*, and *C. auris* included in this study

Isolates	VS02–4′ethyl		Fluconazole	Itraconazole	Terbinafine	Griseofulvin
	MIC (μg/ml)	MFC (μg/ml)	MIC (μg/ml)	MIC (μg/ml)	MIC (μg/ml)	MIC (μg/ml)
T. rubrum						
TRCBS	15.62	31.25	4	0.5	< 0.015	1
TR6284	7.81	31.25	32	0.25	< 0.015	1
TR7984	7.81	31.25	32	0.12	< 0.015	1
GM	9.84					
T. mentagrophytes						
TMATCC	31.25	> 250	4	0.5	< 0.015	0.5
TM6007	7.81	31.25	128	0.5	< 0.015	0.5
TM6085	15.62	62.50	128	0.12	< 0.015	1
GM	15.62					
M. canis						
MCATCC	15.62	62.50	16	0.25	< 0.015	0.06
MC8168	15.62	250	32	0.25	< 0.015	0.25
MC8295	15.62	250	32	0.12	< 0.015	0.5
GM	15.62					
E. floccosum						
EF6050	31.25	250	8	0.12	0.5	2
EF6069	15.62	125	32	0.12	< 0.015	0.5
EF8233	15.62	62,50	32	0.12	< 0.015	2
GM	19.68					
C. glabrata						
CGATCC	500	1000	32	2	16	-
CG6930	1000	> 1000	32	2	16	-
GM	707.11					
C. auris						
CAUCBS	1000	> 1000	8	0.06	2	-
CAU491	1000	> 1000	> 128	0.25	> 16	-
GM	1000					

MIC minimum inhibitory concentration, *MFC* minimum fungicidal concentration, *GM* geometrical mean

Table 3 Sorbitol and ergosterol supplementation tests. Minimum inhibitory concentration (MIC) values (in μg/ml) of the VS02–4′ethyl chalcone-derived compound, caspofungin, and amphotericin B, before and after medium supplementation with sorbitol or ergosterol, against *T. rubrum*, *T. mentagrophytes*, *M. canis*, *E. floccosum*, *C. glabrata*, and *C. auris* isolates

Isolate	Sorbitol supplementation				Ergosterol supplementation			
	MIC Before		MIC After		MIC Before		MIC After	
	VS	CSP	VS	CSP	VS	AMB	VS	AMB
TRCBS	31.25	0.12	31.25	0.5	31.25	0.5	250	2
TMATCC	31.25	0.03	31.25	0.06	31.25	0.25	250	8
MCATCC	7.81	0.12	7.81	0.25	15.62	0.12	125	0,5
EF6050	62.5	0.06	62.5	0.25	31.25	0.25	250	0,5
CGATCC	500	4	500	8	500	16	> 2.000	> 16
CAUCBS	1.000	16	1.000	> 16	1.000	8	> 2.000	> 16

VS VS02–4′ethyl chalcone-derived compound, *CSP* caspofungin, *AMB* amphotericin B

figure shows that the decrease was gradual over time, until the end of the analysis. Contrarily, the analyses evidenced that the compound does not have high activity against the *Candida* isolates tested, since no reduction could be observed after 5 days, even at a higher concentration of the compound (4.000 μg/ml) (Fig. 2).

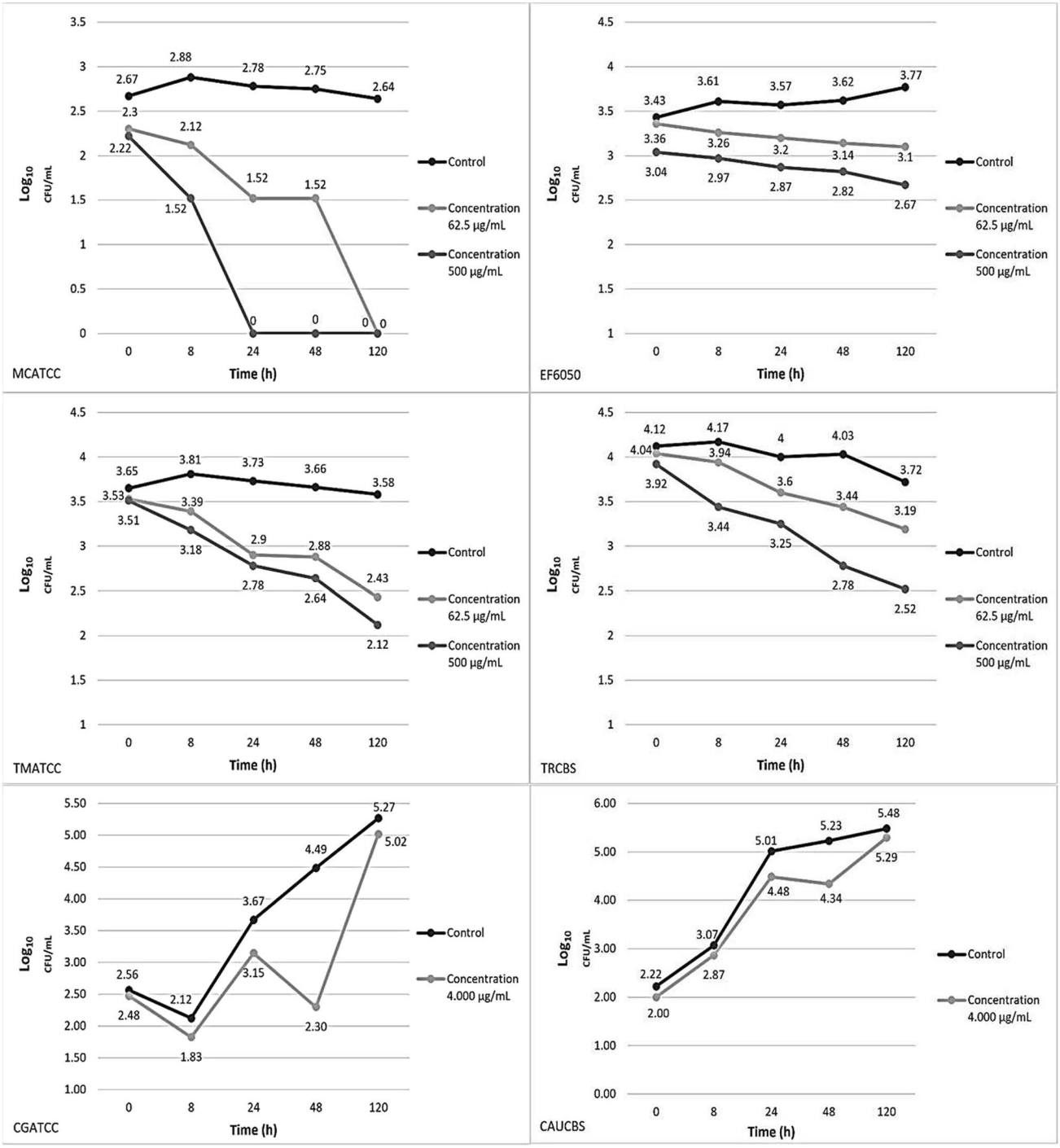

Fig. 2 Time-kill kinetics assay. Log10 CFU/ml of isolates of *T. rubrum*, *T. mentagrophytes*, *M. canis*, *E. floccosum*, *C. glabrata*, and *C. auris* after 0, 8, 24, 48, and 120 h of exposure to the chalcone-derived compound and in its absence (control)

Toxicity analysis

The tests with VS02–4′ethyl concentration of 125 and 250 mg/kg showed low toxicity, with survival rates of 60% of the *G. mellonella* larvae, after 5 days. At 500 mg/kg, 40% of the larvae survived after 5 days. Concentrations of 1000 and 2000 mg/kg of VS02–4′ethyl killed all larvae after three and 4 days, respectively (Fig. 3).

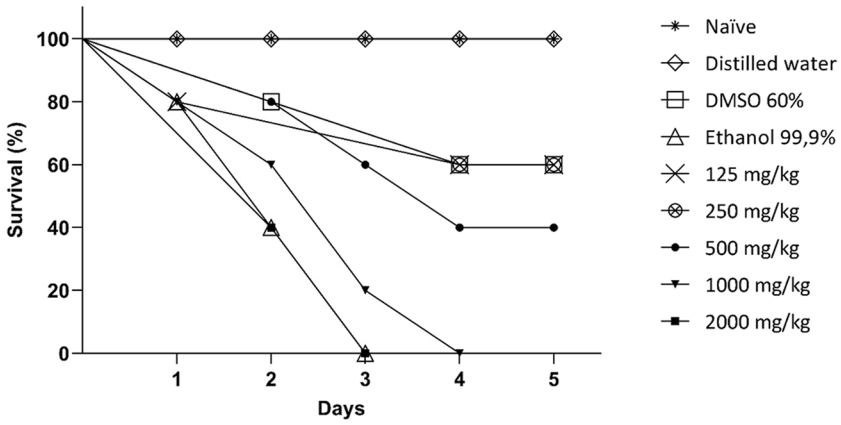

Fig. 3 Survival rate (%) of *Galleria mellonella* larvae exposed to different concentrations of the VS02–4′ethyl chalcone-derived compound, and distilled water, 60% DMSO, 99.9% ethanol

Checkerboard assay

When combined, all tests showed MIC reduction of the antifungal drugs and the VS02–4′ethyl compound. Values of FICI index varied from 0.1 to 0.5, all in the range of synergistic combinatory effect (Table 4).

Discussion

In this study, we report the antifungal activity of the chalcone-derived compound VS02–4′ethyl against the main etiological agents of dermatomycoses. Chalcones are among the main secondary metabolites of edible plants. Most chalcones are polyhydroxylated aromatic compounds and bioprecursors of open-chain flavonoids and isoflavonoids (Rudrapal et al. 2021). They can be obtained from natural or synthetic sources, which can form different derivative compounds from their main structure (Mirzaei et al. 2020). Modification of the structure of chalcones enhances their biological activity, reduces toxicity, and increases their pharmacological effects (Nawaz et al. 2023).

Some of the well-known natural chalcone containing drugs are Butein (anticancer and anti-inflammation), Xanthohumol (antibacterial and anti-HIV agent), Isoliquiritigenin (anti-cancer, chemoprotective, anti-inflammatory and antioxidant), Cardamonin (ATP diphosphohydrolase), licochalcone (anti-inflammatory, anti-cancer), Metochalcone

Table 4 Checkerboard assays results. Minimum inhibitory concentration (MIC) values (in µg/ml) of the VS02–4′ethyl chalcone-derived compound and fluconazole, itraconazole, griseofulvin, and terbinafine, alone and combined, against *T. rubrum*, *T. mentagrophytes*, *C. glabrata*, and *C. auris* isolates; and fractional inhibitory concentration index (FICI), which indicates the nature of the combinatory effect of the compounds

Isolate	Single agent MIC		In combination MIC		FICI	Combinatory effect
	VS	FCZ	VS	FCZ		
TRCBS	31.25	4	0.12	2	0.5	Synergistic
TR7984	15.62	32	0.12	16	0.5	Synergistic
CGATCC	1000	64	0.97	16	0.2	Synergistic
CAUCBS	1000	32	0.97	16	0.5	Synergistic
	VS	ITZ	VS	ITZ		
TRCBS	31.25	0.5	0.12	0.12	0.3	Synergistic
TR7984	15.62	0.5	0.12	0.25	0.5	Synergistic
CGATCC	1000	16	0.97	2	0.1	Synergistic
CAUCBS	1000	0.5	0.97	0.25	0.5	Synergistic
	VS	TBF	VS	TBF		
TRCBS	31.25	0.007	0.12	0.003	0.4	Synergistic
TR7984	15.62	0.015	0.12	0.007	0.4	Synergistic
CGATCC	1000	64	250	2	0.2	Synergistic
CAUCBS	1000	64	250	8	0.4	Synergistic
	VS	GFV	VS	GFV		
TRCBS	31.25	1	0.12	0.5	0.5	Synergistic
TR7984	15.62	1	0.12	0.5	0.5	Synergistic

MIC minimum inhibitory concentration, *VS* VS02–4′ethyl chalcone-derived compound, *FCZ* fluconazole, *ITZ* itraconazole, *TBF* terbinafine, *GFV* griseofulvin, *FICI* fractional inhibitory concentration index

(choleretic agent), and Sofalcone (anti-ulcer agent) (Narwal et al. 2024). Due to the presence of phenolic groups and their property of scavenging radicals, plant extracts rich in chalcones have been extensively studied in the search for new therapeutic compounds (Ouyang et al. 2021).

Regarding their antimicrobial activity, it has been reported that chalcones and other flavonoids are lipophilic, which may lead to disruption of the cell membrane and leakage of nucleic acids (Thebti et al. 2023), which corroborates the data obtained in the ergosterol experiment of this study. Additionally, the activity of a chalcone-derived compounds may result from the downregulation of genes encoding virulence factors, such as isocitrate lyase, citrate synthase and malate synthase (Cantelli et al. 2017), efflux pumps (Komoto et al. 2015; Nematollahi et al. 2023), and inhibition of fatty acid synthesis (Nematollahi et al. 2023). However, chalcones may act differently depending on the fungal species or genera (Lahtchev et al. 2008; Mellado et al. 2020; Morão et al. 2020).

The VS02–4′ethyl compound showed higher activity against dermatophytes when compared to *Candida* isolates, with MIC ranging from 7.81 to 31.25 µg/ml for filamentous fungi, versus MIC values ranged from 500 to 1000 µg/ml for yeast (Table 2). A previous study showed that yeast's intracellular glutathione and cysteine molecules act as defense barriers against chalcones (Lahtchev et al. 2008). These proteins are related to vitality, cellular development, and pathogenesis (Wangsanut and Pongpom 2022). In a metabolomic study by Ciesielska et al. (2021), the authors report that, during keratin degradation by dermatophytes, cysteine levels increased but glutathione molecules were not detected in the experiment. This glutathione deficiency may help elucidate why the VS02–4′ethyl presented higher activity against dermatophytes.

It is crucial the discovery and development of new treatments for dermatomycoses because of their great incidence worldwide. Moreover, treatment failure and antifungal resistance are being increasingly reported (Gupta and Venkataraman 2022). Antifungal resistance is often related to mutations that modifies the target of the drug or regulation of efflux pumps (Ksiezopolska and Gabaldón 2018) and can be associated to long term use. A 2-year study demonstrated that excessive use of fluconazole promoted resistance in *Candida* species, with approximately 98% of the *C. albicans* isolates, 93% of *C. parapsilosis*, 91% of *C. tropicalis*, and 68% of *C. glabrata* resistant to fluconazole (Beardsley et al. 2018). *Candida auris*, although is not a common agent of superficial mycoses, colonizes the skin and it was included in this study due to the high incidence of resistance to multiple classes of antifungals and growing concern about outbreaks (Spivak and Hanson 2018).

Regarding dermatophytes, it is estimated that 19% of infections are caused by azole-resistant strains (Ghannoum 2016). Although resistance to azoles is more likely, resistance to terbinafine has also been reported in *Microsporum* spp. and *Trichophyton* spp., and is associated with mutations in genes encoding the enzyme squalene epoxidase (Lindsø Andersen et al. 2020; Gupta et al. 2021). Failures in treatment may still be associated with insufficient dose and duration regimes, in addition to low patient adherence (Gupta and Venkataraman 2022).

In this study, we highlight the antifungal activity of the VS02–4′ethyl chalcone-derived compound in association with the conventional antifungal drugs (Table 4). Synergistic interaction occurred in all experiments (FIC values less than or equal to 0.5), causing a significant decrease of MIC values for both antifungal, with reductions of up to five-fold in MIC value, and the compound itself, with reductions of up to ten-fold. This may be incredibly beneficial, especially against *C. glabrata* and *C. auris*, which presented elevated MIC values for the compound and the antifungals alone.

The best performance of the compound was observed against the *C. glabrata* strain in synergistic action with itraconazole. Individually, the MIC value of the compound was 1000 µg/mL, while that of itraconazole was 16 µg/mL. However, when combined, there was a significant reduction in the MIC values to 0.97 and 2 µg/mL, respectively. Considering the high resistance rates of *C. glabrata* to itraconazole and other azoles (Kaan et al. 2021; Frías-De-León et al. 2021; Dunaiski et al. 2024), these results indicate a potent synergistic interaction of the compound VS02–4′ethyl, which enhances the action of itraconazole, providing a promising approach for the treatment of resistant infections.

Synergistic interactions can improve drug pharmacokinetics, slow down their metabolism and elimination by the body, and decrease toxicity effects (Ahmad et al. 2017). Different possible mechanisms for synergy activities have already been proposed: one compound may act alone and the second bind to the target, facilitating the binding of the first compound; two compounds may reach different locations or biological pathways and exert a collective effect; or two compounds can act on the same biological pathway at two different stages, increasing activity (Ahmad et al. 2017; Spitzer et al. 2017).

Synergistic interactions between chalcone-derived compounds and fluconazole have already been reported. Ahmad et al. (2017) investigation indicated that the chalcone–fluconazole interaction reversed fluconazole resistance causing a downregulation of the ERG11 gene, which is important in the ergosterol biosynthesis pathway and is crucial for fluconazole resistance. Nonetheless, this may not be the only mode of action, since the VS02–4′ethyl also showed synergy with griseofulvin, which acts in the process of fungal mitosis by interacting with microtubules, disrupting the mitotic spindle (Yesudian et al. 2021). Unfortunately, most data available

on the interaction of antifungal drugs and chalcone-derived compounds reference fluconazole.

In conclusion, the main finding of this study was the highly synergistic activity of VS02–4′ethyl chalcone-derived compound with conventional antifungal drugs against dermatophytes and *Candida* spp. The VS02–4′ethyl in concentration equal to or less than 250 mg/kg showed low toxicity, study report that some chalcones can be toxic at high concentrations, leading to cytotoxicity, genotoxicity, and other harmful effects. However, chalcones are generally considered safe at lower doses (Jesus et al. 2022). Further investigations are needed to elucidate the mode of action and the synergistic interaction of the VS02–4′ethyl with the different classes of antifungals. In addition, further studies should reveal the best administration route and the in vivo effects of the compound. Nevertheless, these results revealed the great potential of chalcone-derived compounds against fungal infections for which treatments are long and laborious.

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Author contributions TML and MTGA the study conception and design. PTC and LOR helped synthesize the compound. TML and BGA performed the literature search and data analysis. TML, THL and MDRM performed the material preparation and data collection. GRC and MRVZK carried out the toxicity test. TML and JPZS wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval Not applied, that no ethical approval is required.

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