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Combined Antifungal Activity of Fluconazole and Berberine Against a Fluconazole-Resistant Clinical Isolate of *Candida albicans*: An In Vitro Agar Well Diffusion Study

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Abstract

Oral candidiasis caused by *Candida albicans* is a common opportunistic fungal infection, and reduced susceptibility or resistance to fluconazole can complicate treatment in repeatedly exposed or otherwise high-risk patients. Berberine, an isoquinoline alkaloid found in *Berberis*, *Coptis*, and related plants, has previously been reported to enhance fluconazole activity against resistant *Candida* isolates. This in vitro experimental study screened fluconazole and berberine, alone and in combination, against a fluconazole-resistant clinical isolate of *C. albicans*. A collection of 40 fluconazole-resistant *C. albicans* isolates had been obtained from 50 clinical specimens and identified by conventional mycological methods together with chromogenic media and the Vitek 2 system; the combination experiment reported in this article was performed on one resistant clinical isolate. Resistance status was established by antifungal susceptibility testing, while the quantitative outcome reported here was inhibition-zone diameter in an agar well diffusion assay. Each reported diffusion condition was tested in triplicate. Fluconazole produced a detectable zone only at 25 μg per well, with a mean

diameter of 13.00 ± 0.00 mm. Berberine alone produced no detectable zone at any tested amount. The nominal combination of fluconazole 25 μg with berberine 250 μg produced a zone of 32.50 ± 2.50 mm, corresponding to a mean difference of 19.50 mm compared with fluconazole alone (95% CI 13.29–25.71 mm by the Welch approach). The three highest-dose comparison groups differed overall (one-way ANOVA, $F(2,6) = 385.32$, $p < 0.0001$; Kruskal–Wallis $H = 7.71$, $p = 0.021$), and the combination differed from fluconazole alone (Welch $t = 13.51$, $df = 2.0$, $p = 0.0054$; pooled-variance Student $t = 13.51$, $df = 4$, $p = 0.00017$). Because berberine alone produced no measurable inhibition zone, the larger zone observed with the combination is consistent with potentiation of fluconazole activity. However, agar well diffusion cannot formally establish pharmacodynamic synergy, and confirmation by checkerboard broth microdilution with a fractional inhibitory concentration index and by time–kill analysis is required.

Keywords: *Candida albicans*, fluconazole, berberine, antifungal resistance, drug combination, agar well diffusion

1. INTRODUCTION

Candida albicans commonly colonises the oral, gastrointestinal, and genitourinary mucosae of healthy individuals. Disease develops when the balance among the organism, the resident microbiota, and host defences is disrupted. Oral candidiasis therefore occurs more frequently in recognisable risk groups, including people living with HIV, patients receiving cytotoxic chemotherapy or head-and-neck radiotherapy, individuals with poorly controlled diabetes mellitus, recipients of broad-spectrum antibacterial therapy, and denture wearers [1, 2]. In profoundly immunocompromised patients, mucosal candidiasis may extend to the oesophagus, and disruption of mucosal barriers can permit bloodstream invasion. Invasive candidiasis consequently remains an important contributor to the global burden of severe fungal disease [3].

Fluconazole remains an important treatment option for mucosal candidiasis [4]. Its principal target is lanosterol 14 α -demethylase, encoded by *ERG11*; inhibition of this enzyme disrupts ergosterol biosynthesis and results in the accumulation of abnormal sterol intermediates, thereby impairing fungal membrane structure and function [5]. Fluconazole is predominantly fungistatic against *Candida*, and prolonged or repeated exposure can select populations with reduced susceptibility when fungal clearance is incomplete.

Fluconazole resistance in *C. albicans* is usually multifactorial. Point mutations in *ERG11* can reduce azole binding, increased expression of *ERG11* can raise target abundance, and gain-of-function alterations affecting transcriptional regulators such as *TAC1* and *MRR1* can increase expression of the ABC transporters Cdr1p and Cdr2p or the major facilitator Mdr1p [6–8]. Clinical isolates from patients with oral candidiasis may contain several resistance mechanisms simultaneously [9]. Growth in biofilms on mucosal or acrylic surfaces can provide an additional layer of tolerance that further complicates treatment [10, 11].

One strategy for addressing azole resistance is to identify adjuvant compounds that restore or increase azole activity rather than replacing the azole entirely. Berberine is a quaternary isoquinoline alkaloid present in *Berberis vulgaris*, *Coptis chinensis*, and related species. Interest in berberine as an antifungal adjuvant increased after Quan and colleagues demonstrated potent in vitro synergism between berberine chloride and fluconazole against fluconazole-resistant clinical isolates of *C. albicans* using checkerboard microdilution, agar diffusion, and time–kill methods [12]. Subsequent studies have reported antifungal activity of berberine against resistant yeasts, although the concentration required for inhibition varies substantially among species, isolates, experimental media, and assay formats [13, 14].

Several mechanisms have been proposed to explain the interaction. Berberine can interfere with drug-efflux responses, including inhibition of induced *CDR1* up-regulation [15], and it can interact with the Mdr1p efflux system in ways that increase intracellular exposure [16]. Other work indicates that the fluconazole–berberine combination can augment endogenous reactive oxygen species [17] and that berberine can disrupt mitochondrial function and iron homeostasis [18]. Ergosterol status also appears to influence cellular tolerance to berberine, providing a mechanistic basis for reciprocal enhancement between fluconazole-mediated sterol disruption and berberine activity [19, 20]. More recent studies continue to report enhanced activity of berberine or berberine hydrochloride with fluconazole against resistant *C. albicans*, including effects on efflux, biofilm-related traits, and stress-response pathways [21, 22].

The present study therefore screened fluconazole and berberine, individually and in combination, against a fluconazole-resistant clinical isolate of *C. albicans*. The primary objective was to determine whether the combination produced a larger inhibition zone than fluconazole alone under the agar well diffusion conditions used. The study was designed as an exploratory in vitro experiment. Because diffusion-zone enlargement cannot by itself establish pharmacodynamic synergy, the interaction is interpreted as potentiation or enhanced activity unless confirmed by a standard quantitative combination method such as checkerboard microdilution with calculation of the fractional inhibitory concentration index (FICI) or by time–kill analysis [12, 22].

2. MATERIALS AND METHODS

2.1. STUDY DESIGN AND ISOLATE SOURCE

This work was an exploratory in vitro laboratory study conducted between April 2025 and February 2026. The laboratory collection described in the study comprised 40 fluconazole-resistant *C. albicans* isolates obtained from 50 clinical specimens. The combination experiment reported in the present article was performed on one fluconazole-resistant clinical isolate, consistent with the single-isolate focus stated in the title and the Results and Discussion sections. The clinical material was associated with the microbiology laboratory of the College of Science, University of Basrah, and specimens were obtained from patients attending Al-Fayhaa Hospital. A previously published study from the same institutional setting is cited for related isolate collection and antifungal laboratory procedures [23]; however, the present fluconazole–berberine experiment and its numerical results are reported independently here.

2.2. IDENTIFICATION OF *CANDIDA ALBICANS* AND CONFIRMATION OF RESISTANCE

Clinical specimens were cultured on Sabouraud dextrose agar (SDA) and incubated at 37 °C for 24–72 h. Colonies were examined microscopically after Gram staining. Presumptive identification included the germ-tube test and chromogenic differentiation on HiCrome™ *Candida* Differential Agar, followed by automated identification with the Vitek 2 system. The germ-tube test was interpreted as a presumptive test rather than a species-specific confirmation because *C. dubliniensis* may also be germ-tube positive; the chromogenic and automated identification steps therefore provided additional species-level discrimination [28]. Antifungal susceptibility testing was performed by broth microdilution in accordance with the principles of the CLSI reference method for yeasts [25]. The isolate used in the combination experiment had been classified as fluconazole resistant. The exact fluconazole MIC for that isolate is not included in the outcome dataset presented in this article; consequently, the current Results focus on the diffusion-zone experiment rather than presenting a new MIC-based resistance analysis.

2.3. PREPARATION OF FLUCONAZOLE AND BERBERINE

Pharmaceutical-grade fluconazole and berberine powders were used, and dimethyl sulfoxide (DMSO) served as the solubilising vehicle. Stock solutions were prepared aseptically and diluted to generate the working preparations used in the diffusion experiment. Fluconazole conditions were designated as 25, 12.5, and 6.25 µg per well, whereas berberine conditions were designated as 250, 125, and 25 µg per well. The preparations were mixed until visually homogeneous and were kept in sterile tubes until use.

Combination preparations were made immediately before application by mixing corresponding fluconazole and berberine working preparations. The three nominal pairings were designated 25 µg + 250 µg, 12.5 µg + 125 µg, and 6.25 µg + 25 µg. These designations are retained because they are the amounts recorded in the laboratory dataset and used throughout the Results. The original laboratory record states that equal volumes were mixed, but it does not document sufficient post-mixing concentration information to reconstruct independently the exact delivered mass after mixing. For that reason, the pairings are treated as nominal experimental designations rather than as independently verified final well concentrations. This limitation is considered explicitly in the Discussion.

2.4. INOCULUM PREPARATION AND AGAR WELL DIFFUSION

A fresh SDA subculture of the selected isolate was suspended in sterile saline. Turbidity was adjusted by visual comparison with the laboratory standard routinely used for antimicrobial susceptibility work, and the suspension was used immediately. For the diffusion experiment, Mueller–Hinton agar plates were inoculated with the *C. albicans* suspension. Wells were prepared aseptically and loaded with the single-agent or combination preparations. A DMSO solvent control was included. Agar well diffusion is an established screening approach for antimicrobial activity, but zone size is affected by both biological potency and the ability of the tested compound to diffuse through the agar matrix [24]. Consequently, the absence of a visible zone was recorded as “no zone detected” rather than interpreted as a formal susceptibility category.

Each condition was tested in triplicate. Plates were incubated overnight at approximately 35–37 °C, after which inhibition-zone diameters were measured in millimetres. The three replicate values were retained for statistical analysis. Standardised CLSI yeast zone-diameter interpretive criteria apply only when the relevant CLSI disk-diffusion methodology is followed [26, 27]; they therefore cannot be applied directly to the non-standard agar well diffusion measurements reported in this study.

2.5. BROTH MICRODILUTION CONTEXT AND INTERPRETATION OF COMBINATION ACTIVITY

Broth microdilution was used in the broader susceptibility workflow to establish the fluconazole-resistance status of the isolate according to CLSI principles [25, 27]. The present article does not report a complete checkerboard concentration matrix for the fluconazole–berberine combination and therefore does not calculate a FICI. Formal synergy was accordingly not assigned from the agar well diffusion result. Published fluconazole–berberine studies that used checkerboard and time–kill methods provide an appropriate methodological benchmark for future confirmation of the interaction observed here [12, 20, 22].

2.6. STATISTICAL ANALYSIS

Data are expressed as mean ± standard deviation (SD). Analyses were carried out in Python 3 using SciPy, and the reported calculations were checked against standard statistical formulae. For the three conditions contributing the highest-dose comparison data—fluconazole 25 µg, berberine 250 µg, and the fluconazole 25 µg + berberine 250 µg

combination—overall differences were examined using one-way analysis of variance (ANOVA). The Kruskal–Wallis test was also calculated as a distribution-free check. The primary pairwise comparison between fluconazole alone and the combination used Welch’s t-test because it does not require equal variances. The pooled-variance Student t-test is reported only as a secondary conventional comparison. All tests were two-sided, and $p < 0.05$ was considered statistically significant. Because each condition had only three replicate observations and one comparison group had zero variance, the inferential results should be interpreted as descriptive support for a large experimental contrast rather than as precise population estimates.

3. RESULTS

Fluconazole produced a detectable zone of inhibition only at 25 μg , where the mean diameter was 13.00 ± 0.00 mm across three replicates. At 12.5 μg and 6.25 μg , no zone was visible. Berberine tested alone at 250, 125, and 25 μg produced no detectable zone at any amount in any replicate. These single-agent results are summarised in Table 1 and Figure 1; representative fluconazole and berberine plates are shown in Figures 3 and 4, respectively.

The nominal combination of fluconazole 25 μg with berberine 250 μg produced a zone of 32.50 ± 2.50 mm, with replicate values of 30.0, 32.5, and 35.0 mm, compared with 13.00 ± 0.00 mm for fluconazole alone. The mean difference was 19.50 mm (95% CI 13.29–25.71 mm by the Welch approach; 15.49–23.51 mm using the pooled-variance calculation). The two lower nominal pairings produced no detectable zone, and the DMSO solvent control also produced no zone. All treatment conditions are presented in Table 1 and Figure 1. A representative plate directly comparing fluconazole, berberine, and the highest combination is shown in Figure 5.

Table 1. Inhibition-zone diameters (mean $\pm$ SD, $n = 3$ replicate measurements per condition) for fluconazole, berberine, and their combination against the *Candida albicans* isolate. Amounts are the nominal experimental designations used for each well. “No zone detected” means that no visible inhibition was observed around the well and is not equivalent to a categorical resistant result. CLSI interpretive zone-diameter criteria are defined for standardised disk-diffusion methodology and do not apply directly to the agar well diffusion format used here [26, 27].

Treatment group	Amount per well	Inhibition zone (mm, mean $\pm$ SD)	Outcome
Fluconazole	25 μg	13.00 ± 0.00	Zone present
Fluconazole	12.5 μg	0.00	No zone detected
Fluconazole	6.25 μg	0.00	No zone detected
Berberine	250 μg	0.00	No zone detected
Berberine	125 μg	0.00	No zone detected
Berberine	25 μg	0.00	No zone detected
Fluconazole + berberine	25 μg + 250 μg	32.50 ± 2.50	Zone present
Fluconazole + berberine	12.5 μg + 125 μg	0.00	No zone detected
Fluconazole + berberine	6.25 μg + 25 μg	0.00	No zone detected
DMSO 10%	—	0.00	No zone detected

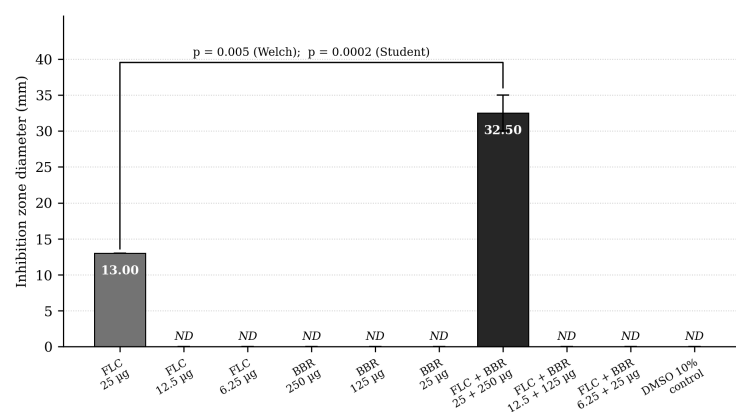


Figure 1. Inhibition-zone diameters for all treatment conditions (mean $\pm$ SD, $n = 3$ replicate measurements per condition), corresponding to Table 1. ND = no zone detected. Error bars show one standard deviation; conditions with zero variance therefore have no visible error bar. FLC = fluconazole; BBR = berberine.

One-way ANOVA across the three highest-dose comparison groups—fluconazole 25 μg , berberine 250 μg , and the nominal combination—gave a grand mean of 15.17 mm and the decomposition shown in Table 2 and Figure 2. Between-group variation accounted for approximately 99.2% of the total sum of squares. The inferential results are collected in Table 3. Given the triplicate design and the zero variance in the fluconazole-alone and berberine-alone conditions, these

tests primarily quantify the magnitude of the observed experimental separation and should not be interpreted as a substitute for replication across independent clinical isolates.

Table 2. One-way ANOVA of inhibition-zone diameters across the fluconazole-alone (25 µg), berberine-alone (250 µg), and nominal combination (25 µg + 250 µg) groups, with *n* = 3 replicate measurements per group.

Source of variation	Sum of squares	df	Mean square	F	p
Between groups	1605.50	2	802.75	385.32	< 0.0001
Within groups (error)	12.50	6	2.08	–	–
Total	1618.00	8	–	–	–

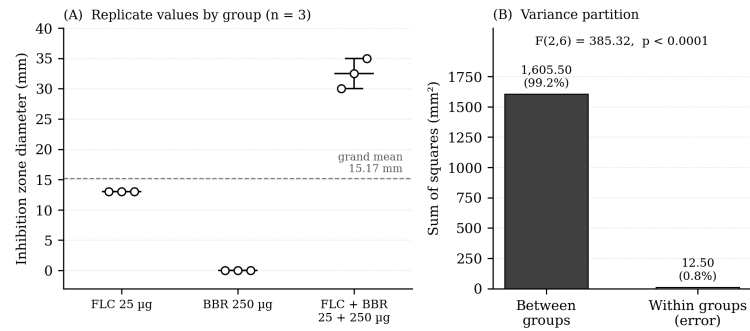


Figure 2. Data underlying the ANOVA in Table 2. (A) Individual replicate values (open circles) with group mean and standard deviation; the dashed line marks the grand mean. (B) Partition of the total sum of squares into between-group and within-group components. FLC = fluconazole; BBR = berberine.

Table 3. Summary of the statistical tests comparing the treatment groups. The Welch test is the primary pairwise comparison; the Student result is shown for completeness only, because one group has zero variance. Kruskal–Wallis is the distribution-free check across all three groups

Test	Statistic	df	p
One-way ANOVA (3 groups)	F = 385.32	2, 6	< 0.0001
Kruskal–Wallis (3 groups)	H = 7.71	2	0.021
Welch t-test (primary pairwise)	t = 13.51	2.0	0.0054
Student t-test (pooled variance)	t = 13.51	4	0.00017



Figure 3. Representative agar well diffusion plate showing the response of the *Candida albicans* isolate to fluconazole. A₁ = fluconazole 25 µg; A₂ = fluconazole 12.5 µg; A₃ = fluconazole 6.25 µg. A zone of inhibition is visible around A₁ only



Figure 4. Representative agar well diffusion plate showing the response of the *Candida albicans* isolate to berberine. B_1 = berberine 250 μg ; B_2 = berberine 125 μg ; B_3 = berberine 25 μg . No zone of inhibition is visible around any well; the coloured halo at the well margins is berberine pigment and is not a zone of inhibition

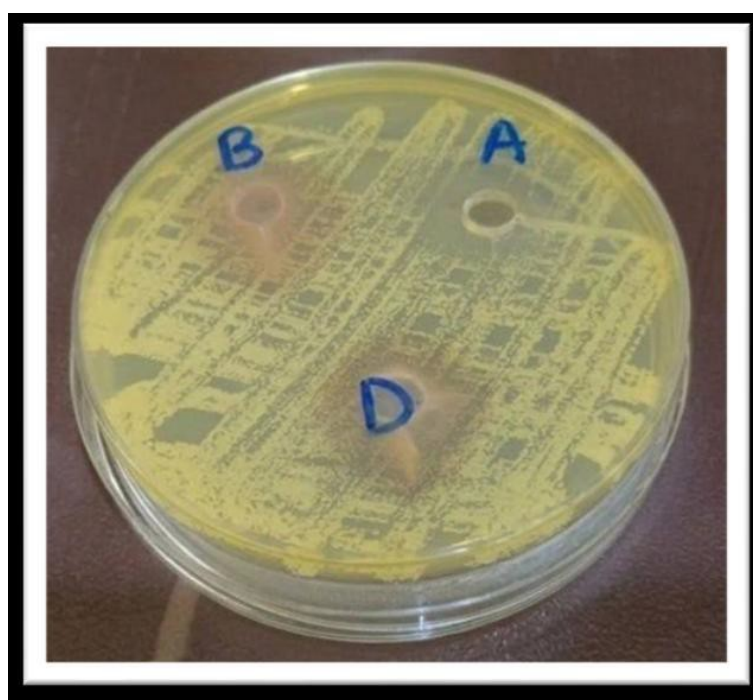


Figure 5. Representative agar well diffusion plate comparing the three highest-dose treatment conditions on a single plate. A = fluconazole 25 μg ; B = berberine 250 μg ; D = the nominal fluconazole 25 μg + berberine 250 μg combination. Each condition was tested in triplicate ($n = 3$ replicate measurements per condition).

4. DISCUSSION

This exploratory study compared fluconazole, berberine, and their nominal combinations against one fluconazole-resistant clinical isolate of *C. albicans*. Fluconazole alone produced a measurable zone only at the highest amount tested, berberine alone produced no detectable zone at any tested amount, and the highest fluconazole–berberine pairing produced a substantially larger zone than fluconazole alone. The pattern is therefore compatible with potentiation of fluconazole activity by berberine under the conditions of the agar well diffusion assay.

Fluconazole produced a 13.00-mm zone only at 25 μg . The isolate had been classified as fluconazole resistant in the

broader susceptibility workflow, although the exact MIC value is not reported in the present dataset. For that reason, the 13-mm well-diffusion zone should not itself be used to assign a CLSI susceptibility category. Current CLSI performance standards provide MIC and disk-zone breakpoints only for assays conducted according to the corresponding standardised M27 and M44 methodologies [25–27]. The agar well diffusion result is therefore best interpreted as an internal experimental comparison among the conditions used in this study rather than as a clinical susceptibility measurement.

Berberine alone produced no detectable zone from 25 to 250 μg . This observation does not demonstrate that berberine lacks antifungal activity. Reported berberine susceptibility values vary considerably among *Candida* isolates and experimental systems [13, 14]. In addition, diffusion assays are influenced by molecular size, solubility, agar composition, and diffusion kinetics as well as biological activity [24]. The representative berberine plate in Figure 4 shows pigment close to the well without a measurable clear zone, a pattern compatible with limited movement through the agar matrix. Accordingly, the appropriate conclusion is that berberine produced no detectable inhibition zone in this assay format; the study does not establish absence of activity in broth or in other experimental systems.

The principal finding was observed with the highest nominal combination. Fluconazole 25 μg plus berberine 250 μg produced a mean inhibition zone of 32.50 ± 2.50 mm, compared with 13.00 ± 0.00 mm for fluconazole alone, for a mean difference of 19.50 mm. The direct plate comparison is illustrated in Figure 5, while the replicate-level statistical pattern is shown in Figure 2. The magnitude of the difference was large relative to the variation among the three replicates, which explains the small p-values in Table 3. Nevertheless, statistical significance in this triplicate diffusion experiment does not establish pharmacodynamic synergy. Zone diameter is not a linear function of drug concentration, the agents may diffuse through agar at different rates, and no checkerboard FICI was calculated. Published work has demonstrated fluconazole–berberine synergy using checkerboard microdilution and time–kill methods [12, 20, 22]; the present result is consistent with that literature but should be described as enhanced activity or potentiation until a standard combination assay confirms synergy.

The nominal dosing of the combination arm also warrants careful interpretation. The laboratory record describes the combination solutions as being prepared by mixing equal volumes of the corresponding working solutions, while the recorded experimental groups are labelled 25 μg + 250 μg , 12.5 μg + 125 μg , and 6.25 μg + 25 μg . Because the documentation available for this article does not permit independent reconstruction of the final post-mixing mass delivered from the working-solution concentrations, these values should be understood as the nominal designations used in the experiment. This does not remove the observed zone enlargement, but it limits dose-response interpretation and is an important reason to repeat the experiment in broth using explicitly defined final concentrations.

The mechanistic literature provides biologically plausible explanations for the observed enhancement, although none was tested directly in the present isolate. Berberine has been shown to interfere with *CDR1*-related efflux responses [15] and to interact with Mdr1p-mediated multidrug resistance [16]. Other studies report increased oxidative stress during fluconazole–berberine exposure [17], disruption of mitochondrial function and iron acquisition by berberine [18], and an important role for ergosterol homeostasis in berberine tolerance and fluconazole-mediated sensitisation [19, 20]. More recent experimental work also supports combined effects on efflux, adhesion, morphogenesis, and biofilm-associated phenotypes in resistant *C. albicans* [21, 22]. These mechanisms make potentiation plausible, but assigning any particular mechanism to the isolate studied here would require direct molecular measurements.

The enhancement was confined to the highest nominal pairing. Neither of the two lower combinations produced a visible inhibition zone. One possible explanation is a threshold phenomenon in which insufficient intracellular berberine or fluconazole exposure is achieved below a particular concentration. Another is methodological: a smaller amount of a compound with limited agar diffusion may fail to establish an inhibitory concentration at a sufficient distance from the well. The present experiment cannot distinguish pharmacodynamic threshold behaviour from diffusion limitations. A checkerboard dilution series in broth would be more suitable for determining whether the interaction changes gradually across concentrations or appears only within a narrow concentration range.

Several aspects of the design strengthen the internal comparison. The same isolate, inoculum preparation, medium, incubation conditions, and solvent system were used across the single-agent and combination conditions, and the DMSO control produced no detectable inhibition. Triplicate measurements were retained rather than reporting only a single representative zone. The analysis also included a variance-robust Welch comparison and a distribution-free Kruskal–Wallis test in addition to conventional parametric statistics. These features support the conclusion that the large zone observed with the highest combination was reproducible within this experimental run.

At the same time, the limitations are substantial. The central experiment involved only one clinical isolate, so biological variability among resistant *C. albicans* strains was not evaluated. Replication was technical rather than replication across independent isolates. The resistance genotype was not determined, and the study did not quantify efflux activity, ergosterol content, intracellular drug accumulation, reactive oxygen species, or mitochondrial function. The agar well method is a screening assay rather than a CLSI standard for assigning yeast susceptibility categories, and the exact final combination concentrations require clearer documentation in a repeat experiment. No checkerboard FICI, minimum

fungicidal concentration, or time–kill curve was generated for the reported fluconazole–berberine pairing. These limitations prevent conclusions about formal synergy, mechanism, clinical efficacy, or dosing.

If the effect is reproduced across a larger isolate panel using standardised quantitative methods, the potential relevance would be as an adjuvant strategy: berberine or a related compound might increase the activity of an existing azole against selected resistant strains. Such a strategy is conceptually attractive because it seeks to recover activity of an established antifungal rather than relying solely on a new drug class. However, the present in vitro screening result is far from demonstrating therapeutic usefulness. Berberine has pharmacokinetic limitations, and efficacy, toxicity, formulation, achievable tissue exposure, and drug interactions would all have to be addressed before clinical translation could be considered.

5. CONCLUSION

Against the single fluconazole-resistant clinical isolate of *C. albicans* tested in this agar well diffusion experiment, fluconazole alone produced a detectable zone only at the highest tested amount and berberine alone produced no detectable zone. The highest nominal fluconazole–berberine pairing produced a mean zone of 32.50 ± 2.50 mm compared with 13.00 ± 0.00 mm for fluconazole alone, yielding a mean difference of 19.50 mm. The contrast was statistically large within the triplicate experiment. Because berberine alone produced no measurable zone, the finding is consistent with potentiation of fluconazole activity. It should not, however, be described as proven synergy because the experiment did not include a checkerboard FICI or a time–kill confirmation and used a non-standard agar well format for the principal quantitative endpoint. The study therefore provides a preliminary experimental rationale for more rigorous combination testing rather than evidence of a therapeutic effect.

6. RECOMMENDED FURTHER WORK

The identity and resistance phenotype of the experimental isolate should first be reconfirmed using a current standardised yeast susceptibility workflow. Fluconazole MIC testing should follow CLSI M27 methodology, with interpretation using the current CLSI M27M44S performance standard and an appropriate quality-control strain [25, 27]. Fluconazole and berberine should then be tested individually and in a full checkerboard concentration matrix so that FICI values can be calculated, followed by time–kill experiments to confirm the interaction dynamically. Exact final concentrations in every combination well should be documented directly rather than inferred from premixing designations.

Subsequent experiments should extend the analysis to multiple independent fluconazole-resistant and susceptible *C. albicans* isolates, followed by selected non-*albicans* *Candida* species where scientifically appropriate. Resistance mechanisms should be characterised by examining *ERG11*, *TAC1*, *MRR1*, *CDR1*, *CDR2*, and *MDR1* as relevant, together with functional measurements such as intracellular fluconazole accumulation, membrane permeability, ergosterol content, and reactive oxygen species. Biofilm assays on surfaces relevant to oral candidiasis would also strengthen translational relevance. Only after reproducible activity has been demonstrated in well-controlled in vitro systems should efficacy, pharmacokinetics, formulation, and tolerability be considered in an appropriate animal model.

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AUTHOR CONTRIBUTIONS

Both authors contributed to the conception, laboratory work, analysis, interpretation, manuscript preparation, and critical revision of the study. Both authors reviewed and approved the final version of the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY

The replicate-level data supporting the findings of this study are available from the corresponding author upon reasonable request.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

In the course of preparing this manuscript, the authors employed ChatGPT to improve the linguistic quality and readability of the content. After utilizing this AI tool, the authors conducted comprehensive review and necessary revisions to ensure the accuracy, integrity, and scientific rigor of the manuscript. All authors bear full responsibility for the final content of the published work, including any errors or inconsistencies that may arise.

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