

Berberine from Coptidis Rhizoma enhances host antifungal immunity against dermatophytosis and cutaneous candidiasis via JNK signaling and autophagy

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Abstract

Background: Dermatophytosis and cutaneous candidiasis are prevalent fungal skin diseases worldwide, with rising antifungal resistance rendering conventional therapies increasingly inadequate. *Coptidis Rhizoma* (Huanglian) is a traditional Chinese medicinal herb historically used for treating skin infections and inflammatory conditions. Berberine, its major active alkaloid, exhibits broad-spectrum antimicrobial activity, yet its host-directed immunomodulatory mechanisms against fungal infections remain poorly understood.

Methods: We employed network pharmacology and molecular docking to identify core targets of berberine against dermatophytosis and cutaneous candidiasis. In vitro antifungal activity was assessed by broth microdilution, checkerboard synergy assays, scanning electron microscopy, crystal violet biofilm staining, and confocal laser scanning microscopy with live/dead staining. In vivo efficacy was evaluated using *Galleria mellonella* survival assays, time-kill curves, and mouse models of ordinary and itraconazole-refractory *Trichophyton rubrum* and *Candida albicans* skin infections. Host immune responses, JNK pathway activation, and autophagic flux were examined by qPCR, histopathology, and immunohistochemistry.

Results: Network pharmacology identified 19 core targets, with JNK signaling prominently enriched. Molecular docking showed stable berberine binding to MAPK8 (JNK) and other targets (≤ -7.0 kcal/mol). Berberine MICs ranged 16–128 $\mu\text{g/mL}$ and synergized with itraconazole (FICI 0.125–0.375). Berberine induced severe ultrastructural damage, significantly inhibited biofilm formation ($P < 0.0001$), and increased dead cell proportions to 40.4% (*T. rubrum*) and 54.9% (*C. albicans*). In vivo, berberine activated JNK (increased p-JNK), enhanced autophagic flux (elevated LC3-II/I, reduced p62), and suppressed excessive inflammation (downregulated *TNF*, *PTGS2*). In refractory models, berberine alone or combined with itraconazole reduced fungal burden by $\sim 2.5\text{--}3.4 \log_{10}$ CFU/g ($P < 0.01$) and ameliorated histopathological damage.

Conclusions: Berberine from *Coptidis Rhizoma* enhances host antifungal immunity by activating the JNK-autophagy axis, while exerting direct fungicidal and antibiofilm effects. Its synergy with itraconazole against refractory infections provides a mechanistic rationale for the ethnopharmacological use of *Coptidis Rhizoma* and supports its development as a promising antifungal sensitizer.

Introduction

Dermatophytosis and cutaneous candidiasis are among the most common skin diseases worldwide, affecting an estimated 20–25% of the global population (Havlickova et al., 2008). These conditions not only cause pruritus, erythema, and scaling but also lead to severe secondary infections in immunocompromised individuals and diabetic patients (Ameen, 2010). Dermatophytosis, mainly caused by filamentous fungi such as *Trichophyton rubrum*, represents an "active invasion" mode through keratinase secretion (Arya et al., 2025), whereas cutaneous candidiasis, predominantly caused by *Candida albicans*, represents an "opportunistic infection" mode following host immune dysregulation

(Mayer et al., 2013). Developing broad-spectrum antifungals effective against both morphologically and pathogenetically distinct fungi is of high scientific and clinical value.

Current clinical treatments rely heavily on azoles and allylamines. However, antifungal resistance is rising dramatically. Resistance rates of *T. rubrum* to terbinafine have increased explosively in some regions (Song et al., 2025), while susceptibility of *Candida* species to fluconazole continues to decline (Angiolella et al., 2008). Moreover, biofilm formation limits the efficacy of single-target drugs, leading to high recurrence rates. Thus, multi-target, multi-pathway strategies covering both filamentous fungi and yeasts are urgently needed.

Coptidis Rhizoma (Huanglian) has a long history in dermatology for clearing heat, drying dampness, and detoxifying fire. Berberine, its most abundant and representative active component, exhibits broad-spectrum antifungal activity by disrupting fungal cell walls, inhibiting mitochondrial function, and interfering with biofilm formation (da Silva et al., 2016). Recent studies also suggest berberine modulates host immunity. Duan et al. reported that berberine enhances innate immunity via the p38 MAPK pathway (Xiao et al., 2025), and berberine is recognized as an autophagy modulator (Mohammadinejad et al., 2019).

The c-Jun N-terminal kinase (JNK) pathway is a central regulator of host antifungal immunity. JNK1-dependent MAPK signaling is essential for IL-17A/IL-17F-mediated mucosal immunity against *Candida* (Chen et al., 2021); JNK1 deficiency leads to chronic mucocutaneous candidiasis (Li et al., 2019). JNK activation also promotes autophagy-related gene expression (e.g., *Beclin-1*, *ATG5*), enhances autophagic flux, and facilitates pathogen clearance (Yao et al., 2025). In macrophages, JNK cooperates with Dectin-1 to activate the Syk-JNK-AP-1 axis, regulating antifungal cytokine production (Huang et al., 2015). JNK is also indispensable for neutrophil antifungal function (Hopke et al., 2025). However, whether berberine activates host JNK signaling to enhance antifungal immunity remains unknown.

Here, we integrated network pharmacology, molecular docking, in vitro susceptibility testing, biofilm assays, ultrastructural analysis, *Galleria mellonella* and mouse infection models, and itraconazole-refractory models to demonstrate that berberine enhances host antifungal immunity by activating the JNK-autophagy axis, providing a mechanistic basis for its clinical application.

Materials and Methods

Detailed methods are provided in the supplementary materials. Key procedures are summarized below.

Network pharmacology

Active compounds of *Coptidis Rhizoma* were retrieved from TCMSP (OB $\geq$ 30%, DL $\geq$ 0.18). Targets were predicted using SwissTargetPrediction. Tinea and cutaneous candidiasis targets were obtained from the CTD database. Intersecting targets were analyzed by Venny 2.1. A "drug-component-target" network and PPI network (STRING 12.0, confidence > 0.40) were constructed using Cytoscape 3.10.2. GO and KEGG

enrichment were performed using Metascape. Core targets were identified by intersecting the top-20 degree targets from the three networks.

Molecular docking

Five major compounds [(R)-Canadine, quercetin, berberine, palmatine, Moupinamide] were energy-optimized with Chem3D. Core target 3D structures were obtained from PDB. Docking was performed with AutoDock Vina 1.2.0 and visualized with PyMOL. Binding energy ≤ -5.0 kcal/mol indicated good binding; ≤ -7.0 kcal/mol indicated very stable binding.

In vitro susceptibility and synergy assays

Broth microdilution (CLSI M27-A3 and M38-Ed3) was used to determine MICs of berberine and seven conventional antifungals against six clinical isolates each of *T. rubrum* and *C. albicans*. The resulting susceptibility profiles were visualized using R (version 4.2.1) with the ggplot2, tidyr, dplyr, and stringr packages to generate heatmaps, where blue and red indicated susceptibility and resistance, respectively. Checkerboard assays were used to evaluate combinations, with synergy defined as FICI ≤ 0.5 . For the checkerboard data, a bubble plot was generated in R, where bubble size and color represented the FICI value, and bubble shape indicated the qualitative interaction (SYN, synergism; PS, partial synergy; AD, additive; IN, indifference).

Scanning Electron Microscopy (SEM)

Exponentially growing *T. rubrum* and *C. albicans* were treated with berberine at the MIC concentration or with medium alone (control) for 24 h at 35°C. Samples were fixed with 2.5% glutaraldehyde, post-fixed with 1% osmium tetroxide, dehydrated through a graded ethanol series, critical-point dried, and gold-sputtered. Specimens were examined using a scanning electron microscope (Zeiss Sigma 300) at an accelerating voltage of 5 kV.

Biofilm Formation Assay

The effect of berberine on biofilm formation was assessed using crystal violet staining. Fungal cells (1×10^6 cells/mL) were incubated in 96-well plates with or without berberine at the MIC for 48 h at 35°C. After incubation, non-adherent cells were removed by washing with PBS. Adherent biofilms were fixed with methanol, stained with 0.1% crystal violet for 15 min, washed, and destained with 33% glacial acetic acid. Absorbance was measured at 590 nm. Experiments were performed in triplicate and repeated three times.

Confocal Laser Scanning Microscopy (CLSM) and Live/Dead Staining

Biofilms were cultured on glass-bottom dishes under the same conditions. After 48 h, samples were stained using the LIVE/DEAD® FungaLight™ Yeast Viability Kit (Invitrogen) according to the manufacturer's instructions. Stained biofilms were imaged using a Zeiss LSM 880 confocal microscope

with a 40× objective. For each merged image, ZEN software was used to extract profile lines along four dimensions across the biofilm. The fluorescence intensity profiles were quantified and analyzed using R to generate circular statistical plots, calculating the proportion of dead (red) and live (green) fungal cells.

Galleria mellonella model: Larvae were infected with 1×10^6 CFU/larva of *T. rubrum* or *C. albicans*. After 3 hours, they received PBS, berberine (20 mg/kg), itraconazole (5 mg/kg), or berberine+itraconazole (4:1). Survival was monitored for 10 days (n = 20/group).

Time-kill curves

C. albicans was sampled at 0, 4, 8, 24, 48, 72 h; *T. rubrum* at 0, 1, 2, 3, 7, 14 days. Synergy was defined as $\geq 2 \log_{10}$ CFU/mL reduction vs. the most effective single agent.

Mouse skin infection models

Immunosuppressed C57BL/6 mice were infected intradermally with 2×10^7 CFU of *T. rubrum* or *C. albicans* and treated with berberine (20 mg/kg/d, gavage) for 7 days. mRNA expression of core targets was quantified by qPCR.

Itraconazole-refractory models

After fungal infection, mice received itraconazole (5 mg/kg/d) for 1 week. Refractory mice (still positive by microscopy) were then treated with PBS, berberine, itraconazole, or berberine+itraconazole for another week. Fungal burden, qPCR, histopathology (H&E, PAS), and p-JNK immunohistochemistry were evaluated.

Statistics

Data are mean $\pm$ SD. Comparisons used one-way ANOVA with Tukey's post-hoc test or Student's t-test. Survival was analyzed by Kaplan-Meier with log-rank test. $P < 0.05$ was considered significant.

Results

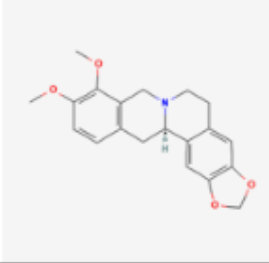
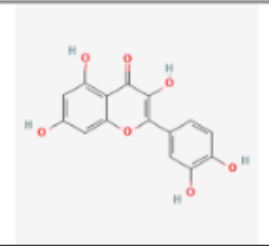
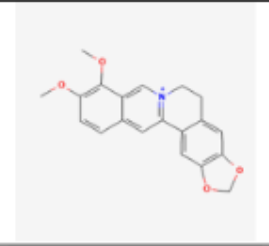
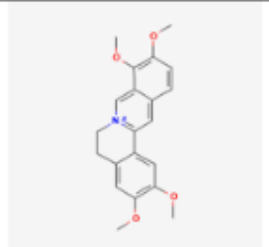
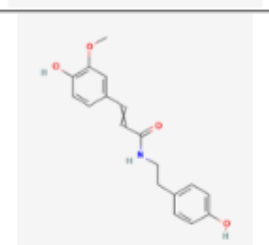
1. Network Pharmacology Identifies Core Targets and Pathways

From TCMSP, 48 compounds were retrieved; 13 satisfied $OB \geq 30\%$ and $DL \geq 0.18$, and SwissADME filtering. A total of 445 *Coptidis Rhizoma* targets were predicted. CTD yielded 1,803 tinea-related and 1,354 cutaneous candidiasis-related targets. Intersection produced 87 potential targets (Fig. 1A). The petal-shaped Venn diagram showed detailed overlaps with disease subtypes (Fig. 1B).

The "drug-component-target" network (Fig. 1C) contained 101 nodes and 221 edges. The top five active compounds were (R)-Canadine, quercetin, berberine, palmatine, and Moupinamide (Table 1). The PPI network (Fig. 1D) contained 83 nodes and 1,300 edges. GO enrichment (Fig. 1E) and KEGG enrichment

(Fig. 1F) revealed significant enrichment in FoxO, HIF-1, PI3K-Akt, and MAPK-related pathways. The target-pathway network (Fig. 1G) highlighted key genes such as MAPK8, AKT1, and TNF.

Table 1. Top 5 active compounds of *Coptis chinensis* for treating cutaneous mycoses.

Molecule ID	Molecule Name	Structural Formula	Degree	Betweenness Centrality	Closeness Centrality
MOL002903	(R)-Canadine		29	2201.2578	0.4347826
MOL000098	quercetin		29	2566.4182	0.4347826
MOL001454	berberine		26	1295.5104	0.42372882
MOL000785	palmatine		26	1461.6394	0.42372882
MOL008647	Moupinamide		23	2080.9597	0.41322315

Intersecting the top-20 degree targets from the three networks yielded 19 core targets: CDK2, CDK4, PTGS2, CYP19A1, PARP1, ABCB1, MET, ROCK2, RPS6KB1, MAPK8, AKT1, TNF, STAT3, BCL2, EGFR, HSP90AB1, EP300, CYP1A1, and MAPK14 (Fig. 1H).

Molecular docking showed that all five major compounds bound stably to these 19 core targets (binding energy ≤ -5.0 kcal/mol). Notably, CDK2, HSP90AB1, PTGS2, PARP1, and MAPK8 exhibited very stable

binding (≤ -7.0 kcal/mol) with all five compounds (Table 2). Representative binding modes are shown in Fig. 2.

Table 2
Binding energies of major active compounds of *Coptis chinensis* with core targets

Core targets	Binding energy (kcal/mol)				
	(R)_Canadine	berberine	Moupinamide	palmatine	quercetin
ABCB1	-8.7	-8.3	-6.8	-7.6	-8.3
AKT1	-6.8	-6.6	-6.1	-6.4	-7.0
BCL2	-8.5	-8.0	-6.7	-7.2	-7.9
CDK2	-8.5	-9.4	-9.4	-9.1	-9.8
CDK4	-7.6	-7.3	-6.0	-6.8	-8.5
CYP1A1	-7.4	-7.6	-6.5	-7.2	-10.5
CYP19A1	-8.6	-8.5	-7.4	-8.0	-8.1
EGFR	-8.0	-7.5	-6.6	-7.2	-7.9
EP300	-9.7	-9.3	-6.2	-8.0	-9.1
HSP90AB1	-8.7	-10.3	-8.4	-8.8	-9.8
MAPK8	-8.4	-9.5	-7.9	-9.1	-9.7
MAPK14	-7.8	-7.5	-7.0	-7.1	-8.3
MET	-7.8	-8.1	-6.4	-7.3	-7.4
PARP1	-9.9	-9.1	-8.2	-8.2	-9.7
PTGS2	-10.0	-10.1	-8.3	-8.1	-9.1
ROCK2	-8.4	-8.7	-7.1	-8.1	-8.0
RPS6KB1	-8.3	-9.5	-8.5	-8.8	-9.0
STAT3	-7.0	-7.3	-5.9	-6.4	-8.2
TNF	-7.1	-8.0	-5.8	-6.0	-8.8

2. In Vitro Antifungal and Antibiofilm Activities of Berberine

Berberine MICs ranged 32–128 µg/mL against *T. rubrum* isolates and 16–128 µg/mL against *C. albicans* isolates. To provide an intuitive overview of the susceptibility patterns, we visualized the MIC data for each clinical isolate against the panel of seven conventional antifungals. As shown in Fig. 3A, the six *T.*

rubrum clinical isolates displayed variable susceptibility, with a notable resistance to fluconazole and, in some strains, to itraconazole. In contrast, the *C. albicans* isolates (Fig. 3B) exhibited a broader and more severe resistance profile, particularly against terbinafine, itraconazole, and fluconazole, highlighting the critical need for alternative therapeutic strategies.

Checkerboard synergy assays demonstrated that berberine combined with itraconazole showed potent synergistic effects (FICI 0.25–0.375 for *T. rubrum*; 0.125–0.5 for *C. albicans*). Synergy or partial synergy was also observed with fluconazole and voriconazole. The comprehensive FICI results for all isolate-drug combinations are presented in Fig. 3C, where bubble size and color represent the FICI value, and shape indicates the qualitative interaction type. This analysis revealed that synergism was most frequently observed for combinations of berberine with itraconazole, voriconazole, and posaconazole across both species.

To investigate the direct morphological effects of berberine, we performed SEM on fungal cells treated with the MIC of berberine. As depicted in Fig. 3D, untreated *T. rubrum* and *C. albicans* cells exhibited smooth, intact, and regular surfaces. In stark contrast, berberine-treated cells showed pronounced ultrastructural damage, including surface collapse, wrinkling, and disruption of the cell wall integrity, consistent with its known mechanism of action.

Given that biofilm formation is a major contributor to antifungal resistance, we assessed the anti-biofilm activity of berberine. Crystal violet staining (Fig. 3E) revealed that berberine at the MIC concentration significantly inhibited biofilm formation in both *T. rubrum* and *C. albicans* compared to untreated controls ($P < 0.0001$). This was further corroborated by CLSM live/dead staining (Fig. 3F). Quantitative analysis of the confocal z-stack profiles using ZEN software and R-generated circular plots showed that berberine treatment led to a substantial increase in dead cells within the biofilm: 40.4% dead (red) vs. 59.6% live (green) for *T. rubrum*, and 54.9% dead vs. 45.1% live for *C. albicans*. These results confirm that berberine not only inhibits planktonic growth but also effectively disrupts biofilm formation and reduces biofilm viability.

3. Berberine Enhances Host Antifungal Immunity in vivo and Synergizes with Itraconazole

Having established the direct antifungal and antibiofilm effects of berberine, we next evaluated its in vivo efficacy and host-directed immunomodulatory activity. In *Galleria mellonella*, berberine+itraconazole significantly improved 10-day survival to 65% vs. 15% (berberine alone) and 35% (itraconazole alone) for *T. rubrum* (Fig. 4A), and similarly for *C. albicans* (Fig. 4B) (log-rank $P < 0.05$).

Time-kill curves showed that single agents had limited and transient killing; regrowth occurred after 48 h for *C. albicans* (Fig. 4D) and day 7 for *T. rubrum* (Fig. 4C). In contrast, the combination achieved a

sustained $> 2 \log_{10}$ CFU/mL reduction vs. single agents and $> 3 \log_{10}$ CFU/mL reduction vs. initial inoculum by day 14.

In mouse skin infection models (Fig. 4E), qPCR of 19 core targets (Fig. 4F) showed that berberine significantly suppressed infection-induced *TNF* and *PTGS2* overexpression ($P < 0.0001$). Notably, while *MAPK8* (JNK) and *MAPK14* (p38) were moderately elevated by infection alone, berberine further increased their expression to 3.8-fold and 3.2-fold (vs. blank) for *T. rubrum*, and 3.0-fold and 2.6-fold for *C. albicans* (all $P < 0.0001$). In contrast, *AKT1* and *RPS6KB1* were unchanged in *T. rubrum* infection but upregulated in *C. albicans* infection and reversed by berberine.

4. Berberine Effectively Treats Itraconazole-Refractory Infections via the JNK-Autophagy Axis

Refractory models were successfully established after 1 week of itraconazole (Fig. 5A). In *T. rubrum*-refractory mice, berberine reduced fungal burden by $\sim 2.3 \log_{10}$ CFU/g vs. PBS ($P < 0.01$), and berberine+itraconazole reduced it by $\sim 3.4 \log_{10}$ CFU/g ($P < 0.0001$) (Fig. 5B). Similar results were obtained for *C. albicans* (Fig. 5C).

qPCR in refractory tissues (Figs. 5D and 5E) showed that berberine upregulated *Mapk8*, *Mapk14*, *Atg5*, *Beclin-1*, and *Map1lc3b*, and downregulated the autophagy substrate *Sqstm1* (p62).

Berberine+itraconazole further enhanced these effects.

Histopathology (H&E and PAS, Figs. 5F and 5G) revealed that berberine alone markedly reduced inflammation and fungal elements, and the combination nearly eliminated fungal structures.

Immunohistochemistry for p-JNK showed that berberine increased p-JNK-positive cells from $\sim 8\%$ (PBS) to $\sim 32\%$ (berberine) in *T. rubrum*-refractory skin ($P < 0.0001$), and further to $\sim 46\%$ with the combination ($P < 0.001$) (Figs. 5H and 5I). Similar results were observed for *C. albicans*.

Discussion

In this study, we integrated network pharmacology, molecular docking, in vitro susceptibility testing, advanced imaging and biofilm assays, *Galleria mellonella* survival assays, time-kill curves, and mouse models of both ordinary and itraconazole-refractory fungal skin infections to systematically elucidate a previously unrecognized mechanism: berberine, the core active component of *Coptidis Rhizoma*, significantly enhances host antifungal immunity by activating the JNK signaling pathway and its downstream autophagic process. This discovery not only deepens the understanding of the traditional Chinese medicine principle of "treating different diseases with the same method" at the molecular level but also provides a potential novel combination strategy for clinically challenging drug-resistant fungal infections. Moreover, our direct antifungal and antibiofilm findings provide a comprehensive portrait of berberine's multifaceted anti-infective activity. Below, we discuss in detail the pathogen-dependent differences in host immune pathway activation, the regulatory mechanism of the JNK-autophagy axis,

the synergistic effects of berberine with conventional antifungals, the implications of its antibiofilm activity, and the study's limitations.

1. Pathogen-Dependent Differences in Host Signaling Pathway Activation: Distinct Roles of PI3K-Akt and MAPK Pathways

Fungal infections elicit host immune responses that are highly dependent on the pathogen species and their mode of interaction with host cells. Our network pharmacology analysis showed that targets from different pathways, especially those in the PI3K-Akt and MAPK (including JNK/p38) pathways, were highly enriched in the target-pathway network. However, subsequent animal experiments revealed a key and intriguing phenomenon: the activation status of these two pathways differed markedly between infection models.

In the *Candida albicans* infection model, the PI3K/Akt pathway-related genes *AKT1* and *RPS6KB1* were significantly upregulated compared with the blank control group (3.4-fold and 2.6-fold, respectively; $P < 0.0001$), indicating that this pathway is activated during *Candida* infection. After berberine treatment, their expression levels returned to near-normal levels. In contrast, in the *Trichophyton rubrum* infection model, no significant differences in these PI3K/Akt pathway genes were observed among groups ($P > 0.05$). This pathogen-dependent difference is strongly supported by existing literature. Moyes et al. found that PI3K/Akt signaling is significantly activated in human oral epithelial cells infected with *C. albicans*, acting independently of NF- κ B and MAPK pathways and playing a critical role in protecting epithelial cells from fungal-induced damage (Moyes et al., 2014). They further demonstrated that the PI3K/Akt/mTOR axis is essential for protecting epithelial cells during mucosal fungal infection, independent of NF- κ B or MAPK signaling (Moyes et al., 2014). In contrast, Achterman et al. reported that when human primary keratinocytes were infected with *Microsporum canis*, MAPK pathways (especially p38 and JNK) were strongly activated, whereas NF- κ B and PI3K pathways were largely unaffected (Achterman et al., 2015). Our experimental results in the *T. rubrum* infection model are highly consistent with that finding, further supporting the view that PI3K/Akt pathway activation may be pathogen- and tissue-dependent.

These differences primarily arise from the following mechanisms. First, the pathogenic strategies and host recognition receptors differ. *C. albicans*, as a facultative opportunistic pathogen, secretes the toxin candidalysin during its hyphal phase, which triggers PI3K/Akt-mediated protective immune responses by damaging epithelial cells. In contrast, dermatophytes are keratinophilic filamentous fungi whose pathogenesis relies mainly on the secretion of keratinases that directly erode and destroy the stratum corneum; the defensive response of host keratinocytes relies more on MAPK pathways. A review by Hube et al. also noted that in fungal skin diseases, different fungal species have distinct abilities to induce host reactions: yeasts such as *C. albicans* cause relatively low levels of host tissue damage and inflammation during pathogenic infection, whereas dermatophytes may induce higher levels of tissue damage and inflammation (Hube et al., 2015). Second, differences in the infection site

microenvironment and epithelial cell type, oral mucosal epithelium versus skin keratinocytes, in terms of tissue structure, degree of keratinization, immune cell composition, and microbiome composition collectively influence the host's choice of signaling pathways in response to different fungal infections.

These findings have two important implications for our study. First, the JNK pathway was clearly identified by Achterman et al. as one of the major activated pathways in keratinocytes infected by dermatophytes (Achterman et al., 2015); molecular docking showed that berberine has a very strong binding affinity for MAPK8 (JNK) (binding energy – 9.5 kcal/mol); and our in vivo experiments verified that berberine significantly increases p-JNK levels. This provides strong support for our focus on the JNK pathway. Second, the enrichment of the PI3K-Akt pathway predicted by network pharmacology is not contradictory to the selective activation observed in vivo; rather, it reflects the forward-looking nature of network pharmacology in screening potential targets and the fine differences in actual pathway responses to different fungal infections.

2. Berberine Activates the JNK Signaling Pathway to Enhance Host Antifungal Immunity

JNK, a core member of the MAPK family, plays a pivotal role in host anti-infective defense by regulating cell proliferation, apoptosis, autophagy, and inflammation (Chen et al., 2021). Recent studies have revealed that the integrity of the human JNK1-dependent MAPK signaling pathway is essential for IL-17A/IL-17F-mediated mucosal anti-*Candida* immunity, and that loss of JNK1 function leads to chronic mucocutaneous candidiasis (Li et al., 2019). In macrophages, JNK signaling cooperates with the Dectin-1 receptor to activate the Syk-JNK-AP-1 axis, regulating the production of antifungal cytokines (Huang et al., 2015). Moreover, JNK activation is indispensable for neutrophils to exert their antifungal functions (Hopke et al., 2025). During fungal infection, activation of the JNK signaling pathway also promotes the expression of autophagy-related genes, enhances autophagic flux, and facilitates pathogen clearance (Yao et al., 2025). Together, these studies establish the JNK signaling pathway as a key node in antifungal immunity.

The direct antifungal effects of berberine, the core active component of *Coptidis Rhizoma*, have been reported previously. Zhang et al. found that berberine exhibits significant in vitro antifungal activity against *C. albicans*, *Cryptococcus neoformans*, *T. rubrum*, and *Trichophyton mentagrophytes* (MIC = 64–128 µg/mL), and confirmed by scanning and transmission electron microscopy that berberine induces marked disruption of fungal cell walls and membranes, while also exerting antifungal effects through CYP51 inhibition (Zhang et al., 2024). Our study further validated the in vitro activity of berberine against *T. rubrum* (MIC range 32–128 µg/mL) using the broth microdilution method, which is highly consistent with the above reports. We further extended these observations using SEM, which provided clear visual evidence of berberine-induced cell wall collapse and surface disruption in both *T. rubrum* and *C. albicans* (Fig. 3D).

A critical and novel finding of our study is the potent antibiofilm activity of berberine. Biofilm formation is a key virulence factor that shields fungi from host immune attack and dramatically increases tolerance to conventional antifungals. Our crystal violet assay demonstrated that berberine significantly inhibited biofilm formation in both species (Fig. 3E). More importantly, CLSM live/dead staining and subsequent quantitative profile analysis revealed that berberine not only prevented biofilm formation but also effectively killed a substantial proportion of cells within established biofilms (Fig. 3F). This is clinically significant, as it suggests berberine could help eradicate biofilm-associated infections, a major challenge in dermatology.

More importantly, our study found that berberine significantly activates the host JNK signaling pathway. In the *T. rubrum* and *C. albicans* infection models, after berberine treatment, the mRNA expression of *MAPK8* (JNK) was further increased to 3.8-fold and 3.0-fold, respectively; in the refractory infection model, the relative expression of *Mapk8* in the berberine monotherapy group increased approximately 2.78-fold ($P < 0.0001$). Immunohistochemical analysis showed that the percentage of p-JNK-positive cells in the berberine group increased significantly from about 8% in the PBS group to about 32% (in the *T. rubrum* model, $P < 0.001$). Mechanistically, this finding echoes research on berberine in the field of oncology. Zhao et al. found that berberine combined with curcumin induces autophagic cell death in breast cancer cells through the JNK/Bcl-2/Beclin-1 pathway (Wang et al., 2016). The core of this mechanism is that JNK activation phosphorylates Bcl-2, causing its dissociation from Beclin-1, and the released Beclin-1 then activates the downstream autophagic process. Duan et al. also reported that berberine enhances host innate immunity to protect against bacterial infection by activating the p38 MAPK pathway (Xiao et al., 2025). These findings corroborate our discovery regarding the JNK pathway and collectively suggest that berberine can regulate multiple members of the MAPK family.

At the level of autophagy regulation, this study provides the first in vivo validation in the context of antifungal immunity that berberine activates the JNK-autophagy axis. After berberine treatment, the relative expression levels of the autophagy-related genes *Atg5*, *Beclin-1*, and *Map1lc3b* increased by 2.21-fold, 2.35-fold, and 2.60-fold, respectively ($P < 0.0001$), while the expression of the autophagy substrate p62-encoding gene *Sqstm1* decreased to 0.52-fold that of the PBS group, indicating a significant increase in autophagic flux. In antifungal immunity, autophagy protects the host through multiple mechanisms, including enhancing the killing efficiency of phagocytes against fungi, promoting fungal antigen presentation, and regulating the secretion of inflammatory cytokines (Kanayama and Shinohara, 2016). Our study found that after berberine activated the JNK-autophagy axis, the clearance of both *T. rubrum* and *C. albicans* was significantly enhanced, providing direct in vivo evidence for the function of this regulatory axis in the treatment of skin fungal diseases.

Furthermore, the inhibitory effect of berberine on excessive inflammatory responses is noteworthy. Our study showed that after berberine treatment, the expression of the pro-inflammatory factors *TNF* and *PTGS2* (COX-2) was significantly reduced compared with the infection model group (both $P < 0.0001$), indicating that berberine not only activates host immune defense but also effectively suppresses the excessive inflammation induced by infection, thus achieving a balance between immune defense and

tissue protection. The molecular basis of this "bidirectional regulation" may involve the fine-tuning of inflammatory factor expression by the JNK-downstream transcription factor AP-1, a possibility that warrants further investigation.

3. Synergistic Effects of Berberine with Conventional Antifungals and Clinical Translation Potential

Berberine combined with itraconazole exhibited significant synergistic effects against both *T. rubrum* and *C. albicans* (FICI = 0.25–0.375 and 0.125–0.5, respectively). This synergy was prominently visualized in our comprehensive bubble plot analysis (Fig. 3C), which confirmed that the combination of berberine with itraconazole, voriconazole, and posaconazole yielded the most consistent synergistic interactions across a large panel of clinical isolates. This result is strongly supported by the literature. Previous studies have shown that berberine combined with fluconazole, itraconazole, voriconazole, amphotericin B, or caspofungin achieves synergy rates of 52.38% to 71.43% against the yeast form of *Talaromyces marneffe*, with no antagonism (Luo et al., 2019). Cong et al. also reported that berberine combined with amphotericin B, fluconazole, or caspofungin exhibits synergistic effects against planktonic cells of *Trichosporon asahii* (Cong et al., 2017).

The clinical relevance of these findings is further underscored by our antibiofilm data. The ability of berberine to reduce biofilm formation and kill cells within biofilms, coupled with its synergy with azoles, offers a promising strategy to overcome biofilm-associated resistance, which is a major cause of treatment failure and recurrence in superficial mycoses.

The time-kill curves further validated the synergistic effect of the combination: in the monotherapy groups, colony counts began to rise again by the end of the observation period (day 14), whereas the combination group maintained sustained killing activity, with colony counts reduced by $> 2 \log_{10}$ CFU/mL compared with the monotherapy groups. In the *Galleria mellonella* survival experiment, the combination treatment group achieved a 10-day survival rate of 65%, significantly better than the monotherapy groups (berberine 15%, itraconazole 35%). In vivo mouse experiments further showed that in the itraconazole-refractory models, the berberine+itraconazole combination reduced fungal burden by approximately $3.4 \log_{10}$ CFU/g compared with the PBS group ($P < 0.0001$), and histopathological staining revealed intact epidermal structure and almost no fungal elements in the combination group.

Mechanistically, the synergy between berberine and itraconazole may arise from two aspects. First, they act on different targets: itraconazole inhibits the synthesis of ergosterol in the fungal cell membrane, whereas berberine both directly damages the fungal cell wall and inhibits CYP51, while simultaneously modulating the host JNK signaling pathway to activate autophagy and enhance immune clearance, creating a "two-pronged" effect. Second, berberine may affect the pharmacokinetics of itraconazole at the infection site by modulating host drug-metabolizing enzymes (such as CYP450 family members); this hypothesis requires further validation.

From a clinical translation perspective, berberine has promising potential as an antifungal sensitizer. For topical formulations, berberine can be developed into gels or creams for combination with azoles to treat superficial skin mycoses. For systemic administration, the dose used in this study (20 mg/kg/d, equivalent to approximately 100–150 mg/d in humans) is well below the conventional clinical dosage of *Coptidis Rhizoma* crude drug, providing a large safety window. Biocompatibility experiments have further verified the low toxicity profile of berberine. Given the continuously increasing incidence of drug-resistant dermatophytosis, especially the rapidly growing resistance of dermatophytes to terbinafine reported in recent decades, this multi-target strategy that combines "direct antifungal" and "host immunomodulatory" effects is particularly clinically valuable.

4. Limitations

This study has several limitations. First, the upstream activation mechanism of the JNK pathway is not yet fully defined. Whether berberine directly binds to JNK protein to exert kinase activation or indirectly activates JNK signaling by affecting upstream kinases such as MKK4/MKK7 (Zeng et al., 2022) requires further confirmation by surface plasmon resonance (SPR) and in vitro kinase activity assays. Second, autophagy has a dual role in antifungal immunity: overactivated autophagy may, under certain conditions, promote inflammatory responses or aggravate tissue damage (Oikonomou et al., 2018). Although we did not observe obvious exacerbation of tissue damage in this study, the safety of long-term use still requires observation over a longer time window. Third, the skin tissue homogenization method used in this study cannot distinguish the main cell types (keratinocytes, macrophages, or neutrophils) in which berberine regulates the JNK-autophagy axis. Future studies should combine lineage-specific gene knockout mouse models or single-cell sequencing technology (Balakumar et al., 2024) to explore the contributions of different cell types. Fourth, this study mainly focused on *T. rubrum* and *C. albicans*; the therapeutic effects of berberine on other clinically relevant fungi (such as *T. mentagrophytes*, *C. neoformans*, and drug-resistant *Candida* clinical isolates) and its regulation of the JNK-autophagy axis require further validation. Fifth, the resistance mechanism of the itraconazole-refractory model has not been fully elucidated; subsequent studies could clarify the specific resistance characteristics of the fungi through genome sequencing or antifungal susceptibility profiling. Sixth, while our in vitro and in vivo data strongly suggest a direct antibiofilm effect, the exact molecular targets involved in this process and the potential for berberine to disrupt mature biofilms in a clinical setting warrant further investigation.

Conclusion

Berberine from *Coptidis Rhizoma* enhances host antifungal immunity by activating the JNK signaling pathway and promoting autophagy. Furthermore, berberine exerts direct fungicidal activity and possesses potent antibiofilm properties. Berberine exhibits direct antifungal activity and potentiates itraconazole efficacy, including against refractory infections. These findings unravel a novel host-directed mechanism for berberine and support its clinical development as an antifungal sensitizer.

Abbreviations

AMB, Amphotericin B

ANOVA, Analysis of Variance

ATCC, American Type Culture Collection

Ber, Berberine

BP, Biological Process

CC, Cellular Component

CFU, colony-forming unit

CLSI, Clinical and Laboratory Standards Institute

CLSM, Confocal Laser Scanning Microscopy

CTD, Comparative Toxicogenomics Database

DL, drug-likeness

FCZ, Fluconazole

FICI, fractional inhibitory concentration index

GO, Gene Ontology

H&E, hematoxylin and eosin

IHC, immunohistochemistry

ITR, Itraconazole

JNK, c-Jun N-terminal kinase

KEGG, Kyoto Encyclopedia of Genes and Genomes

MAPK, mitogen-activated protein kinase

MCF, Micafungin

MF, Molecular Function

MIC, minimum inhibitory concentration

OB, oral bioavailability

PAS, periodic acid-Schiff

PBS, phosphate-buffered saline

PDB, Protein Data Bank

POS, Posaconazole

PPI, protein-protein interaction

qPCR, quantitative polymerase chain reaction

SEM, Scanning Electron Microscopy

TRB, Terbinafine

VCZ, Voriconazole

WB, Western Blot

Declarations

Funding

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Clinical trial registration

This study is not a clinical trial. All experiments were conducted in vitro and in animal models (mouse and *Galleria mellonella*), and did not involve human participants.

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Figures

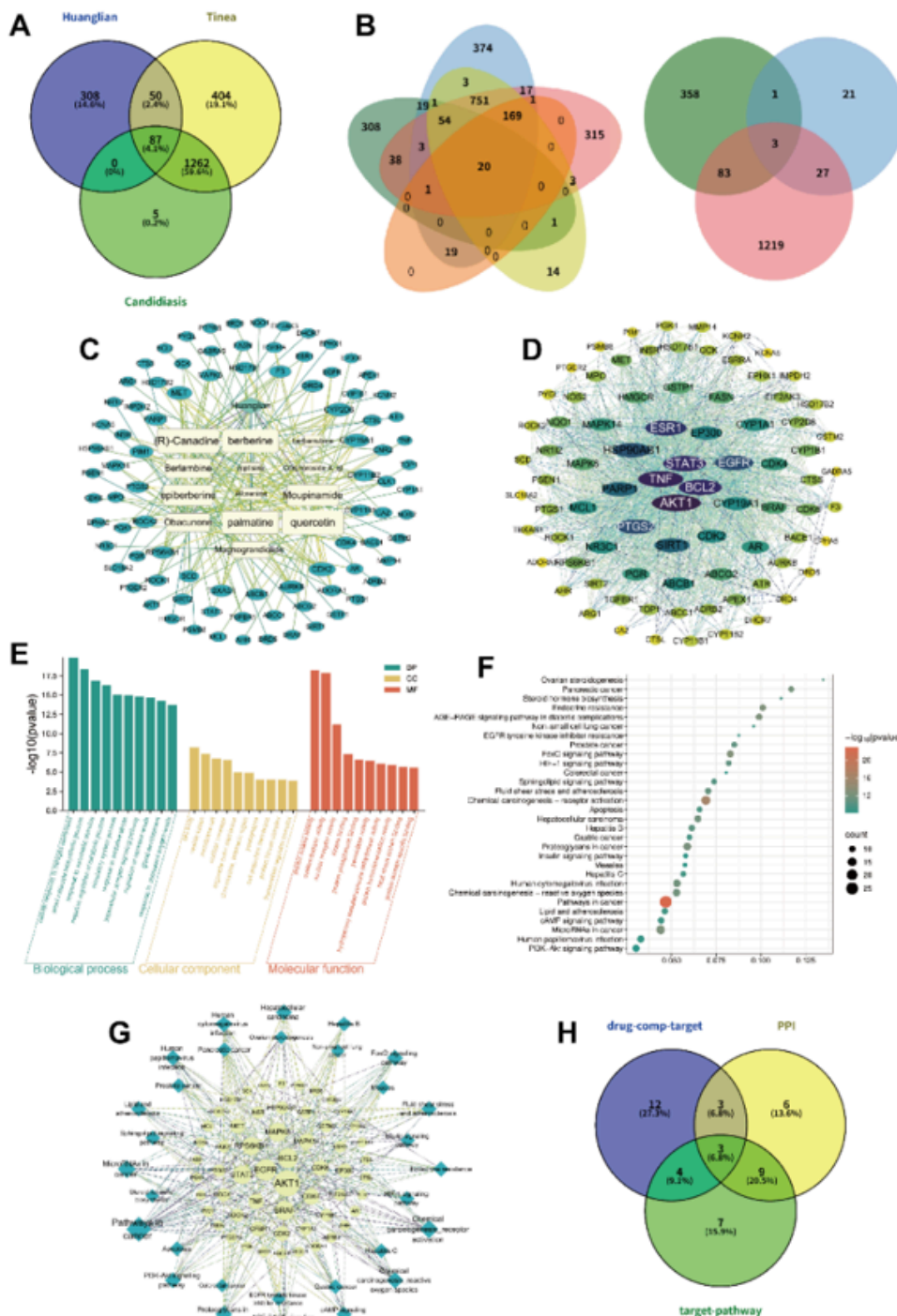


Figure 1

Network pharmacology analysis reveals potential targets and pathways of *Coptidis Rhizoma* against dermatophytosis and cutaneous candidiasis. (A) Venn diagram showing 87 intersecting targets among *Coptidis Rhizoma*-related targets (445), tinea-related targets (1,803), and cutaneous candidiasis-related targets (1,354). **(B)** Petal-shaped interactive Venn diagram illustrating the intersection of *Coptidis Rhizoma* targets with subdivided tinea and candidiasis subtypes. **(C)** “Drug-component-target” network

of *Coptidis Rhizoma*. Nodes represent drugs, active compounds, or targets; edges represent interactions. Node size reflects degree centrality. **(D)** Protein-protein interaction (PPI) network of the 87 intersecting targets constructed using STRING database and visualized in Cytoscape. Edge thickness indicates confidence scores. **(E)** Gene Ontology (GO) enrichment analysis of the 87 targets, including biological processes (BP), cellular components (CC), and molecular functions (MF). **(F)** KEGG pathway enrichment analysis of the 87 targets. Dot size indicates gene count; color represents adjusted *P*-value. **(G)** “Target-pathway” network showing connections between top-ranked targets and enriched KEGG pathways. **(H)** Venn diagram of the top 20 degree-ranked targets from the drug-component-target network, PPI network, and target-pathway network, yielding 19 core targets.

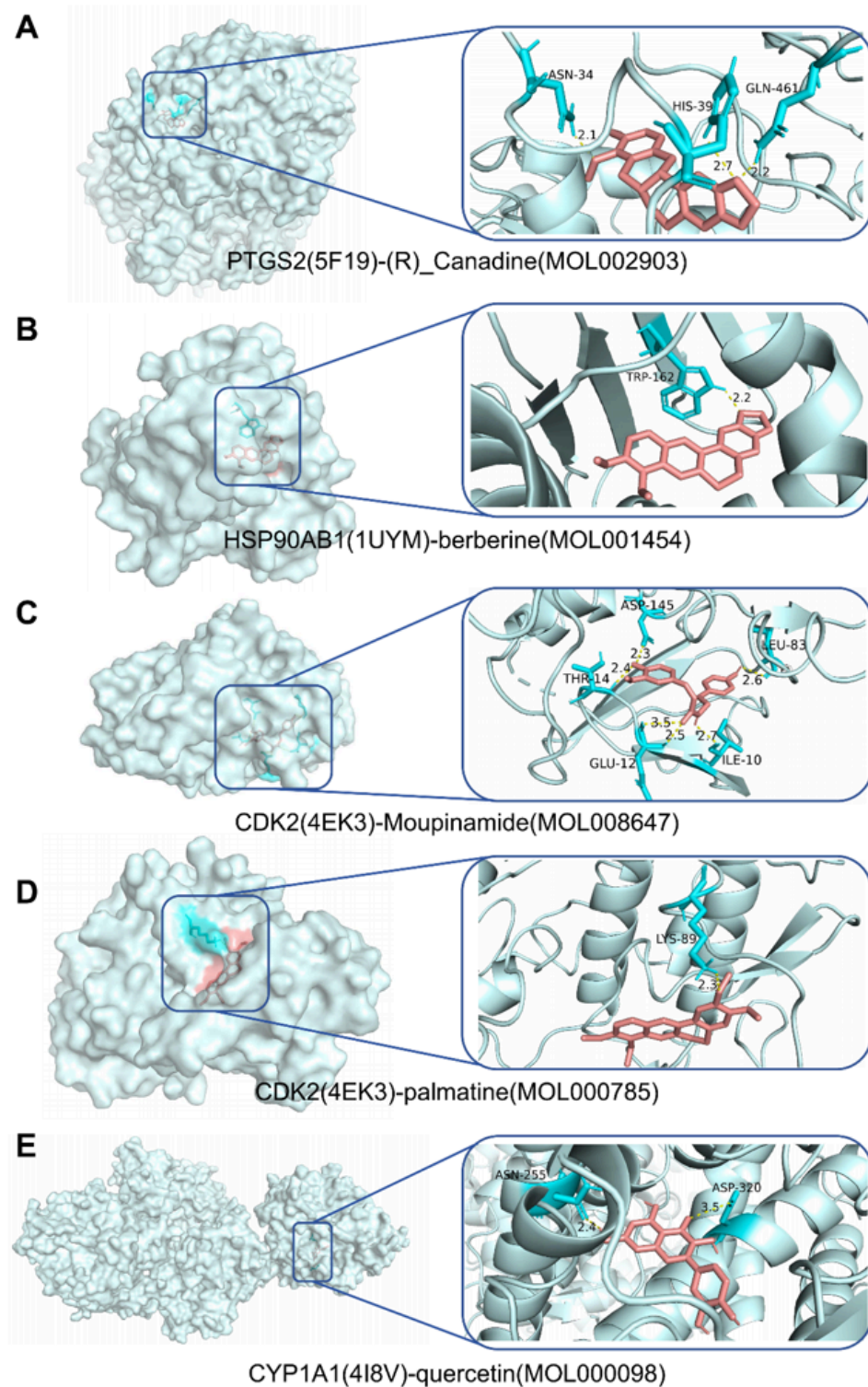


Figure 2

Molecular docking validation of representative binding modes between the five major active compounds from *Coptidis Rhizoma* and core targets.

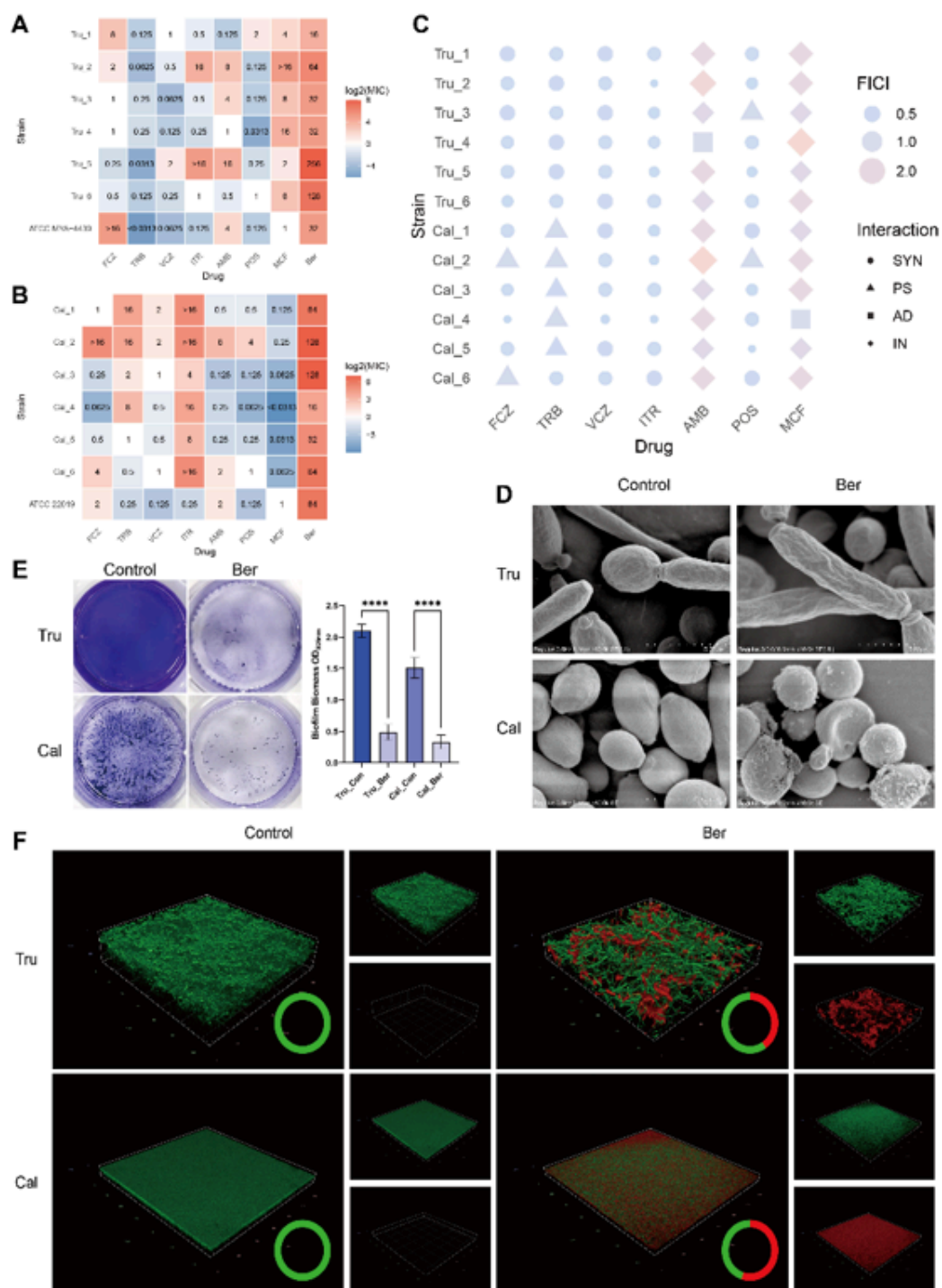


Figure 3

In vitro antifungal susceptibility, synergy, and antibiofilm activities of berberine against clinical isolates. (A) Heatmap visualizing the MICs of seven conventional antifungals against six clinical isolates of *T. rubrum*. Blue indicates susceptibility (S) and red indicates resistance (R). (B) Heatmap visualizing the MICs of seven conventional antifungals against six clinical isolates of *C. albicans*. (C) Bubble plot summarizing the checkerboard synergy assay results for berberine combined with seven antifungals

across all 12 clinical isolates. Bubble size and color represent the FICI value, while bubble shape denotes the qualitative interaction (SYN, synergism; PS, partial synergy; AD, additive; IN, indifference). **(D)** Scanning electron microscopy (SEM) images of *T. rubrum* and *C. albicans* treated with medium alone (Control) or berberine at the MIC (Berberine). Berberine treatment caused severe morphological damage, including surface collapse and wrinkling. Scale bars = 2 μ m. **(E)** Crystal violet staining of biofilms formed by *T. rubrum* and *C. albicans* with or without berberine treatment, and the corresponding quantitative absorbance at 590 nm. Berberine significantly inhibited biofilm formation ($P < 0.0001$). **(F)** Confocal laser scanning microscopy (CLSM) images of live/dead staining of biofilms. Green indicates live cells, red indicates dead cells. The right panels show circular quantitative plots of the fluorescence profiles analyzed using ZEN software and R, demonstrating that berberine treatment resulted in 40.4% dead cells for *T. rubrum* and 54.9% dead cells for *C. albicans*.

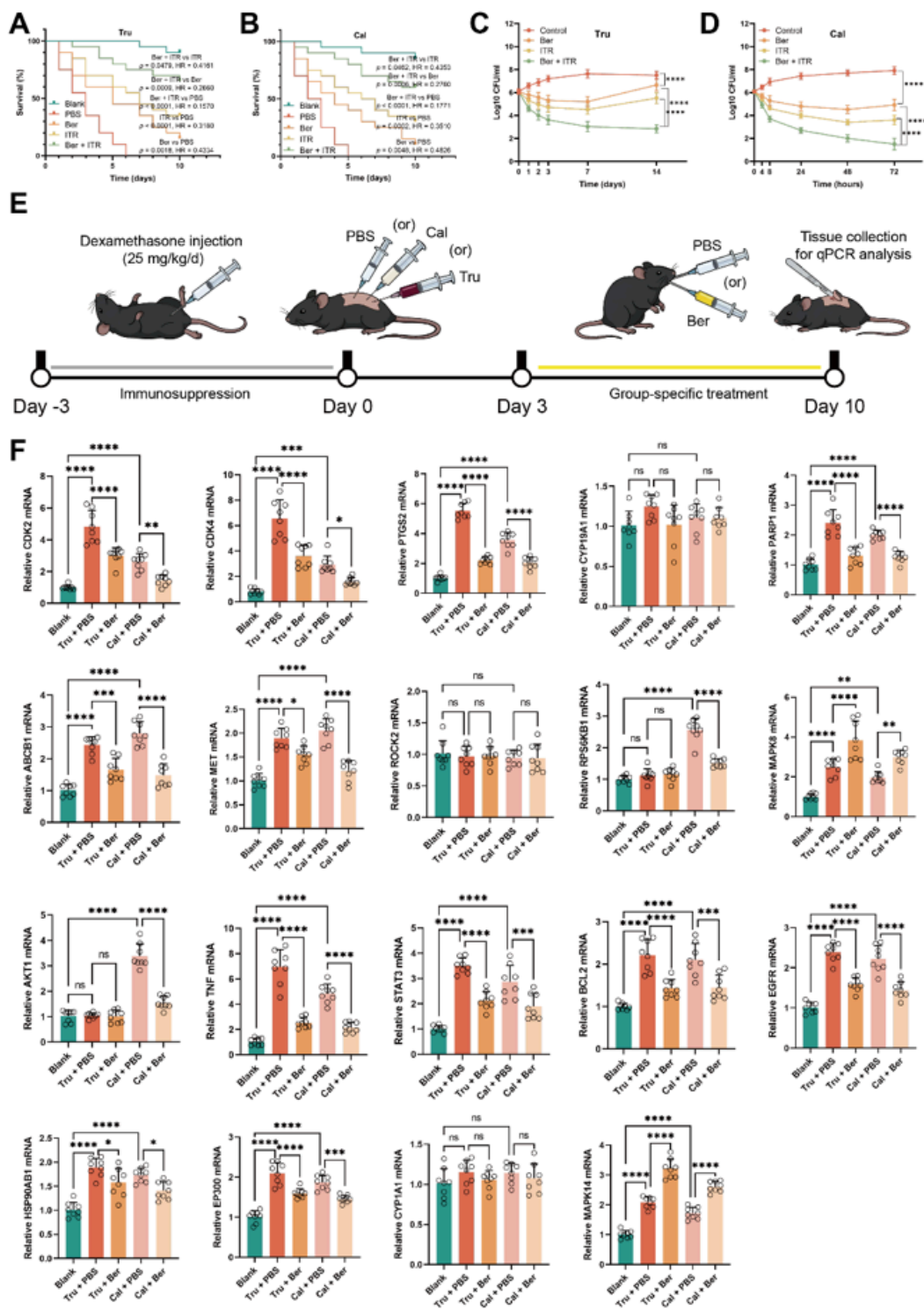


Figure 4

Berberine enhances host antifungal immunity by activating the JNK signaling pathway in mouse models of *Trichophyton rubrum* and *Candida albicans* infection. (A, B) Kaplan-Meier survival curves of *Galleria mellonella* larvae infected with *T. rubrum* (A) or *C. albicans* (B) and treated with PBS, berberine (Ber, 20 mg/kg), itraconazole (ITR, 5 mg/kg), or Ber+ITR (n=20/group). (C, D) Time-kill curves of *T. rubrum* (C) and *C. albicans* (D) treated with control, Ber (64 μ g/mL), ITR (16 μ g/mL), or Ber+ITR (4+1 μ g/mL).

Data are mean $\pm$ SD (n=4). The dashed line indicates 2 log₁₀ CFU/mL reduction from the most effective single agent. **(E)** Schematic illustration of the mouse back skin infection model and treatment regimen. **(F)** qPCR analysis of mRNA expression of 19 core targets in mouse skin tissues from Blank, infection (PBS), and berberine-treated (Ber) groups. Data are presented as fold change relative to Blank (mean $\pm$ SD, n=8). **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001; ns, not significant, *P* > 0.05.

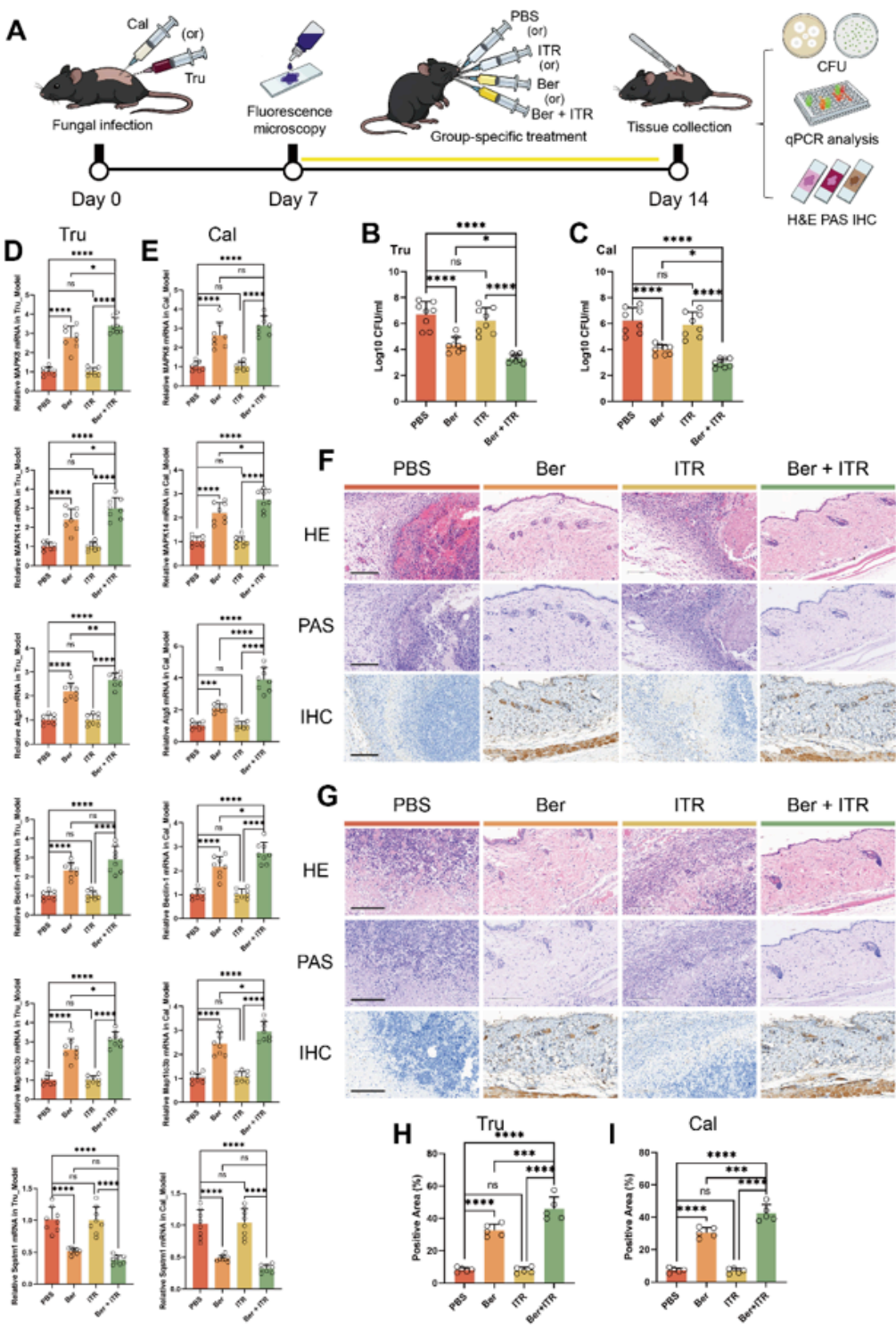


Figure 5

Berberine effectively treats itraconazole-refractory fungal infections by activating the JNK-autophagy axis. **(A)** Schematic diagram of the establishment of itraconazole (ITR)-refractory *T. rubrum* and *C. albicans* infection models. **(B, C)** Fungal burden ($\log_{10}$ CFU/g) in skin tissues of ITR-refractory mice treated with PBS, Ber (20 mg/kg), ITR (5 mg/kg), or Ber+ITR (5+1.25 mg/kg) for 7 days, infected with *T. rubrum* (B) or *C. albicans* (C). $n=8/\text{group}$. **(D, E)** qPCR analysis of *Mapk8*, *Mapk14*, and autophagy-related genes (*Atg5*, *Beclin-1*, *Map1lc3b*, *Sqstm1*) in ITR-refractory models of *T. rubrum* (D) and *C. albicans* (E). **(F, G)** Representative histopathology images (H&E and PAS staining) and immunohistochemistry (p-JNK) of skin sections from ITR-refractory mouse models infected with *T. rubrum* (F) or *C. albicans* (G). Scale bars = 200 μm . **(H, I)** Quantitative analysis of p-JNK-positive cell percentages in skin tissues from the *T. rubrum* (H) and *C. albicans* (I) ITR-refractory models. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$; ns, not significant, $P > 0.05$.