Antifungal compounds of Chinese prickly ash against drug-resistant *Candida albicans*

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**A B S T R A C T**

The leaf of Chinese prickly ash, a unique spice having typical pungent sensation, is a popular food in Southwest China with antipruritic, insecticidal and fungicidal functions, but its bioactive constituents of fungistatic capacity remain unknown. In present investigation, twenty-nine compounds were isolated from leaf of Chinese prickly ash, and their antifungal bioactivity against drug-resistant *Candida albicans* were evaluated *in vitro* and *in vivo*. As a result, three compounds 3, 10, 29 showed antifungal bioactivity by damage of the fungal biofilm, and they might recover sensitive of drug resistant *C. albicans* to Fluconazole. Then Chinese prickly ash leaf was proved to be a functional food against fungus for the first time in experiment.

**ARTICLE INFO**

**1. Introduction**

Chinese prickly ash (*Zanthoxylum bungeanum*) is the best known condiment belongs to the *Zanthoxylum* genus in the Rutaceae family which has become a very popular product throughout the world due to its rich variation in aroma and nutritional together with its health benefits (Luo et al., 2021). Originally from warm temperate and subtropical regions of the world, this genus is widely distributed in some parts of southwest China and southeast Asia (Yoro, Franck, Jean, Alas-sane, & Julien, 2017). The pericarp of this plant is considered a traditional food and used in a wide range of hot pot because of its distinctive aromatic, tongue-numbing sensation, and its pungency (Luo et al., 2021). Chinese prickly ash leaf, the main byproducts of Chinese prickly ash, gain increasing attention due to their unique flavor, wide sources and health-care effects (Ma, Tian, Wang, Huang, & Wei, 2021). In the central part of Yunnan Province, fresh Chinese prickly ash leaf is consumed in salads, cooking, and making tea as a functional food to clear away heat, detoxicate, kill insects, relieve itching (Wu, 2017).

Chinese prickly ash leaf contain various types of bioactive compounds, such as flavonoids, amides, lignans, and coumarins, which have anti-inflamatory, antioxidant, antitumor, and antimicrobial properties (Zhang, Luo, Wang, He, & Li, 2014), and then it is consider to be an important resource for the discovery the development of novel functional foods and cosmetics (Wang, 2021). However, in contrast to Chinese prickly ash seeds, Chinese prickly ash leaf is sometimes consumed as fertilizer or discarded, which may not only cause environmental pollution, but also lead to a waste of natural resources.

*C. albicans* is a common commensal fungus in humans that can cause mucosal and deep tissue infections with a mortality rate of up to 35 % (Nett & Andes, 2006). This commensal fungus is able to proliferate when locally or systemically immunosuppressed, of which oropharyngeal candidiasis (OPC) being the most common infection (Millet, Solis, & Swidergall, 2020). The widespread use of Fluconazole has led to resistance of *C. albicans* to Fluconazole, persistent and repeated invasive infection with Fluconazole resistant *C. albicans* increased health care costs and led to higher morbidity and mortality, so need a

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comprehensive measure in treatment and prevention. Refractory C. albicans infection is often related to the formation of biofilms, which is an important mechanism of fungal drug resistance (Hasan, Xess, Wang, Jain, & Fries, 2009; Yu, Wei, Ma, Chen, & Xu, 2011). In the current situation where a number of highly effective and less toxic antifungal drugs was urgently required, combined antifungal therapy has become one of the hotspots (Ahmad, Abida, & Iqbal, 2012). Chinese prickly ash leaf showed antibacterial effect against pathogenic bacteria, Bacillus cereus, B. subtilis and Staphylococcus aureus (Nongmai et al., 2021), but lack of fungistatic capacity and bioactive constituents report.

2. Materials and methods

2.1. General experimental procedures

All compounds were separated by column chromatography and concentrated by vacuum rotary evaporators (OSB-2200 EYELA). Separate materials included silica gel (200–300 mesh), LiChroprep RP-C18 (40–63 μm), and Sephadex HL-20 (40–70 μm). Partial compounds were purified by an Agilent 1260 Infinity II (USA) with a diode array detector using a semipreparative RP-C18 (250 mm × 10 mm, 5.0 μm). TLC was performed using silica gel GF 254 (Hg/T 2354–2010) on a silica gel plate before separation. The isolated compounds were determined by NMR spectroscopy (BRUKER AVANCE III 400 MHz, Switzerland) and processed with Mestrenova software. ESI-MS spectra were recorded on an Agilent 1290/6545 Q-TOF (Agilent, Santa, Clara, USA).

2.2. Plant materials

Chinese prickly ash leaf was purchased from Yuxi city, China, in September 2020, and was determined by Professor Li-Xing Zhao of Yunnan University. A specimen was stored in the Key Laboratory of Medicinal Chemistry for Natural Resource, Ministry of Education and Yunnan Province, School of Chemical Science and Technology, Yunnan University, Kunming, P.R. China.

2.3. Strains and reagents

C. albicans (08030401, drug resistant) was isolated strains presented from the Second People’s Hospital of Yunnan Province. Fluconazole, cyclophosphamide, crystal violet, and MTT were purchased from Macklin Reagents (Shanghai, China). Ethyl acetate, petroleum ether, chloroform, methanol, aceton and acetone were purchased from Tianjin Chemical Reagents Co (Tianjin, China). DMEM, fetal bovine serum, streptomycin, and penicillin were purchased from Gibco, and the human keratinocyte cell (HaCaT) line were generous gifts received from Yunnan University of Traditional Chinese Medicine. All aseptic operations were carried out in a biosafety cabinet (BSC, Thermo Scientific).

2.4. Animals experimental conditions

10–12 weeks old male Kunming mice (n = 36, 37 ± 2 g) were purchased from Kunming Medical University. All animals were housed at room temperature (20–26 °C) and constant humidity (50–70 %) under a 12 h light–dark cycle in a specific-pathogen-free grade laboratory. Mice were allowed to acclimate to their environment for a week before being randomly assigned to experimental groups. The experiment was reviewed and approved by the Institutional Animal Care and Use Committee of Yunnan Institute of Materia Medica.

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FICI = \frac{MIC \text{ of drugA, tested in combination}}{MIC \text{ of drugA, tested alone}} + \frac{MIC \text{ of drugB, tested in combination}}{MIC \text{ of drugB, tested alone}}
\]

2.5. Characterisation of extracts

The fresh Chinese prickly ash leaf (25 kg) was reflux extracted with EtOH (150 L × 3) at 50 °C three times (48 h × 3), and the EtOH solvent was evaporated under vacuo to give a crude extract (1.8 kg). The residue was suspended in water and successively partitioned with ethyl acetate (15 L × 3) and water (10 L × 3) to yield the ethyl acetate fraction (510 g) and water fraction (1.26 kg). Then the ethyl acetate fraction was subjected to column chromatography (CC, 200–300 mesh, 2.5 kg) on silica gel eluting with petroleum ether-acetone (from 50:1, 30:1, 15:1, 10:1, 7:1, 3:1, 0:1, 10 L for each ratio) to give eight fractions (Y-1 to Y-8), the detailed method is presented in the Supporting Information.

2.6. Identification of compounds from the ethyl acetate fraction by UHPLC-QTOF-MS/MS

The ethyl acetate fraction showed antifungal bioactivity against C. albicans, (08030401, drug resistant) and then its constituents was analyzed by an Agilent 1290 UHPLC instrument coupled and G6545B Q-TOF/MS system (Agilent Technologies). The samples were separated by using an Agilent SB-C18 column (2.1 × 100 mm, 1.8 μm; Agilent Technologies) at a constant temperature (38 °C). The solvent system consists of water containing 0.1 % (v/v) formic acid (A) and methanol (B) at a flow rate of 0.15 ml/min. The injection volume is 2 μL. The gradient elution was 0–20 min, 5 %–100 % B; 20–25 min, 100 % B. The scanning range in the positive ion mode was m/z 50–1200. The ESI voltage in the positive ion mode was 3.5 kV. A nebulizing gas of 35 (psig) and a drying gas (7 L/min) were applied for ionization, using nitrogen in the positive ion mode. These conditions were used to identify compounds of ethyl acetate fraction. By comparing fragment ions with the literatures, and the retention time from MS and MS/MS data with the isolated compounds, compounds of ethyl acetate fraction can be identified. By using MS, NMR and comparing with the data of literature to characterize the structure.

2.7. Evaluation of antifungal activity in vitro

2.7.1. MICs/MFCs determinations

The samples were diluted into seven concentration gradients (128, 64, 32, 16, 8, 4, 2 μg/mL) by the double dilution method in 96-well plates. To ensure the accuracy and reliability of the experiment, each of the samples was repeated six times. Then, 96-well plates were incubated for 8 h at 37 °C, and read at 600 nm OD. The MIC was reported as the lowest concentration (µg/mL) of the drug and visually inhibited the growth of the organism. The fungi incubated in TSB were used as blank controls, Fluconazole as positive controls, final DMSO concentration<1 %.

After determining the MIC, 10 μL was inoculated onto the supplemented TSA plates containing serial dilutions (1 × MIC, 2 × MIC, 4 × MIC and 8 × MIC), and the plate was incubated at 37 °C for 12 h. Each of the samples was repeated three times. The lowest concentration without fungal growth was the MFC.

2.7.2. Checkerboard methods

The microdilution checkerboard method to detect the relationship between compounds and antibiotics synergistic combination of two antifungal agents can be expressed as fractional inhibitory concentration index (FICI), FICI calculated as follows:
FICI \leq 0.5, synergistic effect (SY); 0.5 < FICI \leq 2.0, additive effect (AD); 2.0 < FICI \leq 4.0, no interaction effect (NI).

Using a twofold dilution series for fungistatic drugs in TSB, the concentration of the two drugs was distributed between \( \frac{1}{16} \times \text{MIC} \) and \( 2 \times \text{MIC} \). Join the fungistatic drugs (50 \( \mu \text{L} \)) decrease in the order of concentration in wells of a 96-well plate among the rows of the board, each of the concentrations repeated six times. Then, the same method was used to decrease the compound solution in order of the concentration join it to each column of the 96-well plate. Each of the concentrations was repeated six times, and 100 \( \mu \text{L} \) of fungus solution diluted to \( 10^6 \) CFU/mL was added to each well until a final volume of 200 \( \mu \text{L} \) was obtained. Then, 96-well plates were incubated for 8 h at 37 \( ^\circ \text{C} \) and read at 600 nm OD using a 96-well plate to determine the MIC value of each drug when used in combination. The way is shown in the Supporting Information Fig. S1.

2.7.3. Fungicidal activity was determined by the time-kill curve method

First, samples of different concentrations were added to the 24-well plate so that the final concentrations were \( 1 \times \text{MIC}, 2 \times \text{MIC} \) and \( 4 \times \text{MIC} \), and then TSB was added to 0.9 mL in each well. Finally, 100 \( \mu \text{L} \) of \( C. \text{albicans} \) (08030401, drug resistant) solution (\( 10^6 \) CFU/mL) was added to each well until a final volume of 200 \( \mu \text{L} \) was obtained. Then, 96-well plates were incubated for 8 h at 37 \( ^\circ \text{C} \) and read at 600 nm OD using a 96-well plate to determine the MIC value of each drug when used in combination. The way is shown in the Supporting Information Fig. S1.

2.7.4. Fungal biofilms activity and scanning electron microscope (SEM)

As described previously, \( C. \text{albicans} \) (08030401, drug resistant) solution (\( 10^6 \) CFU/mL) was grown in 96-well plates at 37 \( ^\circ \text{C} \) for 24 h to allow biofilm formation. Then, the medium was removed from each well and washed three times with sterile PBS to remove planktonic fungi that had not formed biofilms. Finally, different drugs diluted with TSB were added and incubated at 37 \( ^\circ \text{C} \) for 24 h. All treatments were repeated 5 times. The quality of the biofilm was assessed using the crystalline violet assay (OD at 600 nm) and the viable cells in the formed biofilm were determined using the MTT assay (OD at 570 nm).

SEM was used to analyze the biofilm morphology as described, keeping concentration samples into 24-well plates to make the final concentrations \( 1/2 \times \text{MIC}, 1 \times \text{MIC} \) and \( 2 \times \text{MIC} \). Each of the samples was repeated three times at 37 \( ^\circ \text{C} \) for 3 h, and centrifuged (3500 rpm, 20 min). The supernatant was removed, washed three times with PBS, then fixed with 4 \% paraformaldehyde for 24 h. Finally, the samples were dehydrated with gradient concentrations of ethanol (30 \% for 10 min, 50 \% for 10 min, 70 \% for 10 min, 90 \% for 10 min, and 100 \% for 10 min), dried, and sprayed with gold. The samples were observed and photographed under SEM.

2.7.5. Cell culture and cell viability assay

The cytotoxic activities of isolated compounds were evaluated by the MTT method. The HaCaT cells were cultured in DMEM supplemented with 10 \% heat-inactivated FBS, 100 U/mL benzylpenicillin/streptomycin at 37 \( ^\circ \text{C} \) in a humidified incubator with 5 \% \( \text{CO}_2 \). In order to determine the influence of additive to HaCaT cells, they were seeded in 96-well plates and cultured for 48 h with MIC of the tested compound alone or in combination with Fluconazole, or DMSO (control). Then, 10 \( \mu \text{L} \) MTT solution (5 mg/mL) was added and incubated at 37 \( ^\circ \text{C} \) for 4 h. The absorbance was measured at 490 nm by a microplate reader.

2.8. Antifungal activity in vivo

Model groups for a total of 36 samples (weighing 37 \( \pm 2 \) g each sample) were randomly divided into six groups, with six replicates for each group: blank group, control group, positive drug group (5 mg/kg of Fluconazole), high-dose group (5 mg/kg of compound 29 and 20 mg/kg of Fluconazole), low-dose group (1.25 mg/kg of compound 29 and 5...
3. Results and discussion

3.1. Isolation of compounds from the Chinese prickly ash leaf

29 known compounds were isolated and identified as (-)-qin bun amide A (1) (Tian et al., 2016), qin bun amide B (2) (Tian et al., 2016), γ-sanshool (3) (Yasuda, Takeyn, & Inoue, 1981), (-)-asaradin (4) (Jooseok et al., 2010), (-)-kobusin (5) (Abe, 1974), (-)-sanshool-4′-O-γ,γ-dimethylallyl ethyl (6) (Bastos, Gottlieb, Sarti, & Fillard, 1996), (+)-magnolin (7) (Hua, He, Ryang, & Ho, 2004), (+)-esusmin (8) (Latip, Harlley, & Waterman, 1999), planisipine A (9) (Lin, Min, Huang, & Chen, 2019), (+)-piperitol (10) (Terreaux, Maillard, Hostettmann, Lod, & Hakimzamungu, 2010), nodakenetin (11) (Saeed & Sabir, 2007), bargapten (12) (Li, 2009), imperatorin (13) (Wang et al., 2008), demethylberosin (14) (Masuda, Takasugi, & Anet, 1998), pubnieren A (15) (Huang et al., 2006), linallyl-γ-glucopyranoside (16) (Omar, Noman, Mohamed, Aluntas, & Demirtas, 2018), (3S,5R,6S,7E)-5,6-epoxy-3-hydroxy-7-megastigmen-9-one (17) (D’Abrósca et al., 2004), (3R,6R,7E)-3-hydroxy-4,7-megastigmen-9-one (18) (D’Abrósca et al., 2004), linally-O-β-glucoside (19) (Sang, Min, Lee, & Kang, 2007), (-)-epicatechin (20) (Zhou & Yang, 2000), queretin (21) (Zhou & Yang, 2000), 4′-O-methyl catechin (22) (Docampo-Palacios et al., 2020), 1H-indole-3-carbaldehyde (23) (Wang, Yang, Mei, & Yang, 2013), N4-formylhidrouracilacarape (24) (Yang, Zhang, & Hu, 2008), 2,6,2′-6′-tetratetrahydroxy-4,4′-bis(2,3-epoxy-1-hydroxypropyl)biphenyl (25) (Shiow-Hwa et al., 2001), cinnaamic acid (26) (Yang, Zhao, Ren, Mei, & Liang, 2003), loliolide (27) (Willuhn & Westhaus, 1987), 4-

mg/kg of Fluconazole), and single compound group (1.25 mg/kg of compound 29). Six groups of mice established hypo-immunity by intraperitoneal injection of cyclophosphamide (30 mg/kg) one day in advance. Except for the blank group, the remaining five groups were treated with sterile cotton swabs dipped in C. albicans (08030401, drug resistant) (10⁶ CFU) and kept on the oral mucosa of the mice for 1 min. Different drug treatments were administered at 3, 24 and 27 h after the start of infection, and the control group was treated with the same amount of normal saline, and the results were tested on day 3. Using a sterile cotton swab of the applicator mouse mouth for 1 min, the cotton tip was cut off and placed into an EP tube containing 0.99 mL of PBS, the EP tube was vortexed for 1 min and 100 µL of the mixture was serially diluted 10-fold with PBS. 10 µL of the mixture was transferred to TSA plates, and each sample was repeated three times. After incubation at 37 °C for 24 h, the colonies on the TSA plates were enumerated. All operations were carried out in a biosafety cabinet (BSC).
hydroxy-3-methoxy-benzaldehyde (28) (Ito et al., 2001), 2, 4-dihydroxy-6-methoxy acetophenone (29) (Singh, Pathak, & Agrawal, 1997) by comparison with literatures.

3.2. Phytochemical analysis

Forty compounds were identified from the ethyl acetate fraction of Chinese prickly ash leaf (Fig. 1), and the main ingredients can be formally divided into the five types, amides, flavonoids, lignans, coumarin and volatile oils. The secondary mass spectrometry analysis of the compound gave a series of fragments, which can be used as a reference for identification of the same type of compound by LC-MS. In the positive ion mode and negative ions mode, the peaks were identified by comparison with standard compounds and MS data statistical analysis in Table 1.

3.3. Antifungal activity in vitro

3.3.1. Determination of MICs and MFCs

The antifungal activity of 29 compounds against C. albicans (08030401, drug resistant) were test by the microdilution broth susceptibility assay, the results were the MICs of compounds 2, 3, 10, 25, 26, 28 and 29 were 32 μg/mL, and the MIC of compounds 1, 9, 11, 20 and 21 were 64 μg/mL. The detailed results shown in the Supporting Information Table S1.

Seven compounds with MIC of 32 μg/mL were tested for synergistic effects with Fluconazole used a checkerboard method. (Table 2).

To describe the combined effects of the seven compounds and antibiotics, the checkerboard experiments were performed followed by FICI numerical calculations and interval definitions. The results of the checkerboard experiment showed that the combination of three compounds (3, 10, 29) with Fluconazole had a synergistic effect on C. albicans (08030401, drug resistant) (FICI ≤ 0.5). The MIC of Fluconazole and compounds (3, 10, 29) against C. albicans (drug resistant) was reduced to a quarter after the combination. In subsequent experiments, the MIC of the compounds in combination with Fluconazole was further investigated including the antifungal pattern in vivo and in vitro.

3.3.2. The time-kill curve technique

To gain insight into the fungistatic mechanism of antibiotic combinations compound, fungistatic and synergistic effects of the tested antibiotic combinations compound were assessed by using the time-kill curve technique. This experiment can judge whether the combination of compounds and antibiotics has concentration-dependent and time-dependent properties, and can further judge whether the compound is a fungicidal compound or a fungistatic compound. Adding 1/4 × MIC, 1/2 × MIC, 1 × MIC (compounds 3, 10, 29 + Fluconazole), C. albicans (08030401, drug resistant) were in a zero-growth state within 6 h. C. albicans began to grow after 3 h after treatment with 1/4 MIC compound alone and Fluconazole, and the control group continued to grow.

Table 2

| Strain          | Compounds | Alone     | Combined       | FICI | Remark |
|-----------------|-----------|-----------|----------------|------|--------|
|                 |           | MICs compound (μg/mL) | MICs Fluconazole (μg/mL) | MICs compound (μg/mL) | MICs Fluconazole (μg/mL) |
| C. albicans (08030401) albicans(08030401) | 2         | 32        | 128            | 16   | 32     | > 0.5 | AD     |
|                 | 3         | 32        | 128            | 8    | 32     | ≤ 0.5 | SY     |
|                 | 10        | 32        | 128            | 8    | 32     | ≤ 0.5 | SY     |
|                 | 25        | 32        | 128            | 16   | 32     | > 0.5 | AD     |
|                 | 26        | 32        | 128            | 16   | 32     | > 0.5 | AD     |
|                 | 28        | 32        | 128            | 16   | 32     | > 0.5 | AD     |
|                 | 29        | 32        | 128            | 8    | 32     | ≤ 0.5 | SY     |

Additive, (AD), synergistic, (SY), no interaction, (NI).

Fig. 2. Time-kill kinetics of compound 3 (A), compound 10 (B), and compound 29 (C) alone and combined with Fluconazole against resistant C. albicans.
2 h later. The results indicated that Fluconazole resistant C. albicans could be recovered its sensitive by three compounds (Fig. 2).

3.3.3. Antibiofilm activity and SEM.

Biofilms produced by fungal is one of the reasons for the development of disease pathogenicity and drug resistance. In order to explore the most possible antifungal mechanism of compounds, fluorescence microscopy imaging and SEM method were used. The detection results are shown in Fig. 3 and Figs. S2–S3.

In the Fig. 3, compound 3 and Fluconazole were used alone or combined to explore the antibiofilm activities of C. albicans (08030401, drug resistant). visualization of C. albicans biofilms under fluorescence microscopy (a) the control showed significant clumpy green fluorescence indicating that there was more biofilm. This part of the biofilm was visible microscopically when the compounds and fluconazole were used alone compared to the control group. As the concentration of the (compounds + Fluconazole) group increases, the green fluorescence gradually decreased to almost nothing, this implied that fluconazole alone could not destroy biofilm while in combination with the compound could destroy biofilm. Meanwhile, biofilms and viable cells were quantified by the crystal violet staining method or MTT staining method. The results showed that compared with control the (compounds + Fluconazole) group at 1/4 MIC and 1/2 MIC significantly reduced the amount of biofilm (c) and viable cells (d). When the compounds and Fluconazole were used alone, there was also a small clearance effect on biofilms and viable cells. Therefore, it was concluded that the compounds (3, 10, 29) in combination with Fluconazole not only disrupt biofilms but also rupture cell structures.

3.3.4. Cytotoxic assay

The cytotoxicity of compounds (3, 10, 29) and Fluconazole was determined using the HaCaT cells by the MTT method. The relative viability of the cells incubated with the extract was calculated taking as reference the untreated cultures (control). The results indicated that 128 μg/mL Fluconazole showed 48.69 % cell viability, and the dosage was reduced when combined with the compounds (3, 10, 29), the toxicity was significantly reduced, and no cytotoxic effects of three compounds (3, 10, 29), as shown as shown in Fig. S4.

3.4. Antifungal activity in vivo

In humans, the most common Candida species found in both healthy oral mucosa and in Oral candidiasis is C. albicans due to its adherence properties and greater level of pathogenicity. Therefore, established a mouse model of oral infection of C. albicans (08030401, drug resistant) who’s oral can be treated by compound and Fluconazole to evaluate whether the compound and fluconazole alone or in combination can effectively remove oral infection of C. albicans. As a result, the C. albicans growth was significantly inhibited in the co-administration group.
Values were mean ± SD for all mice. Six replicates for each group. **P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05, P = 0.05, ##P < 0.05, ###P < 0.01.

compared to the alone administration group. Similar to the findings in vitro, after being with C. albicans for three days, in the control group (wiped with normal saline), C. albicans in the oral cavity grew to 10³ CFU in mice, and C. albicans in the give different doses drugs mice were between 10³ and 10⁷ CFU, compared with control the inhibition rate were 40.76 % (1.25 mg/kg compound 29 and 5 mg/kg Fluconazole), 48.82 % (5 mg/kg compound 29 and 20 mg/kg Fluconazole), 27.5 % (1.25 mg/kg compound 29), 27.79 % (5 mg/kg Fluconazole), respectively (Fig. 4). The results further supported that Fluconazole resistant C. albicans could be recovered its sensitive by three compounds in vivo.

4. Conclusions

It was the first time to examine antifungal activity of Chinese prickly ash leaf, in which three active compounds (3, 10, 29) showed significant antifungal activity against drug-resistant C. albicans. Moreover, three compounds disrupted preformed biofilms of drug-resistant C. albicans, and they had significant synergistic effect combined with fluconazole. The finding extended the potential value of Chinese prickly ash leaf as a functional food to reduce the use of antibiotics in treating drug-resistance fungi.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

X.D. Luo conceived the projects, revised the manuscript and provided financial support. Dan-Yu Ma writing-original draft.; Zhao-Jie Wang writing-review & editing, Yi-Chi Chen finished data curation, Zi-Heng Qi, Huan Wang and Yan-Yan Zhu implemented supervision and validation; Xiao-Dong Luo writing-review & editing and funding acquisition; and all authors approved the final version of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100400.

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Fig. 4. The combination of compound 29 and Fluconazole inhibited C. albicans in mice. (Oral mucosa mice were infected with C. albicans and treated with positive drug group (5 mg/kg of Fluconazole), high-dose group (5 mg/kg of compound 29 and 20 mg/kg of Fluconazole), low-dose group (1.25 mg/kg of compound 29 and 5 mg/kg of Fluconazole), single compound group (1.25 mg/kg of compound 29), control group and blank group (wiped with saline). Data were expressed as means ± SD for all mice. Six replicates for each group.)

Values were mean ± SD, ****P < 0.0001 versus control. **P < 0.05, ***P < 0.01, **P < 0.01, and ***P < 0.001.
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