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Original Article

Fungicidal Kinetics of *Calotropis procera* and *Bridelia ferruginea* Against Multidrug-Resistant *Candida* Species

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Abstract

Background and aims: Multidrug-resistant (MDR) *Candida auris* and *Candida albicans* have emerged as serious hospital-acquired fungal pathogens and are responsible for persistent and hard-to-manage infections worldwide. The increasing prevalence of antifungal resistance, coupled with the limited availability and effectiveness of current therapeutic options, underscores the urgent need to explore alternative antifungal agents. In this context, medicinal plants and their bioactive constituents have gained attention as potential sources of novel antifungal compounds. This study aimed to investigate the antifungal efficacy and time-kill kinetics of leaf extracts from *Calotropis procera* and *Bridelia ferruginea* against MDR *C. auris* and *C. albicans*.

Methods: Leaf materials of both plants were processed and extracted using the maceration technique. Antifungal activity was evaluated through agar well diffusion assays to measure zones of inhibition and by minimum inhibitory concentration determination (MIC). Additionally, time-kill kinetic studies were conducted over a 42 hours at varying extract concentrations to assess the rate and extent of fungicidal activity.

Results: Both plant extracts demonstrated inhibitory effects against the tested *Candida* species (MIC values of 3.125 mg/mL for both). However, *C. procera* exhibited stronger antifungal activity against *C. albicans* (16.33 ± 0.33 mm) compared with *B. ferruginea* (12.67 ± 0.33 mm). Conversely, *B. ferruginea* showed superior inhibition of *C. auris* (16.33 ± 0.33 mm) relative to *C. procera* (12.67 ± 0.33 mm). Time-kill analyses confirmed concentration-dependent and time-dependent fungicidal effects for both extracts. Notably, *C. procera* rapidly reduced *C. albicans* viability, achieving a ≥3-$\log_{10}$ reduction within 26–30 hours, whereas *B. ferruginea* produced a gradual yet sustained decline in *C. auris* counts throughout the 42-hour assay.

Conclusion: The findings revealed species-specific fungicidal properties of the extracts. The rapid action of *C. procera* against *C. albicans* and the prolonged activity of *B. ferruginea* against *C. auris* suggest complementary antifungal potential, supporting the need for further phytochemical characterization and in vivo evaluation.

Keywords: *Candida auris*, *Candida albicans*, Multidrug Resistance, *Calotropis procera*, *Bridelia ferruginea*, Time-Kill Kinetics

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Introduction

Invasive fungal infections are an escalating global health concern, particularly among immunocompromised individuals. In addition, *Candida* species represent the most prevalent fungal pathogens that are associated with bloodstream and mucosal infections, contributing substantially to morbidity and mortality worldwide (1-3). While *Candida albicans* remains the predominant cause of candidiasis, *Candida auris* has emerged as a formidable multidrug-resistant (MDR) yeast that is responsible for numerous healthcare-associated outbreaks (4, 5). These pathogens not only prolong hospital stays and increase healthcare costs but also pose serious treatment challenges due to resistance to multiple antifungal drug classes. Notably, the World Health Organization has listed *C. auris* as a critical priority pathogen (6), underscoring an urgent need for the discovery of new antifungal molecules and therapeutic strategies.

A limited number of available antifungal drug classes, including primarily azoles, echinocandins, and polyenes, whose effectiveness is increasingly compromised by resistance, constrain the clinical management of candidiasis (7). *C. albicans* exhibits adaptive resistance through several mechanisms, such as efflux pump overexpression, biofilm formation, and ergosterol pathway mutations (8), whereas *C. auris* displays both intrinsic and acquired resistance, often affecting multiple drug classes simultaneously (9). These resistance profiles contribute to therapeutic failures, especially among high-risk patients. Furthermore, biofilms formed on medical devices (e.g.,

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catheters and ventilators) serve as reservoirs for persistent infections, significantly reducing antifungal susceptibility (10). With global reports documenting *C. auris* outbreaks characterized by high case-fatality rates, the development of alternative or complementary antifungal therapies has become a critical research priority.

Medicinal plants have gained increasing attention as valuable sources of bioactive compounds with potent antimicrobial properties (11). For centuries, traditional medicine systems have utilized plant extracts to manage infectious diseases across Africa, Asia, and Latin America. Plant-derived secondary metabolites, including terpenoids, alkaloids, saponins, tannins, and flavonoids, exert diverse mechanisms of action (e.g., disrupting cell membranes, inhibiting efflux pumps, and interfering with virulence factors), making them promising candidates against resistant fungal pathogens (12). Importantly, these compounds may also act synergistically with conventional antifungal drugs, potentially restoring drug efficacy and delaying the development of resistance. The systematic evaluation of medicinal plants, therefore, provides a viable pathway for the discovery of novel antifungal leads and the development of more effective therapeutic formulations.

Within this context, *Calotropis procera* (the Apocynaceae family) and *Bridelia ferruginea* (the Phyllanthaceae family) are two ethnomedicinal plants with well-documented antimicrobial properties. *C. procera*, commonly known as the sodom apple, is widespread throughout tropical and subtropical regions and is traditionally used to treat skin infections, fevers, and inflammatory conditions (13, 14). Based on phytochemical analyses, it contains cardenolides, flavonoids, alkaloids, tannins, and terpenoids, which are associated with antimicrobial, anti-inflammatory, and antioxidant activities (15, 16). Similarly, *B. ferruginea*, native to West Africa, is traditionally utilized to manage dysentery, wounds, and infectious diseases. In addition, its stem bark and leaves contain flavonoids, tannins, saponins, and alkaloids, which exhibit antimicrobial and anti-inflammatory activities (17-20). Reports indicate that extracts from both plants possess broad-spectrum antimicrobial activities, including activity against fungi, highlighting their potential as sources of antifungal agents.

Despite the pressing need for effective antifungal agents against MDR *Candida* species, the mechanisms of action and dynamic activities of *C. procera* and *B. ferruginea* remain insufficiently understood. Although standard susceptibility assays, such as inhibition zone diameter, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC), provide valuable baseline information, they offer limited insight into the rate and extent of fungal killing. In contrast, time-kill assays capture these dynamics more comprehensively (21, 22), offering data on killing rates, post-antifungal effects, and potential regrowth, thereby improving the prediction of in vivo efficacy.

Accordingly, this study addresses this gap by investigating the time-kill kinetics of *C. procera* and *B. ferruginea* extracts against MDR *C. albicans* and *C. auris*. By generating detailed profiles of their fungicidal activity, the study will provide new insights into their antifungal potential, identify promising candidates for phytochemical isolation, and support the development of plant-based antifungal therapeutics.

Materials and Methods

Collection of Plant Materials and Preparation of Extracts

The leaves of *Calotropis procera* (Apple of Sodom, the Apocynaceae family) and *Bridelia ferruginea* (the Phyllanthaceae family) were collected from a local site. The samples were identified and authenticated at an institutional herbarium, and voucher specimens were deposited under the numbers PSBHT-285 (*C. procera*) and PSBHT-213 (*B. ferruginea*). Furthermore, the dried leaves were finely milled using a mechanical grinder to obtain uniform powders.

For each plant, 550 g of powdered material was soaked in 2.5 L of ethanol in an airtight container for 72 hours, with intermittent shaking to enhance phytochemical extraction. After maceration, the mixtures were sieved through muslin cloth and subsequently filtered using Whatman No. 1 filter paper in order to obtain clear filtrates. The filtrates were then concentrated under reduced pressure using a rotary evaporator. Next, the resulting crude extracts were weighed, transferred into clean containers, and stored at 4°C until further use. It should be noted that the extraction procedure followed a modified method described by Oluyele and Oladunmoye (23).

Antifungal Assay

The agar well diffusion technique (24) was used to assess the extract's antifungal activity. To this end, 1 mL aliquot of each standardized test organism suspension was transferred onto well-dried sterile Sabouraud dextrose agar (SDA) plates and evenly spread using sterile swab sticks. The plates were then allowed to dry, and a standard sterile cork borer (6 mm diameter) was employed to cut uniform wells into SDA plates. Additionally, appropriate labeling was applied to the underside of each plate to correspond to the respective test samples. Subsequently, 50 µL of the extract (100 mg/mL) prepared in **5% dimethyl sulfoxide (DMSO, Sigma-Aldrich, Germany)** was added to the corresponding wells. In addition, **a 5% DMSO solvent control** was included in one well to ensure that the solvent did not affect fungal growth, and fluconazole was utilized as a standard antifungal control in another well. Next, the plates were left at room temperature for 15 minutes to allow the proper diffusion of the extract. All plates were incubated at 35°C for 24 hours, followed by measuring the zones of inhibition. It was confirmed that the DMSO control had no inhibitory effect on the fungal cells, indicating that the observed antifungal activity was attributable solely to the extract.

Assessment of Minimum Inhibitory Concentration

The MIC of the extracts was determined using the standard tube dilution method (25). A series of extract concentrations ranging from 50 mg/mL to 3.125 mg/mL was prepared in sterile Sabouraud dextrose broth (SDB). A 0.1 mL aliquot of the fungal inoculum, standardized to a 0.5 McFarland turbidity equivalent, was added to each tube. The control tubes included SDB only (the negative control) and SDB with fungal inoculum but without the extract (the positive control). All tubes were incubated at 35°C for 48 hours. After incubation, fungal growth was assessed both visually and spectrophotometrically at 600 nm using a Beckman Model 35 spectrophotometer.

Percentage growth inhibition at each concentration was calculated using the following formulae:

OD test, corr = OD test – OD extract blank

$$\% \text{ inhibition} = \frac{(\text{OD pos} - \text{OD test, corr})}{(\text{OD pos} - \text{OD neg})} \times 100$$

OD test, corr = Corrected test optical density

OD test = Optical density of the broth containing both inoculum and the extract at a given concentration (SDB + inoculum + extract)

OD extract blank = Optical density of the broth containing the extract only without inoculum (SDB + extract)

OD pos = Optical density of the broth containing test inoculum without the extract (SDB + inoculum)

OD neg = Optical density of the broth containing only sterile medium (SDB) without inoculum or the extract (SDB only)

The MIC was defined as the lowest extract concentration that elicited ≥90% growth inhibition relative to the control.

Time-Kill Kinetics Assay

Time-kill kinetics of the extract against the test *Candida* isolates were assessed using standard procedures (21). Standardized inocula (~1×10^6 CFU/mL) prepared from overnight cultures were exposed to the extract at concentrations ranging from 1 × MIC to 16 × MIC in SDB. Control sets included growth control (SDB with inoculum only), sterility control (SDB only), solvent control, and extract blank. All inoculated tubes were incubated at 35°C with gentle shaking, and aliquots were withdrawn at 6 hours, 18 hours, 22 hours, 26 hours, 30 hours, and 42 hours. Moreover, each sample was serially diluted in sterile phosphate-buffered saline, plated on SDA, then incubated at 35°C for 24–48 hours. Then, colonies from plates yielding 30–300 colonies were counted to determine viable counts (CFU/mL), which were converted to log10 values and graphed against exposure time to generate time-kill curves. It should be noted that fungicidal activity was defined as a ≥3 log10 CFU/mL (≥99.9%) reduction from the starting inoculum, while a reduction of < 3 log10 indicated fungistatic activity.

Statistical Analysis

All quantitative data are expressed as means ± standard deviation (SD) of three independent experiments. Statistical analyses were performed using SPSS software, version 22.0. Differences between groups were evaluated by one-way analysis of variance, followed by Duncan's new multiple range test. A P value < 0.05 was considered statistically significant.

Results

Antifungal Susceptibility of Calotropis procera and Bridelia ferruginea Extracts

Based on the results (Table 1), both extracts exhibited inhibitory activity against *C. auris* and *C. albicans*. Unlike *C. auris*, *B. ferruginea* produced a larger inhibition zone (16.33 ± 0.33 mm) compared with *C. procera* (12.67 ± 0.33 mm), although both extracts shared the same MIC value (3.125 mg/mL). In contrast, *C. procera* produced a greater inhibition zone against *C. albicans* (16.33 ± 0.33 mm) than *B. ferruginea* (12.67 ± 0.33 mm), while both exhibited identical MICs (3.125 mg/mL). These results suggest that while the extracts displayed comparable overall antifungal potency, each demonstrating species-specific activity, with stronger inhibition toward different *Candida* species.

Time-Kill Kinetics of Extracts Against Candida Species

The time-kill assays demonstrated concentration-dependent and time-dependent reductions in viable *Candida* cell counts following exposure to *C. procera* and *B. ferruginea* extracts (Figures 1–4). Unlike *C. auris*, *C. procera* produced gradual reductions in viability, with 8 × MIC decreasing counts from 1.53 ± 0.17 $\log_{10}$ CFU/mL at 6 hours to 1.00 ± 0.18 $\log_{10}$ CFU/mL at 42 hours, in comparison to 2.31 ± 0.18 $\log_{10}$ CFU/mL in the control. *C. albicans* exhibited greater susceptibility, with 4 × MIC and 8 × MIC reducing viable counts to 1.00 ± 0.17 $\log_{10}$ CFU/mL by 22–26 hours and further to 0.60 ± 0.17 $\log_{10}$ CFU/mL by 42 hours, achieving fungicidal activity (a ≥3-$\log_{10}$ reduction) between 26 hours and 30 hours. Similar trends were observed for *B. ferruginea*, where *C. auris* showed steady declines to 1.01 ± 0.18 $\log_{10}$ CFU/mL at 42 hours for 8 × MIC, whereas *C. albicans* again demonstrated marked susceptibility, with 4 × MIC and 8 × MIC decreasing counts to 1.00 ± 0.17 $\log_{10}$ CFU/mL by 22–26 hours and to 0.60 ± 0.17 by 42 hours, meeting the fungicidal threshold between 26 hours and 30 hours. Overall, both extracts displayed substantially greater killing against *C. albicans* than *against C. auris*, with fungicidal activity detected only at higher concentrations against the former.

Discussion

The global rise in MDR fungi, such as *C. auris* and *Candida albicans*, has created a serious therapeutic challenge, underscoring the need for alternative antifungal strategies (2, 4, 26). This study evaluated the time-kill kinetics of *C. procera* and *B. ferruginea* extracts against the MDR isolates of these species.

Our findings demonstrated that both extracts possess notable fungicidal activity against *C. auris* and *C. albicans*. Although the extracts exhibited identical MIC values

Table 1. Antifungal Activity of *Calotropis procera* and *Bridelia ferruginea* Extracts Against Multidrug-Resistant *Candida* Species

Organism	*Calotropis procera* Extract		*Bridelia ferruginea* Extract	
	Zone of Inhibition (mm)	MIC (mg/mL)	Zone of Inhibition (mm)	MIC (mg/mL)
Candida auris	12.67 ± 0.33	3.125	16.33 ± 0.33	3.125
Candida albicans	16.33 ± 0.33	3.125	12.67 ± 0.333	3.125

Note. MIC: Minimum inhibitory concentration.

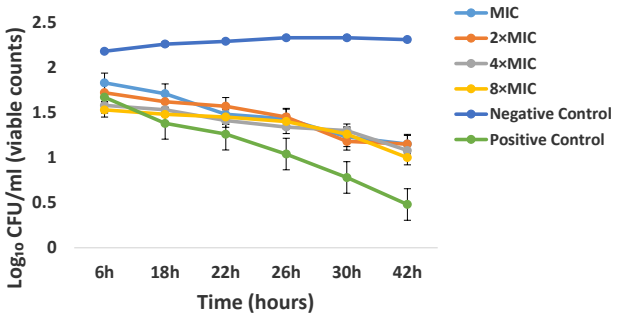


Figure 1 *C. procera* Kill Kinetics Against *Candida auris*
Note. C. procera: *Calotropis procera;* MIC: Minimum inhibitory concentration.

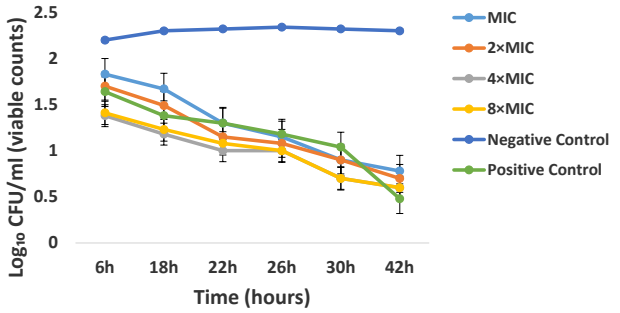


Figure 2. *C. procera* Kill Kinetics Against *Candida albicans*
Note. C. procera: *Calotropis procera;* MIC: Minimum inhibitory concentration.

(3.125 mg/mL), differences in inhibition zone diameters represented varying phytochemical affinities for fungal targets. The time-kill assays revealed clear concentration-dependent and time-dependent reductions in viable cell counts, with declines exceeding 3 $\log_{10}$ CFU/mL over 42 hours, which is consistent with the fungicidal action. Based on the findings, viable counts approached or fell below ~1.0 $\log_{10}$ CFU/mL at concentrations ≥4 × MIC, highlighting strong antifungal potential. Similar time-kill patterns have been reported for other natural products; for example, the essential oil from *Phoenix dactylifera* rapidly eliminated *Streptococcus pneumoniae* within 6 hours at peak concentrations compared to the slower, incomplete killing observed in *Klebsiella pneumoniae* (21).

Kinetic responses varied between extracts and fungal species. *C. procera* displayed rapid fungicidal action against *C. albicans*, achieving ≥3 $\log_{10}$ reductions within 26–30 hours at 4–8 × MIC. Contrarily, its activity against *C. auris* was slower and more progressive, with viable counts declining to approximately 1.0 $\log_{10}$ CFU/mL by 42 hours. Likewise, *B. ferruginea* produced steady reductions in *C. auris* counts to ~1.0 $\log_{10}$ CFU/mL at 42 hours, while *C. Candida albicans* showed faster declines, reaching ~0.60 $\log_{10}$ CFU/mL at similar concentrations. These species-

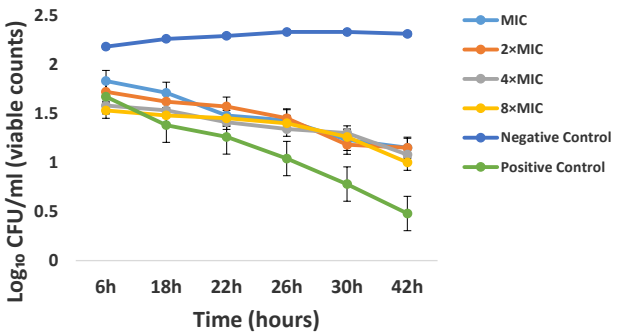


Figure 3. *B. ferruginea* Kill Kinetics Against *Candida auris*
Note. B. ferruginea: *Bridelia ferruginea;* MIC: Minimum inhibitory concentration.

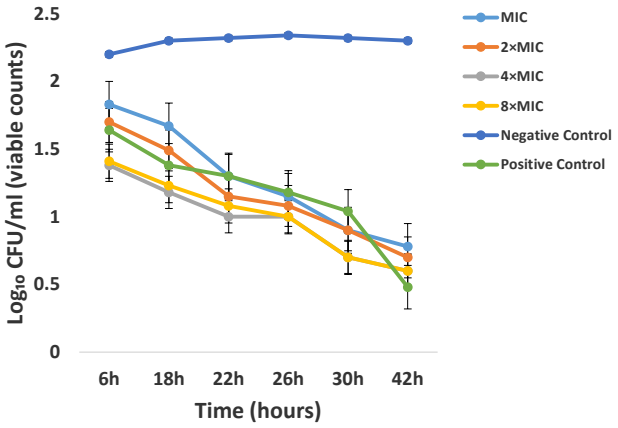


Figure 4. *B. ferruginea* Kill Kinetics Against *Candida albicans*
Note. B. ferruginea: *Bridelia ferruginea;* MIC: Minimum inhibitory concentration.

dependent and extract-dependent differences likely reflect variations in phytochemical composition and fungal cell biology (11, 27).

The rapid fungicidal activity of *C. procera* against *C. albicans* may be attributed to its steroidal glycosides, cardenolides, and triterpenes, which are known to disrupt cell membranes and induce oxidative stress (28-30). Previous studies support this mechanism, reporting the broad antimicrobial activity of leaf extracts rich in phenols, flavonoids, and p-coumaric acid, with *Staphylococcus aureus* exhibiting greater susceptibility than *Escherichia coli* (31). Leaf extracts generally display more potent antifungal activity than stem bark or root preparations (32). Other investigations have shown activity from methanolic extracts at 5 mg/mL (33) and from ethyl acetate fractions against dermatophytes, such as *Trichophyton rubrum* and *Epidermophyton floccosum* (34).

Conversely, the sustained fungicidal activity of *B.*

ferruginea against *C. auris* may reflect its rich tannin and flavonoid content, which inflict cumulative membrane and metabolic damage. Supporting studies indicate that methanolic and aqueous extracts inhibit microbial growth at concentrations below 50 mg/mL (18, 20). Moreover, its phytochemicals are known to disrupt membranes, inhibit essential enzymes, generate oxidative stress, and suppress virulence factors, such as adhesins and biofilms (19, 35-37). These mechanisms likely contribute to the prolonged antifungal effects observed in this work.

Overall, our findings demonstrated that both *C. procera* and *B. ferruginea* exert strong, concentration-dependent and time-dependent fungicidal effects against MDR *Candida* species, mediated by distinct phytochemical mechanisms. Their complementary kinetic profiles, that is, rapid activity by *C. procera* against *C. albicans* and sustained suppression by *B. ferruginea* against *C. auris*, indicate promising potential for development as natural antifungal agents.

Conclusion

The findings of this study confirmed that *C. procera* and *B. ferruginea* extracts exhibit clear fungicidal activity against MDR *C. auris* and *C. albicans*. *C. procera* demonstrated faster kill kinetics against *C. albicans*, whereas *B. ferruginea* produced more sustained fungicidal effects against *C. auris*. These differences probably reflect variations in phytochemical composition and intrinsic resistance mechanisms of the fungal species. Collectively, the results highlight these medicinal plants as promising sources of antifungal agents, particularly amidst increasing rates of multidrug resistance.

Nonetheless, further studies are warranted to isolate, characterize, and evaluate the specific phytochemicals responsible for the observed activity. In vivo efficacy and toxicity assessments are essential to establish safety and therapeutic potential. Additionally, exploring synergistic interactions between these plant extracts and conventional antifungal agents may offer improved treatment strategies for resistant *Candida* infections. Such investigations can facilitate the development of plant-derived antifungal formulations for future clinical applications.

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Authors' Contribution

Conceptualization: Olumide Oluyele.
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Formal analysis: Olumide Oluyele, Comfort Agboola, Victoria Udoh.
Investigation: Comfort Agboola, Victoria Udoh.
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Software: Olumide Oluyele.
Supervision: Olumide Oluyele.
Validation: Olumide Oluyele.
Visualization: Olumide Oluyele.
Writing–original draft: Olumide Oluyele, Comfort Agboola, Victoria Udoh.
Writing–review & editing: Olumide Oluyele, Comfort Agboola, Victoria Udoh.

Competing Interests

The authors declare that there is no conflict of interests.

Ethical Approval

Not applicable.

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