Evaluating the Anti-Fungal Activity of Green-Stemmed and Red-Stemmed *Basella alba* Leaf Extract against *Candida tropicalis*

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Abstract

Background: *Candida tropicalis* holds clinical significance and is identified as the second or third leading cause of candidemia, particularly prevalent in Latin American nations and Asia.

Objectives: The primary goal of this study is to significantly advance the field of antifungal therapies, especially in relation to diseases caused by *C. tropicalis*. The study aims to investigate novel treatments and develop alternative therapeutic approaches that may open up new avenues for future research.

Results: The phytochemical screening of Green-stemmed and Red-stemmed *Basella alba* leaf extracts revealed the presence of flavonoids at 25.5 and 28.8 µg/ml, tannins at 32.2 and 32.5 µg/ml, and phenols at 11.8 and 12.6 µg/ml, respectively, indicating potential antifungal properties. However, when assessed against *Candida tropicalis* using Kirby-Bauer Susceptibility Testing, both extracts showed no antifungal activity, as indicated by the 0 mm zone of inhibition.

Conclusion: This ineffectiveness can be attributed to factors including temperature, the decomposition of phenols by *Candida tropicalis*, the presence of oxygen, acidity, and transport.

Keywords*: Basella alba, Candida tropicalis, Kirby-Bauer Susceptibility Testing, Philippines.*

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Introduction

Candida tropicalis is a clinically significant opportunistic yeast responsible for candidemia and other systemic infections, particularly among immunocompromised and neutropenic patients (Chai et al., 2010). As one of the most prevalent non-albicans *Candida* species, its incidence has notably increased in tropical regions such as India and the Philippines, where it frequently causes nosocomial infections (Kothavade et al., 2010; Itable et al., 2014). The emergence of azole-resistant strains, particularly those resistant to fluconazole, underscores the urgent need for novel antifungal agents (Zuza-Alves et al., 2017).

In light of these challenges, plant-derived bioactive compounds are being explored as promising alternatives to conventional antifungal drugs. *Basella alba* (Malabar spinach), a tropical vine utilized in traditional medicine, contains diverse phytochemicals—flavonoids, tannins, phenols, and mucilage—that contribute to its pharmacological properties, including anti-inflammatory, antioxidant, and antimicrobial activities (Reddy et al., 2023; Arya et al., 2021; Chowdhury et al., 2022). Previous studies have attributed the antifungal effects of *B. alba* to secondary metabolites such as quercetin and other flavonoids, which disrupt fungal cell structures and enzymatic functions (Chandra et al., 2014; Li et al., 2021). Similarly, tannins and phenolic compounds have been reported to interfere with microbial adhesion and membrane integrity (Harborne, 1998; Dewi et al., 2014).

Despite evidence supporting the antimicrobial potential of *B. alba*, data on its specific antifungal efficacy against *C. tropicalis* remain limited and inconclusive (De Cabral Sobreira, 2018). To address this research gap, the present study investigates the antifungal activity of green- and red-stemmed *B. alba* leaf extracts against *C. tropicalis.* Through phytochemical quantification of flavonoids, tannins, and phenols—using aluminum chloride colorimetric, $FeCl_3$, and Folin–Ciocalteu assays—and antifungal assessment via the Kirby–Bauer disk diffusion method, this study seeks to elucidate the potential of *B. alba* as a natural antifungal agent. Findings from this research aim to contribute to the development of plant-based alternatives to conventional antifungal therapies and to inform future pharmacological investigations.

Materials and Methods

Research Design

This study employed a proper experimental design to determine the antifungal activity of green-stemmed and red-stemmed *Basella alba* leaf extracts against *Candida tropicalis*. A proper experimental approach was suitable since the independent variables—types of *Basella alba* leaf extracts—were manipulated to observe their effect on the dependent variables, which included the phytochemical content and the zone of inhibition (Pelz, 2024). Three replicates of each plant extract were used to ensure statistical reliability.

Research Locale

Plant samples were collected from Sitio Helmet, Barangay Dado, Alamada, North Cotabato, a location noted for its clean, pollution-free environment and authenticated by experts. The antifungal assays and phytochemical analyses were conducted in the laboratories of the University of the Immaculate Conception, Main Campus, located at Father Selga Street, Davao City.

Research Procedure:

The research procedure included plant authentication, extraction, phytochemical screening, and antifungal testing, as described below:

Plant Authentication:

All plant samples underwent taxonomic identification prior to experimentation. Authentication was conducted to ensure accurate species usage, which is critical for reproducibility and validity in phytochemical and pharmacological studies (Shirvani et al., 2024).

Maceration Method

Freshly collected leaves were washed, dried, chopped, and soaked in ethanol at room temperature for 76 hours. After soaking, the solution was filtered, and the filtrate was concentrated using a rotary evaporator. The concentrated extracts were stored in airtight containers under refrigeration to prevent decomposition (Azmir et al., 2016).

Aluminum Chloride Colorimetric Assay

This assay was used to determine the total flavonoid content. The method involves reacting flavonoid compounds with aluminum chloride to form a color complex, which was then measured spectrophotometrically at 415 nm (Shraim et al., 2021; Chang et al., 2002; Harborne, 1998). Quercetin was used as the standard for preparing the calibration curve.

Quantitative Test for Tannins Using $FeCl_3$

Tannin levels were determined by preparing the plant extract in a sodium acetate buffer, reacting it with ferric chloride, and measuring the resulting color complex at 510 nm. A standard tannic acid solution was used to establish a calibration curve (Hagerman, 2002).

Folin-Ciocalteu Method

Phenolic content was quantified using the Folin-Ciocalteu reagent in an alkaline medium. The reaction produced a blue complex whose absorbance was read at 750 nm. Gallic acid served as the calibration standard (Perez et al., 2023; Irfan et al., 2022).

Kirby-Bauer Disk Diffusion Susceptibility Test

Antifungal activity was assessed using the Kirby-Bauer disk diffusion method. Standardized agar plates were inoculated with *Candida tropicalis*, and sterile disks soaked with the plant extracts were applied. A 10% phenol solution served as the positive control, while sterile distilled water was used as the negative control (Bauer et al., 1966; da Silva et al., 2018). Zones of inhibition were measured and compared to control groups.

Data Analysis

Mean was used to determine the antifungal activity of Green-Stemmed and Red-Stemmed *Basella alba* Leaf Extract against Candida tropicalis (LibGuides: SPSS Tutorials).

Ethical Considerations

The study was conducted in accordance with the ethical standards for laboratory research. Safety protocols were strictly followed in the handling of plant extracts and fungal cultures. Biohazard disposal was performed using sterilization methods such as

autoclaving, in compliance with institutional and environmental safety regulations. Though human or animal subjects were not involved, ethical diligence was applied in all phases of the study.

Results

The phytochemical screening results of both green-stemmed and red-stemmed *Basella alba* leaf extracts revealed the presence of bioactive compounds that are commonly associated with antifungal properties. As shown in Table 1, the flavonoid content, determined via aluminum chloride colorimetric assay, was 25.5 µg/mL for green-stemmed and 28.8 µg/mL for red-stemmed extracts. Tannin levels were found to be 32.2 µg/mL and 32.5 µg/mL, respectively, using the $FeCl_3$ colorimetric method. Similarly, phenolic content measured via the Folin-Ciocalteu method was 11.8 µg/mL in green-stemmed and 12.6 µg/mL in red-stemmed *Basella alba*.

Table 1.

Phytochemical compounds present in Basella alba leaf extracts

TEST PARAMETER	Green Alugbati (Crude Extract)	Red Alugbati (Crude Extract)	Methods
Flavonoids, ug/ml (as quercetin)	25.5	28.8	Colorimetric - $AlCl_3$
Tannins, ug/ml (as tannic acid)	32.2	32.5	Colorimetric - $FeCl_3$
Phenolic, ug/ml (as gallic acid)	11.8	12.6	Colorimetric - Folin–Ciocâlteu

These values confirm the phytochemical richness of the extracts, aligning with prior studies that have demonstrated the abundance of flavonoids, phenols, and tannins in *Basella alba* (Kumar et al., 2021; Arya et al., 2021). Such compounds are known to contribute to antimicrobial activities by targeting fungal cell walls, membrane integrity, and enzymatic systems (Li et al., 2021; Chandra et al., 2014). Nonetheless, despite the confirmed presence of these bioactive constituents, the in vitro antifungal assay using the Kirby-Bauer disk diffusion method did not demonstrate inhibitory activity against *Candida tropicalis.*

Table 2a.

Zone of Inhibition of Green-stemmed Basella alba leaf extracts

Sample	Diameter of Zone of Inhibition (mm)			
	1	2	3	Mean
Green-stemmed *Basella alba*	0.00	0.00	0.00	0.00
Positive Control: 10% Phenol	26.0	26.0	25.2	25.7
Negative Control: Sterile Distilled Water	0.00	0.00	0.00	0.00

Table 2a and 2b detail the results of the zone of inhibition tests for green- and red-stemmed *Basella alba* leaf extracts. Both variants showed no antifungal activity, with a 0.00 mm zone of inhibition recorded in all three replicates. In contrast, the positive control (10% phenol) yielded average inhibition zones of 25.7 mm and 24.0 mm, respectively, validating the test system. These results indicate that the *Basella alba* leaf extracts lacked fungistatic or fungicidal effects against *C. tropicalis* at the tested concentration.

Table 2b.

Zone of Inhibition of Red-stemmed Basella alba leaf extracts

Sample	Diameter of Zone of Inhibition (mm)			
	1	2	3	Mean
Red-stemmed *Basella alba*	0.00	0.00	0.00	0.00
Positive Control: 10% Phenol	24.6	24.2	23.2	24.0
Negative Control: Sterile Distilled Water	0.00	0.00	0.00	0.00

The absence of antifungal activity may be attributed to multiple experimental and biological factors. Temperature sensitivity, oxidation, and enzymatic degradation are known to affect the integrity of phenolic and flavonoid compounds (Warnasih & Hasanah, 2019; Palvai et al., 2014). Moreover, environmental conditions such as pH, oxygen exposure, and degradation during storage or transport likely reduced the potency of the phytochemicals in the extracts (Dwivedi et al., 2020; Verma & Shukla, 2015; Kumar, 2020).

Thus, while the tested *Basella alba* extracts were phytochemically rich, the failure to inhibit *C. tropicalis* growth under in vitro conditions does not definitively negate their potential as antifungals. It suggests the need for further investigations involving different extract concentrations, alternative solvents, modified preparation methods, or testing against other *Candida* species. It also emphasizes the importance of optimizing post-harvest processing and storage conditions to preserve the bioactivity of phytochemical compounds.

Conclusion

The findings of this study demonstrated that both green-stemmed and red-stemmed *Basella alba* leaf extracts contain measurable amounts of flavonoids, tannins, and phenolic compounds—bioactive constituents known for their potential antimicrobial properties. However, when tested in vitro against *Candida tropicalis* using the Kirby-Bauer disk diffusion method, neither extract showed antifungal activity, as evidenced by a 0.00 mm zone of inhibition. This suggests that under the conditions and concentrations used in this study, the extracts lacked the specific properties required to inhibit the fungal growth of *C. tropicalis.*

The absence of antifungal activity may be influenced by various external factors, including degradation of active compounds due to temperature, oxygen exposure, pH, and inadequate lyophilization during storage and handling. Additionally, the metabolic capacity of *C. tropicalis* to degrade phenolic compounds may have contributed to the outcome. Further studies involving different extraction techniques, higher concentrations, and other parts of *Basella alba* are recommended to evaluate its antifungal potential fully.

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