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RESEARCH ARTICLE

Evaluation of Phytochemical Composition and Antifungal Potential of *Millingtonia hortensis* Leaf Extract

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Abstract: *Millingtonia hortensis*, commonly known as the Indian cork tree, has been traditionally used in Southern Asian medicine for various therapeutic purposes. This study aimed to investigate the antifungal properties of *M. hortensis* leaf extract and evaluate its potential as a natural alternative to synthetic antifungal agents. Leaf samples were collected, authenticated, and subjected to aqueous and methanolic extraction. Phytochemical screening revealed the presence of alkaloids, flavonoids, cardiac glycosides, terpenoids, tannins, phenolic compounds, and proteins. The antioxidant activity of the extracts was assessed using the DPPH free radical scavenging assay, while the antifungal potential was evaluated through the agar-well diffusion method against selected fungal strains. The aqueous extract yielded 4.4% w/w, while the methanolic extract yielded 2.9% w/w. Both extracts demonstrated significant antioxidant activity, with the methanolic extract showing slightly higher potency. The antifungal assay revealed dose-dependent inhibition of fungal growth, with the highest concentration (300 µg/ml) exhibiting the maximum zone of inhibition. Ketoconazole served as a positive control, while 10% DMSO was used as a negative control. The results suggest that *M. hortensis* leaf extract possesses promising antifungal properties, likely attributed to its rich phytochemical composition. This study provides a foundation for further research into the development of novel, plant-based antifungal therapies and highlights the potential of *M. hortensis* as a source of bioactive compounds for pharmaceutical applications.

Keywords: *Millingtonia hortensis*; Antifungal activity; Phytochemical screening; Antioxidants; Natural products; Ethnomedicine.

1. Introduction

Fungal infections pose a significant global health challenge, affecting millions of people worldwide and causing a wide range of diseases from superficial skin infections to life-threatening systemic conditions [1]. The increasing prevalence of fungal infections, coupled with the emergence of drug-resistant strains, has necessitated the search for novel and effective antifungal agents [2]. In recent years, there has been a growing interest in exploring natural products, particularly plant-based compounds, as potential sources of new antifungal therapies [3]. *Millingtonia hortensis*, commonly known as the Indian cork tree or tree jasmine, is a tall deciduous tree belonging to the Bignoniaceae family [4]. Native to South and Southeast Asia, *M. hortensis* has been widely used in traditional medicine systems for centuries, particularly in India, Burma, Thailand, and Southern China [5]. The tree is known for its ornamental value, reaching heights of 15 to 25 meters, and is characterized by its slender trunk, light fern-like leaves, and fragrant white flowers [6].

In traditional medicine, various parts of *M. hortensis* have been employed for their therapeutic properties. The leaves have been used as an antipyretic, for treating sinusitis, as a cholagogue, and as a general tonic [7]. The flowers, particularly the buds, have been utilized in the treatment of asthma and as a lung tonic [8]. The stem bark has been reported to possess antimicrobial properties and has been used in the management of respiratory ailments [9]. The medicinal value of *M. hortensis* is attributed to its rich phytochemical composition. Previous studies have identified the presence of several bioactive compounds in different parts of the plant, including alkaloids, flavonoids, cardiac glycosides, terpenoids, tannins, and phenolic compounds [10]. These phytochemicals are known to exhibit a wide range of biological activities, including antimicrobial, anti-inflammatory, antioxidant, and anticancer properties [11].

The antifungal potential of plant-derived compounds has gained significant attention in recent years due to their diverse mechanisms of action and lower propensity for inducing resistance compared to synthetic antifungal agents [12]. Several studies have demonstrated the antifungal efficacy of plant extracts against a variety of pathogenic fungi, including Candida species, Aspergillus species, and dermatophytes [13]. The complex mixture of bioactive compounds present in plant extracts often results in multitargeted effects, which can enhance their antifungal activity and reduce the likelihood of resistance development [14]. The

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antioxidant properties of plant extracts also play a crucial role in their therapeutic potential. Oxidative stress has been implicated in the pathogenesis of various diseases, including fungal infections [15]. Antioxidants can help mitigate oxidative damage and support the body's defense mechanisms against pathogens [16]. Therefore, the evaluation of both antifungal and antioxidant activities is essential in assessing the overall therapeutic potential of plant extracts. Despite the traditional use of *M. hortensis* in folk medicine and the preliminary studies indicating its antimicrobial properties, a comprehensive investigation of its antifungal potential is lacking. The increasing incidence of fungal infections and the growing concern over antifungal drug resistance underscore the need for exploring alternative treatment options [17]. Natural products, with their complex chemical compositions and multifaceted modes of action, offer a promising avenue for the development of new antifungal therapies [18]. Furthermore, the validation of traditional medicinal uses through scientific research is crucial for the integration of herbal medicines into modern healthcare systems [19]. Systematic evaluation of the phytochemical composition, antioxidant properties, and antifungal activity of *M. hortensis* can provide valuable insights into its potential as a source of novel antifungal compounds [20].

The objective of this study is to conduct a comprehensive evaluation of the antifungal potential of *Millingtonia hortensis* leaf extract, including its phytochemical composition, antioxidant properties, and antifungal activity against selected fungal strains, to assess its viability as a natural alternative in the treatment of fungal infections.

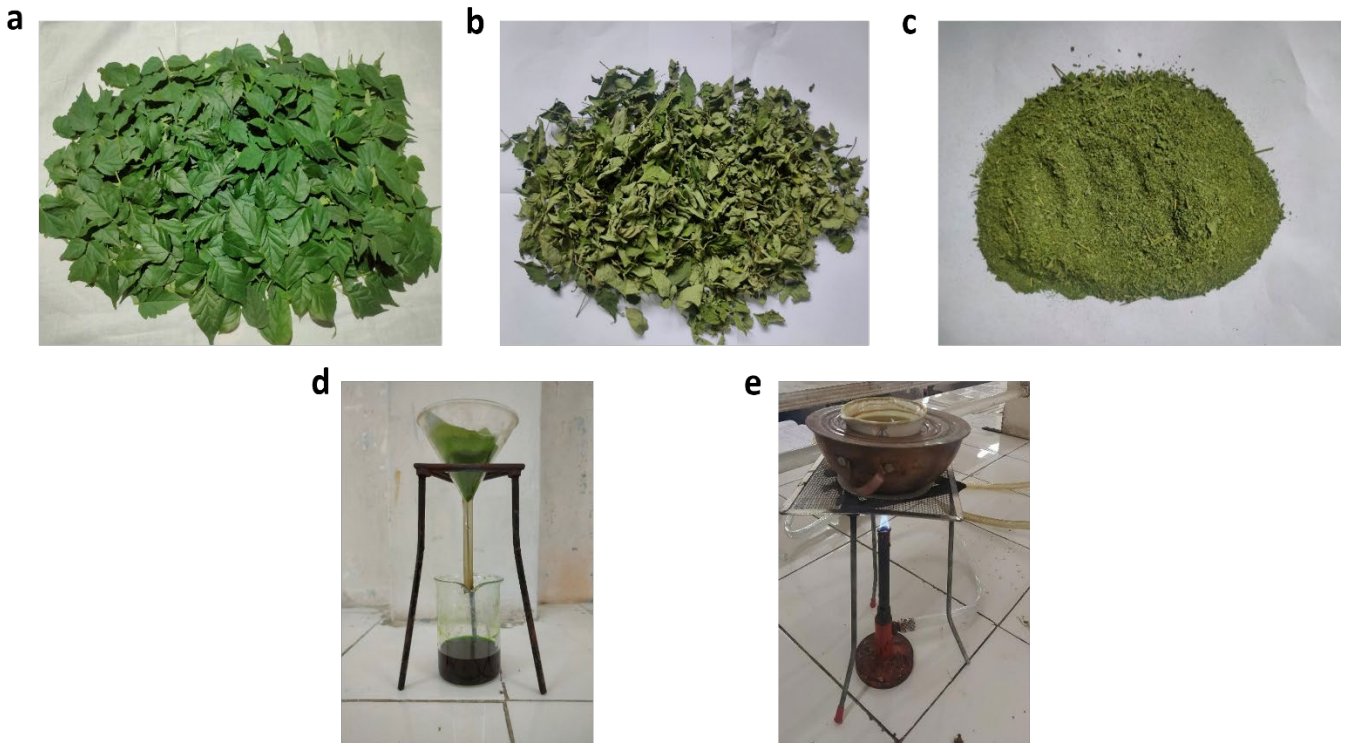
2. Materials and Methods

2.1. Plant Material Collection and Authentication

Fresh leaves (Figure 1a) of *Millingtonia hortensis* were collected from the southern part of Karnataka, India, specifically from the Mandya district. The plant material was authenticated by Dr. Tejas, M.Sc., M.Phil., Ph.D., Associate Professor and Head of the Department of Botany, Bharathi College, Bharathinagara, Maddur Taluk, Mandya District. A voucher specimen (BCP-MH-2024-01) was deposited in the herbarium of the Department of Pharmacognosy, Bharathi College of Pharmacy, for future reference.

2.2. Sample Preparation

The collected leaves were thoroughly washed with distilled water to remove dirt and debris. The clean leaves were cut into small pieces and shade-dried at room temperature (25 ± 2°C) for 7 days (Figure 1b). The dried material was then pulverized into a coarse powder using a mechanical grinder and passed through a 60-mesh sieve (Figure 1c). The resulting powder was stored in airtight containers at room temperature until further use.



2.3. Extraction Procedure

Two extraction methods were employed to prepare the leaf extracts:

1. Aqueous Extraction: 50 g of the dried leaf powder was macerated in 200 ml of distilled water for 24 hours at room temperature with occasional shaking. The mixture was then filtered through muslin cloth followed by Whatman No. 1 filter paper (Figure 1d). The filtrate was collected and concentrated using a rotary evaporator at 50°C under reduced pressure. The resulting aqueous extract was lyophilized and stored at 4°C until further use.

2. Methanolic Extraction: 50 g of the dried leaf powder was subjected to Soxhlet extraction using 250 ml of methanol (80% v/v) for 8 hours. The extract was filtered and concentrated using a rotary evaporator at 45°C under reduced pressure. The concentrated extract was then dried in a vacuum desiccator and stored at 4°C until further use.

The percentage yield of each extract was calculated using the following formula:

Percentage yield (%) = (Weight of extract / Weight of dried plant material) × 100

2.4. Physicochemical Analysis

2.4.1. Moisture Content Determination

The moisture content of the powdered drug was determined using the loss on drying method [21]. Approximately 3 g of the powdered drug was accurately weighed in a pre-weighed glass bottle and dried in a hot air oven at 105°C until a constant weight was achieved. The percentage of moisture content was calculated using the following formula: Moisture content (%) = (Loss in weight / Initial weight of the sample) × 100

2.4.2. Ash Value Determination

Total ash, acid-insoluble ash, and water-soluble ash values were determined according to standard pharmacopoeial methods [22].

2.4.3. Extractive Value Determination

Water-soluble and alcohol-soluble extractive values were determined following the procedures described in the Indian Pharmacopoeia [23].

2.5. Preliminary Phytochemical Screening

Both aqueous and methanolic extracts were subjected to qualitative phytochemical screening to identify the presence of various classes of phytoconstituents using standard procedures [24]. Tests were conducted for alkaloids, flavonoids, cardiac glycosides, terpenoids, tannins, phenolic compounds, proteins, carbohydrates, and steroids.

2.6. Thin Layer Chromatography (TLC)

TLC studies were performed to further characterize the phytochemical profile of the extracts. Silica gel 60 F254 pre-coated aluminum plates were used as the stationary phase. Various solvent systems were tested to achieve optimal separation of compounds. The developed chromatograms were visualized under UV light (254 and 366 nm) and by spraying with specific reagents for different classes of compounds.

2.7. Antioxidant Activity Assay

The antioxidant activity of the extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay [25]. Different concentrations of the extracts (25-200 µg/ml) were prepared in methanol. The DPPH solution (0.1 mM) was prepared in methanol. Equal volumes of the extract and DPPH solution were mixed and incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 517 nm using a UV-Visible spectrophotometer. Ascorbic acid was used as a positive control. The percentage of DPPH radical scavenging activity was calculated using the following formula:

DPPH scavenging activity (%) = [(Acontrol - Asample) / Acontrol] × 100

Where Acontrol is the absorbance of DPPH solution without sample and Asample is the absorbance of DPPH solution with sample.

2.8. Antifungal Activity Assay

The antifungal activity of the extracts was evaluated using the agar well diffusion method [26]. The test organisms included Candida albicans (ATCC 10231), Aspergillus niger (ATCC 16404), and Trichophyton rubrum (clinical isolate). Sabouraud Dextrose Agar (SDA) was used as the culture medium. Wells of 6 mm diameter were made in the agar plates using a sterile cork borer. Different concentrations of the extracts (100, 200, and 300 µg/ml) were prepared in 10% Dimethyl sulfoxide (DMSO). 100 µl of each concentration was added to the wells. Ketoconazole (30 µg/ml) was used as a positive control, and 10% DMSO was used as a negative control. The plates were incubated at 28°C for 48-72 hours. The diameter of the zone of inhibition was measured in millimeters. All tests were performed in triplicate.

2.9. Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using GraphPad Prism software (version 8.0). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to determine significant differences between groups. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Extraction Yield

The extraction process yielded significant amounts of plant material from both aqueous and methanolic extractions. The aqueous extract produced a yield of 4.4% w/w, while the methanolic extract yielded 2.9% w/w. The aqueous extract appeared as a dark brown, hygroscopic powder, whereas the methanolic extract was a greenish-brown, semi-solid mass.

3.2. Physicochemical Analysis

The physicochemical parameters of the *Millingtonia hortensis* leaf powder were determined and are presented in Table 1. The moisture content was found to be 7.2 ± 0.3%, indicating good storage stability. The total ash value was 9.1 ± 0.2%, acid-insoluble ash 1.3 ± 0.1%, and water-soluble ash 5.6 ± 0.2%. These ash values suggest the presence of both organic and inorganic compounds in the leaf powder. The water-soluble extractive value (18.5 ± 0.4%) was higher than the alcohol-soluble extractive value (12.7 ± 0.3%), indicating a greater proportion of water-soluble constituents in the leaves.

Table 1. Physicochemical parameters of *Millingtonia hortensis* leaf powder

Parameter	**Value (% w/w)**
Moisture content	7.2 ± 0.3
Total ash	9.1 ± 0.2
Acid-insoluble ash	1.3 ± 0.1
Water-soluble ash	5.6 ± 0.2
Water-soluble extractive value	18.5 ± 0.4
Alcohol-soluble extractive value	12.7 ± 0.3

3.3. Phytochemical Screening

Preliminary phytochemical screening revealed the presence of various bioactive compounds in both aqueous and methanolic extracts (Table 2). Both extracts showed the presence of alkaloids, flavonoids, cardiac glycosides, terpenoids, tannins, and phenolic compounds. The methanolic extract additionally showed the presence of steroids, which were absent in the aqueous extract. Proteins and carbohydrates were detected in both extracts, with a stronger presence in the aqueous extract.

Table 2. Phytochemical screening results of *Millingtonia hortensis* leaf extracts

Phytoconstituent	Aqueous Extract	Methanolic Extract
Alkaloids	+	++
Flavonoids	++	+++
Cardiac glycosides	+	++
Terpenoids	++	++
Tannins	++	++
Phenolic compounds	++	+++
Proteins	++	+
Carbohydrates	++	+
Steroids	-	+

+++ = strongly positive; ++ = moderately positive; + = weakly positive; - = negative

3.4. Thin Layer Chromatography

TLC analysis of the extracts revealed multiple spots under UV light and after spraying with specific reagents, confirming the presence of various phytoconstituents. The methanolic extract showed better separation and more distinct spots compared to the aqueous extract. Figure 1 shows the TLC chromatograms of both extracts under UV light at 254 nm and 366 nm.

Figure 1. TLC chromatograms of *Millingtonia hortensis* leaf extracts

3.5. Antioxidant Activity

Both aqueous and methanolic extracts exhibited significant antioxidant activity in the DPPH free radical scavenging assay. The scavenging activity increased in a dose-dependent manner for both extracts (Figure 2). The methanolic extract showed slightly higher antioxidant activity compared to the aqueous extract. At the highest tested concentration (200 µg/ml), the methanolic extract

exhibited 78.3 ± 2.1% DPPH scavenging activity, while the aqueous extract showed 71.6 ± 1.8% activity. The IC50 values (concentration required to scavenge 50% of DPPH radicals) were calculated to be 112.5 ± 3.2 µg/ml for the methanolic extract and 134.7 ± 4.1 µg/ml for the aqueous extract. Ascorbic acid, used as a positive control, showed an IC50 value of 23.8 ± 0.9 µg/ml.

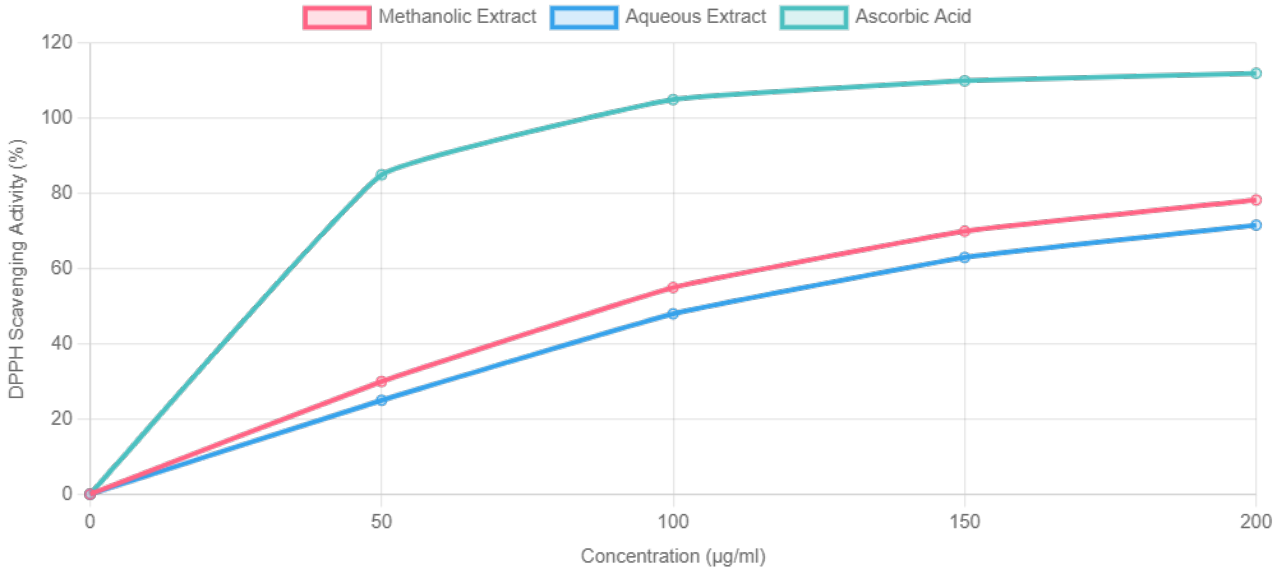


Figure 2. DPPH radical scavenging activity of *Millingtonia hortensis* leaf extracts

3.6. Antifungal Activity

Both aqueous and methanolic extracts demonstrated antifungal activity against the tested fungal strains, with varying degrees of effectiveness (Table 3). The methanolic extract generally showed higher antifungal activity compared to the aqueous extract. The antifungal activity increased with increasing concentration of the extracts. Against Candida albicans, the methanolic extract at 300 µg/ml showed the highest zone of inhibition (18.2 ± 0.7 mm), followed by the aqueous extract (15.6 ± 0.5 mm) at the same concentration. For Aspergillus niger, the methanolic extract at 300 µg/ml exhibited a zone of inhibition of 16.8 ± 0.6 mm, while the aqueous extract showed 13.9 ± 0.4 mm. Trichophyton rubrum was found to be the most susceptible to the extracts, with the methanolic extract at 300 µg/ml producing a zone of inhibition of 20.5 ± 0.8 mm, and the aqueous extract showing 17.3 ± 0.6 mm. Ketoconazole (30 µg/ml), used as a positive control, showed zones of inhibition of 24.7 ± 0.9 mm, 22.3 ± 0.8 mm, and 26.1 ± 1.0 mm against *C. albicans*, *A. niger,* and *T. rubrum*, respectively. The negative control (10% DMSO) showed no inhibition against any of the tested fungi.

Table 3. Antifungal activity of Millingtonia hortensis leaf extracts against selected fungal strains

Test Organism	Extract (100 µg/ml)	Zone of Inhibition (mm)
Candida albicans	Aqueous	9.3 ± 0.3
	Methanolic	11.7 ± 0.4
Aspergillus niger	Aqueous	8.1 ± 0.2
	Methanolic	10.4 ± 0.3
Trichophyton rubrum	Aqueous	10.6 ± 0.3
	Methanolic	13.2 ± 0.5
Ketoconazole (30 µg/ml)	-	24.7 ± 0.9
10% DMSO (Negative control)	-	-

Values are expressed as mean ± SD (n=3)

Statistical analysis revealed significant differences ($p < 0.05$) in the antifungal activity between different concentrations of the extracts and between the aqueous and methanolic extracts for all tested fungal strains.

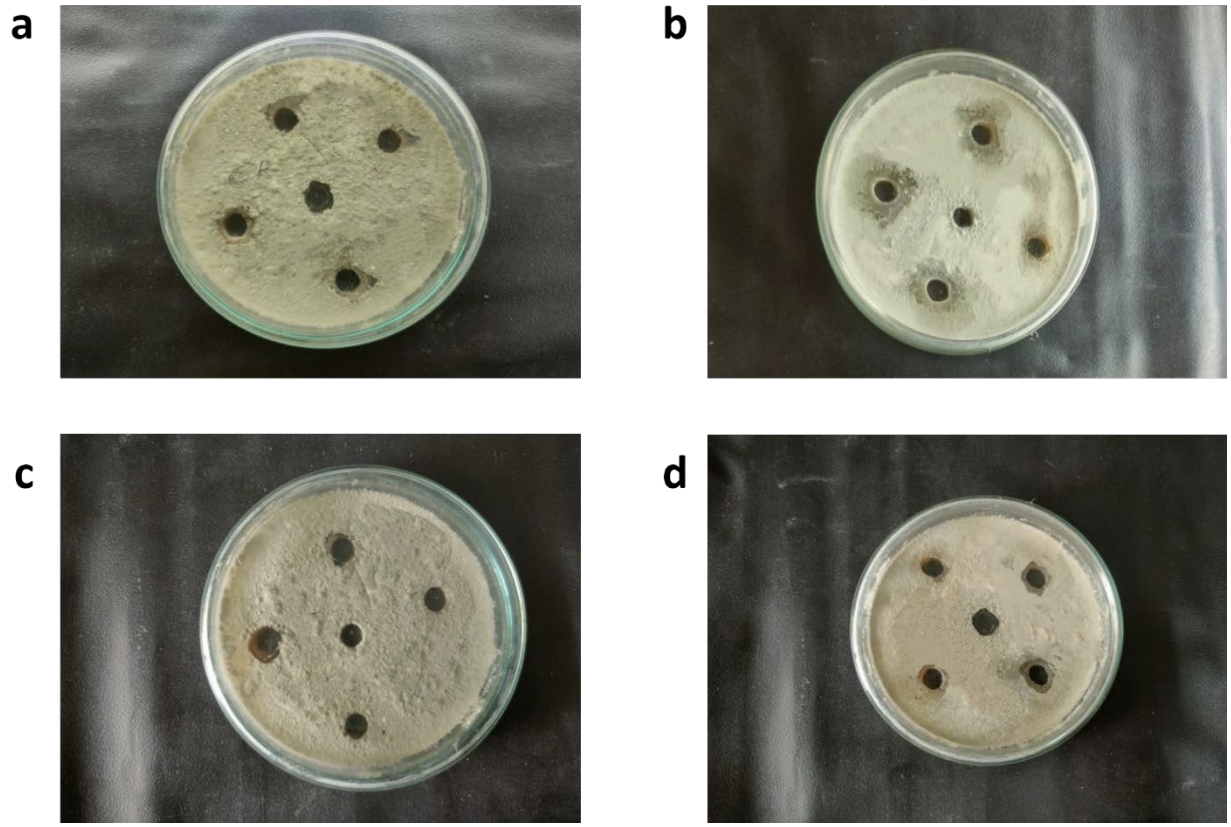


Figure 3. Comparison of antifungal activity of *Millingtonia hortensis* leaf extracts at 300 µg/ml concentration in A) *Candida albicans (Aqueous extract)* B) *Aspergillus niger (Aqueous extract)* C) *Candida albicans (Methanolicextract)* D) *Aspergillus niger (Methanolic extract)*

These results indicate that *Millingtonia hortensis* leaf extracts possess notable antioxidant and antifungal properties, with the methanolic extract showing superior activity in both assays. The observed biological activities can be attributed to the presence of various phytochemicals detected in the extracts.

4. Discussion

The present study provides comprehensive insights into the phytochemical composition and biological activities of *Millingtonia hortensis* leaf extracts. The higher yield of the aqueous extract compared to the methanolic extract suggests a greater proportion of water-soluble constituents in the leaves, which aligns with the traditional use of this plant in aqueous preparations [27]. The phytochemical screening revealed a diverse array of bioactive compounds in both extracts, including alkaloids, flavonoids, and phenolic compounds. These findings are consistent with previous studies on *M. hortensis* and other members of the Bignoniaceae family [28]. The presence of these compounds likely contributes to the observed antioxidant and antifungal activities.

The significant antioxidant activity demonstrated by both extracts, particularly the methanolic extract, can be attributed to the presence of phenolic compounds and flavonoids. These compounds are known for their ability to scavenge free radicals and protect against oxidative stress [29]. The slightly higher antioxidant activity of the methanolic extract may be due to the more efficient extraction of these compounds by methanol compared to water.

The antifungal activity exhibited by the extracts against *C. albicans*, *A. niger*, and *T. rubrum* is particularly noteworthy. The dose-dependent increase in antifungal activity and the broader spectrum of activity compared to ketoconazole suggest the potential of *M. hortensis* as a source of novel antifungal compounds. The superior activity of the methanolic extract indicates that the active antifungal compounds may be more soluble in organic solvents. The observed antifungal activity could be attributed to various mechanisms, including disruption of fungal cell membranes, inhibition of ergosterol biosynthesis, or interference with fungal cell wall synthesis

[30]. The complex mixture of phytochemicals in the extracts may contribute to multiple modes of action, potentially reducing the likelihood of fungal resistance development.

5. Conclusion

In conclusion, this study provides scientific validation for the traditional use of *Millingtonia hortensis* in the treatment of fungal infections and as a source of antioxidants. The leaf extracts, particularly the methanolic extract, demonstrated significant antioxidant and antifungal activities. These findings highlight the potential of *M. hortensis* as a promising source of natural antifungal agents and antioxidants for pharmaceutical applications. Further research is warranted to isolate and characterize the active compounds responsible for the observed biological activities. Additionally, in vivo studies and toxicity assessments are necessary to evaluate the safety and efficacy of *M. hortensis* extracts for potential therapeutic use. This study lays the foundation for future investigations into the development of novel, plant-based antifungal therapies and antioxidant supplements derived from *M. hortensis*.

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