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Antifungal Activity of *Mitracarpus hirtus* Leaf Extract against Dermatophytes Isolated from Ringworm Patients

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A B S T R A C T

Objective: This study aimed to evaluate the antifungal activity of aqueous leaf extracts of Mitracarpus hirtus against dermatophytes isolated from patients with clinically diagnosed ringworm.

Methods: Fresh *M. hirtus* leaves were collected, extracted in water, and subjected to phytochemical screening. Dermatophytes were isolated from skin scrapings of patients in Birnin Kebbi and identified using morphological and microscopic techniques. The antifungal activity of the extracts was assessed in vitro using the agar well diffusion method at concentrations of 20, 40, 80, and 100 mg/mL. Ketoconazole (5 mg/mL) was used as a positive control.

Results: Phytochemical analysis revealed the presence of alkaloids, tannins, flavonoids, steroids, and cyanogenic glycosides, while glycosides and volatile oils were absent. A total of 34 dermatophyte isolates were recovered, with Trichophyton mentagrophyte (29.4%) being the most prevalent, followed by T. beigelii, Microsporum canis, Epidermophyton floccosum, and T. rubrum. The extract exhibited a concentration-dependent antifungal effect, with inhibition zones ranging from 12 mm at 20 mg/mL to 26 mm at 100 mg/mL. The highest activity was observed against *T. rubrum* and *M. canis*, while E. floccosum showed the least susceptibility.

Conclusion: M. hirtus aqueous leaf extract demonstrates significant antifungal activity against common dermatophytes, supporting its ethno medicinal use. The findings highlight its potential as a natural source for antifungal drug development. Further studies focusing on the isolation of active compounds, elucidation of mechanisms of action, and clinical validation are recommended to develop standardized, plant-based antifungal therapies.

Keywords: Mitracarpus, Dermatomycoses, Plant Extracts, Phytotherapy, Antifungal Agents

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Introduction

The use of medicinal plants in treating various ailments dates back to ancient times, forming the foundation of traditional healthcare systems across cultures. Over time, this reliance has persisted, particularly in developing regions, due to the accessibility, affordability, and relatively low side effects of herbal remedies compared to synthetic drugs [1,2]. In recent decades, there has been a global resurgence of interest in phytotherapy, driven by the limitations of conventional treatments and the alarming rise in antimicrobial resistance [3,4]. The continued reliance on traditional medicine in many communities is increasingly driven by the limitations of conventional pharmaceuticals. One major factor is the global rise in antimicrobial resistance, which has rendered many standard treatments less effective or even obsolete. This has prompted both practitioners and patients to seek alternative therapies, particularly those derived from natural sources [5]. Additionally, synthetic drugs often come with adverse effects, including gastrointestinal disturbances, allergic reactions, and long-term toxicity, which can discourage compliance and prompt a return to plant-based remedies perceived as safe [6]. In many low-resource settings, where access to quality healthcare is restricted, traditional medicine remains a primary or complementary option [7]. As a result, medicinal plants are not only culturally significant but also function as practical, accessible, and potentially effective alternatives in the face of modern therapeutic challenges [8].

Fungal infections, once considered minor health concerns, have now become significant causes of morbidity, especially among immunocompromised individuals, such as organ transplant recipients and patients with chronic diseases [9]. Fungal infections, though historically overlooked compared to bacterial diseases, pose a growing global health threat. Recent estimates suggest around 6.5 million invasive fungal infections occur annually, leading to 3.8 million deaths, with 2.5 million directly attributable [10]. Unlike bacterial infections, which benefit from structured surveillance and established treatments, fungal diseases remain underdiagnosed and underreported due to limited diagnostics and public health attention [11].

Dermatophytosis, commonly known as ringworm, is a superficial fungal infection that affects keratinized tissues such as skin, hair, and nails. It is primarily caused by keratin-loving fungi belonging to the genera Trichophyton, Microsporum, and Epidermophyton. These infections are transmitted through contact with infected individuals, animals, or contaminated environments and are exacerbated by factors such as poor hygiene, humid climates, crowded living conditions, and the widespread use of immunosuppressive drugs [12,13]. Dermatophytosis is considered a highly transmissible infection in humans. It often follows a chronic course and, if not properly treated, can lead to permanent damage to the skin, hair, and nails [14].

The genus Mitracarpus, part of the Rubiaceae family, includes over 50 known species. Originally native to Brazil, these plants thrive in tropical and subtropical forest ecosystems. Mitracarpus hirtus, in particular, is widely distributed across tropical and Neotropical regions, including countries such as India, Myanmar, Thailand, and the United States. It is also prevalent in subtropical areas such as East and West Africa, Malaysia, and parts of the USA. It is an erect plant that can be either unbranched or extensively branched, and it typically grows to a height of approximately 60 centimetres [15,16]. Mitracarpus hirtus L. has long been utilized in traditional medicine for managing a wide range of ailments, including ringworm, rashes, toothaches, itching, eczema, venereal diseases, boils, measles, and other dermatological conditions. It is also traditionally applied as a remedy for insect bites and stings. Beyond its medicinal uses, the plant's leaves are edible and serve as fodder for livestock, while the dried leaves are commonly used to accelerate the healing of chronic ulcers [15,17]. Mitracarpus hirtus is rich in various bioactive constituents, notably flavonoids, iridoid glycosides, and coumarins [15]. Research on the aerial parts of the plant has identified several specific compounds, including a newly discovered

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naphthoquinone diglycoside, three isopentenyl isoflavones, four distinct flavonoids, three iridoid glycosides, and two coumarin derivatives [18].

Mitracarpus hirtus is a medicinal plant traditionally used in various parts of Nigeria for treating skin infections, including ringworm. Given the traditional use of Mitracarpus hirtus in the treatment of fungal skin infections, this study aims to scientifically evaluate its antifungal activity against dermatophytes isolated from clinical cases of ringworm. The findings are anticipated to offer scientific validation for the traditional use of M. hirtus in managing fungal skin infections and may contribute to the development of effective plant-derived antifungal therapies.

Materials and Methods

Study area

The study was carried out at Kebbi State University of Science and Technology, Aliero (KSUSTA), situated in Aliero Local Government Area, south-eastern Kebbi State, Nigeria. The geographical coordinates of Aliero are 12°16′42″N latitude and 4°27′6″E longitude. It spans a land area of approximately 350 km^2 and had a population of 65,973 as of the 2006 census. The area is predominantly rural and is characterized by a tropical climate with high humidity and warm temperatures, which create favourable conditions for the growth and transmission of dermatophytes. These environmental factors likely contribute to the high incidence of fungal skin infections in the region.

Collection and Identification of Plant Material

Fresh leaves of Mitracarpus hirtus were collected in August 2024 during the rainy season, a period known to support vigorous plant growth and the accumulation of phytochemicals due to increased moisture and favourable environmental conditions. The collection took place around the central mosque area within the KSUSTA campus. The plant was identified and authenticated by a botanist from the Department of Plant Science and Biotechnology, Faculty of Life Sciences, Kebbi State University of Science and Technology, Aliero (KSUSTA). A voucher specimen was prepared and deposited for future reference under the code KSUSTA/PSB/H/219L.

Preparation of Plant Extracts

The harvested leaves were thoroughly rinsed with distilled water to eliminate dust and surface contaminants, then air-dried at ambient room temperature in a shaded environment to preserve heat-sensitive phytochemicals. Once dried, the leaves were finely ground using a sterilized wooden mortar and pestle. Exactly 100 grams of the powdered material was macerated in 500 mL of cold distilled water to prepare the aqueous extract. Cold aqueous extraction was employed in this study to preserve heat-sensitive phytochemical compounds that might degrade at elevated temperatures, thereby enhancing the integrity and bioactivity of the extract. The mixtures were left to stand undisturbed for 24 hours to allow thorough extraction, after which they were filtered sequentially using sterile muslin cloth and Whatman No. 1 filter paper. The resulting filtrates were concentrated in a drying cabinet set at 40°C until complete dryness was achieved. The dried extracts were subsequently weighed and reconstituted in distilled water to obtain working concentrations of 20 mg/mL, 40 mg/mL, 80 mg/mL, and 100 mg/mL [19].

Phytochemical Screening

A preliminary phytochemical analysis of the aqueous extracts was carried out to identify key bioactive constituents such as alkaloids, tannins, flavonoids, saponins, steroids, glycosides, cyanogenic glycosides, volatile oils, and phenols. The screening was performed following standard qualitative procedures as outlined by (20).

Collection of Clinical Samples

Skin scrapings were collected from patients with suspected ringworm lesions attending Almajiri School in Gwadangaji Town, Birnin Kebbi. Samples were collected from the active margins of ringworm lesions using sterile blunt forceps and placed into labelled sterile paper envelopes. These

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were subsequently enclosed in sample bottles and transported to the Microbiology Laboratory at Kebbi State University of Science and Technology, Aliero, for further analysis. All clinical samples were collected after obtaining informed consent from the participants or their guardians and in accordance with institutional ethical guidelines.

Fungal Isolation and Identification

The skin scrapings were aseptically inoculated onto Sabouraud Dextrose Agar (SDA) plates and incubated at room temperature (28 °C) for a period of 7 days to allow for fungal growth. Fungal growth was monitored daily. To ensure the validity of the results and rule out contamination, uninoculated SDA plates were included as negative controls and incubated under the same conditions. Fungal colonies were subcultured onto fresh SDA to obtain pure isolates. Identification of fungal isolates was performed through microscopic examination using Lactophenol Cotton Blue stain. Fungal isolates were identified by comparing their morphological and microscopic characteristics with standard descriptions provided in the Practical Guide and Atlas for the Diagnosis of Fungal Infections by focusing on distinguishing features of Trichophyton, Microsporum, and Epidermophyton species [21].

Antifungal Susceptibility Testing

The antifungal activity of *Mitracarpus hirtus* leaf extracts was assessed using the agar well diffusion technique, following the procedure described by [15]. Sabouraud Dextrose Agar (SDA) plates were inoculated with fungal isolates, and wells were created using a sterile cork borer. Each well was filled with 20 µL of the extract at concentrations of 20, 40, 80, and 100 mg/mL. The plates were then incubated at room temperature for 7 days. Zones of inhibition were measured in millimetres to determine antifungal efficacy. Ketoconazole (5 mg/mL) served as the positive control for comparison. Each test was conducted in triplicate, and the average inhibition zone was calculated to ensure accuracy and reproducibility of results.

Results

Table 1 shows the results of the phytochemical screening of the aqueous leaf extract of Mitracarpus hirtus. The analysis confirmed the presence of several bioactive constituents such as alkaloids, tannins, flavonoids, steroids, and cyanogenic glycosides, all of which are known for their antimicrobial effects and may be responsible for the extract's antifungal activity. In contrast, glycosides and volatile oils were not detected in the extract.

Table 1: Phytochemical Screening Results of Mitracarpus hirtus Leaf Aqueous Extract

Phytochemical Compound	Presence (+/−)
Alkaloids	+
Tannins	+
Flavonoids	+
Steroids	+
Glycosides	−
Cyanogenic glycosides	+
Volatile oils	−

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Table 2 summarizes the dermatophyte species isolated from patients with ringworm attending the Almajiri School in Gwadangaji Area, Birnin Kebbi. Eight different skin conditions were sampled, revealing diverse clinical presentations ranging from red scaly lesions and inflamed skin to discoloured nails and black patches. The most frequently identified fungal species included Trichophyton mentagrophyte, T. rubrum, Microsporum canis, Epidermophyton floccosum, and T. beigelii. T. mentagrophyte was the most commonly isolated dermatophyte, occurring in multiple lesion types.

Table 2: Dermatophyte Isolates from Skin Samples of Patients at Almajiri School, Gwadangaji Area, Birnin Kebbi

Sample Code	Skin Description	Fungal Species Isolated
A	Circular, red scaly lesions	T. mentagrophyte, E. floccosum
B	Scaly loose hair and inflamed skin	T. rubrum, T. mentagrophyte, M. canis
C	Fine-scale eruption	T. mentagrophyte, T. rubrum
D	Thick discoloured nail	T. mentagrophyte, E. floccosum, T. rubrum
E	Cracking and peeling of the skin	T. mentagrophyte, E. floccosum, T. beigelii
F	Ring-shaped with red raised border	T. rubrum, T. mentagrophyte, M. canis
G	Itchy red patches	T. beigelii, T. mentagrophyte
H	Black patches on the skin	M. canis, T. beigelii

Figure 3 presents the distribution of dermatophyte species isolated from ringworm-infected patients in the study area. A total of 34 fungal isolates were identified, with Trichophyton mentagrophyte being the most prevalent species (29.4%), followed by T. beigelii (20.6%), Microsporum canis (17.6%), and Epidermophyton floccosum (17.6%). T. rubrum showed the lowest frequency at 14.7%. The results indicate that T. mentagrophyte is the dominant etiological agent of dermatophytosis among the study population.

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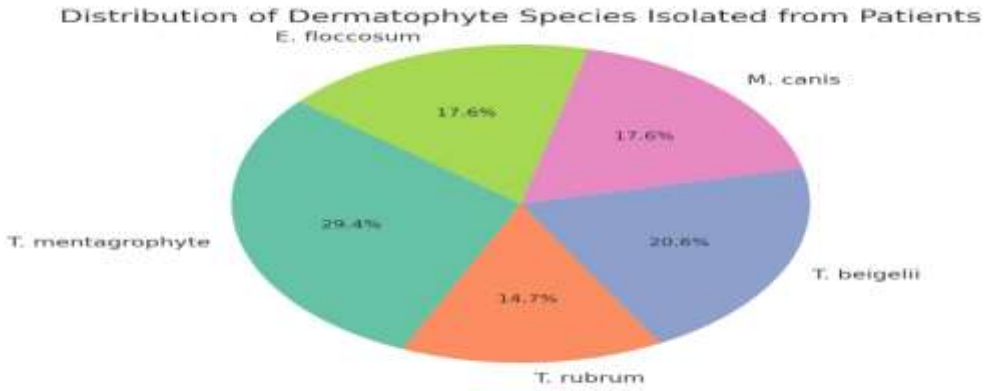


Figure 1: Distribution of dermatophyte species isolated from patients

Table 4 displays the antifungal activity of the aqueous leaf extract of Mitracarpus hirtus against five dermatophyte species, assessed at four concentration levels: 20 mg/mL, 40 mg/mL, 80 mg/mL, and 100 mg/mL. The activity was determined by measuring the diameter of the zones of inhibition in millimetres (mm), indicating the extract's effectiveness against each fungal isolate. Results indicate a concentration-dependent increase in antifungal activity across all tested species. The highest inhibition (26.0 ± 1.0 mm) was observed against T. rubrum and M. canis at 100 mg/mL. E. floccosum exhibited the lowest sensitivity to the extract, with a maximum inhibition of 21.0 ± 1.0 mm. Ketoconazole (5 mg/mL) used as a positive control produced a comparable zone of inhibition (27.0 ± 0.6), indicating that the plant extract possesses promising antifungal potential, especially at higher concentrations.

Table 4: Mean Zones of Inhibition (mm ± SD) of M. hirtus Aqueous Extract against Dermatophytes

Fungal Isolate	20 mg/mL	40 mg/mL	80 mg/mL	100 mg/mL	Ketoconazole (5 mg/mL)
T. mentagrophyte	15.0 ± 1.0	18.0 ± 1.2	20.0 ± 0.8	25.0 ± 1.0	27.0 ± 0.6
T. rubrum	15.0 ± 0.9	20.0 ± 1.1	21.0 ± 0.7	26.0 ± 1.0	27.0 ± 0.6
T. beigelii	15.0 ± 1.0	18.0 ± 1.3	20.0 ± 0.9	25.0 ± 1.0	27.0 ± 0.6
M. canis	14.0 ± 0.8	22.0 ± 1.0	23.0 ± 0.7	26.0 ± 1.0	27.0 ± 0.6
E. floccosum	12.0 ± 1.1	15.0 ± 1.2	20.0 ± 0.8	21.0 ± 1.0	27.0 ± 0.6

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Discussion

Phytochemical analysis of the aqueous leaf extract of *Mitracarpus hirtus* revealed the presence of several bioactive secondary metabolites, including alkaloids, tannins, flavonoids, steroids, and cyanogenic glycosides (Table 1). Flavonoids were particularly abundant, while glycosides and volatile oils were absent. These findings are consistent with previous reports on the phytochemical composition of *M. hirtus* [22,23]. Among the identified constituents, flavonoids and tannins are especially recognized for their antimicrobial and antifungal activities, mediated through mechanisms such as inhibition of spore germination, disruption of fungal cell wall integrity, and interference with critical enzymatic pathways [16,24]. The presence of these compounds may account for the notable antifungal activity of *M. hirtus* and supports its traditional use in the treatment of skin infections. Furthermore, the abundance of antifungal phytochemicals underscores the potential of *M. hirtus* extracts for further pharmacological investigation and possible development as standardized herbal antifungal agents.

Table 2 presents the clinical characteristics and dermatophyte species isolated from eight patients with ringworm infections. Among these, *Trichophyton mentagrophyte* emerged as the most prevalent species, followed by *T. beigelii*, *M. canis*, *E. floccosum*, and *T. rubrum*. Figure 3 further illustrates that *T. mentagrophyte* accounted for 29.4% of all isolates, underscoring its predominance in the study region. This finding carries notable clinical relevance, as *T. mentagrophyte* is widely recognized as a major causative agent of *Tinea corporis*, *Tinea pedis*, and other superficial mycoses, particularly in tropical and subtropical settings [25]. Previous reports [26], subsequently reinforced by additional studies [27], confirm its continued role as a dominant etiological agent of dermatophytosis in both Africa and parts of Asia. This distribution aligns with the findings of previous studies [28,29], which similarly reported *Trichophyton mentagrophyte* and *Microsporum canis* as the most frequently isolated dermatophytes. The wide range of clinical manifestations, from nail discoloration to red, scaly skin, highlights the extensive pathogenic potential of these fungi [30]. The predominance of *T. mentagrophyte*, along with the presence of multiple dermatophyte species in individual patients, underscores the necessity for broad-spectrum antifungal therapies and comprehensive hygiene education in community settings [14]. Factors such as inadequate hygiene, communal living, and the humid tropical environment likely contribute to fungal proliferation and facilitate transmission. The antifungal activity results (Table 4) demonstrated that M. hirtus aqueous extracts exhibited concentration-dependent inhibition of all five dermatophyte isolates. The highest activity (26.0 ± 1.0 mm at 100 mg/mL) was observed against T. rubrum and M. canis at 100 mg/mL, while the lowest inhibition (12.0 ± 1.1 mm) was seen with E. floccosum at 20 mg/mL. These findings indicate that the extract is most effective at higher concentrations.

This observation partially agrees with the findings of [31] and [28] who reported that ethanol extracts of Mitracarpus hirtus had increasing antifungal activity with increasing concentrations. Interestingly, the zones of inhibition at higher extract concentrations (100 mg/mL) were comparable to those produced by the standard antifungal drug ketoconazole (27.0 ± 0.6 mm), reinforcing the therapeutic potential of M. hirtus. Furthermore, the antifungal activity observed is likely attributed to the presence of phytochemicals such as flavonoids and tannins, which are known to exert their effects by disrupting fungal cell wall integrity and interfering with ergosterol synthesis an essential component of fungal cell membranes. (24,32). The results provide promising evidence for the antifungal efficacy of M. hirtus, particularly at higher concentrations, and justify its use in traditional medicine for managing fungal skin infections. With further purification and clinical testing, the plant may serve as a basis for developing topical antifungal formulations.

This study was limited by the use of crude aqueous extracts without standardization or isolation of specific active compounds, making it difficult to

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attribute antifungal activity to individual phytochemicals. The absence of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) determinations limits the precise evaluation of the extract's antifungal potency. Furthermore, the antifungal assessments were restricted to in vitro conditions, with no accompanying in vivo or clinical validation, thereby constraining the applicability of the findings to real-world therapeutic contexts. Antioxidants and bioactive compounds in medicinal plants not only reduce oxidative stress but also exhibit antimicrobial and antifungal properties, playing a key role in preventing and managing infections caused by pathogenic organisms [33-37].

Conclusion

This study confirmed that aqueous leaf extracts of Mitracarpus hirtus exhibit significant antifungal activity against clinical isolates of dermatophytes, including Trichophyton mentagrophyte, T. rubrum, T. beigelii, Microsporum canis, and Epidermophyton floccosum. The extract demonstrated a clear concentration-dependent inhibitory effect, with the highest activity observed at 100 mg/mL comparable to the standard antifungal agent, ketoconazole. Phytochemical analysis revealed the presence of flavonoids, tannins, alkaloids, and steroids, compounds known for their antimicrobial properties, which likely contribute to the observed antifungal effects. While these in vitro findings support the traditional use of M. hirtus in managing fungal skin infections and highlight its potential as a plant-derived antifungal agent, they remain preliminary. Future research should focus on isolating and characterizing the specific active constituents, elucidating their mechanisms of action, evaluating toxicity and safety profiles, and validating therapeutic efficacy through in vivo and clinical studies. These steps are crucial for advancing M. hirtus toward the development of standardized, evidence-based antifungal formulations. However, it is important to note that these results are based solely on in vitro assays and should be considered preliminary. Further studies are needed to isolate and characterize the specific active compounds, elucidate their mechanisms of action, and assess their safety and efficacy through in vivo and clinical evaluations.

Statements and Declarations

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This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing Interests

The authors declare no competing interests related to the content of this study.

Ethics Approval

Ethical approval was obtained from the Research and Ethics Committee of Kebbi State University of Science and Technology, Aliero. Informed consent was obtained from all participants or their guardians prior to sample collection, in accordance with institutional and national ethical guidelines.

Author Contributions

Farida Abubakar Tomo conceptualized the study and designed the research. Sadiq Riskuwa collected plant materials and performed laboratory experiments. Shamsudeen Muhammad Muhammad conducted data analysis and literature review. All authors contributed to manuscript drafting, reviewed the final version.

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