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Phytochemical Composition, FT-IR Characterization, and *In Vitro* Antifungal Activity of Methanol Extract of *Mitracarpus villosus*

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Abstract

This study investigated the phytochemical composition and antifungal potential of the methanol extract of *Mitracarpus villosus*, a medicinal plant traditionally used in Nigeria to treat microbial infections. Phytochemical screening identified terpenoids, flavonoids, tannins, and quinones, whereas phlobatannins were absent. The extract's antifungal activity against *Candida albicans*, *Aspergillus niger*, and *Aspergillus flavus* was evaluated using the agar well diffusion method. The extract exhibited concentration-dependent antifungal activity, with the greatest inhibition observed against *C. albicans* (19 mm at 500 µg/mL), followed by *A. niger* and *A. flavus*. Fourier-transform infrared spectroscopy confirmed the presence of bioactive functional groups, including N–H, C=O, and –CHO, which are commonly associated with antimicrobial activity. These findings provide empirical support for the traditional use of *M. villosus* and indicate its potential as a source of natural antifungal agents. Further research should isolate

and characterize the active compounds and evaluate their pharmacological profiles.

Keywords: *Mitracarpus villosus*; Antifungal Activity; Fourier-Transform Infrared Spectroscopy; Natural Antifungal Agents; Phytochemical Composition

INTRODUCTION

The utilization of medicinal plants has been fundamental in shaping global healthcare, particularly in developing countries where traditional medicine remains a primary source of treatment. The therapeutic efficacy of these plants is largely attributed to the presence of diverse phytochemicals, which serve as natural sources for drug discovery and pharmaceutical development (Lazarus et al., 2025). Among the numerous medicinal species, *Mitracarpus*—a genus in the Rubiaceae family—comprises annual herbs that have been extensively investigated for their pharmacological properties, including antioxidant, antifungal, antibacterial, anti-inflammatory, sedative, anti-diabetic, and even anticancer effects. Despite these benefits, certain *Mitracarpus* species have shown moderate toxicity, warranting comprehensive pharmacological evaluation (Ekalu et al., 2021).

Mitracarpus villosus found predominantly in tropical regions of Africa, especially Nigeria, is traditionally used to treat skin conditions such as eczema, wounds, and ringworm, as well as internal ailments including diarrhea and dysentery. The aerial parts of the plant are used both topically and orally, with reported hepatoprotective, anti-inflammatory, and antimicrobial activities—particularly against *Pseudomonas aeruginosa*. Additionally, the extract is applied in veterinary practice to manage infections such as *Dermatophilus congolensis* in cattle (Aboh et al., 2014). Despite its broad ethnomedicinal applications, comprehensive studies on its phytochemical composition and antifungal mechanisms remain limited.

Emerging studies have further highlighted the significance of *Mitracarpus* species in antifungal research. Hassan et al. (2022) reported that methanol extracts of *Mitracarpus hirtus* contained various bioactive compounds including tannins, flavonoids, alkaloids, and terpenoids, which produced inhibition zones up to 23 mm against fungal strains. Similarly, Chimbekujwo and Ya (2022) demonstrated strong antifungal effects of both ethanol and aqueous extracts of *Mitracarpus scaber* against dermatophytes such as *T. mentagropytes* and *E.*

floccosum. These activities were linked to the presence of secondary metabolites found in both solvent fractions.

Namadina et al. (2020) observed that methanol and aqueous extracts of *M. scaber* showed differential antifungal activity, suggesting variation in the mechanism of action. Other research, such as that by Kwembe et al. (2020), found that although *M. villosus* showed antifungal activity against *Lasiodiplodia theobromae*, it was less potent compared to *Basella alba* and *Ageratum conyzoides*, emphasizing the importance of solvent type and extraction method in determining bioactivity.

Further supporting evidence is provided by Aboh et al. (2018), who found that crude tannin and saponin extracts from *M. villosus* exhibited marked antifungal effects against both yeasts and molds. Itohan et al. (2014) identified ethyl acetate extracts of the plant as particularly potent, with a wide range of bioactive constituents. Ekwealor et al. (2012) and Cimanga et al. (2004) similarly validated the antifungal capabilities of *M. villosus* and *M. scaber* against pathogens including *Candida albicans*, *Trichophyton rubrum*, and *Staphylococcus aureus*.

These accumulating findings suggest that *M. villosus* is a promising candidate for antifungal drug development due to its reservoir of bioactive compounds with known therapeutic potential. However, scientific validation of its antifungal properties, particularly using controlled in vitro assays, remains essential.

Fungal infections continue to pose a major public health threat, particularly with the rising incidence of resistance to conventional antifungal agents. This necessitates the exploration of alternative treatments derived from natural sources. Thus, the present study aims to investigate the phytochemical constituents and in vitro antifungal activity of crude methanol extract of *M. villosus*, offering insights into its potential as a natural antifungal agent.

MATERIALS AND METHODS

Materials

Apparatus and Equipment

Test tubes, spatulas, separating funnels, glass funnels, conical flasks, measuring cylinders, Bunsen burner, water bath, Whatman No. 1 filter paper, beakers, glass column (75

cm × 35 mm), mini-spatula, steel mortar and pestle, Petri dishes, microscope, digital electronic balance, and oven were used for the experimental procedures.

Solvents and Reagents

Methanol, phosphate chloride, acetic acid, ferric chloride, iodine solution, hydrochloric acid, Mayer's reagent, agar medium, and distilled water were obtained from standard suppliers and used without further purification.

Sample Collection and Authentication

Whole plant samples of *Mitracarpus villosus* were collected from Kuri, Yamaltu/Deba Local Government Area of Gombe State, Nigeria. Botanical identification and authentication were conducted at the herbarium of the Botany Department, Gombe State University by Prof. Halima Muhammad Abba, and the specimen was assigned voucher number GSU283.

Sample Preparation

The leaves and stems were separated, shade-dried at room temperature, and ground into a fine powder using a motorized milling machine. The powder was stored in airtight containers and refrigerated until needed for extraction.

Extraction Procedure

A total of 800 g of the powdered plant material was macerated in 7 L of methanol at room temperature for 72 hours with occasional shaking. The extract was filtered using cotton wool and Whatman No. 1 filter paper. The resulting filtrate was concentrated using a rotary evaporator under reduced pressure to yield the crude methanol extract.

Phytochemical Screening

Phytochemical screening was conducted on the crude methanol extract using standard qualitative methods (Ali et al., 2020). For screening, 1.0 g of the extract was dissolved in 10 mL of methanol.

Tannins: 2 mL of 5% ferric chloride was added to 1 mL of the extract. A dark blue or greenish-black color indicated the presence of tannins.

Phlobatannins: A few drops of 2% hydrochloric acid were added to 1 mL of the extract. Formation of a red precipitate indicated phlobatannins.

Flavonoids: 1 mL of 2N sodium hydroxide was added to 2 mL of the extract. A yellow coloration indicated flavonoids.

Quinones: 1 mL of concentrated sulfuric acid was added to 1 mL of the extract. The development of a red color confirmed quinones.

Terpenoids: 0.5 mL of extract was treated with 2 mL of chloroform and concentrated sulfuric acid. A red-brown interface confirmed terpenoids.

Antifungal Screening

Fungal Strain Collection

Fungal strains (*Candida albicans*, *Aspergillus niger*, and *Aspergillus flavus*) were sourced from the Department of Microbiology, Gombe State University. The organisms were subcultured and maintained on Sabouraud Dextrose Agar (SDA) slants at 4°C.

Identification of Fungal Isolates

Fungal identification was based on macroscopic characteristics (e.g., hyphae, pigmentation, conidiophores) and microscopic observation using lactophenol cotton blue staining. Slides were examined under x10 and x40 objective lenses and compared with standard taxonomic keys and the Dermatophyte Atlas (Namadina et al., 2020).

Preparation of Sabouraud Dextrose Agar (SDA)

SDA was prepared by dissolving 42 g of powder in 1 L of distilled water. The medium was sterilized at 121°C for 15 minutes, dispensed into bottles, and slanted to form agar slopes.

Preparation of Sabouraud Dextrose Liquid Medium (SDLM)

30 g of SDLM powder was dissolved in 1 L of distilled water, distributed into sterile Petri dishes, and autoclaved at 121°C for 15 minutes. The medium was cooled to 45°C and allowed to solidify.

Preparation and Maintenance of Fungal Cultures

Test organisms were cultured on SDA containing 0.5% cycloheximide and 0.45% chloramphenicol and incubated at 30°C for 7 days. Stock cultures were stored at 4°C for subsequent use.

Spore Suspension Preparation

Spore suspensions were prepared by washing fungal colonies grown on SDA plates. Spores were harvested using sterile saline, centrifuged, and resuspended in harvesting medium. Suspensions were standardized and stored at 4°C for use within two weeks.

Determination of Zone of Inhibition

The antifungal activity of *M. villosus* crude extract was evaluated using the agar well diffusion method (Namadina et al., 2020). SDA plates were inoculated with 0.1 mL of standardized spore suspension. Wells of 6 mm diameter were bored and filled with 100, 50, 25, and 12.5 mg/mL concentrations of the extract. After 10 minutes of diffusion, plates were incubated at 37°C for 24 hours. Zones of inhibition (DIZ) were measured in millimeters. Fluconazole (10 mg/mL) and DMSO served as positive and negative controls, respectively.

RESULTS

Phytochemical Screening

Phytochemical analysis of the methanol extract of *Mitracarpus villosus* revealed the presence of key secondary metabolites including terpenoids, flavonoids, tannins, and quinones, while phlobatannins were absent (Table 1). These compounds are known for their diverse pharmacological properties, including antimicrobial and antifungal activities.

Table 1: Phytochemical Constituents of Methanol Extract of Mitracarpus villosus

Phytochemical Component	Presence
Terpenoids	++
Flavonoids	++
Tannins	+
Quinones	++
Phlobatannins	–

Key: (+) Present in moderate amount **(++)** Present in high amount **(-)** Absent

These findings align with those reported by Hassan et al. (2020), who detected terpenoids, flavonoids, and tannins in *Mitracarpus zucc* extracts. Similarly, Namadina et al. (2020) documented the presence of flavonoids and tannins in methanol extracts of *Mitracarpus villosus*, consistent with our observations. The presence of these phytochemicals may contribute to the plant's traditional use in treating microbial infections.

Antifungal Activity

The methanol crude extract of *Mitracarpus villosus* demonstrated promising antifungal activity against *Candida albicans*, *Aspergillus niger*, and *Aspergillus flavus* in a dose-dependent manner (Table 2). The zone of inhibition was greatest against *C. albicans*, followed by *A. niger* and *A. flavus*, with activity generally decreasing with lower extract concentrations.

Table 2: Zone of Inhibition (mm) of Mitracarpus villosus Extracts against Fungal Isolates

S/N	Organism	500 µg	250 µg	125 µg	62.5 µg	Fluconazole (10 mg/mL)
1	Candida albicans	19 mm	16 mm	13 mm	9 mm	21 mm
2	Aspergillus niger	14 mm	11 mm	9 mm	8 mm	25 mm
3	Aspergillus flavus	13 mm	11 mm	7 mm	6 mm	17 mm

These results confirm the extract's potent antifungal properties, particularly against *C. albicans*, a common pathogen in human infections. The efficacy appears concentration-dependent, which is characteristic of plant-based antimicrobial agents. The outcomes are comparable with findings by Aboh et al. (2018) and Namadina et al. (2020), who both highlighted significant antifungal effects of *Mitracarpus* species.

FT-IR Analysis

Fourier-transform infrared (FT-IR) spectroscopy was employed to analyze the bioactive components of the methanol extract of Mitracarpus villosus. The spectral results revealed functional groups likely responsible for the antifungal activity observed.

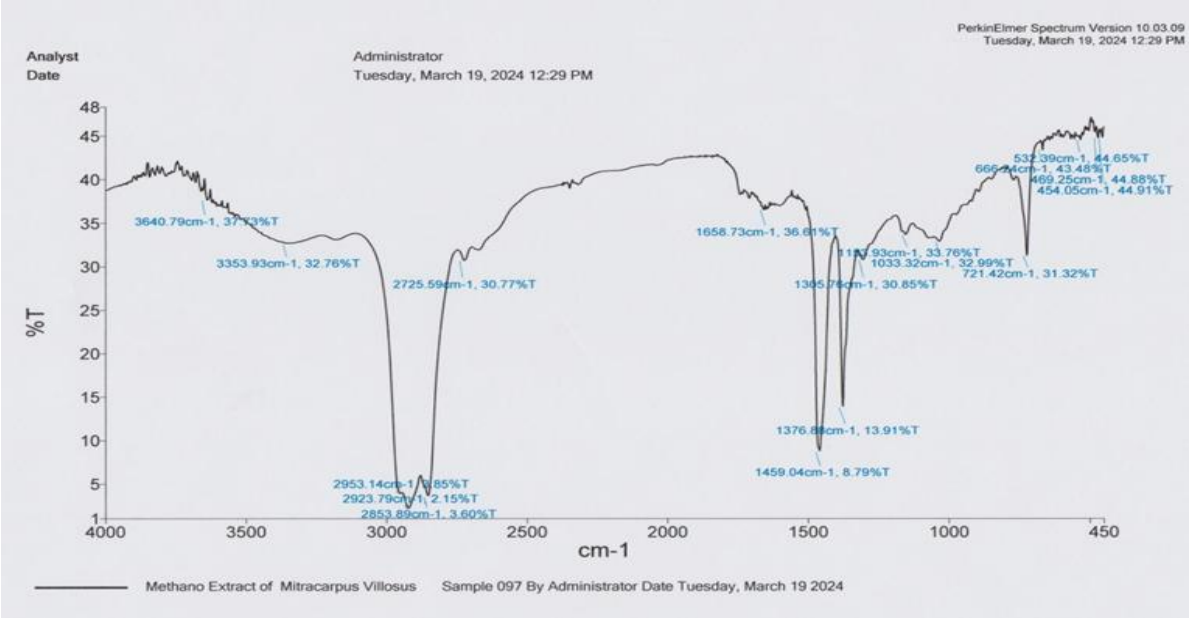

Figure 1: FT-IR spectrum of methanol extract of Mitracarpus villosus. The spectrum reveals peaks corresponding to amines, carbonyls, alkanes, aldehydes, and ethers, suggesting the presence of bioactive functional groups.

Table 3: FT-IR Spectral Data of Methanol Extract of Mitracarpus villosus

Absorption Peak (cm^{-1})	Functional Group Assignment
3640.79	N–H (amine or hydroxyl group)
2953.14	C–H (sp^3 stretching, alkanes)
2725.59	–CHO (aldehyde group)
1658.73	C=O (carbonyl group)
1459.04	C–C (alkane stretch)
1376.88	C–N (amine stretch)
1305.76	C–O (ether or ester group)

These spectral peaks indicate the presence of multiple functional groups such as hydroxyls, amines, carbonyls, and ethers, which are commonly found in bioactive phytochemicals. The peak at 3640.79 cm^{-1} suggests an –NH group, while 2725.59 cm^{-1} and 1658.73 cm^{-1} correspond to aldehydes and ketones, respectively, supporting the phytochemical findings. The presence of these groups implies potential antimicrobial activity due to their ability to interfere with microbial cell functions.

DISCUSSION

The findings of this study reinforce the medicinal potential of *Mitracarpus villosus*, a plant long used in traditional medicine for the treatment of various microbial infections. Phytochemical screening of the methanol extract revealed a rich composition of secondary metabolites such as terpenoids, flavonoids, tannins, and quinones, which are widely reported to possess antimicrobial and antifungal properties. The absence of phlobatannins suggests that these specific tannin derivatives may not play a significant role in the antifungal activity of the plant.

These results are consistent with the observations of Hassan et al. (2022), who found similar bioactive compounds in *Mitracarpus hirtus*, and Namadina et al. (2020), who reported comparable findings in *Mitracarpus villosus*. The presence of these metabolites supports the ethnomedicinal use of *M. villosus* and provides a scientific basis for its therapeutic application.

The antifungal activity assay demonstrated notable inhibition of fungal growth, particularly against *Candida albicans*, followed by *Aspergillus niger* and *Aspergillus flavus*. The highest zone of inhibition was recorded against *C. albicans* (19 mm at 500 μg/mL), which closely approaches that of the positive control, fluconazole (21 mm). This confirms that the methanol extract exhibits significant antifungal potential, comparable to conventional antifungal agents, especially at higher concentrations. These findings mirror those of Aboh et

al. (2018), who also observed strong antifungal effects of *M. villosus*, particularly in crude tannin and saponin extracts.

The dose-dependent response observed further supports the idea that the antifungal effect of the extract is directly related to the concentration of bioactive compounds. Similar trends were documented by Chimbekujwo and Ya (2022) and Itohan et al. (2014), who emphasized the solvent-dependent variability in antifungal activity among *Mitracarpus* species.

The FT-IR spectral analysis confirmed the presence of functional groups such as N–H, C–H, –CHO, C=O, C–C, C–N, and C–O, which are indicative of amines, alkanes, aldehydes, carbonyls, and ethers. These functional groups are characteristic of bioactive plant constituents with known antimicrobial mechanisms. The peak at 3640.79 cm^{-1} (N–H) and 1658.73 cm^{-1} (C=O) support the presence of amino and carbonyl functionalities, which have been implicated in the disruption of microbial membranes and enzymatic systems. This biochemical profile aligns with the proposed modes of action of plant-derived antifungal agents (Cimanga et al., 2004; Ekwealor et al., 2012).

Finally, the results of this study validate previous literature highlighting the antifungal potential of *Mitracarpus* species. The rich phytochemical profile, strong zone of inhibition against common fungal pathogens, and confirmed functional groups via FT-IR analysis collectively suggest that *M. villosus* is a promising candidate for developing plant-based antifungal formulations. Future research should focus on isolating individual compounds responsible for the activity, as well as assessing the safety and efficacy of the extract in vivo.

CONCLUSION

This study confirms that methanol extracts of *Mitracarpus villosus* contain key phytochemicals with significant antifungal activity, particularly against *Candida albicans*. The FT-IR analysis supports the presence of bioactive functional groups, highlighting the potential of this plant as a source of natural antifungal agents. Further research should explore the isolation of individual compounds and assess their pharmacological safety and efficacy in vivo.

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