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Impact of Extraction Solvent on Phytochemical Profile and Antifungal Efficacy of *Vitellaria paradoxa* Leaf Extracts against *Candida albicans*, *Trichophyton rubrum*, and *Microsporum canis*

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Abstract

The increasing resistance of fungal pathogens to conventional antifungal drugs necessitates the search for alternative therapeutic agents. *Vitellaria paradoxa* has been recognised for its medicinal properties, but solvent-dependent variations in its antifungal potency remain underexplored. This study investigated the phytochemical composition and antifungal efficacy of methanolic and aqueous extracts of *V. paradoxa* leaves against *Candida albicans*, *Trichophyton rubrum*, and *Microsporum canis*. Fungal isolates were obtained from the previously stocked samples in the Microbiology Laboratory, Bauchi State University, Gadau, while *V. paradoxa* leaves samples were purchased from Azare Central Market. Phytochemical screening of the leaves was performed using standard qualitative methods, while antifungal efficacy was assessed through the disc diffusion technique. Phytochemical screening revealed both extracts contained alkaloids, flavonoids, tannins, steroids, and phenols, while glycosides and carbohydrates were detected only in the methanolic extract, and saponins were exclusive to the aqueous extract. Antifungal susceptibility testing demonstrated that methanolic extracts exhibited significantly higher potency, with inhibition zones reaching 19.0 ± 2.00 mm against *M. canis* at 200 mg/mL, whereas the aqueous extract showed minimal inhibition. The methanolic extract also had lower minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC), suggesting greater efficacy in disrupting fungal growth. Statistical analysis (ANOVA, $p = 0.049$) confirmed a significant difference between the efficacy of the methanolic and aqueous extracts. These findings highlighted the importance of solvent choice in optimising antifungal activity and suggested that methanolic extracts of *V. paradoxa* may serve as a potential alternative to synthetic antifungal agents.

Keywords: Antifungal, Extraction, Infections, Phytochemicals, Solvent

INTRODUCTION

Plants remain valuable sources of foods and medicines for the management of infectious diseases and nutrition-related syndrome (Lawal *et al.*, 2020a). Herbal preparations have been a reliable source that reclaimed man's health from various mild and life-threatening diseases, before the advent of conventional drugs (Adam and Omogbene, 2020). Also, plant extracts as well as other alternative kinds of medical materials have greatly contributed to human health and well-being (Magaji *et al.*, 2023). Furthermore, plants are used for the management of diverse ailments is general and still sustained among African people (Lawal *et al.*, 2020b). Over a decade, secondary metabolites of plants previously with unidentified pharmacological activities, have been thoroughly investigated as sources of

various kinds medicinal agents (Magaji *et al.*, 2023). *Vitellaria paradoxa* (Shea tree), one of these medicinal plants, is a multipurpose plant widely distributed across the Sahel region of Africa, from Senegal to Uganda (Abdul-Hammed *et al.*, 2020). It is highly valued for its nuts, which yield Shea butter, an essential product in cosmetics, food, and traditional medicine (Abdul-Hammed *et al.*, 2020). The plant thrives in warm climates with temperatures ranging from 24 to 38 °C and adapts to various soil types (Magaji *et al.*, 2023). Two subspecies of *V. paradoxa* differ in their oil composition; “*paradoxa*” (West Africa) and “*nilotica*” (East Africa) due to genetic and environmental factors (Iddrisu *et al.*, 2019).

Beyond its economic value, *V. paradoxa* has a significant medicinal property. Its extracts are traditionally used to treat infections, wounds,

rheumatic pain, and inflammatory conditions (Zhang *et al.*, 2018). Various studies highlight its antibacterial, antifungal, antiviral, and anticancer activities, which are attributed to bioactive compounds such as flavonoids, triterpenoids, and phenolics (Magaji *et al.*, 2023). Recent research suggests that Shea butter and other plant extracts may serve as alternative therapies for resistant microbial infections, including fungal diseases (Catteau *et al.*, 2017).

The antifungal efficacy of *V. paradoxa* is linked to its diverse phytochemical composition, which varies depending on extraction methods (Mahmud *et al.*, 2025). Different solvents influence the yield and concentration of bioactive compounds, ultimately affecting antifungal potency (Mahmud *et al.*, 2025). Previous studies have shown that methanol extracts exhibit strong cytotoxicity and antimicrobial activity, whereas aqueous and essential oil extracts displays lower potency (Mahmud *et al.*, 2025). However, comparative studies on solvent-dependent variations in phytochemical composition and antifungal activity remain limited (Mahmud *et al.*, 2025).

The rising resistance of fungal pathogens to conventional antifungal drugs necessitates the search for alternative treatments (Magaji *et al.*, 2023). While *V. paradoxa* has demonstrated promising antifungal properties, there is a gap in knowledge regarding the impact of different solvents on its bioactive compounds and efficacy. Understanding these variations is crucial for optimising extraction methods and enhancing therapeutic applications (Magaji *et al.*, 2023). This study aimed to compare the phytochemical composition of *V. paradoxa* extracts obtained using water and ethanol, evaluate the antifungal efficacy of these extracts against pathogenic fungi, and determine the most effective solvent for extracting bioactive antifungal compounds.

MATERIALS AND METHODS

Sources of Fungal strains used for the study

Stocked clinical isolates of *Candida albicans*, *Trichophyton rubrum* and *Microsporum canis* were obtained from the Microbiology Laboratory of Bauchi State University, Gadau. These isolates were originally collected from patients with suspected cases of superficial mycoses for diagnostic and research purposes. Prior to their use in this study, the isolates were preserved through periodic subculturing on Sabouraud

Dextrose Agar (SDA) slants and stored at 4°C to maintain viability. In addition, long-term preservation was done by suspending fungal spores and cells in 20% glycerol and storing them at -20°C to ensure the genetic and physiological stability of the isolates for future research and reference purposes.

The confirmation of the isolates was done macroscopically based on the colonies characteristic (color of the surface and reverse, topography, and texture), and microscopically as cited by Magaji *et al.*, (2023), by picking a small portion of the fungal mycelium from the medium, staining it with Lactophenol Cotton Blue, and examining it using 10X and 40X objectives.

Plant's Leaves Sample Collection and Authentication

Fresh leaves of *Vitellaria paradoxa* were purchased from Azare Central Market, Katagum Local Government Area, Bauchi State, Nigeria. The leaves were taxonomically authenticated by Dr. Umar Aminu Muhammad of the Department of Biological Sciences, Bauchi State University, Gadau. The authentication process involved macroscopic and microscopic examination of the plant's morphological features, including leaf shape, venation, margin, and texture, followed by comparison with voucher specimens available in the departmental herbarium. A voucher specimen was deposited for future reference.

The procedure adopted for authentication followed the standard botanical identification method as stated by (Mahmud *et al.*, 2025).

Preparation of the Plant Leaves

The collected leaves of *V. paradoxa* were washed with distilled water to remove debris and surface contaminants, then shade-dried at room temperature (25-30°C) for two weeks to prevent photodegradation of bioactive compounds (Magaji *et al.*, 2023). The dried leaves were ground into a fine powder using an electric blender and stored in airtight containers at 4°C until further analysis (Magaji *et al.*, 2023).

Extraction of Bioactive Compounds from the Leaf Sample

A cold maceration technique was used for the extraction, following the methodology described by Magaji *et al.* (2023). **Methanolic Extraction:** about 100 g of the powdered leaf sample was weighed and soaked in 500 mL of methanol (99%)

for 72 hours at room temperature with occasional shaking. The extract was filtered using Whatman No. 1 filter paper and the filtrate was concentrated using a rotary evaporator at 40°C under reduced pressure and the crude extract was stored at 4°C for further analysis.

Aqueous Extraction: another 100 g of powdered leaf sample was macerated in 500 mL of distilled water for 72 hours. The extract was filtered and concentrated using a water bath at 50°C. The dried extract was stored in airtight vials at 4°C.

Phytochemical Screening

The qualitative phytochemical screening was conducted on the methanol and aqueous extracts to determine the presence of bioactive compounds, following standard procedures (Magaji *et al.*, 2023).

Alkaloids (Mayer's Test): about 2 mL of extract was mixed with 2 mL of 1% HCl and heated. Mayer's reagent was then added dropwise. A creamy white precipitate indicated the presence of alkaloids.

Flavonoids (Shinoda Test): about 2 mL of extract was treated with a few drops of concentrated HCl and magnesium ribbon. A pink or reddish colouration confirmed the presence of flavonoids.

Saponins (Frothing Test): about 2 mL of extract was vigorously shaken with 5 mL of distilled water. Persistent frothing indicated the presence of saponins.

Tannins (Ferric Chloride Test): exactly 2 mL of extract was mixed with a few drops of 1% ferric chloride solution. A blue-black or green colouration indicated tannins.

Phenols (Ferric Chloride Test): about 2 mL of extract was mixed with 2 mL of 5% ferric chloride solution. A deep blue or green colouration confirmed the presence of phenols.

Steroids (Liebermann-Burchard Test): about 2 mL of extract was dissolved in chloroform, followed by the addition of acetic anhydride and concentrated H₂SO₄. A greenish colour indicated the presence of steroids.

Glycosides (Keller-Kiliani Test): about 2 mL of extract was treated with glacial acetic acid and ferric chloride, followed by sulfuric acid. A brown ring at the interface indicated the presence of glycosides.

Carbohydrates (Molish's Test): To 1 mL of the filtrate in a test tube, about 1 mL of Molish's reagent was added. Development of a reddish colour at the interfacial ring on the addition of 1 mL of concentrated sulphuric acid indicated the presence of carbohydrate.

Antifungal Susceptibility Testing

Fungal spore suspensions were standardised to a concentration of 1×10^6 spores/mL before use in antifungal susceptibility testing. This was achieved by harvesting spores from 10-day-old cultures grown on Sabouraud Dextrose Agar using sterile normal saline containing 0.05% Tween 80. The spore suspension was filtered through sterile gauze to remove hyphal fragments, and the turbidity was adjusted to match a 0.5 McFarland standard using a spectrophotometer at 530 nm. The standardised suspension was then used for inoculation in both disc diffusion and broth dilution assays (Magaji *et al.*, 2023).

The disc diffusion method was employed in this study. Different concentrations (200mg/mL, 100mg/mL, 50mg/mL and 25mg/mL) of each of the water and methanolic extracts of the plant's leaves were prepared. Five millimetre (5mm) diameter discs were made from Whatman's No1 filter paper and soaked separately in the different concentrations of each of the extracts and allowed to stay for 24hours for proper absorption. The discs were then air dried (Magaji *et al.*, 2023).

Prepared Sabouraud Dextrose Agar (SDA) was inoculated with the standardised spore suspension, containing 1×10^6 spores/mL. The crude extract discs of different concentrations (200mg/mL, 100mg/mL, 50mg/mL and 25mg/mL) were placed onto each of the appropriately labelled plates. The negative control consisted of distilled water, while the positive control was represented by the standard disk impregnated with fluconazole (50mg). The plates were incubated at ambient temperature for 14 days. Growth was observed, and all tests were conducted in triplicate. The resulting zones of inhibition around the disks were measured and recorded as mean diameters of inhibition zones (Magaji *et al.*, 2023).

Determination of Minimum Inhibitory Concentration and Minimum Fungicidal Concentration of *Vitellaria paradoxa* Leaf Extracts

The broth dilution method, as described by Mahmud *et al.* (2025), was employed to

determine the minimum inhibitory concentration of the *V. paradoxa* extracts. Sabouraud Dextrose Broth was prepared following the manufacturer's instructions, with 1 mL dispensed into individual test tubes. The tubes were then sterilised at 121°C for 15 minutes and allowed to cool. Different concentrations of each of the water and methanolic extracts, ranging from the stock concentration (200mg/mL) to concentrations of 100 mg/mL, 50 mg/mL and 25 mg/mL were prepared with 1 mL added to an appropriately labelled test-tube. About 0,1 mL of the standardised inoculum (1×10^6 spores/mL) was introduced into each concentration in the broth, and the control (broth without extracts). Incubation took place at 30°C for 7 days, and visible colonies growths were monitored. The minimum inhibitory concentration was identified as the lowest concentration without observable growth in the test tube.

To determine the Minimum Fungicidal Concentration (MFC) of the extracts, fresh Sabouraud Dextrose Agar media were used. The content of the tube containing the minimum inhibitory concentrations was subcultured onto the agar media. All plates were then incubated at 30°C for 1-7 days and observed for growth. The Minimum Fungicidal Concentration was identified as the lowest concentration of the extracts without any growth on the agar plate (Mahmud et al., 2025).

Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean \pm standard deviation (SD). Data were analysed using SPSS version 25.0. One-way ANOVA followed by Tukey's post hoc test was used to compare variations in phytochemical composition and antifungal activity among the extracts, and statistical significance was determined at $p < 0.05$.

RESULTS

The phytochemical screening of *V. paradoxa* leaf extracts revealed notable variations between aqueous and methanolic extracts (Table 1). The aqueous extract contained alkaloids, saponins, flavonoids, tannins, steroids, and phenols but lacked glycosides and carbohydrates. In contrast, the methanolic extract contained all the tested phytochemicals except saponins (Table 1).

Table 1: Phytochemical Constituents of *V. paradoxa* Methanolic and Aqueous Leaf Extracts

S/N	Constituents	Methanolic extract	Aqueous extract
1	Alkaloids	+	+
2	Saponins	-	+
3	Glycosides	+	-
4	Tannins	+	+
5	Flavonoids	+	+
6	Steroids	+	+
7	Phenols	+	+
8	Carbohydrate	+	-

Keys: "+" = present "-" = absent

Antifungal susceptibility results showed that all the tested isolates resisted 50 mg/mL of the aqueous extract. However, at 100 mg/mL, a zone of inhibition of 1.58 ± 0.40 mm was observed against *Microsporum canis*. At 200 mg/mL, the aqueous extract demonstrated inhibition against *Candida albicans* (14 ± 0.0 mm), *M. canis* (15.6 ± 0.40 mm), and *Trichophyton rubrum* (12.9 ± 0.27 mm). In contrast, the methanolic extract exhibited greater antifungal potency. At 50 mg/mL, it produced zones of inhibition of 12.33 ± 0.57 mm (*C. albicans*), 13.8 ± 0.53 mm (*M. canis*), and 12.1 ± 0.27 mm (*T. rubrum*). At 100 mg/mL, inhibition zones increased to 16.00 ± 1.00 mm (*C. albicans*), 17.0 ± 0.92 mm (*M. canis*), and 13.5 ± 0.17 mm (*T. rubrum*). The highest concentration (200 mg/mL) resulted in zones of inhibition of 17.67 ± 0.57 mm, 19.0 ± 2.00 mm, and 16.0 ± 1.00 mm against *C. albicans*, *M. canis*, and *T. rubrum*, respectively (Table 2).

The study also revealed that the ANOVA test result (Sig = 0.049) (Table 2) indicated a slight statistically significant difference in the efficacies of aqueous and methanolic leaf extracts at 200 mg/mL, and fluconazole, at the 0.05 significance level.

The MIC of the aqueous extract was found to be 100 mg/mL against *Microsporum canis* and 200 mg/mL against *Candida albicans* and *Trichophyton rubrum*. In contrast, the methanolic extract exhibited significantly lower MIC values (25 mg/mL) across all tested pathogens, indicating stronger antifungal activity (Table 3).

The aqueous extract at 200 mg/mL did not reach the minimum fungicidal concentration required to kill the fungal cells. While the MFC of the methanolic extract was 100 mg/mL for *C. albicans* and *M. canis*, and 200 mg/mL for *T. rubrum* (Table 4).

Table 2: Antifungal Activity of the Leaves Extracts of *V. paradoxa*

Pathogens	Control		Aqueous extract (mg/ml)			Methanolic extract (mg/ml)			p-value
	Fluconazole	Water	50	100	200	50	100	200	
<i>C. albicans</i>	16.00±0.00	0	0±0.00	0±0.00	14.00±0.0	12.33±0.57	16.00±1.00	17.67±0.57	0.049
<i>M. canis</i>	20.00±0.00	0	0±0.00	1.58±0.40	15.6±0.40	13.8±0.53	17.0±0.92	19.0±2.00	
<i>T. rubrum</i>	20.20±1.11	0	0±0.00	0±0.00	12.9±0.27	12.1±0.27	13.5±0.17	16.0±1.00	

Key: (±) = mean standard deviation

DISCUSSION

The phytochemical screening in this study revealed notable differences between the two extracts. While both extracts contained alkaloids, flavonoids, tannins, steroids, and phenols, glycosides and carbohydrates were exclusive to the methanolic extract, whereas saponins were found only in the aqueous extract. These differences can be attributed to the varying polarity of the solvents used, influencing their capacity to dissolve specific classes of compounds. Methanol, being semi-polar, is particularly effective at extracting moderately polar bioactive compounds, which may account for the greater antifungal activity observed (Adam *et al.*, 2023).

These findings also align with previous research demonstrating that solvent choice significantly impacts the extraction efficiency of phytochemicals, ultimately influencing the biological activity of plant-based remedies (Koudou *et al.*, 2020).

The findings of this study underscore the significant influence of extraction solvent on the phytochemical composition and antifungal efficacy of *Vitellaria paradoxa* leaf extracts. Methanolic extracts demonstrated superior antifungal activity compared to aqueous extracts, as evidenced by larger zones of inhibition, lower minimum inhibitory concentrations (MIC), and minimum fungicidal concentrations (MFC) against *Candida albicans*, *Trichophyton rubrum*, and *Microsporum canis*. This enhanced efficacy may be attributed to the broader range of bioactive compounds extracted by methanol, including flavonoids, glycosides, carbohydrates, and phenols.

The enhanced activity of the methanolic extract is consistent with recent studies, which demonstrated that methanol is an effective solvent for extracting a wide array of antimicrobial phytochemicals from *V. paradoxa* and other medicinal plants. For instance, Bertrand *et al.* (2021) reported that methanol-extracted bark fractions of *V. paradoxa* showed strong inhibitory effects against *C. albicans* and *A. niger*, with higher potency than aqueous extracts. Similarly, Babarinde *et al.* (2023) showed that methanolic nut fractions of *V. paradoxa* possessed significant antifungal effects against dermatophytes, supporting the present findings. These studies collectively highlight methanol's ability to solubilize semi-polar and polar compounds, which are often responsible for antimicrobial effects.

Statistical analysis further reinforced the observed differences in efficacy. The ANOVA result ($p = 0.049$) indicated a significant difference between the antifungal activity of methanolic and aqueous extracts at 200 mg/mL. This suggests that the antifungal effect of the plant is dose-dependent and solvent-sensitive, with methanol providing a more suitable medium for extracting therapeutic compounds.

These findings indicated a dose-dependent response, with methanolic extracts demonstrating superior antifungal activity compared to aqueous extracts. Notably, the

inhibition by methanolic extracts at higher concentrations was comparable to fluconazole, suggesting their potential as alternative antifungal agents. This is abreast the findings of [Kalgo et al. \(2019\)](#), who highlighted the effectiveness of *V. paradoxa* in treating both bacterial and fungal infections.

Bioactive compounds in *V. paradoxa* have been identified as key contributors to this antifungal activity. This also agrees with the results of [Fodouop et al. \(2015\)](#) who reported its use in managing skin conditions, especially those of fungal origin.

Table 3 Minimum Inhibitory Concentration of *V paradoxa* Extracts

Pathogens	Aqueous extract (mg/mL)	Ethanolic extract (mg/mL)
<i>C albicans</i>	200	25
<i>M canis</i>	100	25
<i>T rubrum</i>	200	25

Table 4: Minimum Fungicidal Concentration of *V paradoxa* Extracts

Pathogens	Aqueous extract (mg/mL)			Ethanolic extract (mg/mL)		
	50	100	200	50	100	200
<i>C albicans</i>	-	-	-	-	+	+
<i>M canis</i>	-	-	+	-	+	+
<i>T rubrum</i>	-	-	-	-	-	+

Key: (*) minimum fungicidal concentration

The MIC and MFC results showed that methanolic extracts exerted fungistatic and fungicidal effects at significantly lower concentrations compared to aqueous extracts. These findings agree with the report of [Ekhuemelo et al. \(2021\)](#), who observed similar patterns in antifungal activity against wood-decay fungi, where methanol extracts showed high potency with MICs as low as 50 µg/mL. This further emphasises the potential of methanolic extracts of *V. paradoxa* as effective antifungal agents. Also, [Boyejo et al. \(2019\)](#), reported superior antifungal effects of ethanolic extracts against dermatophytes. This supported the findings of the current study.

The observed differences in MIC and MFC values between aqueous and methanolic extracts suggested that solvent choice significantly impacts antifungal potency.

The implication of these findings is twofold. Firstly, methanolic extraction of *V. paradoxa* may be optimised for use in herbal formulations targeting fungal infections, especially in resource-limited settings where synthetic antifungal agents are not readily available. Secondly, the observed antifungal activity supports the ethnomedical use of the plant and

highlights the need for further investigation into the isolation and characterisation of its active components, as proposed by recent pharmacognostic studies ([Babarinde et al., 2023](#)).

CONCLUSION

This study demonstrated that the choice of extraction solvent significantly influences the phytochemical composition and antifungal efficacy of *Vitellariaparadoxa* leaf extracts. The methanolic extract showed a broader range of phytochemicals, including glycosides and carbohydrates, which were absent in the aqueous extract. It also exhibited superior antifungal activity against *Candida albicans*, *Trichophyton rubrum*, and *Microsporum canis*, as evidenced by larger zones of inhibition and lower MIC and MFC values. Statistical analysis confirmed a significant difference in efficacy between the methanolic and aqueous extracts ($p = 0.049$), highlighting methanol as the more effective solvent. These findings suggest that methanolic extracts of *V. paradoxa* leaves may serve as a promising natural alternative to conventional antifungal agents, particularly in settings with limited access to synthetic drugs. Further studies are recommended to isolate and

characterise the specific bioactive compounds responsible for the observed antifungal effects.

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