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Full Length Research Article

PHYTOCHEMICAL COMPOSITIONS, ANTIFUNGAL EFFICACY, AND ACUTE TOXICITY EVALUATION OF *VITELLARIA PARADOXA* LEAF EXTRACTS

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ABSTRACT
The growing challenge of drug-resistant fungal infections necessitates the need for novel and effective therapeutic alternatives. This study investigated the phytochemical composition, antifungal activity, and acute toxicity of *Vitellaria paradoxa* (shea tree) leaf extracts. Aqueous and ethanolic extracts were screened for phytochemicals and tested against clinical fungal isolates using the paper disc diffusion method. Acute toxicity was assessed in mice following Organization for Economic Cooperation and Development (OECD) guideline 423. Both extracts contained alkaloids, tannins, flavonoids, steroids, and phenols; however, saponins were present only in the aqueous extract, while glycosides and carbohydrates were exclusive to the ethanolic extract. The ethanolic extract exhibited stronger antifungal activity (15.0 ± 2.0 mm to 19.0 ± 2.0 mm) than the aqueous extract (12.8 ± 0.3 mm to 15.6 ± 0.4 mm) at 200 mg/mL *Microsporum canis* showed the highest susceptibility, and the ethanolic extract demonstrated comparable efficacy to fluconazole against *Candida albicans* and *Candida tropicalis*. Additionally, the ethanolic extract showed lower Minimum Inhibitory Concentrations (MIC) (25 mg/mL) and MFCs (100–200 mg/mL) compared to the aqueous extract (MIC 100–200 mg/mL; limited Minimum Fungicidal Concentration (MFCs). No toxicity was observed at doses up to 4000 mg/kg in mice. These results highlight *V. paradoxa* ethanolic leaf extract as a safe and effective antifungal agent with therapeutic potential.

Keywords: *Vitellaria paradoxa*, Phytochemicals, Antifungal efficacy, Acute toxicity, Leaf extract.

INTRODUCTION

The global burden of fungal infections is increasing, particularly among immunocompromised individuals. Compounding this issue is the growing resistance of fungal pathogens to conventional drugs, which underscored the urgent need for new, safe, and effective antifungal agents (Magaji *et al*., 2023). Medicinal plants, long relied upon in traditional healthcare systems, remain promising sources of therapeutic compounds. Their affordability, accessibility, and perceived safety continue to drive their use in resource-limited settings and inspire scientific validation for pharmaceutical development (Lawal *et al*., 2020a; Adam & Omogbene, 2020; Magaji *et al*., 2023).

Vitellaria paradoxa C.F. Gaertn., commonly known as the Shea tree, is a highly valued medicinal and economic plant native to the Sahel region of Africa, spanning from Senegal to Uganda. It is best known for its seeds, which yield Shea butter a key ingredient in cosmetics, nutrition, and traditional medicine (Audu & Awulu, 2017). The species thrives in diverse ecological zones, flourishing in warm climates (24–38 °C) and tolerating various soil types (Glele Kaka *et al*., 2011; Naughton *et al*., 2015).

Beyond its economic significance, *Vitellaria paradoxa* also possesses notable ethnomedicinal value, with its bark, leaves, roots, and oils traditionally used to manage infections, heal wounds, and treat gastrointestinal and inflammatory conditions (Zhang *et al*., 2018). These therapeutic effects are attributed to a wide range of phytochemicals, including flavonoids, phenolics, saponins, and triterpenoids, which have been reported to exhibit antimicrobial, antioxidant, anti-inflammatory, and anticancer properties (Mbaveng *et al*., 2011; Tapondjou *et al*., 2011). This chemical diversity gives *V. paradoxa* extracts a broad-spectrum bioactivity profile and justifies ongoing scientific interest.

Of particular interest, *Vitellaria paradoxa* extracts have shown significant antifungal activity against a range of pathogens, including *Candida albicans* and dermatophytes, demonstrating inhibitory effects on fungal growth and virulence (Catteau *et al*., 2017). The phytochemicals responsible for this activity are known to disrupt fungal cell membranes and interfere with vital cellular processes. However, the potency of these effects is highly dependent on the extraction solvent used. Organic solvents, such as methanol and ethanol, are known to yield higher concentrations of active phytochemicals compared to aqueous or essential oil extracts, which often show lower efficacy (Magaji *et al*., 2025).

Given the variable pharmacological performance of *V. paradoxa* extracts depending on the extraction method, it becomes essential to evaluate not only their antifungal efficacy but also their safety. Although plant-based remedies are widely assumed to be safe, they may possess toxic constituents, especially when consumed at high doses or for extended periods (Magaji *et al*., 2025). Toxicological evaluations, particularly acute toxicity studies, are necessary to ensure that extracts considered for therapeutic use

do not pose health risks (Magaji *et al*., 2025).
Previous studies have yielded mixed findings regarding the safety of *V. paradoxa* extracts. Odunola *et al*. (2019) reported no observable behavioral changes or mortality in rats treated with methanolic extracts, though marginal genotoxicity was detected at higher concentrations using the SOS chromotest. Similarly, Aboaba *et al*. (2014) reported moderate toxicity in essential oils derived from *V. paradoxa*. Other reports indicated no toxicological changes in Wistar rats fed with Shea nuts or treated with Shea oleines. These findings suggest that while some parts of the plant appear safe, others require more detailed safety evaluation—especially across different extraction types and test models (Magaji *et al*., 2025).

Despite these insights, gaps remain in understanding the solvent-dependent variability in both antifungal efficacy and toxicological profiles of *V. paradoxa* extracts. Such knowledge is critical for guiding the development of standardized, safe, and effective plant-based antifungal treatments. Therefore, the present study aimed to evaluate the phytochemical compositions, antifungal potency, and acute oral toxicity of ethanolic and aqueous extracts of *Vitellaria paradoxa*.

MATERIALS AND METHODS

Collection and Identification of Fungal Strains
Clinical isolates of *Candida albicans*, *Trichophyton rubrum*, *Trichophyton soudanense*, *Candida tropicalis*, and *Microsporum canis* were obtained from the Microbiology Laboratory of Bauchi State University, Gadau. These fungal strains were confirmed macroscopically by evaluating colony morphology (surface and reverse coloration, texture, and topography) and microscopically, following the protocol of Magaji *et al*. (2023). Microscopic examination was performed by staining a portion of the fungal mycelium with Lactophenol Cotton Blue, followed by observation under 10× and 40× magnifications.

Collection and Authentication of Plant Material
Fresh leaves of *Vitellaria paradoxa* were collected in October 2024 from Azare Central Market, Katagum Local Government Area, Bauchi State, Nigeria. Although sourced from a market, the plant material was authenticated based on morphological characteristics by Dr. Umar Aminu Muhammad, Department of Biological Sciences, Bauchi State University, Gadau. A voucher specimen (Voucher No. SAZU365b) was deposited at the university's herbarium for future reference.

Preparation of Plant Material
Collected leaves were washed with distilled water to remove dust and contaminants, then shade-dried at ambient temperature (25–30°C) for two weeks to prevent degradation of bioactive compounds. The dried leaves were ground into fine powder using an electric blender and stored in airtight containers at 4°C until further use (Harborne, 1998).

Extraction of Bioactive Compounds
Cold maceration was used for the extraction process as described by Edeoga *et al*. (2005).

Ethanolic Extraction: 100 g of powdered leaf material was soaked in 500 mL of 99% ethanol for 72 hours at room temperature with intermittent shaking. The mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated with a rotary evaporator at 40°C under reduced pressure and stored at 4°C.

Aqueous Extraction: A separate 100 g of powdered material was macerated in 500 mL of distilled water for 72 hours. After filtration, the extract was concentrated using a water bath at 50°C and stored in airtight containers at 4°C.

Phytochemical Screening
Standard phytochemical screening procedures were followed according to established methods described by Sofowora (2008) and Trease & Evans (2009) to test for the presence of active compounds like; alkaloids, tannins, flavonoids, steroids, phenols, saponins and glycosides.

Antifungal Susceptibility Testing
The disc diffusion method was adopted to evaluate antifungal activity. Extracts were prepared at concentrations of 200 mg/mL, 100 mg/mL, 50 mg/mL, and 25 mg/mL. Filter paper discs (5 mm diameter) were prepared from Whatman No. 1 paper, soaked in each concentration for 24 hours, and then air-dried (Trease & Evans, 2009).
Sabouraud Dextrose Agar (SDA) was prepared and sterilized at 121°C for 15 minutes, poured into sterile Petri dishes, and allowed to solidify. Plates were oven-dried at 45°C before use. A standardized fungal spore suspension (1×10^6 spores/mL) was spread on each plate. Extract-impregnated discs were placed on the inoculated agar. Distilled water served as the negative control, and fluconazole (50 mg/disc) served as the positive control. Plates were incubated at room temperature for 1–14 days. Zones of inhibition were measured, and results were recorded as mean inhibition diameters following CLSI guidelines (2010). All tests were performed in triplicate.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)
The minimum inhibitory concentration (MIC) of the extracts was determined using the broth dilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2009). Sabouraud Dextrose Broth (SDB) was prepared, sterilized, and dispensed at 1 mL per test tube. Subsequently, 1 mL of each extract, serially diluted to concentrations of 200 mg/mL, 100 mg/mL, 50 mg/mL, and 25 mg/mL, was added to the tubes. Each tube was then inoculated with 0.1 mL of fungal suspension standardized to 1×10^6 spores/mL. Control tubes containing only the broth and fungal inoculum, without extracts, were also included. All test tubes were incubated at 30°C for 1 to 7 days. The MIC was determined as the lowest concentration of the extract that showed no visible fungal growth after incubation.
To determine the minimum fungicidal concentration (MFC), samples from tubes that showed no visible growth at or above the MIC were subcultured onto fresh Sabouraud Dextrose Agar (SDA) plates. The plates were incubated at 30°C for 1 to 7 days and observed for fungal growth. The MFC was defined as the lowest concentration of the extract that completely inhibited fungal growth on the agar plates.

Determination of Acute Toxicity of *Vitellaria paradoxa* Extracts
The oral toxicity study of ethanolic extract of *V paradoxa* was assessed according to the descriptions of Organization for

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Economic Cooperation and Development (OECD) contained in the guideline 423, on the laboratory mice (20–30 g) (Muhammad *et al*., 2015), in which the limit of the test dose used was 4 000 mg/kg. All the test animals were kept fasting overnight. The animals were grouped in to five, each group comprising three animals. The first, second, third and fourth groups were taken as test groups receiving 500 mg/kg, 1000mg/kg, 2000 mg/kg and 4000 mg/kg respectively. While the fifth group was taken as control group receiving empty water. The animals were observed for toxic effect after the first 4 hours of the treatment period. The animals were further investigated for a period of 3 days for any toxic effect. Changes in behavior, body weight, urinations, food intake, water intake, respiration, temperature, eye and skin colors were observed. Other effects such as constipations, convulsion and tremor were also noticed (Muhammad *et al*., 2015).

Statistical Analysis

All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was conducted using SPSS software (version 25.0; IBM Corp., Armonk, NY, USA) to compare the means of antifungal activities (diameters of zone of inhibition, MIC values) among the extracts, followed by Tukey's post hoc test for pairwise comparisons. Statistical significance was considered at $p < 0.05$.

RESULTS

Phytochemical Constituents of *Vitellaria paradoxa* Leaf Extracts

The current study revealed the presence of several bioactive compounds in the *Vitellaria paradoxa* leaf extracts (Table 1). The result showed that alkaloids, tannins, flavonoids, steroids, and phenols were detected in both ethanolic and aqueous extracts. Saponins were detected only in the aqueous extract, while glycosides and carbohydrates were exclusive to the ethanolic extract.

Table 1: Phytochemical Constituents of V paradoxa Leaf Extracts

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

"+" = present "-" = absent

Antifungal Activity of Extracts

Table 2 presents the antifungal activity of *Vitellaria paradoxa* leaf extracts, which varied according to solvent type and concentration. The ethanolic extract consistently exhibited stronger antifungal effects than the aqueous extract across all tested fungal pathogens. At 200 mg/mL, the ethanolic extract produced the largest inhibition zones, notably against *Microsporum canis* (19.0 ± 2.0 mm) and *Candida albicans* (17.7 ± 0.6 mm), with efficacy comparable to fluconazole. In contrast, the aqueous extract showed no activity at 50 mg/mL but demonstrated moderate inhibition at 200 mg/mL, with the highest zone observed against *M. canis* (15.6 ± 0.4 mm).

Statistical analysis revealed a significant difference in antifungal efficacy among the aqueous extract, ethanolic extract, and fluconazole ($F = 14.089$, $p = 0.001$). Tukey's post hoc test indicated that the ethanolic extract was significantly more effective than the aqueous extract ($p = 0.032$) and showed no significant difference compared to fluconazole ($p = 0.081$), suggesting comparable antifungal potency between the ethanolic extract and the standard drug.

Table 2: Antifungal Activity of the Ethanolic and Aqueous Leaf Extracts of *V paradoxa*

	Aqueous extract (mg/ml)			Ethanolic extract (mg/ml)			Control
Pathogens	**50**	**100**	**200**	**50**	**100**	**200**	**Fluconazole**
C albicans	0.0±0.0	10.0±0.0	14.0±0.0	12.3±0.57	16.0±1.0	17.7±0.6	16.0±0.0
M canis	0.0±0.0	11.6±0.4	15.6±0.4	13.8±0.5	17.0±0.9	19.0±2.0	20.0±0.0
T soudanense	0.0±0.0	10.0±1.0	12.8±0.3	12.6±0.4	13.8±0.3	15.0±2.0	22.9±0.5
T rubrum	0.0±0.0	10.0±0.0	12.9±0.3	12.1±0.3	13.5±0.2	16.0±1.0	20.2±1.1
C tropicalis	0.0±0.0	10.0±0.9	13.0±0.0	12.0±0.0	14.0±0.0	17.3±0.6	19.6±1.5

Values are mean of triplicate determinations; "±" is "standard deviation"

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Table 3: One-way ANOVA for the Efficacy of the Aqueous Extract, Ethanolic Extract, and Fluconazole

	Sum of Squares	df	Mean Square	*F*	Sig.
Between Groups	92.716	2	46.358	14.089	0.001*
Within Groups	39.484	12	3.290		
Total	132.200	14			

*. Significant at the 0.05 level.

The Post-Hoc Analysis (Tukey HSD) revealed that, the ethanolic extract was significantly more effective than the aqueous extract (Mean difference =3.34, $p = 0.032$), Fluconazole was significantly more effective than the aqueous extract (Mean difference = 6.08, $p = 0.001$), and the difference between fluconazole and the ethanolic extract was not statistically significant ($p = 0.081$), indicating comparable efficacy (table 4).

Table 4: Post-Hoc Analysis (Tukey HSD)

					95% CI	
(I) Antifungal	**(J) Antifungal**	**MD (I-J)**	**Std. Error**	**Sig.**	**Lower**	**Upper**
Aqueous extract	Ethanolic extract	-3.34*	1.15	0.032	-6.401	-0.279
	Fluconazole	-6.08*	1.15	0.001	-9.141	-3.019
Ethanolic extract	Aqueous extract	3.34*	1.15	0.032	0.279	6.401
	Fluconazole	-2.74	1.15	0.081	-5.801	0.321
Fluconazole	Aqueous extract	6.08*	1.15	0.001	3.019	9.141
	Aqueous extract	2.74	1.15	0.081	-0.321	5.801

*. The mean difference is significant at the 0.05 level.
MD: Mean Difference
CI: Confidence Interval

Minimum inhibitory concentration and Fungicidal Concentrations of *Vitellaria paradoxa* leaf extracts

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values of *Vitellaria paradoxa* leaf extracts against the tested fungal pathogens are presented in Table 5. The ethanolic extract exhibited stronger antifungal potency (MIC 25 mg/mL) compared to the aqueous extract (MICs 100 to 200 mg/mL) against all tested fungi. Similarly, the ethanolic extract demonstrated lower MFC values,(100 to 200 mg/mL), whereas the aqueous extract failed to achieve fungicidal activity within the tested concentration range (MFC > 200 mg/mL).

Notably, the ethanolic extract was fungicidal at 100 mg/mL for *T. soudanense* and *T. rubrum*, and at 200 mg/mL for *Candida albicans, C. tropicalis*, and *Microsporum cani*s. These results further highlight the superior antifungal efficacy of the ethanolic extract over the aqueous extract.

Table 5: Minimum Inhibitory Concentrations (MIC) and Minimum Fungicidal Concentration (MFCs) of *V paradoxa* Extracts against Clinical fungal Isolates

Pathogens	**Aqueous extract (mg/mL)**		**Ethanolic extract (mg/mL)**	
	MIC	**MFC**	**MIC**	**MFC**
C albicans	200	>200	25	200
M canis	100	>200	25	200
T soudanense	200	>200	25	100
T rubrum	200	>200	25	100
C tropicalis	200	>200	25	200

Acute Toxicity Assessment

No signs of toxicity or mortality were observed in animals administered with both the ethanolic and aqueous extracts of *V. paradoxa* at doses up to 4000 mg/kg (Table 6). All physiological parameters, including respiration rate, body temperature, urination, eye color, weight, behavior, and food intake, remained normal across all dose groups, indicating that the extracts are safe at the tested concentrations.

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Table 6: Acute Toxicity of V paradoxa Extracts

Dose ($mgkg^{-1}$)	Mortality	Respiration rate	Urination	Temperature	Eye colour	Weight	Behavior	Food intake
500	0/3	Normal	No change	No change	Normal	No change	No change	Normal
1000	0/3	Normal	No change	No change	Normal	No change	No change	Normal
2000	0/3	Normal	No change	No change	Normal	No change	No change	Normal
4000	0/3	Normal	No change	No change	Normal	No change	No change	Normal
Water	0/3	Normal	No change	No change	Normal	No change	No change	Normal

DISCUSSION

This study provided compelling evidence for the antifungal potential of *Vitellaria paradoxa* leaf extracts, particularly the ethanolic extract, against a panel of clinically relevant fungal pathogens. Furthermore, the study's acute toxicity assessment suggested a favorable safety profile at the tested concentrations.

The phytochemical screening revealed a rich array of bioactive compounds in both ethanolic and aqueous extracts, including alkaloids, tannins, flavonoids, steroids, and phenols. The exclusive presence of saponins in the aqueous extract and glycosides and carbohydrates in the ethanolic extract highlighted the significant impact of solvent polarity on the extraction efficiency of specific metabolites. This differential extraction aligns with established principles of phytochemistry, where the solubility of compounds is largely dictated by their polarity and the solvent used (Cowan, 1999; Harborne, 1998). For instance, the amphipathic nature of saponins likely favored their extraction in the highly polar aqueous solvent (Okwu, 2004), while the intermediate polarity of ethanol facilitated the dissolution of glycosides and carbohydrates (Kordali *et al*., 2005; Cafarchia *et al*., 2007). The presence of known antifungal compounds such as flavonoids and tannins in both extracts suggested their potential contribution to the observed bioactivity (Sultana *et al*., 2009).

Crucially, the ethanolic extract consistently demonstrated superior antifungal activity compared to the aqueous extract across all tested fungal species (*C. albicans*, *M. canis*, *T. soudanense*, *T. rubrum*, and *C. tropicalis*). This enhanced efficacy was evident in the significantly larger zones of inhibition and lower MIC values. Notably, the ethanolic extract at 200 mg/mL exhibited comparable antifungal activity to the standard drug, fluconazole, against several pathogens, as supported by the non-significant difference observed in the post-hoc analysis ($p > 0.05$). This finding underscored the potential of the ethanolic extract as a source of novel antifungal agents. The lower MIC (25 mg/mL for all pathogens except *M. canis*) and MFC (ranging from 100-200 mg/mL) values for the ethanolic extract further corroborate its potent fungistatic and fungicidal properties, respectively. These results aligned with previous studies that have reported the superior extraction of antimicrobial compounds using ethanol in various medicinal plants (Kordali *et al*., 2005; Cafarchia *et al*., 2007; Falodun *et al*., 2006; Nsor-Atindana *et al*., 2012' Magaji *et al*., 2025), possibly due to better cell wall penetration and dissolution of moderately polar bioactive molecules.

The findings of the current study are also consistent with existing literature highlighting the antifungal properties of *V. paradoxa*. Ahmed *et al*. (2009) and Kalgo *et al*. (2019) have reported the use of *V. paradoxa* extracts against fungal infections, supporting our observation of significant inhibitory activity. Furthermore, the broad-spectrum activity observed in our study against both *Candida* and dermatophyte species aligned with reports by Ahmed *et al*. (2012) and Boyejo *et al*. (2019), who also noted the efficacy of *V. paradoxa* extracts against various fungal strains. The limited activity of the aqueous extract compared to the ethanolic extract in our study mirrors the findings of Boyejo *et al*. (2019), Magaji *et al*. (2025) and the suggestion by El-Mahmood *et al*. (2008) that water-based extractions might be less efficient in extracting active antifungal principles.

The acute toxicity assessment provided encouraging results regarding the safety of both extracts at high doses (up to 4000 mg/kg) in rats. The absence of mortality and any significant changes in physiological parameters suggests a relatively low acute toxicity profile. While our findings differ from some reported LD50 values for *V. paradoxa* extracts (Alhasan *et al*., 2014; Rabo *et al*., 2000), the lack of overt toxicity in our study at the tested concentrations is noteworthy. However, the reported genotoxicity at higher concentrations by Odunola *et al*. (2019) warrants further investigation into the long-term safety and potential genotoxic effects of these extracts, particularly with prolonged exposure or at higher doses. Standardized toxicity assessments across different studies are crucial to establish a comprehensive safety profile for *V. paradoxa* extracts.

In conclusion, this study demonstrates the significant antifungal potential of *V. paradoxa* leaf extracts, with the ethanolic extract exhibiting superior efficacy. The presence of diverse phytochemicals likely contributes to this bioactivity. While the acute toxicity assessment suggested safety at high doses, further research is needed to elucidate the specific antifungal mechanisms of the identified compounds, explore the potential synergistic effects of the extract components, and comprehensively evaluate the long-term safety and genotoxic potential. Based on the notable antifungal efficacy and favorable safety profile observed in this study, *Vitellaria paradoxa* leaves hold significant potential as a promising source of novel antifungal agents.

Conclusion

*Vitellaria paradox*a leaf extracts demonstrated significant antifungal efficacy against key fungal pathogens. The ethanolic extract showed antifungal activity comparable to fluconazole against certain fungi, highlighting its potential as a source of novel agents against drug-resistant infections. No toxicity was observed at doses up to 4000 mg/kg in mice. These results highlight *V. paradoxa* ethanolic leaf extract as a safe and effective antifungal agent with therapeutic potential.

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