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ORIGINAL RESEARCH ARTICLE

GC-MS Profiling and In Vitro Antifungal Efficacy of *Vitellaria paradoxa* Oil Against Dermatophytes and Yeasts

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ABSTRACT

The rising concern of antifungal resistance has necessitated the exploration of natural alternatives. This study investigated the in vitro antifungal efficacy of *Vitellaria paradoxa* oil against *Candida albicans, Microsporum canis*, and *Trichophyton rubrum*. The oil's fatty acid composition was analyzed using gas chromatography. The antifungal activity of the oil was evaluated using the agar well diffusion method, and all tests were conducted in triplicates. The results showed that oleic acid (45.56%) and stearic acid (31.91%) were the predominant components of the oil. The minimum inhibitory concentration (MIC) for all tested pathogens (*Trichophyton rubrum*, *Candida albicans,* and *Microsporum canis*) was 25% oil concentration. The minimum fungicidal concentration (MFC) varied among species, with *Trichophyton rubrum* requiring 50% oil concentration, and *Candida albicans* and *Microsporum canis* requiring 100%. Statistical analysis revealed no significant difference between the antifungal efficacy of 100% *V. paradoxa* oil and fluconazole ($p = 0.627$, $F = 0.276$). These findings suggest that *V. paradoxa* oil has promising antifungal properties, warranting further clinical evaluation as a natural therapeutic alternative for fungal infections.

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INTRODUCTION

Fungal infections pose significant global health challenges, affecting millions of individuals annually, particularly in regions with high humidity and poor hygiene conditions (Vitiello *al*., 2023; Mudenda *et al.,* 2023). These infections range from superficial mycoses, which affect the skin, nails, and hair, to systemic mycoses, which can be life-threatening in immunocompromised individuals (Pathadka *et al*., 2022).

The increasing resistance of fungal pathogens to conventional antifungal agents, such as azoles, polyenes, and echinocandins, has necessitated the exploration of alternative therapies (Fisher *et al*., 2022). The indiscriminate and prolonged use of these antifungal drugs has led to the emergence of resistant fungal strains, rendering standard treatments less effective (Hossain *et al*., 2022; Fisher *et al*., 2022). Additionally, synthetic antifungal agents are often associated with adverse effects, including hepatotoxicity, nephrotoxicity, and drug interactions, limiting their long-term use (Bromley *et al*., 2022). These challenges underscored the need for safer and more effective antifungal alternatives derived from natural sources.

Medicinal plants have long been utilized for their therapeutic properties, with numerous studies highlighting their antimicrobial, anti-inflammatory, and antioxidant activities (Magaji *et al*., 2023). Among these plants, *Vitellaria paradoxa,* commonly known as the shea tree, has gained significant attention due to its economic and medicinal importance (Zhang *et al*., 2018). Native to Africa, *Vitellaria paradoxa* is widely cultivated for its seeds, which yield shea butter, a product extensively used in cosmetics, food, and traditional medicine (Lawal *et al*., 2020a). Beyond its commercial applications, *Vitellaria paradoxa* oil has been reported to possess bioactive compounds with antimicrobial properties, making it a potential candidate for antifungal therapy (Mbaveng *et al*., 2011; Tapondjou *et al*., 2011).

Studies have shown that *Vitellaria paradoxa* oil contains several fatty acids, including oleic acid, stearic acid, and palmitic acid, which contribute to its medicinal properties

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(Pohl *et al.* 2011). These acids have demonstrated antifungal activity against various pathogenic fungi, disrupting their cell membranes, inhibiting spore germination, and impairing metabolic functions (Kordali *et al.,* 2005).

Despite existing reports on the general antimicrobial and antifungal activities of *Vitellaria paradoxa*, there remains a significant gap in the literature concerning its standardized efficacy, dose-dependent response, and direct comparison with established antifungal agents such as fluconazole. Most previous studies have used crude extracts or varied plant parts without a focus on the oil component and its quantified fatty acid composition. Furthermore, limited evidence evaluates its minimum inhibitory and fungicidal concentrations against clinically relevant dermatophytes and yeasts under standardized conditions. This study addresses these gaps by investigating the antifungal potential of *V. paradoxa* oil, its fatty acid profile, and its comparative efficacy with fluconazole using well-characterized fungal isolates and standardized laboratory protocols. The findings would contribute to the growing body of knowledge on plant-based antifungal agents and their role in managing fungal infections. By providing scientific validation for the traditional use of *Vitellaria paradoxa* oil, this research may pave the way for the development of novel antifungal formulations derived from natural sources.

MATERIALS AND METHODS

Sample Collection

1. **Test organisms:**

   Isolates of *Candida albicans, Microsporum canis Trichophyton rubrum* were obtained from Microbiology Laboratory of Bauchi State University Gadau, and used for the study.
2. **Nuts of *Vitellaria paradoxa***
   Fresh nuts of *Vitellaria paradoxa* were purchased from Azare Central Market, Katagum Local Government Area, Bauchi State, Nigeria. The nuts were authenticated by Dr. Umar Aminu Muhammad of the Department of Biological Sciences, Bauchi State University, Gadau. A voucher specimen was deposited in the university herbarium for future reference.

Extraction of the Plant's Oil

The nuts of the plant were boiled in water and allowed to dry. They were cracked in a motor to remove the kernel. The kernels were then crushed, and a shea nut pulp was formed. The pulp is then continuously stirred with the addition of hot water. This was then followed by skimming of the milky liquid formed. This was followed by boiling the liquid, after which the oil was decanted from the mixture. While cooling, the oil was homogenized and sieved molten into a clean, empty container. This was then allowed to solidify (Jatto *et al.*, 2010).

Gas Chromatographic Analysis of the *Vitellaria paradoxa* Oil

About 2 mL of the oil sample was diluted with hexane to reduce its viscosity and improve its flow through the chromatograph. The diluted sample was then filtered to remove any particulate matter that might clog the GC system. The analysis was conducted on HP-5890 gas chromatograph built-in with a HP-5 capillary columns of 30m x 0.25mm inner diameter, and 0.25µm film thickness, and HP-Wax. About 2 µL of the sample was injected into the GC system. Flame Ionization Detector (FID) was selected for the analysis. The injector and transfer line temperatures were kept at 220°C and 240°C, respectively. The GC oven temperature was programmed from 60 to 240°C at 3°C/min. Helium was used as a carrier gas at a 1 mL/min flow rate. The peak areas were integrated, and the calibration curves were used to quantify components. The retention times of the peaks were compared to known standards, and their mass spectra were compared with the published spectra of the reference compounds from NIST 98 (Adams, 1995; Aboaba *et al.*, 2014).

Preparation of Different Concentrations of *Vitellaria paradoxa* Oil

Different concentrations of the oil (100%, 50%, and 25%) were prepared from the 100% oil solution (undiluted oil). 50% oil solution was prepared by mixing 5 mL of *Vitellaria paradoxa* oil with 5 mL of 10% (v/v) DMSO. This was then vortex until a uniform emulsion is formed. For the 25% solution, 5 mL of 50% *Vitellaria paradoxa* oil was mixed with 5 mL of 10% (v/v) DMSO and vortex to ensure proper mixing. Sterile 10% (v/v) DMSO was used as negative control, and fluconazole as positive control (Das *et al.*, 2010; Balouiri *et al.*, 2016).

Antifungal Potency of *Vitellaria paradoxa* Oil

Agar well diffusion method was used for this study. The prepared fungal suspension ($1x10^6$ cells/mL) was evenly swabbed onto the prepared agar plate. A sterile cork borer was used to punch 6 mm diameter wells into the agar. 50 µL of each concentration (100%, 50%, 25%) was added into separate wells. 50 µL of DMSO was added into another well as a negative control. 50 µL of fluconazole (25 µg/mL) was used as a positive control. The plates were allowed to stand at room temperature for 30 minutes for proper diffusion. These were then Incubated at 28–30°C for 2–7 days. The diameters of the inhibition zones (mm) were measured using a ruler, and the results were recorded and compared with controls (CLSI, 2017). All tests were performed in triplicate.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The MIC was determined using the broth dilution method (CLSI, 2009). Sabouraud Dextrose Broth was prepared and sterilized. 1 mL of broth was dispensed into test tubes and mixed with 1 mL of each of the oil concentrations

(100%, 50%, 25% and 12.5%), followed by the addition of 0.1 mL of fungal inoculum (1×10^6 spores/mL). Controls without oil were included. The tubes were incubated at 30°C for 1–7 days, and the MIC was determined as the lowest concentration showing no visible growth.

To determine the MFC, the contents of MIC tubes were subcultured onto fresh SDA plates, incubated at 30°C for 1–7 days, and observed for growth. The MFC was the lowest concentration, with no fungal growth on the agar surface (CLSI, 2009).

RESULTS

Gas Chromatographic Analysis

The gas chromatographic analysis of *Vitellaria paradoxa* oil identified the presence of various fatty acids in different proportions. The major components included Oleic acid (45.56%), Stearic acid (31.91%), and Palmitic acid (10.12%), while minor components comprised Linoleic acid (4.47%), Linolenic acid (0.53%), Arachidic acid (0.20%), Eicosanoic acid (0.23%), Behenic acid (0.11%), and Myristic acid (0.12%) (Figure 1a and b).

Antifungal Activity of *V. paradoxa* Oil

The antifungal activity of *V. paradoxa* oil was evaluated at different concentrations (100%, 50%, and 25%) against *Candida albicans*, *Microsporum canis*, and *Trichophyton rubrum*. At full concentration (100%), the oil exhibited inhibition zones of 17.33±0.57 mm, 19.07±0.11 mm, and 17.47±0.50 mm against *C. albicans*, *M. canis*, and *T. rubrum*. A reduction in concentration led to a decrease in antifungal activity. At 50% concentration, the inhibition zones were 14.33±0.57 mm, 14.20±0.34 mm, and 15.08±0.14 mm, while at 25%, they were 11.13±0.11 mm, 8.87±0.11 mm, and 10.2±0.17 mm, respectively (Table 1).

Statistically, the ANOVA results showed no significant difference between the antifungal efficacy of 100% *V. paradoxa* oil and fluconazole ($p = 0.627$, $F = 0.276$) (Table 2).

Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The Minimum Inhibitory Concentration (MIC) for all tested pathogens was found to be 25% of the oil concentration. However, the Minimum Fungicidal Concentration (MFC) varied among pathogens. While *T. rubrum* required 50% oil concentration for fungicidal activity, *C. albicans* and *M. canis* required 100% oil concentration (Table 3).

DISCUSSION

Gas Chromatographic Analysis

The gas chromatographic analysis of *Vitellaria paradoxa* oil identified several fatty acids, with oleic acid (45.56%) and stearic acid (31.91%) as the most abundant. These findings aligned with previous studies, though variations exist due to differences in extraction methods, geographical sources, plant maturity, and environmental conditions. Such factors influence lipid synthesis and may account for the observed disparities in fatty acid composition.

Jatto *et al.* (2010) reported oleic and stearic acid levels similar to that reported in the current study, supporting methodological consistency. However, differences in the least abundant fatty acids, such as behenic acid in the current study versus myristic acid in theirs, suggested variations due to extraction techniques and regional differences in plant growth conditions. Considering the least abundant fatty acids, factors such as extraction solvents and techniques may play a pivotal role. The choice of solvents can selectively influence the extraction of certain fatty acids, contributing to the observed discrepancies in myristic and behenic acid levels. Geographical variations in plant sources may also account for differences in fatty acid composition, as environmental factors influence lipid synthesis. Furthermore, variations in plant maturity, nutrient availability, and cultivation practices could contribute to the nuanced differences in the least abundant fatty acids (Jatto *et al.*, 2010).

Antifungal Activity of *V. paradoxa* Oil

The antifungal efficacy of *V. paradoxa* oil was demonstrated through its dose-dependent inhibitory activity against *Candida albicans, Microsporum canis*, and *Trichophyton rubrum*. At 100% concentration, the oil produced inhibition zones comparable to fluconazole, with no statistically significant difference between them ($p = 0.627$). This suggested that *V. paradoxa* oil may be a potential alternative to synthetic antifungal agents, particularly in resource-limited settings.

The minimum inhibitory concentration (MIC) of *V. paradoxa* oil was determined to be 25%, while the minimum fungicidal concentration (MFC) ranged between 50% and 100% of the oil concentrations. These findings underscored the oil's potential as a natural antifungal agent, are consistent with the previous studies in the different regions of the country and the world at large.

Ahmed *et al.* (2009) and Kalgo *et al.* (2019) documented the antimicrobial activity of various extracts of *V paradoxa*, aligning with the current study's findings. The presence of bioactive compounds with antifungal properties, as highlighted by Fodouop *et al.* (2015), supported the observed inhibitory effects. Additionally, the antifungal activity of fatty acids may contribute to the efficacy of *V. paradoxa* oil (Pohl *et al.*, 2011).

Furthermore, other study by Ahmed *et al.* (2012) highlighted bioactive compounds in various parts of the *V. paradoxa* extracts effective against specific fungal

strains. Similarly, Fodouop *et al.* (2015) discussed various therapeutic uses of *V. paradoxa*, which include managing dermatitis and skin conditions. This also confirmed the current study's findings, which focused more on the idea that natural compounds exhibited antifungal properties.

A study by Prescott *et al.* (2002) documented the medicinal properties of *V. paradoxa* against different kinds of microorganisms, supporting the relevance of investigating its efficacy against fungal infections. On the other hand, Pohl *et al.* (2011) noted the antifungal activity of fatty acids extracted from *V. paradoxa*, which aligned with the observed inhibitory activity of *V. paradoxa* oil in this study.

Additional studies by Kordali *et al.* (2005) reported that essential oils possess various properties, including antibacterial, antifungal, antiviral, insecticidal, and antioxidant properties. Similarly, Ozcan *et al.* (2006) and Cafarchia *et al.* (2007) reported antibacterial and antifungal activity of plant extracts. Also, Vardar-Unlu *et al.* (2003) and Salehi *et al.* (2005) discussed the antimicrobial, anticancer, antiviral, and antioxidant properties of certain compounds from *V paradoxa* against various pathogens, including fungi. Further, Ahmad & Beg (2001) highlighted the potential of natural compounds against drug-resistant pathogens. Additionally, Wahedi & David (2014) discussed the effectiveness of essential oils and Shea butter oil as antifungal agents compared to standard drugs like co-trimoxazole and penicillin. The present study corroborates these findings, by demonstrating the antifungal activity of *V. paradoxa* oil against different fungal species.

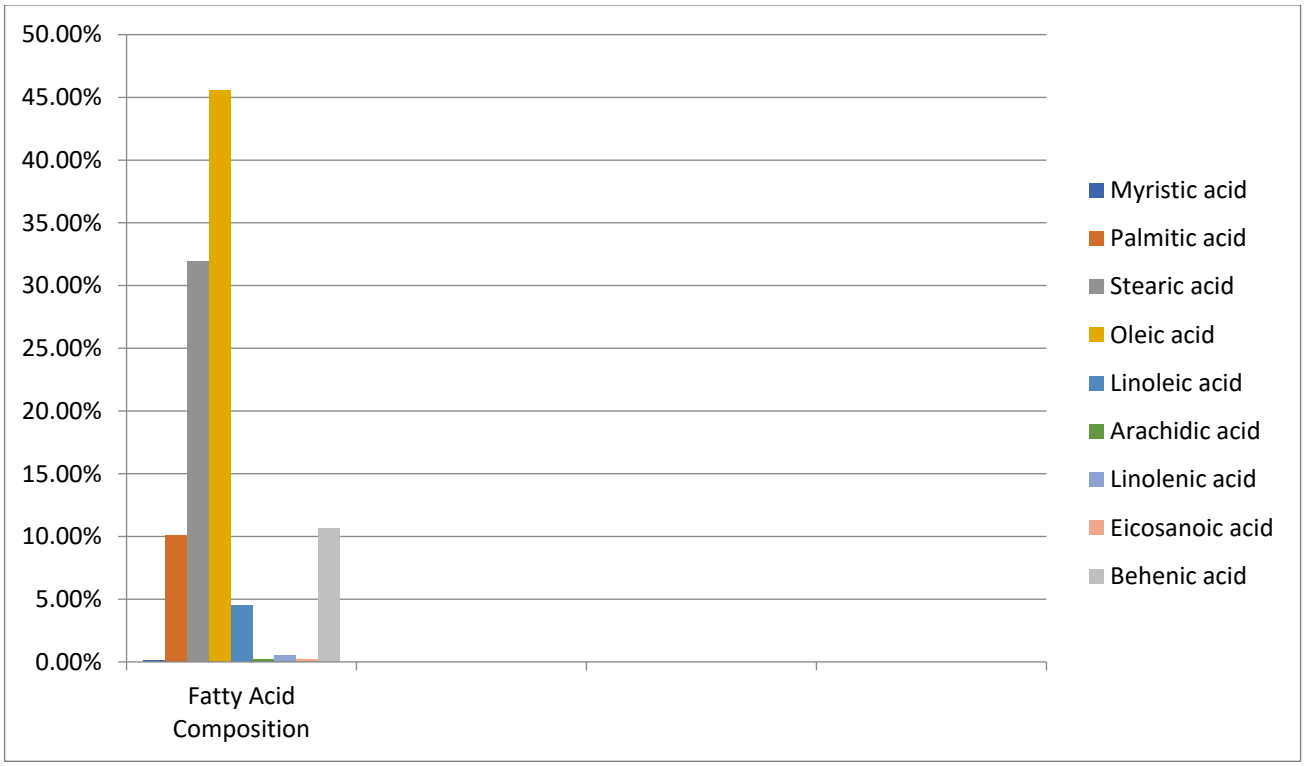

Figure 1a: Constituents of *V paradoxa* Oil

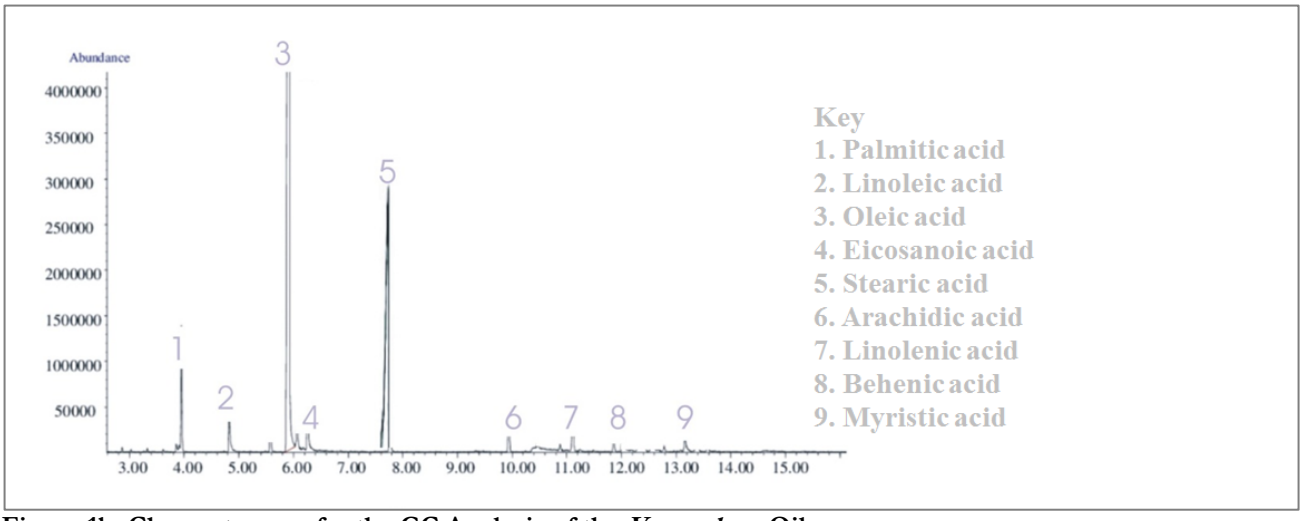

Figure 1b: Chromatogram for the GC Analysis of the *V paradoxa* Oil

Table 1 Antifungal Activity of the Oil of *V paradoxa*

Pathogens	Oil Solutions			Controls	
	100% (mm)	50% (mm)	25% (mm)	DMSO (mm)	Fluconazole (mm)
C albicans	17.33±0.57	14.33±0.57	11.13±0.11	0±0.0	16.00±00
M canis	19.07±0.11	14.20±0.34	8.87±0.11	0±0.0	20.00±0.00
T rubrum	17.47±0.50	15.08±0.14	10.2±0.17	0±0.0	20.20±1.11

Key: (±) = mean standard deviation

Table 2: Comparison between Efficacy of 100% of the *V paradoxa* Oil and Fluconazole Using One Way ANOVA

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	0.905	1	0.905	0.276	0.627
Within Groups	13.096	4	3.274		
Total	14.001	5			

Key: "Df" = degree of freedom, "Sig" = significant at $p<0.05$

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of *V paradoxa* Oil

Pathogens	MIC (%)	MFC (%)
C albicans	25	100
M canis	25	100
T rubrum	25	50

CONCLUSION

This study provides compelling evidence for the antifungal potential of *Vitellaria paradoxa* oil, positioning it as a viable natural alternative to conventional antifungal agents. Gas chromatographic analysis revealed a rich composition of bioactive fatty acids—primarily oleic and stearic acids—which likely contribute to its antimicrobial effects. The oil demonstrated significant inhibitory and fungicidal activity against *Candida albicans*, *Microsporum canis*, and *Trichophyton rubrum*, with inhibition zones at 100% concentration comparable to those of fluconazole. Notably, there was no statistically significant difference between the antifungal efficacy of 100% *V. paradoxa* oil and fluconazole (p = 0.627), underscoring its therapeutic potential. The finding that the minimum inhibitory concentration (MIC) was uniformly 25% for all tested pathogens, while minimum fungicidal concentration (MFC) varied, reflects both the broad-spectrum activity and species-specific response of the oil. These results suggest that *V. paradoxa* oil could serve as an accessible and affordable antifungal treatment, especially in low-resource settings where synthetic drugs may be costly or unavailable.

To fully harness this potential, future research should focus on optimizing the oil's formulation, conducting in vivo studies, and assessing safety through toxicological evaluations and clinical trials. With further development, *V. paradoxa* oil could become a cornerstone in the fight against fungal infections and antifungal resistance, offering a sustainable and locally sourced solution to a growing global health challenge.

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