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PHYTOCHEMISTRY AND ANTIMICROBIAL POTENCY OF VITELLARIA PARADOXA ROOTS METHANOLIC FRACTION AGAINST SELECTED BACTERIA

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ABSTRACT

This study investigated the phytochemical composition and antimicrobial potency of a methanolic root fraction from Vitellaria paradoxa, a plant widely utilized in traditional medicine. Through TLC analysis, multiple fractions with distinct Rf values were identified, indicating a diverse chemical profile. Both the crude extract and its fractions demonstrated significant bacteriostatic activity against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Salmonella typhi, with the F1 fraction (Rf 0.33) showing the highest potency (MIC of 6.25 mg/ml against most tested bacteria). Qualitative and quantitative phytochemical screening confirmed the presence of various bioactive compounds, notably high concentrations of phenols (9.73 mg/g) and tannins (7.44 mg/g), alongside flavonoids, alkaloids, and saponins. These findings validate the traditional use of V. paradoxa roots and highlight its potential as a source of novel antimicrobial agents to combat escalating drug resistance.

Keywords: Antimicrobial, Fraction, Phyto-chemistry, Safe Dose, *Vitellaria paradoxa*

INTRODUCTION

Medicinal plants are known to have a wide range of bioactive compounds that have antimicrobial, antifungal, anticancer, anti-inflammatory, and antioxidant activities (Konappa et al., 2020 and Jagannath et al., 2021). Many researchers have documented the potent activity of plants' bioactive compounds on drug-sensitive and resistant bacteria (Shakeri et al., 2018). Although plants contain a very large number of bioactive compounds, few have been discovered (Konappa et al., 2020). Gakuya et al. (2020), reported that the term "traditional medicine" is a broad term incorporating various systems and forms of indigenous medicine. It is also referred to as folk medicine or ethno-medicine. The world health organization (2023) has defined the term as referred totality of health practices, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercise, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being. Badal and Delgoda (2017) have defined the term as the total of the knowledge skills and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illness. It is a system of health practice based on indigenous knowledge (also called folk knowledge, indigenous knowledge or ancestral knowledge) that has over a long span of time been passed on from generation to generation (Mukhwana, 2021).

Iwuchukwu et al. (2023) reported that over 80% of people in Africa receive care from Traditional African Medicine (TAM), which is part of our socioeconomic and cultural legacy. They further said that Nigerian traditional herbal medicine has made significant contribution to the primary health care needs of Nigerians from time immemorial. However, there is significant room for improvement in its development and utilization. In most communities, they have served as the primary and, in some cases, the only source of healthcare for many millions of people. This is because they are accessible and reasonably priced. They are trusted by a vast majority of the populace, having been embraced culturally. Due to the current economic set back in Nigeria and other developing countries, the affordability of these herbal medicines has made them to be in high demand as alternatives for primary health needs of majority of the populace. A significant number of efforts have been made to translate herbs used locally into standardized dosage forms, however there is still a lot to be done in this regard. Herbal medicines, whose potency, efficacy and safety have been scientifically validated, can help to achieve the goal of ensuring that everyone has access to healthcare.

Vitellaria paradoxa (Shea buter) belongs to the family Sapotaceae it is commonly known as shea tree. Vitellaria paradoxa is used in traditional medicine for treatment of various ailments including inflammation, fever, skin irritation, dermatitis, sunburn, rheumatism, diarrhea, stomachache, and ulcers (Nazifi et al., 2020). However, the efficacy of these plant extracts and their fractions against specific species of bacteria, remains insufficiently characterized. The aim of this study is to assess phytochemical composition and antimicrobial potency of the methanolic root fraction of Vitellaria paradoxa (shea tree) on some species of bacteria. The investigation of this study is critical due to the escalating global challenge of antimicrobial resistance. This study was revealing the best concentration of root extracts and fractions that inhibit the growth of bacteria and also the bioactive constituents that exhibit such control. This enhances place of these species on the ladder of pharmaceutical industries and ethno medicinal as well as enhance studies in microbial.

MATERIALS AND METHODS

The Study Area

The study area is Bayo Local Government Area situated in the southern part of Borno State, northeastern Nigeria, located between Latitude 10025'21'' N to 10042'25'' N and between Longitude 11042'22'' E to 11070'62''E in an elevation of 950 ft (290 m) above sea level. The study area is within the Sudan Savanna ecological zone. The region is characterized by distinct semi-arid climatic conditions with two prominent seasons: the rainy season typically occurs between June and September, with an average annual rainfall of 600–1000 mm and the dry season spanning from October to May, the dry season is dominated by the Harmattan winds, which blow from the northeast across the Sahara Desert, bringing dust-laden and dry air (Adebayo and Tukur, 2020b). This season is characterized by very low humidity and prolonged dry spells. The Temperature of the study area remain consistently high, with peak temperatures exceeding 40°C between March and May, and cooler months (December to January) averaging 25°C to 30°C (Nigerian Meteorological Agency (NiMet), 2025). And the vegetation type is savanna vegetation, typical of northern Nigeria's transition between the Sahel and Guinea Savannah belts (Adedire et al., 2020). This vegetation type is adapted to seasonal rainfall and extended dry periods. Scattered Trees: The landscape is dominated by drought-resistant trees, including: Vitellaria paradoxa (Shea butter), Balanites aegyptiaca (Desert Date), Acacia species, Parkia biglobosa (African Locust Bean), Azadirachta indica (Neem), and Adansonia digitata (Baobab) (Adebayo and Tukur, 2020).

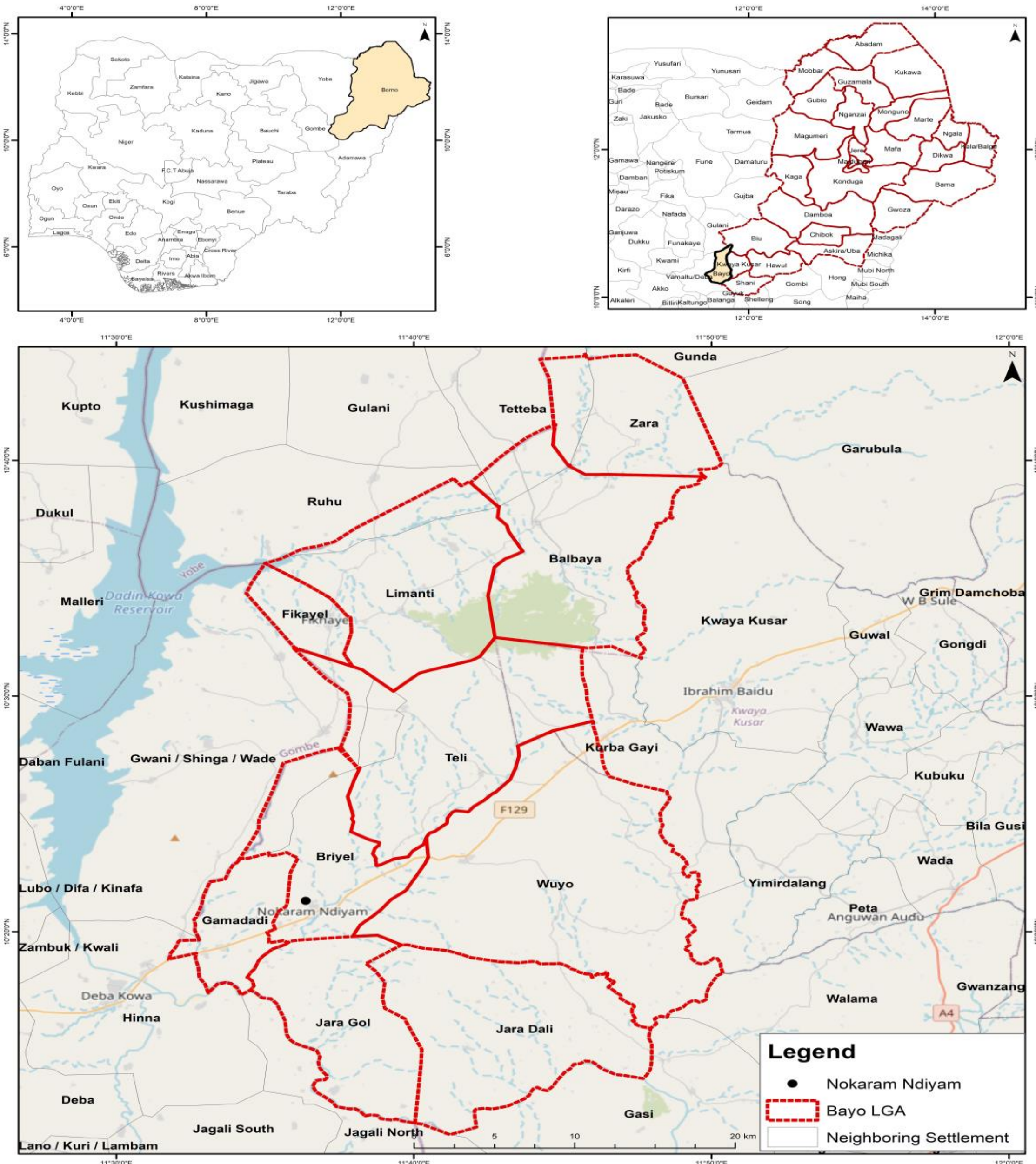

Figure 1: Map of the Study Area
Source: Extracted from www.earth.google.com (2024)

Data Collection

The plant samples of Vitellaria paradoxa roots were collected from the study area, an area well-known for its diverse savanna vegetation where the species is commonly found in the wild and utilized traditionally for medicinal purposes. The selection of the study site was based on the natural abundance and ethnobotanical relevance of the species within the local communities. A purposive sampling technique was employed to identify the mature Vitellaria paradoxa plants that exhibited no visible signs of disease or pest infestation. This method was chosen to ensure that only healthy specimens were selected for phytochemical and antimicrobial analysis. A systematic sample collection procedure was followed in which three replicate samples from each root were collected from multiple mature plants within different sites in the study area to ensure representativeness. The samples were pooled, cleaned, and shade-dried to preserve bioactive compounds before being pulverized for extraction. These procedures ensured that the collected plant materials were of high quality, free from contamination, and sufficiently representative of the species' characteristics in the region. The root was collected based on their traditional medicinal usage and reports of potential bioactivity from previous studies (Badal and Delgoda, 2017; Yakubu et al., 2020). To ensure sample authenticity, the collected plant materials were identified and authenticated at the Herbarium of the Department of Plant Science, Faculty of Life Sciences, Modibbo Adama University (MAU), Yola, where a voucher specimen number MAU/PLS/SB/0047 was assigned for future reference. The research activities, including sample preparation, extraction, phytochemical screening and antimicrobial testing were carried out at the Biochemistry and Microbiology laboratories of Modibbo Adama University.

Extraction of Vitellaria paradoxa Roots

The extraction of the Vitellaria paradoxa roots was carried out following the method described by Jean-Bosco et al. (2020) with slight modifications. The collected root samples were first shade-dried to prevent the degradation of heat-sensitive bioactive compounds. Once completely dried, the samples were ground into fine powder using a wood mortar and piston milling machine to increase the surface area for effective solvent penetration. The powdered root was then subjected to solvent extraction using the maceration method. In this process, 350g of the powdered sample was immersed in 750 mL of 80% methanol. The mixture was allowed to stand for 72 hours with intermittent shaking, facilitating the dissolution of phytochemical constituents into the solvent. After maceration, the extract was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated to dryness under reduced pressure using a rotary evaporator. The concentrated extract was further air-dried to remove residual solvent and then stored in airtight containers at 4°C until required for further phytochemical and antimicrobial analyses. This method ensures the efficient extraction of bioactive compounds and is widely adopted for preparing plant extracts for pharmacological studies.

Bacterial Species

The bacterial strains used in this study were obtained from the Department of Microbiology, Modibbo Adama University, Yola, Nigeria. The strains were initially isolated and maintained on sterile Nutrient Agar (NA) slants under aseptic conditions. The cultures were incubated at an appropriate temperature of 28 ± 2°C to promote optimal growth and were subsequently stored at 4°C for preservation until required for experimental analysis. This method of isolation and storage ensured the viability and purity of the bacterial cultures throughout the research period, in line with standard microbiological practices (Osuntokun et al., 2018). The tests bacteria that were used in this study were Bacillus subtillus, Staphylococcus aureus, Salmollena typhi and Escherichia coli.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) of V. paradoxa root Extracts against Bacillus subtilus, Staphylococcus aureus, Escherichia coli and Selmonella typhi

The antimicrobial activity for the root extracts of V. paradoxa was evaluated using the Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) methods, following the standard procedures outlined by the Clinical and Laboratory Standards Institute (CLSI, 2012).

The MIC activity of V. paradoxa root extracts test on bacteria samples was investigated using agar diffusion method. Six serial dilutions of the root extract were prepared in sterile broth media to achieve varying concentrations of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml 12.5mg/ml and 6.25mg/ml was introduced into each dilution tube. The inoculated tubes were incubated at 28 ± 2°C for 48-72 hours, after which they were examined for turbidity or visible growth. The lowest concentration of the extract that inhibited visible growth was recorded as the MIC. The antibiotics used as control were ciprofloxacin for bacteria according to Ekhuemelo et al. (2018). The MBC was then determined by sub-culturing samples from tubes that showed no growth in the MIC test onto Potato Dextrose Agar (PDA) plates. The plates were incubated under the same conditions. The MBC was defined as the lowest concentration of the extract that resulted in no bacterial growth on the agar plates, indicating a bacterial effect. These methods allowed for a quantitative assessment of the antibacterial efficacy of the root extract.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) of V. paradoxa root Fraction against Bacillus subtilus, Staphylococcus aureus, Escherichia coli and Selmonella typhi

The MIC activity of V. paradoxa root fraction test on bacteria samples was investigated using agar diffusion method. Six serial dilutions of the root fraction were prepared in sterile broth media to achieve varying concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml 6.25mg/ml and 3.12mg/ml was introduced into each dilution tube. The inoculated tubes were incubated at 28 ± 2°C for 48-72 hours, after which they were examined for turbidity or visible growth. The lowest concentration of the extract that inhibited visible growth was recorded as the MIC. The antibiotics used as control were ciprofloxacin for bacteria according to Ekhuemelo et al. (2018). The MBC was then determined by sub-culturing samples from tubes that showed no growth in the MIC test onto Potato Dextrose Agar (PDA) plates. The plates were incubated under the same conditions. The MBC was defined as the lowest concentration of the fraction that resulted in no bacterial growth on the agar plates, indicating a bacterial effect. These methods allowed for a quantitative assessment of the antibacterial efficacy of the root fraction.

Thin Layer Chromatography of Crude Extracts

The Analytical thin layer chromatographic technique was done according to the method described by (Oloke et al., 1988) to spot, separate and determine the Retention factors (Rf) values and a suitable solvent system for fractionation of the phytochemical components by column chromatography on the crude extracts. This was achieved by using the TLC silica gel 60 F254 Aluminum sheet made by Merck KGaA, Millipore Corporation Germany was used as the stationary phase for the most active extracts. The solvent system that gave the best separation based on the Rf values were used to fractionate the crude extracts by column chromatography, while the number of spots seen was recorded.

The Column Chromatography Fractionation of the Vitellaria Paradoxa Root Extracts

The root extracts of Vitellaria Paradoxa were subjected to column chromatography for fractionation. The procedure was carried out following the method described by Ighodaro et al. (2016), with slight modifications to suit the extract characteristics.

Determination Phytochemical Analysis of Vitellaria Paradoxa root Fraction
Qualitative and Quantitative phytochemical test were carried out using Standard Phytochemical Screening Methods of Okoli et al. (2023).

RESULTS AND DISCUSSION

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Root Extracts of *V. paradoxa* against Bacillus subtilus, *Staphylococcus aureus*, *Escherichia coli* and *Selmonella typhi*
The antimicrobial screening results presented in Table 1 indicate that, the root extracts of Vitellaria paradoxa exhibited inhibitory activity against Bacillus subtilus, Staphylococcus aureus, Escherichia coli and Selmonella typhi. The Minimum Inhibitory Concentration (MIC) for all bacterial species were determined to be 6.26 mg/ml, this indicate that it possesses strong bacteriostatic activity, effectively inhibiting bacterial growth at low concentrations while the Minimum Bactericidal Concentration (MBC) exceeded 200 mg/ml indicate that the extract does not exert a bactericidal effect within the tested concentration range. The result of this study aligns with previous findings of Ojo et al. (2023), who reported strong antibacterial activity of V. paradoxa root extracts against S. aureus and E. coli at MICs of 5 to 10 mg/ml, attributed to high concentrations of flavonoids and phenolic compounds. Studies by Adebayo and Ishola (2020) also reported that V. paradoxa root extracts show promising antimicrobial effects against enteric pathogens, highlighting their ethno-medicinal relevance in treating gastrointestinal infections. These findings imply that, the extract was effective in inhibiting the growth of the organisms at relatively low concentrations, it did not exhibit strong bactericidal activity at concentrations up to 200 mg/ml. This large gap between MIC and MBC values suggests that the extract is primarily bacteriostatic rather than bactericidal.

Table 1: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Root Extracts of V. paradoxa against Bacillus subtilus, Staphylococcus aureus, Escherichia coli and Selmonella typhi

Plant Parts	Bacteria	Concentration (Mg/mL)						MIC Mg/mL	MFC Mg/Ml
		200	100	50	25	12.5	6.25		
Root	*S. aureus*	-	-	-	-	-	-	6.25	> 200
	B. subtilus	-	-	-	-	-	-	6.25	> 200
	E. coli	-	-	-	-	-	-	6.25	> 200
	Salmonella typhi	-	-	-	-	-	-	6.25	> 200

Key: - No growth, + Growth, > Greater than

Analysis Thin Layer Chromatography of Extracts
This table presents the results of a Thin Layer Chromatography (TLC) analysis performed on root extracts of Vitellaria paradoxa, specifically using a solvent system of Methanol: Ethyl Acetate: N-hexane in a 2:1:2 ratio. TLC is a common analytical technique used in phytochemistry to separate components of a mixture, allowing for the visual identification of different compounds or "spots" and the calculation of their retention factors (Rf values). Similarly, Tsafe et al. (2019) reported that V. paradoxa is generally has more polar, some saponins might show distinct Rf values depending on the solvent system's polarity and their glycosylation patterns. Tapondjou et al. (2011) reported that V. paradoxa is rich in triterpenoids, with some studies specifically isolating them from root bark. These compounds often have intermediate to high Rf values in less polar solvent systems, depending on their specific functional groups (Table 2).

Table 2: *Vitellaria Paradoxa* Root Extracts

Solven System	NS	DMS Cm	DMF Cm	RF Cm
Methanol: ethyl acetate: N-hexane				
2:1:2		5.4	4.9	0.91
1	F1	5.4	1.8	0.33
	F2	5.4	2.9	0.54
	F3	5.4	4.3	0.79

Key: NS= Number or – Spot, DMS = Distance more by solvent, DMF = Distance move by fraction, RF = Retention factor

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Root of V. paradoxa Fraction against Bacillus subtilus, Staphylococcus aureus, Escherichia coli and Selmonella typhi
The result of the study in Table 3 reveals that, the antibacterial activity of chromatographic fractions (F1, F2, F3) derived from Vitellaria paradoxa root extracts against four pathogenic bacterial species (Staphylococcus aureus, Bacillus subtilus, Escherichia coli, and Salmonella typhi). The assessment was based on Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) using standard broth micro-dilution techniques. Fraction F1 (Rf = 0.33) showed the highest antibacterial potency, exhibiting inhibitory effects at 6.25 mg/ml against all tested bacteria, except S. typhi, which had an MIC of 12.5 mg/ml. However, MBC values for all organisms exceeded 100 mg/ml, indicating that the fraction effectively suppressed bacterial growth, it lacked significant bactericidal activity at the tested doses. The MIC pattern observed, implies that F1 may contain highly active polar bioactive constituents with strong growth-inhibitory but not lethal properties.
Fractions F2 (Rf = 0.54) and F3 (Rf = 0.79) exhibited moderate antibacterial activity, with MICs consistently at 25 mg/ml for all test organisms, and similarly high MBC values (>100 mg/ml). The reduced activity compared to F1 suggests a concentration gradient or loss of synergistic effects among compounds during separation. It also reflects the possible localization of active principles in earlier-eluting polar fractions. The consistent sensitivity of both Gram-positive (S. aureus, B. cereus) and Gram-negative (E. coli, S. typhi) bacteria across fractions supports the broad-spectrum potential of V. paradoxa root-derived compounds. These results are in line with previous findings by Ibrahim et al., (2018) and Adedapo et al., (2020), who reported the presence

of antibacterial compound in V. paradoxa extracts. The notably low MICs but high MBCs once again suggest a bacteriostatic mechanism of action rather than bactericidal. This might be due to the ability of the bioactive components to inhibit bacterial enzyme systems, nucleic acid synthesis, or membrane permeability without directly killing the organisms (Ghosh et al., 2019; Suleiman et al., 2021).

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Root Fraction of V. paradoxa against Staphylococcus aureus, Bacillus subtilus, Escherichia coli and Selmonella typhi

Fraction (Rf)	Bacteria	Concentration (Mg/mL)						MIC Mg/mL	MBC Mg/mL
		100	50	25	12.5	6.25	3.12		
F1 (0.33)	S. aureus	-	-	-	-	-	+	6.25	> 100
	B. subtilus	-	-	-	-	-	+	6.25	> 100
	E. coli	-	-	-	-	-	+	6.25	> 100
	Salmonella typhi	-	-	-	-	+	+	12.5	> 100
F2 (0.54)	S. aureus	-	-	-	+	+	+	25	> 100
	B. subtilus	-	-	-	+	+	+	25	> 100
	E. coli	-	-	-	+	+	+	25	> 100
	Salmonella typhi	-	-	-	+	+	+	25	> 100
F3 (0.79)	S. aureus	-	-	-	+	+	+	25	> 100
	B. subtilus	-	-	-	+	+	+	25	> 100
	E. coli	-	-	-	+	+	+	25	> 100
	Salmonella typhi	-	-	-	+	+	+	25	> 100

Key: +Growth, - No Growth, Greater than >

Phytochemical Profile of Vitellaria Paradoxa Root Fractions

The phytochemical screening of Vitellaria paradoxa root fractions (Table 4) revealed the presence of several classes of bioactive secondary metabolites. Specifically, saponins, tannins, alkaloids, flavonoids, phenols, steroids, and terpenoids were detected, while glycosides were absent. The result of this study is in line with previous reports by Adetutu et al. (2013) and Olaniyan et al. (2020), which documented the presence of similar bioactive components in various parts of V. paradoxa. Interestingly, glycosides were not detected, which may suggest a lower likelihood of certain cytotoxic or cardiac activities often associated with this class, making the extract potentially safer for therapeutic applications.

These phyto-constituents are known for their wide-ranging pharmacological effects. Flavonoids and phenols, for example, are potent antioxidants and antimicrobial agents, capable of scavenging free radicals and disrupting microbial cell membranes (Ghasemzadeh and Ghasemzadeh, 2011). The presence of alkaloids supports the antibacterial activity observed in previous assays, as alkaloids are known to inhibit nucleic acid synthesis and bacterial protein functions (Cushnie et al., 2014). Saponins, known for their surfactant properties, may contribute to antimicrobial efficacy by increasing cellular membrane permeability (Avato et al., 2006). Tannins are astringent polyphenols that bind to microbial proteins and enzymes, thereby inhibiting growth (Scalbert, 1991). The detection of terpenoids and steroids further indicates a complex phytochemical structure, as these compounds are associated with anti-inflammatory and membrane-stabilizing effects (Dwivedi and Tripathi, 2010).

Table 4: Phytochemical Profile of Vitellaria paradoxa Root Fractions

S/N	Phytochemicals	V. paradoxa
1	Saponin	+
2	Tannins	+
3	Alkaloids	+
4	Flavonoids	+
5	Phenols	+
6	Steroids	+
7	Terpenoids	+
8	Glycoside	_

Key: + = present; - = absent

Quantitative Phytochemical Composition of Vitellaria paradoxa Root Fraction

The quantitative phytochemical analysis of Vitellaria paradoxa root fraction (as shown in the table 5) revealed varying concentrations of key bioactive compounds. The most abundant constituents were phenols (9.73 mg/g) and tannins (7.44 mg/g), followed by flavonoids (6.88 mg/g), alkaloids (3.01 mg/g), and saponins (2.53 mg/g). Steroids were detected in lower amounts (0.66 mg/g), while terpenoids and glycosides were not detected.

The result of the study is largely consistent with recent findings by Yakubu et al., (2022), who reported high concentrations of phenolics and flavonoids in the methanolic root extract of V. paradoxa, noting values of 8.95 mg/g and 7.22 mg/g respectively, which are closely to the value obtained in this research. These compounds are known to exert strong antioxidant and antimicrobial effects, mainly by scavenging free radicals and disrupting microbial membranes. Similarly, Adewumi et al., (2023) identified saponins (2.12 mg/g) and alkaloids (3.45 mg/g) in V. paradoxa root extract, which are within the same value range of the current study. The presence of alkaloids is particularly important as they are implicated in bacterial DNA disruption and enzyme inhibition, which aligns with the extract's antimicrobial efficacy against E. coli, S. aureus, and Salmonella typhi. The absence of glycosides and terpenoids in the current analysis diverges slightly from Ogunlade et al., (2021), who reported minor traces of terpenoids in leaf and seed extracts of V. paradoxa. This variation may be attributed to plant part specificity, geographic origin, or seasonal factors, all of which influence phytochemical synthesis and accumulation (Abdullahi et al., 2022). Furthermore, the lower concentration of steroids (0.66 mg/g) is consistent with findings by Tapsoba et al., (2020), who noted that steroidal compounds in V. paradoxa roots are generally less abundant compared to leaves or bark. Despite the low levels, steroids may still contribute to membrane stabilization and anti-inflammatory activity.

Table 5: Quantitative Phytochemical Composition of Vitellaria paradoxa Root Extract

Phytochemicals	Saponins	Tannins	Alkaloids	Flavanoids	Phenols	Steroids	Terpenoids	Glycosides
V. paradoxa	2.53	7.44	3.01	6.88	9.73	0.66	-	-

CONCLUSION

This study comprehensively elucidated the phytochemical profile and antimicrobial efficacy of Vitellaria paradoxa root methanolic fractions, validating its traditional medicinal applications. The presence of significant quantities of bioactive compounds, particularly phenols and tannins, alongside other secondary metabolites like flavonoids, alkaloids, and saponins, underpins its therapeutic potential. Crucially, both the crude extract and its fractions, especially the more polar F1 fraction, exhibited notable bacteriostatic activity against a range of Gram-positive and Gram-negative bacteria, with low MICs suggesting potent growth inhibition. These findings collectively affirm V. paradoxa roots as a promising source of natural antimicrobial agents, offering valuable insights for the development of new pharmaceutical interventions to address the escalating challenge of antimicrobial resistance.

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