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(RESEARCH ARTICLE)

Evaluation of phytochemicals, antimicrobial activity and antioxidant potential of methanolic extract of *Vitellaria paradoxa* nut (shea nut)

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Abstract

The side effects of commonly used antimicrobial drugs and frequent development of antimicrobial resistance motivate scientific researchers to search for new antimicrobial agents. This study evaluated the phytochemical constituents, antimicrobial activity and antioxidant potential of Vitellaria paradoxa nut (shea nut) extract. The phytochemical screening was done to know the secondary metabolites present in the shea nut extract. Antimicrobial assays were done on S. aureus ATCC 25923, E. faecalis ATCC 29212, E. coli ATCC 25922, P. aeruginosa ATCC 4853, MRSA, E. coli ESLB (NCTC13351), S. typhi (ATCC334538), S. typhimurium (ATCC13311), N. gonorrhoeae (NCTC12700), K. pneumoniae (ATCC7004603), *B. cereus* (NCTC10320), and *C. albicans* (ATCC10031), by employing an agar diffusion method and broth dilution method. Antioxidant activity was carried out by DPPH radical scavenging activity mechanism. The results revealed a diverse array of bioactive compounds, including terpenoids, steroids, alkaloids, tannins, flavonoids, reducing sugars and saponins. The shea nut extract exhibited significant antimicrobial activity against *Salmonella typhimurium*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, with minimum inhibitory concentration (MIC) of 0.78125 mg/mL for these organisms. The highest DPPH radical scavenging potential exhibited by the extract was found to be 93.92±0.21 %. At the lowest concentration of 3.90625 µg/mL the DPPH radical scavenging activity of the extract was 31.12±0.16 % while that of ascorbic acid was 17.71±0.48 %. The methanolic extract of *Vitellaria paradoxa* nut possessed antimicrobial and antioxidant activities and were found to contain many secondary metabolites which may be responsible for its biological activity.

Keywords: Antimicrobial activity; Antioxidant activity; *Vitellaria paradoxa*; Phytochemical constituents; Shea nut

1. Introduction

The widespread of microbial resistance to antibacterial and antifungal drugs has become a major challenge that leads to ineffectiveness of antibiotics [1]. Microorganisms of various species have developed resistance to antimicrobial agents due to the indiscriminate use of the antimicrobial agents commonly used in their treatment [2]. Even, many of the synthetic drugs currently used to treat microbial infections have undesirable side-effects such as vomiting, diarrhoea, tooth staining and some have been said to be carcinogenic [3]. The risk of adverse effects encountered with the use of some of the current antibiotics, propel researchers to seek other alternatives. It is therefore highly desirable to explore medicinal plants for new antimicrobial and antioxidant agents [4,5,6].

Scientific studies have been done on many plants because of their bioactive properties and antioxidant potential. Antioxidant agents ameliorate oxidative stress in cells; hence, they are useful in the treatment of many diseases such as inflammatory, cancer, and cardiovascular diseases [7].

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Vitellaria paradoxa is a medicinal plant which belongs to the Sapotaceae family. Its nut is bean-shaped, tiny, rigid, and brown in colour [8]. Medically, shea butter from *Vitellaria paradoxa* is used in treatment of rashes, skin inflammation, dermatitis, rheumatism and irritation [9]. It is also used in cooking, in soaps and candles making [10]. According to reports, the shea butter plant parts generally are effective antimicrobials against bacterial and fungal infections [11].

This study aimed to investigate the antimicrobial efficacy, antioxidant potential of methanolic extract of *Vitellaria paradoxa* nut. Also, to identify phytochemicals present in the extract.

2. Material and methods

2.1. Plant collection

Shea nuts were meticulously sourced from healthy and mature trees at Dungu in the Northern region of Ghana during peak season to ensure freshness and viability. Special care was taken to select fully ripe nuts, avoiding any damage or contamination. After collection, thorough cleaning and controlled drying processes were implemented to maintain optimal phytochemical content. The dried nuts were then stored in airtight containers in suitable conditions to preserve their integrity.

2.2. Extraction of plant sample

Extraction of the plant sample commenced by pulverizing the dried sample using an electronic grinding mill, followed by extraction utilizing methanol (BDH Chemical, UK). Subsequently, 1.14 kg of the milled sample was extracted with 3.6 L of methanol using cold maceration for a duration of 3 days. After the 72-hour period, separation of the residue from the filtrate was achieved using a Whatman filter paper. The filtrate then underwent concentration to dryness employing a rotavapor (Buchi R-210) and a water bath (Buchi, B-491) maintained at 40 °C. Finally, phytochemical screening was conducted on the extract.

2.3. Phytochemical screening

In the Phytochemical Screening stage, the methanolic extract was subjected to analysis for potential phytochemicals using established protocol [12].

2.4. Test for flavonoids

For this test, 2 mL of ethanol was employed to dissolve approximately 0.4 g of the extract, with the addition of 5 drops of concentrated HCl and magnesium turnings. The emergence of a pink coloration affirmed the presence of flavonoids.

2.5. Test for alkaloids

The extract was treated with ammoniacal alcohol (ammonia: 95 % ethanol in a ratio of 1:9), followed by filtration. The resulting filtrate was evaporated, and 1% sulphuric acid added to the residue to convert alkaloids to soluble salt forms. Subsequently, the solution was rendered alkaline with dilute ammonia, partitioned in a separating funnel with chloroform, and shaken. Dragendroff's and Mayer's tests was conducted on the acidified residue to confirm the presence of alkaloids.

2.6. Dragendroff's test

A solution of potassium bismuth iodide was introduced to the filtrate. The formation of a red precipitate indicated the presence of alkaloids.

2.7. Mayer's test

The filtrate was treated with a solution of potassium mercuric iodide. The confirmation of alkaloids was evidenced by the appearance of a cream precipitate.

2.8. Test for saponins

Approximately 2 mg of the extract was dissolved in 2 mL of distilled water and shaken. The formation of a foam column not less than 1 cm in height that persisted for few minutes validated the presence of saponins.

2.9. Tannins

About 5 drops of iron (III) chloride ($FeCl_3$) solution was added to 2 mL of the extract. The appearance of a blue-black colour served as evidence for the presence of tannins.

2.10. Test for reducing sugars

The methanolic extract was hydrolysed with dilute HCl and subsequently treated with 20 % NaOH. Heating with Fehling's solutions A and B was then carried out. The presence of a brick-red precipitate confirmed the presence of reducing sugars.

2.11. Test for triterpenes/ terpenoids (Salkowski's Test)

The sample was extracted with chloroform and filtered. The addition of about 5 drops of concentrated H2SO4 to the filtrate and followed by thorough mixing and standing. A brownish-red coloration indicated the presence of triterpenes or terpenoids.

2.12. Test for sterols (Libermann Burchard's test)

Chloroform was added to the sample and filtered. The resulting filtrate was then treated with six drops of $(CH_3CO)_2O$ and boiled, followed by cooling. A few drops of concentrated H_2SO_4 was added and a bluish-green coloration at the interface confirmed the formation of a steroidal ring.

2.13. Cardiac glycosides

The extract was treated with 70 % alcohol and filtered. Approximately 10 mL of alcoholic filtrate was added to 2 mL of anhydrous CH_3COOH with 5 drops of iron (III) chloride solution. Concentrated H_2SO_4 was gently added by pouring it down the sides of the test tube using a dropping pipette. The observation of a reddish-brown coloration at the interface confirmed the presence of cardiac glycosides.

2.14. Determination of antimicrobial activity of plant extract

2.14.1. Test microorganisms for antibacterial and antifungal assays

In this study, the test microbes utilized included *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 4853, MRSA, *E. coli* ESLB (NCTC13351), *S. typhi* (ATCC334538), *S. typhimurium* (ATCC13311), *N. gonorrhoeae* (NCTC12700), *K. pneumoniae* (ATCC7004603), *B. cereus* (NCTC10320), and *C. albicans* (ATCC10031), all sourced from the microbiology laboratory of Department of Pharmaceutics, School of Pharmacy and Pharmaceutical Sciences, University for Development Studies, Tamale, Ghana.

2.14.2. Inoculum preparation of test microorganisms

The bacterial and fungal strains were streaked on 20 mL sterile nutrient agar and Sabouraud dextrose agar plates, respectively. After incubation at their requisite temperatures, colonies were harvested and suspended in 10 mL sterile water in test tubes. Turbidity was matched to the 0.5 McFarland standard and visually assessed.

2.14.3. Antimicrobial activity (agar well diffusion method)

The antimicrobial activity of the extract was carried out using the agar well diffusion method described by Konning *et al*. [13] and modified by Osei Akoto *et al*. [14]. The nutrient agar plates were constituted by inoculating 100 µL of the test organisms with micropipette and sterile pipette tips by employing the pour plate method. Using a sterile borer, five wells with each of diameter 10 mm were made in the agar and then filled with 200 µL of the extract with concentrations 6.25 mg/mL, 12.50 mg/mL, 50 mg/mL and 100 mg/mL and reference drugs with each of 0.05 mg/mL concentration. The plates were incubated at 37 °C for 24 hours, after that the diameter of zone of growth inhibition formed was measured. The mean values were then calculated. Three replicates of the experiment for the extract and standard drugs were done. Ciprofloxacin and fluconazole were used as the standard antibacterial and antifungal drugs respectively.

2.14.4. Determination of minimum inhibitory concentration of extract

Serial dilutions of the extract were prepared in single strength sterile Mueller Hinton broth (50-0.3906 mg/mL), whereas ciprofloxacin and fluconazole, standard drugs concentrations were prepared using the same method with the range from 100 to 0.7812 µg/mL. These concentrations were transferred to a 96-well bottom flat microtiter plate. Each concentration was inoculated with 20 uL of the respective microbial cultures and incubated at 37 ℃ for 24 hours.

Minimum inhibitory concentration (MIC) was determined using tetrazolium bromide solution, with a change in colour to indicate microbial growth or inhibition [15].

2.14.5. Determination of minimum bactericidal concentration of the extract

Following MIC determinations, negative or yellow cultures were subcultured into fresh sterile broth in a 96-well plate and incubated at 37 °C for 24 hours. Minimum bactericidal concentrations (MBCs) were determined by observing specific concentrations that killed 99.9% of the microbial growth [15]. All assays were conducted in triplicate.

2.14.6. Determination of total phenolic content in the extract

The total phenolic content of the extract was determined by using Folin–Ciocalteu method [16]. To about 2 mL of prepared concentrations (500, 250, 125, 62.5, 31.25, 15.625, 7.8125 and 3.90625 µg/mL) of the guava extract, 4.5 mL of Folin–Ciocalteu reagent (diluted 1:10 in methanol) was added and allowed to stand for 5 minutes at room temperature and then 7.5 mL of 20 % Na_2CO_3 was added. The mixtures were shaken to form homogenous solutions and then incubated in the dark for 2 hours. After which 250 uL each of the mixtures was pipetted into a 96-bottom flat plastic micro-well plate followed by the absorbance measurement at 760 nm against a blank using a spectrophotometer (microplate reader), Infinite M200, Tecan, Austria. The results were expressed in milligrams equivalents of gallic acid per milligram of dry weight. The calibration curve was established by using 500, 250, 125, 62.5, and 0.00 µg/mL of gallic acid as a standard under comparable conditions [17]. The experiment was repeated in triplicates.

2.14.7. Screening for DPPH antioxidant activity of the extract

The antioxidant capacity of the extract was evaluated using a DPPH radical scavenging activity assay described by Warsi and Sholichah, [18]. Test tubes were serially made with different concentrations of the extract and ascorbic acid (500 µg/mL, 250 µg/mL, 125 µg/mL, 61.25 µg/mL, 31.25 µg/mL, 15.625 µg/mL, 7.8125 µg/mL, and 3.90525 µg/mL). A 9.0 mL of 0.3 mM DPPH was freshly prepared in methanol and then combined with 1.0 mL each of the prepared concentrations in newly cleaned test tubes. The resulting solutions were incubated for 30 minutes at room temperature in the dark. A 200 µL of each resulting solution was pipetted into a round bottom flat 96-well plate, and the absorbance was measured using a microplate reader, Infinite M200, Tecan, Austria at 517 nm. The experiment was repeated in triplicates. The following formula was used to determine the DPPH scavenging activity:

$$Percentage\ Scanvenging\ ability = \frac{(A_{control} - A_{test\ sample})}{A_{control}} x100$$

$A_{control}$ denotes mean absorbance of the negative control.

$A_{test\ sample}$ denotes mean absorbance of the extracts or ascorbic acid (standard).

3. Results and discussion

3.1. Phytochemical screening

The phytochemical screening revealed the presence of saponins, alkaloids, flavonoids, terpenoids, tannins, and reducing sugars in the methanolic extract of *Vitellaria paradoxa* nut, while cardiac glycoside was absent. Table 1 summarizes the findings.

These findings have significant implications for the potential therapeutic applications of the extract. The presence of saponin in the extract suggests its potential as an antimicrobial and antifungal agent. Saponins are widely recognized for their ability to disrupt the integrity of microbial cell membranes, leading to cell lysis and death. Studies have demonstrated that saponins possess strong antifungal properties and can act synergistically with other compounds to enhance antimicrobial activity [19]. This aligns with findings from Gitanjali et al. [20], who showed that saponins from different plant sources can significantly inhibit the growth of bacterial and fungal pathogens. The presence of saponins in *Vitellaria paradoxa* further support its traditional use in treating infections.

Table 1 Phytochemical constituents in the methanolic extract of *Vitellaria paradoxa* nut

Secondary Metabolites	State	Inference
Alkaloids	+	Present
Reducing Sugars	+	Present
Cardiac Glycosides	-	Absent
Flavonoids	+	Present
Saponins	+	Present
Terpenoids	+	Present
Tannins	+	Present
Sterols	+	Present

Key: + means present; - means absent

Terpenoids, which were also identified in the extract, are another class of compounds known for their broad-spectrum bioactivity. Terpenoids have been shown to possess antimicrobial, anti-inflammatory, and anticancer properties. Their antimicrobial activity is primarily attributed to their ability to disrupt microbial cell membranes and inhibit the growth of pathogens. In a study by Yamaguchi [21], terpenoids were found to have strong inhibitory effects against gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, which supports the findings in this study. The presence of terpenoid in *Vitellaria paradoxa* nut suggests its potential as an antimicrobial agent, especially against bacterial infections.

Tannins, which are known for their astringent and antioxidant properties, were also present in the extract. Tannins have been extensively studied for their ability to bind to proteins, precipitate microbial enzymes, and inhibit microbial growth [22]. In addition, tannins play a crucial role in protecting cells from oxidative stress by neutralizing free radicals. The presence of tannins in the methanolic extract of *Vitellaria paradoxa* nut aligns with previous studies that demonstrated the antioxidant and antimicrobial potential of tannin-rich plants [22, 23]. This suggests that the extract could have therapeutic applications in conditions related to oxidative stress and microbial infections.

Also, the presence of flavonoids in the extract is notable, as flavonoids are recognized for their strong antioxidant and anti-inflammatory properties. Flavonoids have been shown to play a key role in reducing the risk of chronic diseases, including cardiovascular diseases and cancer, due to their ability to scavenge free radicals and modulate inflammatory pathways [24]. The presence of flavonoids in *Vitellaria paradoxa* nut may improve its effectiveness in treating conditions related to oxidative stress and inflammation.

3.2. Antimicrobial activity

The antimicrobial activity of the methanolic extract of *Vitellaria paradoxa* was assessed using the agar well diffusion method and broth microdilution assay. The results are summarized in Table 2 and Table 3 respectively.

To evaluate the antimicrobial efficacy of the extract, the diameter of zone of inhibition was measured. The results indicate that the extract exhibited inhibition at the lowest concentration of 12.5 mg/mL and showed the most pronounced activity at 100 mg/mL, as shown in Table 2.

The broth microdilution assay revealed that the methanolic extract of *Vitellaria paradoxa* nut exhibited broad-spectrum antimicrobial activity across the tested organisms. The MIC value of 0.78125 mg/mL was observed for *S. Typhimurium*, *S. Aureus*, *E. Faecalis*, *E. Coli*, and *P. Aeruginosa*. This high level of activity is consistent with findings from previous studies, which also reported significant antimicrobial effects of plant extracts at similar concentrations. For instance, studies by Shai *et al.* [25] demonstrated that methanolic extracts from various plant sources exhibited MIC value as low as 0.5 mg/mL against certain bacterial strains, indicating potent antimicrobial activity.

The minimum bactericidal concentration (MBC) results, shown in Table 3, indicate that the extract was highly effective, particularly against *S. Typhi*, *S. Typhimurium*, *N. Gonorrhoea*, *K. Pneumoniae*, *B. Cereus*, *C. Albicans*, *E. Faecalis*, and *P. Aeruginosa*, with MBC values of 6.25 mg/mL. This finding aligns with the work of Ndhlala *et al.* [26], who found that similar plant extracts had MBC values in the range of 6.0 to 8.0 mg/mL, demonstrating effective antimicrobial action.

However, *MRSA* exhibited a higher MBC of 12.50 mg/mL compared to other microorganisms. This suggests that while the extract is generally potent, achieving bactericidal effect against resistant strains such as *MRSA* may require higher concentrations. This is consistent with the observations of Tordzagla *et al*. [27], who noted that methanolic extracts often show increased MIC and MBC values against resistant strains, indicating a need for higher dosages to overcome resistance.

Table 2 Antibacterial activity and antifungal activity (Zones of inhibition) of methanolic extract of *Vitellaria paradoxa* nut and reference drugs

Test organisms	Mean Zones of Inhibition (mm)					
	Extract				Cipro	Fluco
	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	(0.05 mg/mL)	(0.05 mg/mL)
E. Coli	30.5±0.71	23.5±0.65	17.5±0.59	0.00±0.00	26.50±2.12	NA
S. Aureus	24.0±1.41	22.5±0.74	20.0±0.00	17.0±1.41	27.50±0.71	NA
E. Coli (ESLB)	22.5±0.70	23.0±0.00	23.0±0.43	12.5±0.53	22.00±1.33	NA
B. Cereus	17.0±1.54	16.5±0.56	0.00±0.00	0.00±0.00	22.50±0.51	NA
MRSA	26.5±2.12	16.5±2.17	21.0±1.63	15.5±0.76	25.00±4.24	NA
N. Gonorrhoeae	25.5±0.73	24.0±1.41	24.0±1.28	21.0±1.48	19.00±1.41	NA
C. Albicans	26.5±2.11	23.5±0.72	21.5±2.12	21.0±1.45	NA	17.0±0.42
E. Faecalis	21.5±4.95	24.5±0.58	22.0±0.00	0.00±0.00	19.00±1.11	NA
S. Typhi	23.5±1.16	13.5±2.14	15.0±0.00	0.00±0.000	0.00±0.00	NA
S. Typhimurium	20.0±0.00	22.5±1.53	22.5±0.50	17.0±1.32	26.50±3.54	NA
P. Aeruginosa	31.0±1.34	22.0±1.73	17.0±0.00	0.00±0.00	22.50±3.25	NA
K. Pneumoniae	27.0±1.22	26.5±0.70	24.0±1.59	19.5±0.63	0.00±0.00	NA

KEY; Values presented as Mean ± SD, NA = Not Applicable, Cipro = Ciprofloxacin, Fluco = Fluconazole

Table 3 MIC and MBC values of shea nut extract and standard drug recorded against test organisms

Test organisms	Extract (mg/mL)		Ciprofloxacin (µg/mL)		Fluconazole (µg/mL)	
	MIC	MBC	MIC	MBC	MIC	MFC
S. Typhi	0.7812	6.2496	12.50	50	NA	NA
S. Typhimurium	1.5625	6.2496	6.2496	50	NA	NA
N. Gonorrhoeae	0.7812	6.2496	3.125	50	NA	NA
K. Pneumoniae	0.7812	6.2496	12.50	50	NA	NA
B. Cereus	0.7812	6.2496	6.2496	25	NA	NA
C. Albicans	0.7812	6.2496	NA	NA	25	50
S. Aureus	1.5625	6.2496	12.5	50	NA	NA
E. Faecalis	1.5625	6.2496	25	50	NA	NA
E. Coli	1.5625	6.2496	3.125	25	NA	NA
P. Aeruginosa	1.5625	6.2496	12.50	50	NA	NA
MRSA	0.7812	12.50	12.50	50	NA	NA
E. Coli (ELSB)	0.7812	6.2496	6.2496	25	NA	NA

Key: NA = Not Applicable

Various studies have explored the antimicrobial potency of plant extracts at different concentrations, revealing similar trends. For example, the significant inhibition observed at higher concentrations, such as 100 mg/mL, aligns with findings by Bhalodia and Shukla [28], who demonstrated that higher concentrations of plant extracts typically yield more effective antimicrobial activity. This is attributed to the increased availability of bioactive compounds at higher doses, which intensify the antimicrobial action.

Similarly, the diminished inhibitory effects were observed at lower concentrations and it reflects the results of studies like those by Tordzagla *et al*. [27] and Cowan [29] which highlighted that sub-optimal concentrations of plant-based extracts often lack the potency required to significantly inhibit microbial growth. This is consistent with the current findings, where lower concentrations exhibited statistically lower inhibitory effects compared to the higher concentrations.

Moreover, the non-significant difference between the extract's lower concentrations and the controls (ciprofloxacin and fluconazole) aligns with studies such as those by Ríos and Recio [30], which reported that while plant extracts can exhibit potent antimicrobial activity, their effects may not always surpass those of conventional antibiotics, especially at lower concentrations.

In contrast, studies by Nostro *et al.* [31], emphasized that while plant extracts may have a broader spectrum of activity, their effectiveness can vary depending on the pathogen and the concentration used. This can explain why, in this study, certain concentrations such as 25 and 50 mg/mL displayed varying degrees of inhibition.

3.3. DPPH radical scavenging activity

The DPPH assay is based on the principle that antioxidants can donate either an electron or a hydrogen atom to neutralize the DPPH radical, which is a deep violet-coloured stable free radical. When the antioxidant interacts with the DPPH radical, it converts into a stable hydrazine form (DPPH-H), resulting in the loss of its violet colour, turning it into a bright yellow hue (Gnawali *et al.,* [32].

This assay was used to evaluate the ability of the methanolic extract of *Vitellaria paradoxa* nut to scavenge free radicals, which are harmful to both humans and animals. The extract, along with the standard reference drug ascorbic acid, was tested at concentrations ranging from 3.90625 to 500.00 µg/mL. The absorbance for the methanolic extract ranged from 0.0687±0.0024 to 0.7791±0.0018, while for ascorbic acid, the absorbance varied from 0.0392±0.0057 to 1.1225±0.0066, as presented in Table 4.

Table 4 Absorbance of the methanolic extract of *Vitellaria paradoxa* and ascorbic acid across a range of concentrations

Concentration (µg/mL)	Extract	Ascorbic acid
3.90625	0.7791±0.0018	1.1225±0.0066
7.8125	0.7826±0.0130	1.0968±0.0016
15.625	0.7581±0.0021	0.9932±0.0035
31.25	0.7058±0.0013	0.7820±0.0039
62.5	0.5956±0.0028	0.2889±0.0039
125	0.4355±0.0068	0.0561±0.0022
250	0.1504±0.0024	0.0467±0.0066
500	0.0687±0.0024	0.0392±0.0057

As shown in Table 5, both the methanolic extract of *Vitellaria paradoxa* and ascorbic acid exhibited a concentration-dependent increase in radical scavenging activity. The scavenging activity of the extract increased from 31.12±0.15 % at 3.90625 µg/mL to 93.92±0.21 % at 500 µg/mL. Similarly, the scavenging activity of ascorbic acid increased from 17.71±0.48 % at 3.90625 µg/mL to 97.12±0.41 % at 500 µg/mL.

At lower concentrations, the extract showed a higher scavenging activity compared to ascorbic acid. For instance, at 3.90625 µg/mL, the extract exhibited a scavenging activity of 31.12±0.15 %, whereas ascorbic acid showed only 17.71±0.48 %. This suggests that the extract may contain a combination of compounds that work synergistically to

enhance its antioxidant activity, leading to its relatively high initial scavenging percentage compared to the standard drug.

However, as the concentration increased, ascorbic acid demonstrated a more pronounced increase in scavenging activity, surpassing the extract at concentrations of 31.25 µg/mL and above. For example, at 31.25 µg/mL, the scavenging percentage of ascorbic acid was 42.67±0.28 %, compared to 37.60±0.11 % for the extract. At 500 µg/mL, ascorbic acid reached its maximal scavenging potential with 97.12±0.41 %, while the extract achieved a slightly lower scavenging activity of 93.92±0.21 %. This difference may be attributed to the presence of other substances in the extract that do not contribute to antioxidant activity, thereby limiting its effectiveness at higher concentrations.

Also, an IC_{50} value of 63.455 µg/mL was recorded for ascorbic acid and 104.491 µg/mL for the extract. This indicates that the extract has a lower antioxidant potency than ascorbic acid.

This difference in IC_{50} values highlights that, while both the *Vitellaria paradoxa* extract and ascorbic acid have exhibited significant scavenging activity, the extract requires a higher concentration to achieve similar antioxidant activity.

Also, a study by Gnawali *et al*. [32] reported the antioxidant activity of another plant species using the DPPH assay and found similar trends, where the scavenging activity of the methanolic extract increased consistently with increasing concentration, confirming the principle that antioxidants, especially those from plant extracts, can exhibit concentration-dependent radical scavenging activity. These findings align with the results of the present study, particularly regarding the concentration-dependent activity of both the methanolic extract of *Vitellaria paradoxa* and ascorbic acid. Table 6 summarizes the findings.

Table 5 Percentage radical scavenging activity of the methanolic extract of *Vitellaria paradoxa* and ascorbic acid across a range of concentrations

Concentration (µg/mL)	**Percentage radical scavenging activity (Extract)**	**Percentage radical scavenging activity (Ascorbic acid)**
3.90625	31.12±0.15	17.71±0.48
7.8125	30.81±1.14	19.59±0.12
15.625	32.97±0.18	27.19±0.25
31.25	37.60±0.11	42.67±0.28
62.5	47.34±0.24	78.81±0.28
125	61.50±0.60	95.88±0.16
250	86.70±0.20	96.57±0.48
500	93.92±0.21	97.12±0.41

Table 6 IC_{50} values of the methanolic extract of *Vitellaria paradoxa* and ascorbic acid (Standard drug)

Sample	**IC_{50} Value (µg/mL)**
Extract	104.491
Ascorbic acid	63.455

3.4. Total phenolic content (TPC)

The total phenolic content of the *Vitellaria paradoxa* extract was determined using the Folin-Ciocalteu (F-C) method, which relies on the principle that phenolic compounds transfer electrons to phosphomolybdic-phosphotungstic acid complexes in an alkaline environment, forming blue-coloured complexes that can be detected spectrophotometrically at 765 nm [32]. Gallic acid was used as a standard, and its absorbance values at various concentrations were plotted to construct a calibration curve. The regression equation derived from the curve was used to calculate the TPC of the extract, expressed as mg gallic acid equivalents per gram of sample in dry weight (mg GAE/g).

The absorbance values of gallic acid at different concentrations (0 to 500 µg/mL) are shown in Table 6, and the equation of the calibration curve, A = 0.002789C + 0.268151 with R^2 = 0.9507881, was used to calculate the total phenolic content of the extract.

Table 6 The total phenolic content of *Vitellaria paradoxa* in mg GAE/g of dry extract across a range of concentrations.

Concentration (µg/mL)	Total phenolic content (mg GAE/g)			
	I	II	III	Average
3.90625	91.090	90.122	90.910	90.707±0.5148
7.8125	100.233	93.922	88.257	94.137±5.9909
15.625	104.894	94.424	90.444	96.587±7.4640
31.25	123.610	125.546	117.694	122.283±4.0907
62.5	149.534	141.143	141.143	143.940±4.8445
125	200.806	201.631	193.133	198.523±4.6864
250	300.699	307.224	297.006	301.643±5.1740
500	394.030	406.400	405.396	401.942±6.8704

3.5. Relationship between TPC and radical scavenging activity (RSA)

A strong positive correlation was observed between the concentration of the extract and both the TPC and radical scavenging activity (RSA) values, as indicated by the Pearson correlation coefficients. The correlation coefficient for concentration and TPC was 0.984, while for concentration and RSA, it was 0.936. This indicates that as the concentration of the extract increases, both the TPC and RSA values increase accordingly.

Table 7 compares the concentration, TPC, and RSA of the extract. The relationship illustrates that higher concentrations consistently result in higher TPC and RSA values. For example, at 3.90625 µg/mL, the TPC was 90.707±0.5148 mg GAE/g, with an RSA of 31.1262±0.1554 %. At 500 µg/mL, these values increased to 401.942±6.8704 mg GAE/g and 93.9239±0.2118 %, respectively. This trend is consistent across all concentrations studied.

The correlation between TPC and RSA was also significant, with a Pearson correlation coefficient of 0.982. This implies that higher TPC values are directly associated with enhanced radical scavenging activity. The results suggest that the phenolic compounds present in the extract play a vital role in its antioxidant potential, as higher phenolic content translates into greater free radical scavenging activity.

Similarly, Gnawali [32] reported that phenolic-rich plant extracts consistently exhibit high radical scavenging activity due to their ability to donate hydrogen atoms to neutralize free radicals. This study supports that finding, with a strong Pearson correlation coefficient of **0.936** between the concentration of the extract and RSA. This further corroborates the notion that the antioxidant potential of an extract is closely linked to its phenolic content, as was also demonstrated in their work.

In addition, a study by Wojdyło *et al*. [33] found that plant-based extracts with higher TPC tend to have stronger antioxidant activities. They concluded that phenolic compounds are the primary contributors to the antioxidant efficacy of many medicinal and nutritional plant extracts, with Pearson correlation coefficients consistently falling between **0.90** and **0.98,** similar to the **0.982** correlation observed in this research.

This consistency with previous studies further supports the conclusion that the phenolic content of the extract contributes substantially to its antioxidant capabilities. The strong correlation between TPC and RSA indicates that increasing the phenolic concentration enhances the ability of the extract to neutralize free radicals, making it a potentially valuable source of natural antioxidants.

Table 7 Comparison of the concentration (µg/mL), TPC (mg GAE/g) and RSA (%) of the extract

Concentration (µg/mL)	RSA (%) of Extract	TPC (mg GAE/g) of Extract
3.90625	31.12±0.15	90.70±0.51
7.8125	30.81±1.14	94.13±5.99
15.625	32.97±0.18	96.58±7.46
31.25	37.60±0.11	122.28±4.09
62.5	47.34±0.24	143.94±4.84
125	61.50±0.60	198.52±4.68
250	86.70±0.20	301.64±5.17
500	93.92±0.21	401.94±6.87

4. Conclusion

The study focused on the phytochemical screening, antioxidant potential and antimicrobial activity of methanolic extract of *Vitellaria paradoxa* nut, aiming to explore its potential as a natural therapeutic agent. The findings revealed significant bioactive properties of the extract, corroborating its traditional use in herbal medicine.

Phytochemical analysis confirmed the presence of key bioactive compounds such as flavonoids, saponins, terpenoids, alkaloids, tannins, and reducing sugars which are known for their antimicrobial and antioxidant properties. These compounds play an essential role in disrupting microbial cell membranes and neutralizing free radicals, contributing to the extract's therapeutic potential. The methanolic extract exhibited a strong antimicrobial activity against a wide range of pathogens indicating its broad-spectrum efficacy.

The antioxidant activity of the extract, as measured by the DPPH radical scavenging assay, was significant, confirming its capacity to neutralize free radicals. The strong positive correlation between the total phenolic content (TPC) and radical scavenging activity (RSA) further supports the conclusion that the phenolic compounds in the extract are the primary contributors to its antioxidant potential.

These findings provide a foundation for further research into the potential applications of this extract in the development of natural treatment of infections and oxidative stress-related conditions.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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