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Comparative Phytochemical Composition and Antibacterial Activities of Aqueous, Ethanolic and n-hexane Fruit Pulp Extracts of *Adansonia digitata, Tamarindus indica* and *Vitellaria paradoxa*

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Abstract

The increasing prevalence of antimicrobial resistance has intensified the search for alternative therapeutic agents from natural sources. *Adansonia digitata, Tamarindus indica,* and *Vitellaria paradoxa* are important indigenous African fruit species traditionally utilized for nutritional and medicinal purposes. This study evaluated the phytochemical composition and antibacterial activities of aqueous, ethanolic, and n-hexane fruit pulp extracts of these species. Fruit pulps were extracted by percolation using the three solvents. Qualitative and quantitative phytochemical analyses were performed using standard methods, while antibacterial activity was evaluated against *Escherichia coli, Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* using the agar well diffusion method at concentrations of 300, 150, 75, and 37.5 mg/mI, with three replicates per treatment. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also determined. Data were analysed using analysis of variance (ANOVA), with statistical significance set at $p < 0.05$. Qualitative screening revealed the presence of alkaloids, tannins, flavonoids, phenols, glycosides, terpenoids, saponins, and steroids in varying proportions across the extracts. Quantitative analysis showed that phenols were predominant in most extracts, with the ethanolic extract of *A. digitata* exhibiting the highest phenolic content (90.10 mg/g), while *T. indica* showed the highest tannin (80.20 mg/g) and flavonoid (62.40 mg/g) contents. Antibacterial activity varied among extracts, concentrations, and bacterial isolates, with ethanolic and n-hexane extracts generally showing greater activity than aqueous extracts. *V. paradoxa* exhibited the broadest antibacterial spectrum and produced the highest inhibition zone (31 mm) against *K. pneumoniae*. MIC values ranged from 32 to 280 mg/mI, whereas MBC values ranged from 37.5 to 300 mg/mI, with some MBC values exceeding the highest concentration tested for individual extracts. ANOVA revealed significant differences among bacterial isolates for *A. digitata* ($p = 0.000002$) and *T. indica* ($p = 0.00000079$), whereas significant differences among extract treatments were observed for *V. paradoxa* ($p < 0.001$). The findings demonstrate that the fruit

pulps of *A. digitata, T. indica*, and *V. paradoxa* contain diverse bioactive phytochemicals and exhibit antibacterial activity, suggesting that these fruits may serve as promising sources of antibacterial phytochemicals for further pharmaceutical, nutraceutical, and functional-food research.

Introduction

Medicinal plants have long contributed to traditional healthcare and remain important sources of structurally diverse bioactive compounds. Their biological activities have been associated with secondary metabolites such as alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, glycosides, saponins, and steroids. The increasing prevalence of antimicrobial resistance (AMR) has reduced the effectiveness of conventional antimicrobial agents and strengthened the need to explore new sources of antibacterial compounds. Antimicrobial resistance occurs when microorganisms develop the ability to survive exposure to antimicrobial agents that would normally inhibit or kill them. In bacteria, resistance may involve enzymatic inactivation or modification of antibiotics, alteration or protection of antimicrobial targets, reduced cellular permeability, and active efflux. Resistance determinants may arise through genetic variation and may also be acquired through horizontal gene transfer.

The World Health Organization (WHO) 2024 Bacterial Priority Pathogens List identifies critical, high-, and medium-priority antibiotic-resistant pathogens to guide research and development of new antibacterial interventions. Methicillin-resistant *Staphylococcus aureus* is classified as a high-priority pathogen, whereas macrolide-resistant *Streptococcus pneumoniae* is classified as a medium-priority pathogen. The continuing emergence of resistant bacterial pathogens has increased interest in natural products as potential sources of chemically diverse antibacterial compounds. African fruit-producing species are of particular interest because their edible portions may contain phytochemicals with biological activities.

Adansonia digitata L. (baobab), *Tamarindus indica L.* (tamarind), and *Vitellaria paradoxa* (shea butter tree) are important African fruit-producing species with nutritional and traditional uses. Studies involving *A. digitata* fruit have demonstrated the presence of phytochemicals and reported antioxidant and antimicrobial properties, including investigations of fruit pulp extracts (Thompson et al., 2024). Evidence is also available for the antibacterial activity of *T. indica* fruit pulp. Goanar et al. (2024) investigated ethanol and acetone extracts of *T. indica* fruit pulp and reported antibacterial activity against *S. aureus* and *K. pneumoniae,* with the latter showing greater sensitivity to the extracts in their study. Similarly, *V. paradoxa* fruit pulp has been investigated for its nutritional and phytochemical composition. Akoma et al. (2018) reported nutritional and phytochemical constituents in *V. paradox*a fruit pulp, while subsequent research has further examined the functional and antioxidant properties associated with the fruit pulp.

These findings indicate that the fruit pulp represents a source of potentially bioactive constituents and support further investigation of its biological properties. Although these species have been investigated individually, comparative information on the phytochemical composition and antibacterial activity of their fruit pulps extracted using solvents of different polarities remains limited. Differences in solvent polarity can influence the classes and quantities of compounds extracted from plant materials and may consequently affect their biological activity. Comparative evaluation of aqueous, ethanolic, and n-hexane extracts can therefore provide useful information on the phytochemical profiles and antibacterial activities of these fruit pulps. The present study therefore evaluated the qualitative and quantitative phytochemical composition of aqueous, ethanolic, and n-hexane extracts of the fruit pulps of *A. digitata, T. indica,* and *V. paradoxa* and assessed their antibacterial activities against selected pathogenic bacteria. The study further aimed to provide scientific evidence regarding the

antibacterial potential of these indigenous African fruits and their possible value as sources of bioactive phytochemicals.

Materials and Methods

Collection and Authentication of Plant Materials

Fruits of *A. digitata, T. indica,* and *V. paradoxa* were purchased from local markets in Kano State, Nigeria. The fruits were identified and authenticated at the Herbarium, Department of Plant Biology, Bayero University, Kano, Nigeria. Voucher specimens were deposited under accession numbers BUKHAN 0036, BUKHAN 0074, and BUKHAN 0489 for *A. digitata, T. indica,* and *V. paradoxa*, respectively.

Preparation and Extraction of Fruit Pulp Samples

The fruits were processed according to the method described by Ali et al. (2017). Tamarind fruits were manually peeled and the pulp separated from the seeds. The pulp was air-dried at room temperature for two weeks, ground into a fine powder, and stored in airtight containers. Baobab fruit pulp was separated from the fruit, sieved through a 0.9-mm mesh, and stored in sterile containers. Shea fruit pulp was scraped from the fruits, air-dried at room temperature, pulverized, sieved, and stored in airtight containers until extraction. Fifty grams (50 g) of each powdered sample were extracted separately with 500 mI of distilled water, ethanol, and n-hexane using the percolation method for five days. The extracts were filtered through Whatman No. 1 filter paper (11 µm pore size) and concentrated using a rotary evaporator at 40 °C. Evaporation was continued until the respective solvents were removed and concentrated extracts were obtained; the duration varied according to the solvent. The concentrated extracts were stored at 4 °C until further analysis.

Sterility Testing of Extracts

To ensure the absence of microbial contamination, 1 mI of each extract was inoculated onto sterile nutrient agar plates and incubated at 37 °C for 24 h. Plates showing no microbial growth were considered sterile and suitable for subsequent analyses (Fischer et al., 2020).

Qualitative Phytochemical Screening

Qualitative phytochemical screening was performed to determine the presence or absence of alkaloids, glycosides, tannins, saponins, flavonoids, steroids, phenols, and terpenoids using standard colour-reaction and precipitation tests. Alkaloids were detected using Dragendorff's test, in which the formation of an orange or reddish-brown precipitate indicated a positive reaction. Glycosides were detected using the Keller–Killiani test. Tannins and phenols were screened using the ferric chloride test, with development of a characteristic blue-black or greenish colour indicating their presence. Saponins were detected using the frothing test, in which persistent foam following vigorous shaking indicated a positive reaction. Flavonoids were screened using the alkaline reagent test. Steroids were detected using the Liebermann–Burchard test, while terpenoids were detected using the Salkowski test. The qualitative phytochemical screening procedures were based on established methods described by Ali et al. (2017) and standard phytochemical procedures.

Quantitative Phytochemical Analysis

Quantitative phytochemical analysis was performed using spectrophotometric methods. Calibration curves were prepared using the respective reference standards, and the concentrations of phytochemicals in the extracts were calculated from the corresponding calibration curves and expressed as milligrams per gram (mg/g) of extract.

Alkaloids

Alkaloid content was determined following the method described by Nbaeyi-Nwaoha and Onwuka (2014). One gram (1 g) of each extract was dissolved in 20 ml of 20% sulfuric acid (H_2SO_4) in ethanol (1:1) and filtered. One millilitre (1 mI) of the filtrate was transferred into test tubes, followed by the addition of 5 ml of 40% H_2SO_4. The mixture was thoroughly mixed and allowed to stand for 4 h. Absorbance was measured at 568 nm using a UV–Visible spectrophotometer, and alkaloid concentration was calculated from the atropine standard calibration curve.

Glycosides

Glycoside content was determined according to Nbaeyi-Nwaoha and Onwuka (2014). Fifteen percent lead acetate (2.5 mI) was added to 1 g of each extract and mixed thoroughly before filtration. Chloroform (2 mI) was added to the filtrate, mixed thoroughly, and allowed to stand. The lower portion was collected and evaporated to dryness. Glacial acetic acid (3 mI), 0.1 mI of 5% ferric chloride, and 0.25 ml of concentrated H_2SO_4 were added to the dried residue, mixed vigorously, and allowed to stand for 3 h. Absorbance was measured at 568 nm, and glycoside concentration was calculated using the arbutin standard calibration curve.

Tannins

Tannin content was determined using the Folin–Denis method described by Nbaeyi-Nwaoha and Onwuka (2014). Ten millilitres (10 mI) of distilled water was added to 1 g of each extract and shaken for 30 min at 5-min intervals. The mixture was filtered, and 2.5 mI of the filtrate was treated with 1 mI of Folin–Denis reagent and sodium carbonate (Na_2CO_3). The mixture was thoroughly mixed and allowed to stand for 90 min at room temperature. Absorbance was measured at 720 nm, and tannin concentration was calculated from the tannic acid standard calibration curve.

Saponins

Saponin content was determined according to Nbaeyi-Nwaoha and Onwuka (2014). Petroleum ether (10 mI) was added to 1 g of each extract and allowed to drain. Ethanol (5 mI) was added to the dried residue and mixed thoroughly. Approximately 2 mI of the resulting mixture was transferred into test tubes and allowed to stand for 30 min. Absorbance was subsequently measured at 550 nm, and saponin concentration was calculated from the saponin standard calibration curve.

Flavonoids

Flavonoid content was determined following Nbaeyi-Nwaoha and Onwuka (2014). One gram (1 g) of each extract was dissolved in 200 mI of ethyl acetate and filtered. Five millilitres (5 mI) of the filtrate was transferred into test tubes, followed by the addition of 5 of dilute ammonia. The mixture was mixed vigorously and allowed to stand before absorbance was measured at 490 nm. Flavonoid concentration was calculated from the quercetin standard calibration curve.

Other quantitative phytochemicals

Total phenolic, steroid, and terpenoid contents were determined spectrophotometrically using gallic acid, cholesterol, and limonene, respectively, as reference standards. Absorbance was measured at 765 nm, 240 nm, and 220 nm, respectively, and concentrations were calculated from the corresponding calibration curves. All spectrophotometric measurements were performed using a UV–Visible spectrophotometer.

Antibacterial Activity

Bacterial Strains and Inoculum Preparation

Four bacterial isolates, *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli,* and *Klebsiella pneumoniae,* were used in the study. The isolates were obtained from Aminu Kano Teaching Hospital, Kano, Nigeria, and identified by Gram staining, colony morphology, and appropriate biochemical tests. Fresh cultures were grown on Mueller–Hinton agar at 37 °C for 24 h. Bacterial inocula were prepared in sterile normal saline and adjusted to a 0.5 McFarland turbidity standard according to standard antimicrobial susceptibility-testing procedures.

Agar Well Diffusion Assay

Antibacterial activity was evaluated using the agar well diffusion method as described by Balouiri et al. (2016). Standardized bacterial inocula were uniformly swabbed onto Mueller–Hinton agar plates. Wells measuring 6 mm in diameter were aseptically bored and filled with 100 µL of plant extract at concentrations of 300, 150, 75, and 37.5 mg/mI. The extract concentrations were prepared by two-fold serial dilution from a 300 mg/mI stock solution using DMSO as the solvent. DMSO at the concentration used for extract preparation served as the negative control, while ciprofloxacin at 100 µg/ml served as the positive control. Plates were incubated at 37 °C for 24 h, after which the zones of

inhibition were measured in millimetres. All assays were performed in triplicate.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) was determined using the broth dilution method. Extract concentrations were prepared in Mueller–Hinton broth and inoculated with 100 μL of standardized bacterial suspension. Appropriate broth and growth controls were included. The tubes were incubated at 37 °C for 24 h, and the MIC was recorded as the lowest extract concentration showing no visible turbidity.

For MIC determination, the extracts were initially tested at concentrations of 300, 150, 75, and 37.5 mg/mI. Based on the inhibitory responses observed at these concentrations, additional intermediate concentrations were prepared within the relevant inhibitory ranges to obtain a more precise MIC. The intermediate concentrations were prepared using the standard dilution equation: $C_1V_1 = C_2V_2$, where C_1 represents the initial concentration, V_1 the volume of the stock solution required, C_2 the desired concentration, and V_2 the final volume of the prepared solution. The intermediate concentrations tested were 280, 260, and 240 mg/mI from the 300 mg/mI concentration; 140, 130, and 120 mg/mI from the 150 mg/mI concentration; 70, 65, and 60 mg/mI from the 75 mg/mI concentration; and 36, 34, and 32 mg/mI from the 37.5 mg/mI concentration. These concentrations were experimentally tested to determine the lowest concentration capable of inhibiting visible bacterial growth. The MIC was recorded as the lowest concentration showing complete inhibition of visible growth.

For determination of the minimum bactericidal concentration (MBC), 100 μL from tubes showing no visible growth in the MIC assay were subcultured onto nutrient agar plates. The plates were incubated at 37 °C for 24 h. The MBC was recorded as the lowest extract concentration showing no bacterial colony growth following subculture.

Statistical Analysis

All experimental data were analysed using Microsoft Excel. Results were expressed as mean ± standard deviation (SD) of triplicate determinations (n = 3). Analysis of variance (ANOVA) was used to assess differences among bacterial isolates and among extract treatments. For the antibacterial analysis, each extract treatment represented an individual solvent–concentration combination. Differences were considered statistically significant at $p < 0.05$.

Results

Qualitative Phytochemical Composition of Fruit Pulp Extracts

The qualitative phytochemical screening revealed a diverse array of secondary metabolites in the fruit pulp extracts of *Adansonia digitata*, *Tamarindus indica*, and *Vitellaria paradoxa*. Alkaloids, tannins, flavonoids, phenols, glycosides, and terpenoids were detected across the aqueous, ethanolic, and n-hexane extracts of the three fruits. Saponins and steroids varied among the fruit species and extraction solvents.

Quantitative Phytochemical Composition of Fruit Pulp Extracts

The quantitative phytochemical analysis showed variation in the measured concentrations among fruit species and extraction solvents (Table 1, 2, 3). *A. digitata* recorded its highest phenolic content in the ethanolic extract (90.10 ± 0.10 mg/g), while *T. indica* showed its highest tannin (80.20 ± 0.08 mg/g) and flavonoid (62.40 ± 0.22 mg/g) contents in the ethanolic extract. *V. paradoxa* generally recorded lower numerical concentrations for several quantified phytochemicals, although its n-hexane extract showed relatively high terpenoid and steroid values.

The one-way ANOVA results showed no statistically significant differences among the solvent groups for *A. digitata* ($F = 0.2800$, $p = 0.758536$), *T. indica* ($F = 0.9606$, $p = 0.398864$), or *V. paradoxa* ($F = 3.2946$, $p = 0.056956$) at $p = 0.05$. Thus, although individual phytochemical means differed numerically among solvents, the overall differences tested by the ANOVA were not statistically significant.

Antibacterial Activity of the Fruit Pulp Extracts

The antibacterial activities of the fruit pulp extracts were evaluated using the agar well diffusion method at 37.5, 75, 150, and 300 mg/ml against *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae*. Ciprofloxacin (100 µg/ml) served as the positive control (Table 4, 5, 6, 7).

A. digitata extracts showed varying zones of inhibition depending on solvent and concentration (Table 4). The highest activity was observed with ethanolic extract against *E. coli* at 18 mm. *T. indica* extracts exhibited strong activity, particularly ethanolic extract against *K. pneumonia* with 18.67 mm inhibition zone (Table 5). *V. paradoxa* demonstrated the broadest antibacterial spectrum and produced the highest zone of inhibition of 31 mm against *K. pneumonia* (Table 6).

Analysis of variance showed that the bacterial-isolate effect was significant for *A. digitata* ($p = 0.000002$) and *T. indica* ($p = 0.00000079$), but not for *V. paradoxa* ($p = 0.220989$). The extract-treatment effect, in which each treatment represented a specific solvent–concentration combination, was not significant for *A. digitata* ($p = 0.557575$) *or T. indica* ($p = 0.3725$), but was highly significant for *V. paradoxa* ($p < 0.001$). Because solvent and concentration were treated as a single extract-treatment factor, these analyses do not establish the independent effects of solvent or concentration.

Table 1: Qualitative phytochemical composition of the fruit pulp extracts.

Phytochemical	*A. digitata* Aq.	*A. digitata* EtOH	*A. digitata* n-Hex	*T. indica* Aq.	*T. indica* EtOH	*T. indica* n-Hex	*V. paradoxa* Aq.	*V. paradoxa* EtOH	*V. paradoxa* n-Hex
Alkaloids	+	+	+	+	+	+	+	+	+
Saponins	+	-	+	+	-	-	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Steroids	+	+	-	-	-	-	+	+	+
Phenols	+	+	+	+	-	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+	+	+

Key: + = present; − = absent.

Table 2: Quantitative phytochemical constituents of the fruit pulp extracts (mg/g).

Phytochemical	*A. digitata Aq.*	*A. digitata EtOH*	*A. digitata n-Hex*	*T. indica Aq.*	*T. indica EtOH*	*T. indica n-Hex*	*V. paradoxa Aq.*	*V. paradoxa EtOH*	*V. paradoxa n-Hex*
Alkaloids	3.55 ± 0.10	5.67 ± 0.67	3.45 ± 0.45	5.20 ± 0.05	10.40 ± 0.18	3.60 ± 0.32	0.20 ± 0.20	1.80 ± 0.80	0.80 ± 0.07
Saponins	40.13 ± 0.53	ND	20.45 ± 0.45	25.50 ± 0.30	ND	ND	1.20 ± 0.20	5.60 ± 0.60	0.80 ± 0.12
Tannins	20.56 ± 0.56	35.67 ± 0.67	12.34 ± 0.34	40.80 ± 0.55	80.20 ± 0.08	6.40 ± 0.19	0.45 ± 0.45	3.45 ± 0.45	0.45 ± 0.07
Flavonoids	15.67 ± 0.67	25.34 ± 0.34	10.23 ± 0.23	31.20 ± 0.15	62.40 ± 0.22	14.80 ± 0.35	0.80 ± 0.20	4.20 ± 0.20	2.00 ± 0.06
Steroids	1.10 ± 0.10	2.45 ± 0.45	ND	ND	ND	ND	0.50 ± 0.50	3.00 ± 0.00	5.00 ± 0.00
Phenols	60.12 ± 0.12	90.10 ± 0.10	40.12 ± 0.12	60.15 ± 0.07	ND	24.60 ± 0.27	3.50 ± 0.50	12.10 ± 0.10	2.50 ± 0.07
Glycosides	8.90 ± 0.90	14.12 ± 0.12	5.67 ± 0.67	17.86 ± 0.52	35.60 ± 0.33	9.60 ± 0.18	1.00 ± 0.00	4.50 ± 0.50	2.50 ± 0.14
Terpenoids	3.50 ± 0.50	5.60 ± 0.60	20.33 ± 0.33	11.20 ± 0.07	22.40 ± 0.29	36.07 ± 0.05	2.00 ± 0.00	10.50 ± 0.50	14.34 ± 0.07

Key: ND = Not Detected. Values are mean ± standard deviation (SD) of triplicate determinations (n = 3).

Table 3: One-way ANOVA summary for quantitative phytochemical composition.

Fruit	Source of variation	SS	Df	MS	F	p-value	Decision (α=0.05)
A. digitata	Between groups	280.2462	2	140.1231	0.280034	0.758536	Not significant
T. indica	Between groups	965.5357	2	482.7678	0.960563	0.398864	Not significant
V. paradoxa	Between groups	78.8473	2	39.42365	3.294607	0.056956	Not significant

Table 4: Antibacterial activity of *Adansonia digitata* fruit pulp extracts using the agar well diffusion method.

TI	Aq. 300	Aq. 150	Aq. 75	Aq. 37.5	EtOH 300	EtOH 150	EtOH 75	EtOH 37.5	n-Hex 300	n-Hex 150	n-Hex 75	n-Hex 37.5	CPR
EC	14.67 ± 0.58	13.00 ± 0.00	12.00 ± 0.00	10.33 ± 0.58	18.00 ± 1.00	6.0	6.0	6.0	15.00 ± 1.00	14.00 ± 0.00	11.00 ± 1.00	6.0	25.0
KP	12.00 ± 1.00	6.0	6.0	6.0	18.67 ± 0.58	6.0	6.0	6.0	13.00 ± 1.00	6.0	6.0	6.0	26.0
SA	8.33 ± 0.58	6.0	6.0	6.0	6.0	6.0	6.0	6.0	14.00 ± 1.00	6.0	6.0	6.0	21.0
SP	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	20.0

Key: TI: Test isolates, EC: E. coli, KP: *K. pneumonia, SA: S. aureus, SP: S. pneumonia.* Values are mean ± SD of triplicate determinations. A value of 6.00 mm represents the diameter of the well and indicates no measurable inhibition beyond the well. CPR = ciprofloxacin (100 µg/ml)

Table 5: Antibacterial activity of *T. indica* fruit pulp extracts using the agar well diffusion method.

TI	Aq. 300	Aq. 150	Aq. 75	Aq. 37.5	EtOH 300	EtOH 150	EtOH 75	EtOH 37.5	n-Hex 300	n-Hex 150	n-Hex 75	n-Hex 37.5	CPR
EC	17.00 ± 1.00	14.33 ± 0.58	6.0	6.0	18.67 ± 0.58	10.00 ± 1.00	6.0	6.0	18.00 ± 1.00	15.00 ± 1.00	13.67 ± 0.58	11.33 ± 0.58	29.0
KP	14.67 ± 0.58	13.33 ± 0.58	6.0	6.0	17.00 ± 1.00	9.33 ± 0.58	6.0	6.0	13.00 ± 1.00	12.00 ± 1.00	10.00 ± 1.00	6.0	28.0
SA	16.00 ± 2.00	6.0	6.0	6.0	16.00 ± 0.00	6.0	6.0	6.0	16.00 ± 0.00	14.0 ± 2.00	6.0	6.0	23.0
SP	13.00 ± 0.00	9.67 ± 0.58	6.0	6.0	14.33 ± 0.58	6.0	6.0	6.0	6.0	6.0	6.0	6.0	22.0

Key: TI: Test isolates, EC: E. coli, KP: *K. pneumonia, SA: S. aureus, SP: S. pneumonia*. Values are mean ± SD of triplicate determinations. A value of 6.00 mm represents the diameter of the well and indicates no measurable inhibition beyond the well. CPR = ciprofloxacin (100 µg/ml).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results further confirmed the antibacterial potential of the extracts (Table 8). The MIC values ranged from 32 to 280 mg/mI, while the MBC values ranged from 37.5 to 300 mg/mI among the extracts and susceptible isolates. Lower MIC values were recorded for *A. digitata* aqueous and ethanolic extracts against *E. coli* and *K. pneumoniae* at 32.0 mg/mI. *T. indica* showed MIC values of 70 mg/mI for ethanolic extract against *E. coli*, while *V. paradoxa* had MIC of 37.5 mg/mI for n-hexane extract against *E. coli*. MBC values were generally higher than the corresponding MIC values, indicating that greater extract concentrations were required to achieve bacterial killing than to inhibit bacterial growth.

The ANOVA showed that antibacterial activity of *A. digitata* differed significantly among bacterial isolates ($F = 7.2318$, $p = 0.000002$), whereas the

extract-treatment effect was not significant (F = 0.5957, p = 0.557575). For *T. indica,* differences among bacterial isolates were significant (F = 7.9297, p = 0.00000079), whereas the extract-treatment effect was not significant (F = 1.0208, p = 0.3725). For *V. paradoxa*, the difference among bacterial isolates was not significant (F = 1.2532, p = 0.220989), whereas the extract-treatment effect was highly significant (F = 82.2601, p < 0.001).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC results further confirmed the antibacterial potential of the fruit pulp extracts. In general, MBC values were equal to or higher than the corresponding MIC values, indicating that higher concentrations were often required to achieve bacterial killing than to inhibit bacterial growth.

Table 6: Antibacterial activity of *V. paradoxa* fruit pulp extracts using the agar well diffusion method.

TI	Aq. 300	Aq. 150	Aq. 75	Aq. 37.5	EtOH 300	EtOH 150	EtOH 75	EtOH 37.5	n-Hex 300	n-Hex 150	n-Hex 75	n-Hex 37.5	CPR
EC	22.00 ± 1.00	18.00 ± 2.00	15.00 ± 0.00	11.00 ± 2.00	26.00 ± 2.00	18.00 ± 0.00	15.00 ± 0.00	9.00 ± 1.00	27.00 ± 1.00	20.33 ± 1.53	14.67 ± 1.53	8.67 ± 1.53	32.0
KP	18.00 ± 0.00	15.00 ± 1.00	12.00 ± 1.00	8.00 ± 0.00	31.00 ± 0.00	27.00 ± 1.00	21.00 ± 0.00	15.00 ± 1.00	20.00 ± 0.00	17.00 ± 1.00	11.00 ± 0.00	9.00 ±1 .00	30.0
SA	25.00 ± 0.00	20.00 ± 0.00	11.00 ± 1.00	8.67 ± 1.53	28.00 ± 0.00	20.00 ± 2.00	11.00 ± 0.00	6.0	17.00 ± 1.00	12.00 ± 0.00	8.00 ± 1.00	6.0	26.0
SP	19.00 ± 0.00	16.00 ± 1.00	13.00 ± 0.00	10.00 ± 1.00	26.00 ± 1.00	19.00 ± 0.00	11.00 ± 1.00	6.0	15.00 ± 1.73	12.00 ± 1.00	9.00 ± 1.00	6.0	24.0

Key: TI: Test isolates, EC: E. coli, KP: *K. pneumonia, SA: S. aureus, SP: S. pneumonia*. Values. Well diameter = 6.0 mm; CPR = ciprofloxacin (100 μg/ml).

Table 7: ANOVA summary for antibacterial activity of the fruit pulp extracts.

Fruit	Factor (ANOVA source)	F	p-value	Decision (α=0.05)
A. digitata	Rows	7.231817	0.000002	Significant
A. digitata	Columns	0.595681	0.557575	Not significant
T. indica	Rows	7.929685	0.00000079	Significant
T. indica	Columns	1.020751	0.3725	Not significant
V. paradoxa	Rows	1.25324	0.220989	Not significant
V. paradoxa	Columns	82.26011	0.000000	Significant

Table 8: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the fruit pulp extracts.

Fruit	Test isolate	Aqueous MIC	Aqueous MBC	Ethanol MIC	Ethanol MBC	n-Hexane MIC	n-Hexane MBC
A. digitata	*E. coli*	32.0	37.5	260	280	70	75
A. digitata	*K. pneumoniae*	260	280	260	280	260	280
A. digitata	*S. aureus*	280	300	-	-	260	280
A. digitata	*S. pneumoniae*	-	-	-	-	-	-
T. indica	*E. coli*	130	150	70	75	37.5	>37.5
T. indica	*K. pneumoniae*	130	140	70	75	70	75
T. indica	*S. aureus*	260	280	130	150	130	150
T. indica	*S. pneumoniae*	150	>150	140	150	-	-
V. paradoxa	*E. coli*	32.0	37.5	32.0	37.5	32.0	37.5
V. paradoxa	*K. pneumoniae*	32.0	37.5	32.0	37.5	32.0	>37.5
V. paradoxa	*S. aureus*	32.0	>37.5	70.0	75.0	75.0	>75.0
V. paradoxa	*S. pneumoniae*	37.5	>37.5	70.0	>75.0	70.0	>75.0

Key: MIC = minimum inhibitory concentration; MBC = minimum bactericidal concentration; − = not tested because there was no inhibition. Concentrations are expressed in mg/mI.

Discussion

The present study revealed that the fruit pulps of *Adansonia digitata, Tamarindu sindica,* and *Vitellaria paradoxa* contain diverse phytochemicals, including alkaloids, tannins, flavonoids, phenols, glycosides, terpenoids, saponins, and steroids (Table 1). The widespread occurrence of these secondary metabolites across the extracts supports the traditional medicinal uses of these fruits and highlights their potential as sources of biologically active compounds. Similar phytochemical profiles have previously been reported for these plant species and other medicinal fruits, where such compounds were associated with antioxidant, antimicrobial, and anti-inflammatory activities (Thompson et al., 2024; Niamketchi et al., *2025).*

Phenols were the predominant phytochemicals in most extracts, with the ethanolic extract of *A. digitata* recording the highest concentration at 90.10 mg/g (Table 2). This finding is consistent with studies that identified baobab fruit pulp as an important source of phenolic compounds and natural antioxidants (Thompson et al., 2024). However, Fischer et al., (2020) reported lower phenolic and flavonoid contents in *A. digitata* fruit pulp. Such variations may be attributed to differences in environmental conditions, fruit maturity, geographical origin, and extraction procedures.

The ethanolic extract of *T. indica* exhibited the highest tannin (80.20 mg/g) and flavonoid (62.40 mg/g) contents among the studied fruits (Table 2). These results further support previous findings that tamarind fruit pulp is rich in polyphenolic compounds with potential health benefits (Niamketchi et al., 2025). Differences reported among studies may be due to variations in solvent systems, extraction conditions, and analytical methods.

Although *V. paradoxa* exhibited comparatively lower concentrations of several quantified phytochemicals, all investigated phytochemical classes were detected (Tables 1 & 2). This contrasts with reports of higher phenolic and flavonoid contents in other parts of the plant (Chegeni et al., 2025), suggesting that phytochemical accumulation varies among plant organs. Since the present study focused exclusively on fruit pulp, such differences are expected.

Extraction solvent influenced the numerical recovery of several phytochemical constituents, with ethanol generally yielding higher concentrations of phenols, tannins, flavonoids, and glycosides than aqueous and n-hexane extracts, likely due to its intermediate polarity and ability to dissolve a wide range of phytochemicals (Table 2). Similar observations have been reported by Rungsung et al. (2024) and Chegeni et al. (2025). Conversely, n-hexane was more effective in extracting terpenoids, reflecting the non-polar nature of these compounds.

The antibacterial assay demonstrated that the fruit pulp extracts possessed varying degrees of activity against *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus,* and *Streptococcus pneumoniae.* (Table 4-6). Among the tested fruits, *Vitellaria paradoxa* exhibited the broadest antibacterial spectrum and produced the highest inhibition zone (31 mm) against *K. pneumonia* (Table 6). The observed inhibition zones were comparable to those reported for several medicinal plant extracts with established antibacterial properties. Although larger inhibition zones were generally observed at higher extract concentrations, statistical analysis indicated that concentration effects were significant only for *V. paradoxa* extracts.

The antibacterial activities observed in the present study were closely associated with the phytochemical constituents detected in the fruit pulp extracts. Ethanolic extracts of *A. digitata* and *T.indica*, which contained relatively high concentrations of phenols, flavonoids, tannins, and glycosides (Table 2), generally exhibited stronger antibacterial activity than the corresponding aqueous and n-hexane extracts (Table 4 & 5). These phytochemicals are known to possess antimicrobial properties through multiple mechanisms of action. Phenols and flavonoids can disrupt bacterial cell walls and membranes, resulting in leakage of intracellular contents and inhibition of essential metabolic processes. Tannins exert antimicrobial effects through protein precipitation and enzyme inhibition, while alkaloids may interfere with DNA

replication and cellular metabolism. Terpenoids have also been reported to alter membrane integrity and inhibit microbial growth (Gomathi et al., 2024; Jeruto et al., 2024). Interestingly, *V. paradoxa* demonstrated comparatively stronger antibacterial activity despite possessing lower concentrations of several quantified phytochemicals. This suggests that antibacterial efficacy may not depend solely on the abundance of phytochemicals but may also result from synergistic interactions among bioactive constituents or the presence of highly potent antimicrobial compounds. Therefore, the antibacterial activities demonstrated by the fruit pulp extracts are likely due to the combined action of multiple phytochemical classes rather than a single bioactive compound.

The MIC and MBC results further confirmed the antibacterial potential of the fruit pulp extracts (Table 8). Lower MIC values obtained for several extracts indicate their ability to inhibit bacterial growth at relatively low concentrations, whereas the corresponding MBC values demonstrate their bactericidal potential. These findings support the agar well diffusion results and provide additional evidence for the antimicrobial efficacy (Table 4-8).

Overall, the results establish a strong relationship between phytochemical composition (Table 2) and antibacterial activity (Tables 4–8) in the studied fruit pulps. The presence of abundant phenols, flavonoids, tannins, alkaloids, and terpenoids likely contributed to the observed antibacterial effects. These findings provide scientific support for the traditional medicinal applications of *A. digitata, T. indica,* and *V. paradoxa* and suggest that these fruits may serve as promising sources of natural antibacterial agents for pharmaceutical and nutraceutical development.

This study was limited to the evaluation of crude fruit pulp extracts and did not identify the specific compounds responsible for the observed antibacterial activities. Furthermore, antibacterial activity was assessed only under in vitro conditions against selected bacterial pathogens. Therefore, the results should be interpreted with caution until further studies involving compound isolation, toxicity assessment, mechanism-of-action investigations, and in vivo evaluations are conducted.

Conclusion

The study demonstrated that the fruit pulps of *Adansonia digitata, Tamarindus indica*, and *Vitellaria paradoxa* contain diverse phytochemical constituents and exhibit in-vitro antibacterial activity against the tested bacterial isolates. Phytochemical profiles varied among the extracts, while *V. paradoxa* exhibited the broadest antibacterial activity and the highest inhibition zone recorded in the study. MIC and MBC findings provided additional evidence of antibacterial activity, although potency varied among extract–isolate combinations. The results indicate that these fruit pulps are promising sources of natural antibacterial phytochemicals; however, because the study evaluated crude extracts under in vitro conditions, further research is recommended to isolate and characterize the active compounds, investigate their mechanisms of action, assess safety and toxicity, and validate their antibacterial potential through appropriate in vivo studies before therapeutic or commercial applications are considered.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

Amina Ibrahim Usman: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing—original draft. Umar Sharif: Supervision, Project administration, Writing—review and editing. Muhammad Yushau: Conceptualization, Methodology, Investigation, Supervision. Surayya Lawan Idris: Writing—review and editing. Ibrahim Muhammad:

Writing—review and editing. All authors reviewed and approved the final version of the manuscript.

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