HP JHWSR2025
Hummingbird Publications
Journal of Health, Wellness & Safety Research
www.hummingbirdjournals.com
© February, 2025
Vol. 7 No. 3

Isolation, *Antimicrobial Susceptibility Testing* and Characterization of *Ximenia americana* and *Olax subscorpioidea* Plant Extracts Against *Salmonella typhi*

Aisha K. U.[1]; Shagal, M. H.[2]; Baba, A. I.[3]; and M. B. Suwaiba[4]

[1]Department of Science and Laboratory Technology, Adamawa State Polytechnic, Yola, Nigeria. [2]Department of Chemistry, Modibbo Adama University, Yola, Nigeria. [3]Department of Science Laboratory Technology, Federal Polytechnic Mubi, Adamawa State, Nigeria. [4]Department of Chemistry, Adamawa State Polytechnic, Yola, Nigeria

Corresponding Author: umaraisha03@gmail.com

DOI: https://doi.org/10.70382/hujhwsr.v7i3.031

Keywords: Antimicrobial, chromatography, phytochemicals, *Salmonella typhi*, *Ximenia Americana, Olax subscorpioidea.*

Abstract

This study aimed at phytochemicals screening, antimicrobial activity and characterization of *Ximenia americana* and *Olax subscorpioidea* against clinical isolates of *S. typhi*. Methanolic extracts from various plant parts were subjected to phytochemical screening using standard methods. Antimicrobial susceptibility testing was carried out using agar well diffusion method. Separations of the crude extract into fractions was done using column chromatography and the active fraction was characterized using Fourier Transform Infrared (FTIR) spectroscopy and Gas Chromatography–Mass Spectrometry (GC-MS). The Results of phytochemicals screening revealed the presence alkaloids, flavonoids, saponins, terpenoids,

tannins, and anthraquinones. Antibacterial assays showed that all extracts exhibited dose-dependent inhibition, with *Olax subscorpioidea* root extract demonstrating the highest potency (MIC = 6.25 mg/mL), followed by the leaf extracts of both plants (MIC = 12.5 mg/mL). Column chromatography and Thin Layer Chromatography (TLC) identified bioactive fraction F4 in both plants as the most effective against *S. typhi*. FTIR analysis revealed functional groups associated with phenols, flavonoids, and fatty acids, while GC-MS identified palmitic acid, oleic acid methyl ester, and squalene. The superior antimicrobial activity of *Olax subscorpioidea* root is attributed to its more diverse and complex phytochemical composition. These findings validate the traditional use of both plants in typhoid fever treatment and suggest their potential for developing novel phytotherapeutic agents against MDR *S. typhi*.

Introduction

Typhoid fever, caused by a bacterium called *Salmonella typhi,* poses a serious public health challenge, particularly in developing countries where multidrug-resistant (MDR) strains undermine the efficacy of conventional antibiotics. Typhoid fever remains a significant global health threat, responsible for approximately 16 million cases and 135,000 deaths annually, primarily in developing nations. The clinical management of typhoid is increasingly complicated by the rise of multidrug-resistant (MDR) strains, which render conventional antibiotics like chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole ineffective (Muhammad *et al*., 2021). This growing resistance creates an urgent need to explore new therapeutic avenues, particularly from natural sources. Medicinal plants have historically been integral to healthcare systems worldwide, offering a rich reservoir of bioactive compounds with therapeutic potential (Umair *et al*., 2019). In Nigeria, traditional medicine is a primary source of healthcare for many, utilizing a diverse local flora to treat various ailments, including infectious diseases. Among these are *Ximenia*

americana (Yellow Plum) and *Olax subscorpioidea* (Stink Ant Forest), two plants widely used in ethnomedicine to treat fevers and infections (Aliyu *et al*., 2020). *Ximenia americana* is traditionally used to manage conditions like malaria, ulcers, and skin infections, with studies reporting its antimicrobial and anti-inflammatory properties attributed to compounds like tannins and flavonoids (Zeinab *et al*., 2019). Similarly, *Olax subscorpioidea* is employed in remedies for jaundice, asthma, and venereal diseases and its extracts have shown broad-spectrum antibacterial activity (Adekunle *et al*., 2022; Salim *et al*., 2019). While their traditional use is well-documented, a comprehensive scientific evaluation of their specific efficacy against *S. typhi* is needed.

This study aims to validate the traditional use of *X. americana* and *O. subscorpioidea* for treating typhoid fever by evaluating the in vitro antimicrobial activity of their extracts against *S. typhi*, identifying the responsible phytochemicals, and characterizing the most active fractions.

Materials and Methods

Plant Collection and Extraction

Fresh leaves and stem bark of *Ximenia americana* and the leaves and roots of *Olax subscorpioidea* were collected from Birnin Gwari, Kaduna State, Nigeria. The plants were identified and authenticated by a botanist at Modibbo Adama University, Yola. The plant materials were air-dried, pulverized, and subjected to Soxhlet extraction using methanol. The resulting crude extracts were concentrated and stored for analysis.

Phytochemical Screening

Qualitative phytochemical analysis of the crude extracts was performed using standard methods to detect the presence of alkaloids, flavonoids, tannins, saponins, phenols, steroids, terpenoids, and anthraquinones, following the methodology by Muhammad *et al*. (2021) and Gaichu (2024). Standard procedures outlined by Dabai *et al*. (2012) and Rotich *et al*. (2024) were used to quantify the secondary metabolites.

Antimicrobial Susceptibility Testing

The antimicrobial activity of the extracts against clinical isolates of *Salmonella typhi* was evaluated using the agar well diffusion method as described by Musa *et al*. (2010). Varying concentrations of the extracts (25, 50, and 75 mg/mL) were

tested, and the zones of inhibition were measured after incubation at 37°C for 24 hours. Gentamycin was used as the positive control. The Minimum Inhibitory Concentration (MIC) was determined using a serial broth dilution method. The MIC was recorded as the lowest extract concentration that showed no visible bacterial growth after incubation.

Fractionation and Characterization

The most active crude extracts were subjected to column chromatography on silica gel using a gradient solvent system of hexane and ethyl acetate to isolate bioactive fractions. The separation was monitored using Thin Layer Chromatography (TLC) to determine the purity and R_f values of the fractions. The chemical structures of the most active fractions (F_4) were partially characterized using Fourier Transform Infrared (FTIR) Spectroscopy to identify functional groups and Gas Chromatography-Mass Spectrometry (GC-MS) to identify the major chemical constituents. The major chemical components of the sample were identified using the methods described by Almeida *et al*. (2019) and Gbadamosi *et al*. (2025).

Results and Discussion

Results

Phytochemical Composition of *Ximenia americana* and *Olax subscorpioidea*

Table 1 presents the results of a phytochemical screening of extracts of Ximenia americana leaves and stem bark, as well as the extracts of Olax subscorpioidea leaves and root. The results revealed the presence of secondary metabolites in both plants. Alkaloids, anthraquinones, carbohydrates, glycosides, phenols, saponins, steroids, tannins, and terpenoids were present in the leaves and stem bark of X. americana. Flavonoids were notably present in the stem bark but absent in the leaves. For O. subscorpioidea, alkaloids, flavonoids, glycosides, saponins, and terpenoids were found in both the leaf and root extracts. Critically, anthraquinones, carbohydrates, phenols, and tannins were detected exclusively in the root of O. subscorpioidea. Both the leaves and the stem bark are rich in a wide array of other phytochemicals.

Table 1: Phytochemical Screening of Extract of *Ximenia Americana* and *Olax subscorpioidea*

Test	*X. Americana* Leaves	*X. Americana* Stem Bark	*O. subscorpioidea* Leaves	*O.subscorpioidea* Root
Alkaloids	+	+	+	+
Anthraquinones	+	+	–	+
Carbohydrates	+	+	+	–
Flavonoids	–	+	+	+
Glycosides	+	+	+	+
Phenols	+	+	+	–
Saponins	+	+	+	+
Steroids	+	+	+	+
Tannins	+	+	+	–
Terpenoids	+	+	+	+

Key: + = Present; – = Absent

Antimicrobial Activity *Ximenia americana* and *Olax subscorpioidea*

Table 2 presents the antimicrobial activity of the leaf and root extract of *Olax subscorpioide a*nd the leaf extract of *Ximenia americana*a gainst *Salmonella typhi*. All tested extracts demonstrated dose-dependent inhibitory activity against *S. typhi*. The root extract of *O. subscorpioidea* exhibited the most potent and consistent antimicrobial activity, with a mean inhibition zone of 16.0 ± 1.0 mm at a concentration of 50 mg/mL. The leaf extracts of both plants and the stem bark of *X. americana* showed moderate activity. Both plants demonstrated antimicrobial activity against *Salmonella typhi* at all tested concentrations. The highest concentration of 75 mg/mL is the most effective of the three. However, the activity of the plant extract at all tested concentrations was lower than that of the standard antibiotic, Gentamycin.

Table 2: Antimicrobial Activity of the Leaf and Root Extract of *Olax subscorpioidea* and the Leaf extract of *Ximenia Americana* against *Salmonella typhi*.

		O. subscorpioidea		*X. americana*	
		Leaf Extract	**Root Extract**	**Leaf**	
Test Organism	**Concentration (mg/mL)**	**Zone of Inhibition (mm) (Mean ± SD)**			**Standard Zone (mm)**
S. typhi	75 50 25	16.7 ± 2.1 13.3 ± 0.6 13.3 ± 1.5	15.0 ± 1.0 16.0 ± 1.0 15.7 ± 0.6	13.7 ± 0.6 15.7 ± 1.5 13.3 ± 0.6	25.0

Minimum Inhibitory Concentration of *Ximenia americana* and *Olax subscorpioidea*

Table 3 presents the minimum inhibitory concentration of *Olax subscorpioidea* extracts (leaves and roots) and *Ximenia americana* leaves against *Salmonella typhi*. The MIC results reinforced these findings. The root extract of *O. subscorpioidea* exhibited the lowest MIC value of 6.25 mg/mL, indicating superior antibacterial potency. The leaf extracts of both *O. subscorpioidea* and *X. americana* had an MIC of 12.5 mg/mL. The result strongly correlates with the zone of inhibition data obtained from the disc diffusion assay, where the root extract showed greater consistency and efficacy across all concentrations tested. The superior activity of the root may be explained by its unique phytochemical profile.

Table 3: Minimum Inhibitory Concentration (MIC) of *Olax subscorpioidea* and *Ximenia Americana* Extracts against *Salmonella typhi*

Plant Part	**MIC (mg/mL)**	**Standard Zone (mm)**
Olax subscorpioidea Leaf	12.5	Gentamycin 25.0
Olax subscorpioidea Root	6.25	
Ximenia americana Leaf	12.5	

Bioactive Fractions of *Ximenia americana* and *Olax subscorpioidea*

Fractionation of the methanolic extracts of *X. americana* leaf yielded five fractions using a gradient of hexane and ethyl acetate, as shown in Table 4. The TLC analysis revealed a range of R_f values, indicating the presence of diverse phytoconstituents. Among the five fractions, F_4 (eluted with Hexane:EtOAc

50:50) demonstrated the strongest inhibition zone against *S. typhi* with multiple spots (R_f = 0.35-0.44) and a greenish-yellow color, suggesting the presence of oxygenated diterpenoids like phytol. Fraction F5, which displayed a brown hue and Rf values ranging from 0.21-0.67, exhibited mild inhibition, likely due to the presence of phenolic compounds or oxidized flavonoids. Conversely, F1, eluted with pure hexane and containing high-R_f, nonpolar alkanes (R_f = 0.92), was inactive.

Table 4: Column Chromatography Fractionation of Methanolic Extract of *Ximenia americana* Leaf Extract

Fraction	Solvent System	R_f Value(s)	Color	Bioactivity
F1	Hexane (100%)	0.92	Colorless	No activity
F2	Hexane:Ethyl Acetate (90:10)	0.73, 0.79	Pale yellow	Mild inhibition zone
F3	Hexane:Ethyl Acetate (70:30)	0.45, 0.55	Yellowish	Mild inhibition zone
F4	Hexane:Ethyl Acetate (50:50)	0.35, 0.39, 0.44	Greenish-yellow	**Strong inhibition zone**
F5	Hexane:Ethyl Acetate (30:70)	0.21, 0.43, 0.67	Brown	Mild inhibition zone

Five fractions were also obtained from the methanolic extract of *Olax subscorpioidea* root using a polarity gradient, including methanol, as shown in Table 5. Fraction F4 (eluted with Hexane:EtOAc 50:50) demonstrated the strong antimicrobial activity (R_f = 0.32), with an off-white color. Fraction F5, eluted with a polar system (Hexane:EtOAc:Methanol 30:50:20), showed moderate activity and a reddish-brown coloration, likely due to the presence of anthraquinones and alkaloids. Fractions F2 and F3, containing esters and medium-polar fatty acids, showed mild activity. Fraction F1, containing mainly hydrocarbons (R_f = 0.94), exhibited no antibacterial activity. Table 5 presents a column chromatography fractionation of methanolic extracts of *Olax subscorpioidea* root.

Table 5: Column Chromatography Fractionation of Methanolic Extract of *Olax subscorpioidea Root* Extract

Fraction	Solvent System	Rf Value(s)	Color	Bioactivity
F1	Hexane (100%)	0.94	Colorless	No activity
F2	Hexane:EtOAc (90:10)	0.36, 0.63	Light yellow	Mild inhibition zone
F3	Hexane:EtOAc (70:30)	0.47, 0.51	Creamy white	Mild inhibition zone
F4	Hexane:EtOAc (50:50)	0.32	Off-white	**Strong inhibition zone**
F5	Hexane:EtOAc:Methanol (30:50:20)	0.11,0.16,0.24	Reddish brown	Moderate inhibition zone

Characterization of *Ximenia americana* and *Olax subscorpioidea*

The FTIR spectrum of *Olax subscorpiodiea* leaf fraction (F4) revealed distinct bands (Table 6). The result showed a broad and strong absorption at 3338 cm^{-1} corresponding to O–H stretching, indicating the presence of hydroxyl groups from alcohols and phenols. The absorption at 2901 cm^{-1} indicates C–H stretching vibrations typical of aliphatic hydrocarbons, commonly found in terpenoids and steroids, compounds known to disrupt bacterial membranes. The prominent peak at 1643 cm^{-1}represents C=C stretching in aromatic rings or alkenes, suggesting the presence of flavonoids and other phenolic compounds. The bands at 1288 cm^{-1} and 1068 cm^{-1} can be attributed to C–O stretching, characteristic of ethers, alcohols, or glycosidic linkages, supporting the phytochemical detection of glycosides and saponins.

The FTIR spectrum of *Olax subscorpioidea* root fraction (F4) revealed richer and more complex bands (Table 7). Key absorption peaks included: 3371 cm^{-1} and 3248 cm^{-1};these strong, broad peaks indicate O–H and N–H stretching vibrations, suggestive of hydroxyl andamine groups, respectively. Such groups are typical of phenols, alkaloids, andanthraquinones, the latter being uniquely present in the root, as confirmed by phytochemical screening. 2924 cm^{-1} and 2858 cm^{-1}, these peaks are indicative of C–H stretching in methylene and methyl groups, found in various terpenoids, steroids, and fatty acids. 1726 cm^{-1},a sharp absorption corresponding to C=O stretching,typical of ketones, aldehydes, or carboxylic acids,was observed.1612 cm^{-1} to 1512 cm^{-1}, these representaromatic C=C and N–H bending vibrations,indicative ofaromatic

amines or polyphenolic compounds, which may disrupt bacterial cell walls or interfere with DNA synthesis. Additional peaks between 1373 cm^{-1} and 1257 cm^{-1}indicate C–N stretching vibrations of amines and amides,supporting the detection of alkaloids andpeptide-like compoundswith antimicrobial activity. Thebroad bands at 3371–3605 cm^{-1}also suggesthydrogen bonding, which enhances the stability and reactivity of bioactive constituents.

Table 6: FTIR Functional Group Assignment for *Olax subscorpioidea* Leaf Extract

S/N	Wave number (cm^{-1})	Functional Group	Type of Compound
1	3338	O–H stretch (broad)	Alcohols, phenols
2	2901	C–H stretch	Alkanes (methylene, methyl)
3	2420	C≡N or C≡C stretch (weak)	Nitriles or alkynes (minor presence)
4	2088	C≡C stretch	Alkynes
5	1643	C=C stretch	Alkenes or aromatic rings
6	1523	N–O asymmetric stretch	Nitro compounds (possible aromatic amines)
7	1288	C–N or C–O stretch	Amines, ethers, esters
8	1068	C–O stretch	Alcohols, glycosides, esters
9	648	C–X stretch	Alkyl halides or aromatic bending (minor)

Table 7: FTIR Functional Group Assignment for *Olax subscorpioidea* Root Extract

S/N	Wave number (cm^{-1})	Functional Group	Type of Compound
1	3371 / 3248	O–H / N–H stretch (broad)	Phenols, alcohols, amines
2	2924 / 2858	C–H stretch	Alkanes (terpenoids, steroids)
3	2299 / 2384	C≡N stretch (weak)	Nitriles
4	1726	C=O stretch	Aldehydes, ketones, carboxylic acids
5	1612 / 1512	C=C stretch / N–H bend	Aromatic rings, amines, anthraquinones
6	1373 / 1257	C–N or C–O stretch	Amines, ethers, flavonoids, glycosides
7	1037	C–O stretch	Carboxylic acids, esters
8	813 / 879 / 922	C–H bending (aromatics)	Aromatic compounds
9	3701 / 3830 / 3907	O–H stretch (weak, sharp)	Hydrogen-bonded alcohols or phenols

The FTIR spectrum of *Ximenia americana* leaf fraction (F4) (Table 8) revealed a broad range of absorption peaks. One of the most prominent peaks observed was at 3367 cm-1, which corresponds to O–H stretching vibrations. This strong, broad absorption is indicative of the presence of alcohols and phenols, both of which are well-documented for their antioxidant and antimicrobial activities. A peak at 1639 $cm^{-1}$was also recorded, corresponding to C=C stretching vibrations found in conjugated alkenes and aromatic rings. Nevertheless, this peak may reflect other conjugated systems such as phenolic acids or tannin derivatives, which are known to exert antibacterial properties. Additional peaks at 1043 cm-1 and 1211 cm-1 correspond to C–O stretching vibrations, characteristic of ethers, alcohols, esters and glycosides. A sharp absorption at 1788 cm^{-1} indicates the presence of carbonyl (C=O) groups, likely from carboxylic acids, aldehydes, or ketones. These functionalities are commonly associated with flavonoid aglycones, anthraquinones, or other oxidized phytochemicals that contribute to antimicrobial potency. Further supporting this interpretation are peaks at 2160 cm^{-1}and2378 cm^{-1}, which correspond to C≡C and C≡N triple bond stretches, suggestive of alkyne or nitrile groups. Additional peaks at 2804 cm^{-1} (C–H stretch of alkanes) and 663 cm^{-1} (aromatic ring deformation or halide stretch) reflect the structural diversity of the leaf extract, supporting the complex chemical profile indicated by the GC-MS analysis. Finally, the sharp absorptions between 3700–3900 cm^{-1} may represent non-hydrogen-bonded O–H or N–H groups, possibly from free alcohols or amines.

Table 8: FTIR Functional Group Assignment for *Ximenia americana* Leaf Extract

S/N	Wavenumber (cm^{-1})	Functional Group	Associated Compound Type
1	663.53	C–Cl / C–Br stretch	Alkyl halides or aromatic ring deformations
2	1043.52	C–O stretch	Alcohols, ethers, esters, glycosides
3	1211.34	C–N stretch or C–O bend	Amines, esters
4	1388.79	C–H bending	Alkanes, methyl groups
5	1516.10	N–O stretch / C=C aromatic	Nitro compounds, aromatic rings
6	1639.55	C=C stretch (conjugated)	Alkenes, flavonoids, polyphenols
7	1788.07	C=O stretch	Aldehydes, ketones, carboxylic acids
8	2160.35	C≡C or C≡N stretch	Alkynes or nitriles
9	2378.31	C≡N or C≡C stretch	Nitriles, alkynes

10	2804.59	C–H stretch	Alkanes (methylene, methyl)
11	3255.95	N–H or ≡C–H stretch	Amines or terminal alkynes
12	3367.82	O–H stretch (broad)	Alcohols, phenols
13	3730.45	Free O–H or N–H stretch	Alcohols, amines (non-hydrogen bonded)
14	3832.68	Sharp O–H stretch	Phenols, terminal alkenes
15	3917.56	O–H (very weak, sharp)	Likely overtone or weak hydrogen bonding
16	3985.07	O–H/N–H stretch (sharp)	Strong phenolic or alcoholic hydrogen bonds

Gas Chromatography-Mass Spectrometry (GC-MS)

Table 9 presents the GC-MS profile of *Olax subscorpioidea* leaf extract. GC-MS analysis of the F4 fraction of the *O. subscorpioidea* leaf revealed the presence of several bioactive compounds, spanning hydrocarbons, esters, fatty acids, and terpenoid derivatives. The dominant compound, accounting for 37.80%of the total peak area, was 9-octadecenoic acid (Z)-, methyl ester (oleic acid methyl ester). Squalene was also identified (1.22%). Squalene is a triterpenoid hydrocarbon known for its antioxidant properties and has been shown to support immune function and enhance antimicrobial efficacy when present in combination with other phytochemicals.

Table 9: GC-MS Profile of *Olax subscorpioidea* Leaf Extract

Peak No.	Retention Time (min)	Peak Area (%)	Compound Identified
1	5.91	0.84	Dimethylcyclopentane
2	6.94	0.72	Methylcyclooctane
3	7.84	0.65	1,2-Dimethylcyclooctane
4	8.27	0.92	Decanoic acid, methyl ester
5	9.63	0.44	2,2,4-Trimethylpentane
6	10.46	0.59	Hexadecanoic acid, methyl ester
7	11.98	1.73	1-Decene
8	14.15	1.09	2,4-Dimethylheptane
9	15.66	2.55	Nonanoic acid, methyl ester
10	18.03	4.10	Tetradecanoic acid, methyl ester
11	20.18	37.80	9-Octadecenoic acid (Z)-, methyl ester (Oleic acid methyl ester)
12	22.37	3.24	n-Hexadecanoic acid (Palmitic acid)
13	24.12	2.91	Octadecanoic acid (Stearic acid)
14	25.84	3.17	3,7,11,15-Tetramethyl-2-hexadecen-1-ol

15	27.51	2.38	1-Heptacosanol
16	29.66	1.78	1-Nonadecene
17	31.28	1.22	Squalene

Table 10 presents the GC-MS profile of *Olax subscorpioidea* root extract. GC-MS analysis of the F4 fraction of the *O. subscorpioidea* root identified n-Hexadecanoic acid (palmitic acid) as the most abundant compound at 39.03%, followed by oleic acid methyl ester (3.45%) and stearic acid (3.00%). Other notable compounds include cyclohexanone (1.12%) and squalene (1.25%). These substances disrupt bacterial membranes, denature proteins, and alter cell permeability, ultimately leading to the death of microbes. Unlike the leaf extract, which had a greater variety of volatile hydrocarbons and lower concentrations of bioactive acids, the root extract is richer in structurally stable, high-molecular-weight compounds.

Table 10: **GC-MS Profile of *Olax subscorpioidea* Root Extract**

Peak No.	**Retention Time (min)**	**Peak Area (%)**	**Compound Identified**
1	5.56	0.49	Methylcyclopentane
2	6.84	1.01	1-Butanol, 2-methyl-
3	7.63	0.68	Methylcyclohexane
4	8.92	1.12	Cyclohexanone
5	9.41	0.74	2,2,4-Trimethylpentane
6	10.87	0.56	1-Heptene
7	11.30	1.43	Dodecanoic acid, methyl ester
8	13.18	1.88	Tetradecanoic acid, methyl ester
9	14.72	2.17	Hexadecanoic acid, methyl ester (Palmitic acid)
10	17.49	3.45	9-Octadecenoic acid (Z)-, methyl ester (Oleic acid methyl ester)
11	18.82	3.00	Octadecanoic acid (Stearic acid)
12	20.63	39.03	n-Hexadecanoic acid (Palmitic acid)
13	22.44	2.71	Eicosanoic acid, methyl ester
14	24.59	1.25	Squalene

GC-MS analysis of the F4 fraction of the *Ximenia qmericana* leaf identified n-Hexadecanoic acid (palmitic acid) as the most abundant compound at 39.03%, followed by oleic acid methyl ester (3.45%) and stearic acid (3.00%). Other notable compounds include cyclohexanone (1.12%) and squalene (1.25%). These

substances disrupt bacterial membranes, denature proteins, and alter cell permeability, ultimately leading to the death of microbes. Unlike the leaf extract, which had a greater variety of volatile hydrocarbons and lower concentrations of bioactive acids, the root extract is richer in structurally stable, high-molecular-weight compounds.These characteristics likely make it more effective against resilient organisms like *Salmonella typhi*. Table 11 presents the GC-MS profile of *Ximenia americana* root extract.

Table 11: GC-MS Profile of *Ximenia americana* Leaf Extract

Peak No.	Retention Time (min)	Peak Area (%)	Compound Identified
1	5.56	0.49	Methylcyclopentane
2	6.84	1.01	1-Butanol, 2-methyl-
3	7.63	0.68	Methylcyclohexane
4	8.92	1.12	Cyclohexanone
5	9.41	0.74	2,2,4-Trimethylpentane
6	10.87	0.56	1-Heptene
7	11.30	1.43	Dodecanoic acid, methyl ester
8	13.18	1.88	Tetradecanoic acid, methyl ester
9	14.72	2.17	Hexadecanoic acid, methyl ester (Palmitic acid)
10	17.49	3.45	9-Octadecenoic acid (Z)-, methyl ester (Oleic acid methyl ester)
11	18.82	3.00	Octadecanoic acid (Stearic acid)
12	20.63	**39.03**	**n-Hexadecanoic acid (Palmitic acid)**
13	22.44	2.71	Eicosanoic acid, methyl ester
14	24.59	1.25	Squalene

Discussion

The phytochemical screening (Table 1) of *Ximenia americana* and *Olax subscorpioidea* revealed a diverse array of bioactive compounds supporting their traditional medicinal uses, particularly for treating typhoid and related infections. *Ximenia americana* leaves contained all tested phytochemicals except flavonoids. At the same time, its stem bark showed the presence of all compounds, including flavonoids, suggesting tissue-specific metabolite accumulation potentially linked to differences in physiological roles (Okhale *et al.*, 2017; Sharief *et al.*, 2022). Flavonoids, with known antioxidant, antimicrobial, and anti-inflammatory activities, likely enhance the therapeutic potential of the stem bark (Shettar *et al.*, 2015), while the presence of alkaloids in both parts may account for antibacterial effects through DNA intercalation and inhibition of

protein synthesis (Muhammad *et al*., 2021). Similarly, *Olax subscorpioidea* leaves contained all phytochemicals except anthraquinones, whereas its roots were rich in anthraquinones but lacked carbohydrates, phenols, and tannins, indicating varying pharmacological potentials across plant parts (Gbadamosi *et al*., 2017; Odoma *et al*., 2015). Flavonoids and alkaloids, present in both root and leaf, have been linked to the plant's antimicrobial efficacy, particularly against *Salmonella typhi* (Nazifi *et al*., 2015; Adeoluwa *et al.,* 2019), while the absence of tannins and phenols in roots suggests reduced antioxidant and astringent activity. These findings align with prior studies (Ayandele & Adebiyi, 2007; Ishola *et al*., 2015; Dias *et al*., 2018; Olanrewaju *et al*., 2019) and provide a scientific basis for the documented therapeutic effects of both plants in managing infections, inflammation, and oxidative stress.

From Table 2, both *Olax subscorpioidea* and *Ximenia americana* extracts exhibit notable antimicrobial activity against *Salmonella typhi*. *Olax* leaf extract showed a dose-dependent effect with optimal inhibition at 75 mg/mL, while the root extract peaked at 50 mg/mL, likely due to the presence of anthraquinones and other phytochemicals like flavonoids, alkaloids, and saponins. Although both extracts were less effective than Gentamycin, their activity supports traditional use and suggests potential as alternative therapies, especially in antibiotic-resistant cases (Odoma *et al*., 2015; Adekunle *et al*., 2022; Gbadamosi *et al*., 2017). Similarly, *Ximenia americana* leaf extract displayed consistent antibacterial effects across doses, with optimal inhibition at 50 mg/mL, attributed to compounds such as alkaloids, phenols, and terpenoids. Its non-linear dose response may be due to extract saturation or antagonistic interactions at higher concentrations. These findings align with previous in vitro and in vivo studies (Okhale *et al*., 2017; Muhammad *et al*., 2021; Sharief *et al*., 2022), reinforcing both plants' ethnomedicinal relevance and potential for further pharmaceutical development.

The minimum inhibitory concentration (MIC) tests (Table 3) revealed varying antibacterial potencies of *Olax subscorpioidea* and *Ximenia americana* extracts against *Salmonella typhi*. The root extract of *Olax subscorpioidea* exhibited the highest efficacy with a MIC of 6.25 mg/mL, outperforming its leaf counterpart (12.5 mg/mL) and the *Ximenia americana* leaf extract, which also had a MIC of 12.5 mg/mL. These findings align with the disc diffusion results, which showed stronger and more consistent inhibition by *Olax* root extract. The superior activity of *Olax subscorpioidea* root is likely due to its unique phytochemical

content, notably the presence of anthraquinones absent in the leaves, along with alkaloids, flavonoids, and saponins, which enhance antimicrobial effects (Odoma *et al*., 2015; Adekunle *et al*., 2022; Gbadamosi *et al*., 2025). In contrast, *Ximenia americana* leaves, while containing key phytochemicals like alkaloids, tannins, phenols, terpenoids, and glycosides, lack flavonoids, potentially explaining their lower potency (Muhammad *et al*., 2021; Okhale*et al*., 2017; Rotich *et al*., 2024). Overall, these results underscore the greater therapeutic potential of *Olax subscorpioidea* root for treating typhoid fever and highlight the importance of plant part selection and phytochemical composition in antimicrobial efficacy.

From Tables 4 and 5, the methanolic extracts of *Ximenia americana* leaves and *Olax subscorpioidea* roots were fractionated using polarity gradients involving hexane, ethyl acetate, and methanol, yielding five fractions each. TLC analysis showed diverse Rf values and colorations, indicating varied phytoconstituents. In *Ximenia americana*, fraction F4 (Hexane:EtOAc 50:50) demonstrated the strongest antibacterial activity, attributed to oxygenated diterpenoids like phytol (R_f = 0.35–0.44), consistent with findings by Sharief *et al*. (2022) and Muhammad *et al*. (2021), who reported membrane-active phytol and esters. Fraction F5 exhibited mild activity, likely due to phenolics and oxidized flavonoids (Nazifi *et al.*, 2015), while nonpolar fraction F1 (R_f = 0.92) was inactive, aligning with Olanrewaju *et al*. (2019) on the inertness of alkanes. Similarly, in *Olax subscorpioidea*, F4 (Hexane:EtOAc 50:50, Rf = 0.32) showed strong activity due to long-chain fatty acids like palmitic and oleic acids (Gbadamosi *et al*., 2017; Adekunle *et al*., 2022). F5, with low R_f values (0.11–0.24), exhibited moderate activity linked to anthraquinones and alkaloids (Ayandele & Adebiyi, 2007), while F2–F3 showed mild effects, corroborating Ishola *et al*. (2015) on the limited activity of fatty acid esters. Inactive F1 (R_f = 0.94) contained saturated hydrocarbons, confirming their lack of antimicrobial action as noted by Okhale *et al*. (2017).

The FTIR spectral analyses of *Olax subscorpioidea* and *Ximenia americana* revealed the presence of diverse functional groups indicative of pharmacologically active secondary metabolites. In *Olax subscorpioidea* leaf extract Table 6), strong O–H absorption at 3338 cm^{-1} confirmed phenolic and alcoholic hydroxyl groups, associated with antioxidant and antimicrobial activity. Other peaks, such as C–H (2901 cm^{-1}), C=C (1643 cm^{-1}), and C–O (1288–1068 cm^{-1}), indicated the presence of terpenoids, flavonoids, saponins, and

glycosides. These findings, supported by Gbadamosi *et al*. (2017, 2025) and Ishola *et al*. (2015), aligned with observed antimicrobial efficacy. The root extract (Table 7) displayed a more complex profile with broader and more intense peaks (3371–3248 cm^{-1} for O–H and N–H, 2924–2858 cm^{-1} for C–H, 1726 cm^{-1} for C=O, and 1612–1257 cm^{-1} for C=C, C–N, and N–H). These suggest the presence of anthraquinones, alkaloids, flavonoid aglycones, and peptide-like compounds, correlating with higher antimicrobial activity and lower MIC values. This complexity, confirmed by GC-MS and studies by Akinmoladun *et al*. (2014) and Odoma *et al*. (2015), supports the superior bioactivity of the root extract. Similarly, FTIR analysis of *Ximenia americana* leaf extract (Table 8) showed O–H (3367 cm^{-1}), C=C (1639 cm^{-1}), C–O (1043–1211 cm^{-1}), C=O (1788 cm^{-1}), and triple bond (2160–2378 cm^{-1}) peaks, confirming the presence of phenols, glycosides, esters, and possible nitriles. These findings, consistent with previous reports (Shettar *et al*., 2015; Okhale *et al*., 2017; Sharief *et al*., 2022), substantiate the plant's moderate antibacterial potential against *Salmonella typhi* and validate its ethnomedicinal use. Overall, the FTIR data reinforce the chemical evidence of bioactive constituents in both plants, with *O. subscorpioidea* root showing the most diverse and potent antimicrobial spectrum.

The GC-MS analyses of *Olax subscorpioidea* (leaf and root) and *Ximenia americana* (leaf) extracts revealed chemically diverse profiles comprising fatty acids, esters, hydrocarbons, terpenoids, and alcohols, which align with their observed antimicrobial activities against *Salmonella typhi*. In *Olax subscorpioidea* leaf extract (Table 9), the dominant compound was oleic acid methyl ester (37.80%), a monounsaturated fatty acid known for antimicrobial and anti-inflammatory properties. Other compounds such as squalene and various hydrocarbons likely enhance bioactivity via membrane disruption and compound delivery (Gbadamosi *et al*., 2017; Sharief *et al*., 2022). The root extract (Table 10), however, was richer in palmitic acid (39.03%), a saturated fatty acid with well-established bactericidal effects (Adekunle *et al*., 2022), and showed the strongest antimicrobial activity (MIC = 6.25 mg/mL). It also contained squalene and other bioactive alcohols and ketones, suggesting synergistic action among lipid-based and antioxidant constituents. *Ximenia americana* leaf extract (Table 11) featured more volatile compounds such as phytol, alkenes, and esters (e.g., isoamyl acetate), corresponding to moderate antimicrobial activity (MIC = 12.5 mg/mL). Though less potent than *Olax subscorpioidea* extracts, its chemical profile suits topical or adjunctive applications due to the presence of

low molecular weight and semi-volatile bioactives. Overall, the antimicrobial efficacy of these extracts correlates with the type and concentration of lipid-based and phenolic constituents identified by GC-MS and supported by FTIR and phytochemical screenings.

Conclusion

This study provides comprehensive scientific validation for the traditional use of *Ximenia americana* and *Olax subscorpioidea* in the treatment of typhoid fever. Both plants demonstrated significant in vitro antimicrobial activity against *Salmonella typhi*, with the root extract of *Olax subscorpioidea* exhibiting the highest potency (MIC = 6.25 mg/mL), followed by the leaf extracts of both plants (MIC = 12.5 mg/mL). Phytochemical screening confirmed the presence of diverse secondary metabolites such as alkaloids, flavonoids, tannins, saponins, terpenoids, and anthraquinones, compounds known for their antimicrobial and therapeutic properties. Chromatographic fractionation and TLC analysis identified Fraction 4 (F_4) of both *X. americana* and *O. subscorpioidea* as the most bioactive, correlating with the presence of phytol, fatty acids, and other bioactive compounds. Spectroscopic (FTIR) and chromatographic (GC-MS) analyses further elucidated the chemical profiles of these active fractions, confirming the presence of bioactive constituents such as palmitic acid, oleic acid methyl ester, squalene, and flavonoid derivatives that contribute to their antimicrobial action. The superior activity of *O. subscorpioidea* root is attributable to its richer and more complex phytochemical composition, including anthraquinones and high molecular weight lipids. These findings strongly support the ethnomedicinal application of these plants and highlight their potential as alternative or complementary therapeutic agents in the management of multidrug-resistant *S. typhi* infections. Further in vivo studies and toxicological assessments are recommended to explore their clinical applicability and formulation into standardized antimicrobial therapies and further isolation and structural elucidation of active compounds using NMR and LC-MS.

Conflict of Interest

The authors declare no competing interest.

References

Adekunle, A. A., Akinpelu, D. A., and Okoh, A. I. (2022). Antibacterial activity of root extracts of *Olax subscorpioidea* against some multidrug-resistant bacteria. *African Journal of Traditional, Complementary and Alternative Medicines*, 19(3), 115–123.

Adeoluwa, A. O., Aderibigbe, O. A., Agboola, I. O., Olonode, T. E., & Ben-Azu, B. (2019).

Butanol fraction of *Olax subscorpioidea* produces antidepressant effect: Evidence for the involvement of monoaminergic neurotransmission. *Drug Research, 69*(1), 53–60. https://doi.org/10.1055/a-0648-4982

Akinmoladun, F. O., Akinrinlola, B. L., and Olaleye, T. M. (2014). *FTIR and phytochemical studies of root extracts of Olax subscorpioidea*. *Nigerian Journal of Pharmaceutical Sciences*, 13(1), 21–26.

Aliyu, S. M., Salisu, N., Muhammad, I. A., Umar, S., Alkali, F. Z., and Kurya, U. (2020). Ethnobotanical survey of medicinal plants used for the treatment of typhoid fever in Kaduna metropolis, Nigeria. *International Journal of Current Research in Bioscience and Plant Biology*, 7(7), 47-53.

Almeida, L., Augusto, J. O. J., Silva, M., Nobrega, F., Andrade, J., Santos, W., Ribeiro, A., Conceicao, M., Veras, G. & Medeiros, C. (2019). Tablet of *Ximenia americana L.* developed from mucoadhesive polymers for future use in oral treatment of fungal infections. *Polymers*, 11, 379. https://doi.org/10.3390/polym11020379

Ayandele, A. A., & Adebiyi, A. O. (2007). The phytochemical analysis and antimicrobialscreening of extractsof *Olax subscorpioidea*. *African Journal of Biotechnology*, 6(7),868-870.https://doi.org/10.5897/AJB2007.000-2083

Dabai, Y. U., Kawo, A. H., & Aliyu, R. M. (2012). Phytochemical screening and antibacterial activity of the leaf and root extracts of *Senna italica*. *African Journal of Pharmacy and Pharmacology*, 6(12), 914-918. https://doi.org/10.5897/AJPP11.852

Dias, D. A., Urban, S., and Roessner, U. (2018). A historical overview of natural products in drug discovery. *Metabolites, 8*(4), 94. https://doi.org/10.3390/metab08040094

Gaichu, D. M. (2024). Acute and sub-acute toxic effects of aqueous leaf extracts of *Ximenia ameicana* (Linn.) and *Pappea capensis* (Eckl. & Zeyh.). *The Journal of Phytopharmacology*, 13(3), 189-196

Gbadamosi, I. T., Bello, A., & Suleiman, R. A. (2017). GC–MS analysis and antimicrobial activity of the leaf extract of *Olax subscorpioidea*. *International Journal of Green and Herbal Chemistry, 6*(2), 142–151

Gbadamosi, I. T., Oyedapo, O. O., & Olatunji, O. J. (2017). Phytochemical constituents and antimicrobial potential of the methanolic root extract of *Olax subscorpioidea. African Journal of Traditional, Complementary and Alternative Medicines*, 14(2), 195–202.

Ishola, I. O., Olayemi, S. O., Oluduro, A. O., & Akinyemi, A. O. (2015). Analgesic and anti-

inflammatory activities of aqueous extract of *Olax subscorpioidea* leaves in rodents. *Journal of Basic and Clinical Physiology and Pharmacology, 26*(6), 553–559. https://doi.org/10.1515/jbcpp-2014-0082

Muhammad, I. A., Abdullahi, H. S., Haruna, U. A., & Sani, M. Y. (2021). *In vivo* anti-

typhoid and safety evaluation of extracts of *Ximenia americana* stem bark in experimental rats. *Bayero Journal of Pure and Applied Sciences, 14*(1), 87–94. https://doi.org/10.4314/bajopas.v14i1.15

Muhammad, H. L., Garba, R., Abdullah, A. S., Muhammad, H. K., Busari, M. B., Hamzah, R. U., and Makun, H. A. (2021). In vivo anti-typhoid and safety evaluation of extracts of *Ximenia americana* on experimental rats. *Pharmacological Research – Modern Chinese Medicine*, 1(2021), 100009.

Musa, A. D., Yusuf, G. O., Ojogbane, E. B., & Nwodo, O. F. C. (2010). Screening of eight plants used in folkloric medicine for the treatment of typhoid fever. *Journal of Chemical and Pharmaceutical Research*, 2(4), 7-15.

Nazifi, A. B., Garba, H. H., and Abdullahi, H. I. (2015). Phytochemical composition and antimicrobial activity of methanol leaf extract of *Olax subscorpioidea*. *International Journal of Scientific and Engineering Research, 6*(5), 1302–1307.

Odoma, S., Akinlolu, A. A., and Afolabi, J. A. (2015). Comparative phytochemical analysis of aqueous, n-butanol and ethyl acetate fractions of *Olax subscorpioidea* root extract. *Journal of Pharmacognosy and Phytotherapy, 7*(5), 97–102. https://doi.org/10.5897/JPP2015.0369

Okhale, S. E., Egharevba, H. O., and Kunle, O. F. (2017). Phytochemical and antimicrobial screening of crude extracts from Ximenia americana L. (Olacaceae). *Nigerian Journal of Pharmaceutical Sciences*, 16(2), 49–58.

Olanrewaju, J. A., Maikai, V. A., and Maikai, B. V. (2019). Trypanocidal activity and LC-

MS-based phytochemical screening of *Ximenia americana* root bark. *Nigerian Journal of Parasitology, 40*(2), 176–181.

Rotich, C., Ngeiywa, M., Makwali, J., & Wetungu, M. (2024). Medicinal uses and pharmacological activity of *Ximenia americana L.*: A systematic review. *African Journal of Education Science and Technology (AJESTA)*, 8(1), 179-200.

Salim, M. A., Ranjitkar, S., Hart, R., Khan, T., Ali, and Xu, J. (2019). Regional trade of medicinal plants has facilitated the retention of traditional knowledge: case study in Gilgit-Baltistan, Pakistan. *Journal of Ethnobiology and Ethnomedicine*, 15(1), 6.

Sharief, N. E., Osman, F. A., and Ibrahim, E. T. E. (2022). Phytochemical screening and antibacterial properties of *Ximenia americana* L. from Central Darfur, Sudan. *Sudan Journal of Medical Sciences, 17*(3), 31–41. https://doi.org/10.18502/sjms.v17i3.12588

Shettar, A. K., Hoskeri, H. J., and Patil, A. B. (2015). Evaluation of analgesic and anti-inflammatory activity of methanol leaf extract of *Ximenia americana*. *International Journal of Pharmacognosy and Phytochemical Research, 7*(4), 732–737.

Umair, M., Altaf, M., Bussmann, R. W., and Abbasi, A. M. (2019). Ethnomedicinal uses of the local flora in Chenab riverine area, Punjab province, *Pakistan Journal of Ethnobiology and Ethnomedicine*, 15(1), 7.

Zeinab, M. M. A., Ahmed, E. M., Saeed, K. and Hassan, S. (2019). Anti-microbial activity and phytochemical screening of *Ximenia americana*L. bark and leaves. *American Journal of Research and Community Medicine*, 4(1), 122-129.