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Original Research Article

Bioactive Components Identification and Characterization of the Mechanisms of Action of Selected Medicinal Plant Extracts Against *Salmonella enterica* Serovar *Typhi*

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ABSTRACT

The rise in antimicrobial resistant bacteria has necessitated the search for alternatives therapy, this study evaluates the antimicrobial potency of five medicinal plants against MDR *S. Typhi*. The leaves of *Azadirachta indica*, *Bambusa vulgaris*, *Cymbopogon citratus*, *Moringa oleifera*, *Carica papaya*, were collected, shed-dry, powdered, and soaked in ethanol for 72 hours, dried to generate the crude extract. The potency was tested on MDR *S. Typhi* isolated from Alheri laboratory. GCMS and Fourier Transform Infrared (FTIR) were performed to identify plant's active components and functional group(s). Scanning Electron Microscope (SEM) and UV spectrophotometer were used to assay effects on bacterial membrane. MDR *S. Typhi* was isolated, confirmed through serological and molecular analysis. Both the *invA* and *tviA* genes were identified. *Azadirachta indica* showed the highest activity with a zone of inhibition of 19.6 ± 0.8 mm at 100 mg/mL. The MIC and MBC of *A. indica* was 50 mg/mL and 100 mg/mL, respectively. GCMS analysis identified cis-13-octadecenoic (19.63%), n-hexadecanoic (17.05%), octadecanoic (6.99%), trans-13-octadecenoic (3.74%) acids, as the major components. FTIR analysis confirmed the presence of hydroxyl, carbonyl, amine, ether, ester, and aromatic functional groups associated with antimicrobial phytochemicals such as phenols, flavonoids, alkaloids, and terpenoids. SEM analysis of treated bacterial cells showed morphological cell deformation. UV spectrophotometric analysis at 260 nm and 280 nm wavelength showed increased absorbance in treated cells, suggesting leakage of nucleic acids and proteins. *Azadirachta indica* possesses significant antibacterial activity against MDR *S. Typhi*, primarily through disruption of bacterial membrane integrity and leakage of intracellular contents.

Keywords: Multidrug-resistant (MDR) *Salmonella Typhi*, *Azadirachta indica*, Antibacterial activity, Membrane disruption, Phytochemicals

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Introduction

For thousands of years, medicinal plants have been of great importance because of their potential to treat and prevent diseases. The ancient civilizations recognized and relied on the healing potential of plants before the discovery of micro-organisms and the role they play in causing diseases and it constitutes the major source of medicine.[1,2] The testing of folk plant remedies for scientific validity is now a major research area in both natural product chemistry and pharmacology. Traditional medicine has continued to flourish and plays an important role in global health care. Of the 374,000 known plant species, 50,000 to 70,000 possess medicinal properties, of which some 5,000 species have specific therapeutic applications uses.[3] The estimate shows that 50-95% of the world's population uses medicinal plants for early in developing countries.[4]

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The World Health Organization (WHO) reported that more than 80% of the world population mainly rely on plant-based medicine for their health care needs.[5,6]
Plant-derived antimicrobial compounds often possess novel mechanisms of action compared to synthetic antibiotics. This is especially valuable in addressing the increasing issue of antimicrobial resistance (AMR) where many conventional antibiotics are failing. The emergence of resistant pathogens has been accelerated by overuse and misuse of antibiotics in human medicine and agriculture. Current projections estimate that without effective solutions, AMR could cause up to 10 million deaths per year by 2050.[7,8]
Among pathogens of great concerns are the Gram-negative bacteria, which have complex cell envelopes that restrict the penetration of antibiotics. *Salmonella enterica* serovar *Typhi*, the causative agent of typhoid fever, has developed resistance to several first-line antibiotics including ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole[9]
The emergence of multidrug-resistant (MDR) strains of *S. Typhi* has significantly reduced treatment options and increased disease burden in regions such as sub-Saharan Africa, South Asia, and Latin America.[10]
The growing problem of antimicrobial resistance has stimulated a growing scientific interest in medicinal plants as possible sources of novel antimicrobial agents. Plant-derived compounds are especially promising because they often have more than one bacterial target. This multi-target activity means that they can interfere with several microbial pathways at the same time and so it is less likely that pathogens will develop resistance as rapidly as they do to conventional antibiotics. Medicinal plants are known to contain a wide array of bioactive secondary metabolites such as alkaloids, flavonoids,

terpenoids, tannins and saponins. These compounds show antimicrobial activity through different mechanisms such as disruption of bacterial cell membranes, inhibition of protein synthesis, nucleic acid synthesis and interference with essential metabolic pathways. Although an increasing number of studies have shown the antimicrobial potential of medicinal plants.[11]

Several studies have demonstrated the antimicrobial potential of medicinal plants, but many species have not been adequately explored, especially in terms of identification of their specific bioactive compounds and elucidation of the exact mechanisms by which they exert antimicrobial effects.[12] Most of the existing literatures have been focused on demonstrating the antimicrobial activity of plant extracts but with relatively less effort to understand the interactions of these compounds with microbial cells at molecular and biochemical levels. Detailed knowledge of these mechanisms is critical to convert promising plant extracts into therapeutics relevant to the clinic. The present study hence aims to bridge the gap between traditional medicinal knowledge and modern biomedical science through identification of the bioactive constituents of selected medicinal plants and characterization of their mechanism of action against *Salmonella typhi.*

Materials and Methods

Plant Collection and Identification

The plants used in this study include: *Azadirachta indica*, *Moringa oleifera*, *Carica papaya;* collected from Jalingo and *Bambusa vulgaris*, *Cymbopogon citratus*; collected from Kurmi and Jalingo Local Government Areas of Taraba State, Nigeria, around November of 2025. The plants were identified by Yermuya Illiya; a botanist at the Department of Biological Sciences, Unit of Botany, Taraba State University, Jalingo. The voucher specimen number of 024, 025, 026, 027 and 028 were assigned to *Azadirachta indica*, *Bambusa vulgaris*, *Cymbopogon citratus*, *Moringa oleifera*, and *Carica papaya* respectively. The specimens were prepared and deposited in the Departmental herbarium for reference and future verification.

Sample Collection and Handling

Using sterile stainless-steel scissors, approximately 1–2 kg of fresh plant leaves was collected and placed in sterile breathable paper bags during the early dry season (November) 2025. The collected samples were, properly labeled, and transported to the laboratory within 24 hours of collection.

Preparation of Plant Extracts

Drying and Pulverization

Collected plant materials were thoroughly washed with clean water to remove debris and air-dried under shade at 25–28 °C for 14 days to preserve heat-sensitive phytochemicals. Dried materials were ground into fine powder using a sterile mechanical grinder and passed through a 0.5 mm mesh sieve to ensure uniform particle size.

Solvent Extraction

Extraction was performed using the maceration method with solvents of increasing polarity:

n-hexane (analytical grade, 99%), ethyl acetate (analytical grade, 99.5%), ethanol (99.9%)

Approximately 200 g of powdered plant material was soaked in 1000 mL of solvent (solid–solvent ratio 1:5 w/v) in sterile conical flasks. The mixtures were agitated on an orbital shaker at 120 rpm for 72 hours at room temperature approximately 29±2 ^{0}C. The extracts were filtered using Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator at 40 °C. The resulting crude extracts were further dried in a vacuum desiccator and stored in sterile amber bottles at 4 °C until analysis.[13]

Microbial Isolates

Clinical isolates of *Salmonella typhi* were obtained from a Alheri diagnostic laboratory and confirmed using biochemical tests, serological and molecular test. Stool samples were collected from febrile patients seeking medical attention at the Alheri Diagnostic laboratory Jalingo. A selective enrichment step was performed to increase the chances of isolating *S. Typhi*, amidst normal intestinal flora by plating a portion of the stool on Selenite F broth; an enrichment medium, then incubated at 37°C for 6 to 8 hours to allow sufficient enrichment. After enrichment, the cultures were sub-cultured onto selective and differential agar; Salmonella-Shigella (SS) agar and MacConkey agar, incubated at 37°C for 18 - 24 hours.[14]

Antibiotic Susceptibility Testing

Antibiotics susceptibility testing (AST) was performed on the 5 isolates to identify MDR strains of *S. Typhi* using the following antibiotics: Ofloxacin 10 µg, Augmentin 30 µg, Peflacine 10 µg, Ceftazidime 30 µg, Ciprofloxacin 10 µg, Ceporex 10 µg, Ceftriaxone 30 µg, Streptomycin 30 µg, Cefutaxime 30 µg (manufactured by optun laboratory Nig LTD).

Identification and Biochemical Characterization

Colonies presumptively identified as *Salmonella* based on colony morphology and confirmed as multidrug-resistant (MDR) according to CLSI standard were subjected to biochemical characterization. The tests performed included Triple Sugar Iron (TSI) agar, urease, citrate utilization, indole, and Lysine Iron Agar (LIA). Typical *Salmonella Typhi* reactions observed were alkaline slant/acid butt with H_2S production on TSI agar (K/A + H_2S), urease negative, citrate positive, indole negative, and lysine decarboxylation with H_2S production on LIA. These biochemical characteristics aided the preliminary identification of *S. typhi.*[15]

Serological Confirmation

Biochemically confirmed isolates were further identified serologically using the slide agglutination technique according to the Kauffmann–White scheme. Fresh colonies grown on nutrient agar at 37°C for 18–24 h were emulsified in sterile normal saline and tested with polyvalent and monovalent antisera. Detection of the O9 somatic antigen, Vi capsular antigen, and phase 1 d flagellar antigen was carried out using specific antisera. Agglutination reactions observed within one minute were considered positive. Isolates exhibiting the antigenic profile O:9, Vi^{+}, H:d were confirmed as *Salmonella enterica* serovar *typhi* (*S. typhi*).[16]

Molecular Identification

Molecular confirmation of *S. Typhi* was performed through DNA extraction, PCR amplification, and agarose gel electrophoresis. DNA was extracted using the boiling method, where bacterial colonies suspended in sterile distilled water were heated at 95–100°C for 10 min, cooled on ice, and centrifuged. The supernatant containing crude DNA served as the PCR template.

PCR amplification targeted the InvA and *tviA* genes within the *viaB* operon using specific primers targeting the *Salmonella* spp invA-secretory protein F: 5′ GTATTGTTGATTAATGAGATCCG-3′ R: 5′-ATATTACGCACGGAAACACGTT-3′[17] and tviA-F (5′-CACGCACCATCATTTCACCG-3′) and tviA-R (5′-AACAGGCTGTAGCGATTTAGG-3′).[18] The reaction mixture contained DNA template, primers, Taq polymerase, dNTPs, and buffer solution, while thermal cycling included denaturation, annealing, and extension steps, then gel electrophoresis for the presence of any visible band 15 – 13, 17-14, 16-15, 18-16, 13- 17, 14-18

Antibacterial Activity Assay

Preparation of Test Extracts and Antibacterial

Agar well diffusion assay from the crude extract, 100mg/mL stock solution was produced by dissolving 1g of each of the plant extract in 10 mL of phosphate buffer saline (PBS). Then 2-fold serial dilution was performed to vary the concentration to 50 mg/mL, 25mg/mL, 12.5mg/mL.

Sterile Mueller–Hinton agar plates were inoculated with standardized bacterial suspensions. Wells of 6 mm diameter were bored into the agar using sterile cork borers. Each well was filled with 100 µL of plant extract at different concentration (12.5mg/mL, 25mg/mL, 50mg/mL, 100 mg/mL and 200mg/mL concentration) from the prepared plant extract. Control treatments included: Positive control: Ceftriaxone (30 µg/mL); Negative control: Phosphate buffer saline (PBS). Plates were then incubated at 37 °C for 24 hours, after which zones of inhibition were measured in millimeters and recorded.

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

MIC and MBC values were determined using the broth microdilution method following guidelines from the Clinical and Laboratory Standards Institute.[15] The MIC was defined as the lowest concentration showing no visible bacterial growth. For MBC determination, 10 µL from wells without visible growth were subcultured onto Mueller–Hinton agar plates. The lowest concentration showing no colony growth after incubation was recorded as the MBC.

Identification of Bioactive Compounds

Column Chromatography

Active extracts were fractionated using silica gel column chromatography (60–120 mesh). Elution was performed, the solvents used were 100% n-hexane, 100% ethyl acetate and 100% ethanol.

Gas Chromatography–Mass Spectrometry (GC–MS)

Bioactive fractions were analyzed using GC–MS equipped with a capillary column (30 m × 0.25 mm × 0.25 µm film thickness). In the GC–MS analysis, the extract was filtered through a membrane filter of 0.22 µm to remove large particles; 1.0 µL of the filtrate was injected into the GC–MS system. Separation was achieved using a capillary column with helium as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature was programmed from 50 °C to 280 °C at 10 °C/min. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV with a scan range of m/z 40–600. Compound identification was achieved by comparing mass spectra with NIST spectral library databases.

Mechanism of Antibacterial Action

Electron Microscopy Analysis

Morphological changes in treated bacterial cells were examined using Scanning Electron Microscopy (SEM). Bacterial cells were fixed with 2.5% glutaraldehyde, 2% osmium tetra-oxide, dehydrated in graded ethanol (30%, 50%, 70%, 90% and 100%), and sputter-coated with gold before imaging.

Cell Membrane Permeability Assay

The cell membrane permeability assay was conducted to evaluate the ability of the test extract to disrupt the cytoplasmic membrane of *Salmonella enterica* serovar *typhi* through leakage of intracellular constituents. *S. typhi* cultures grown to mid-log phase in nutrient broth were exposed to sub-inhibitory concentrations of the extract (0.5× MIC and 0.25× MIC) and incubated at 37°C for selected time intervals. Untreated cultures served as negative controls, while cultures treated with Ceftriaxone; a membrane-disrupting agent served as positive controls.

After incubation, the cultures were centrifuged and the supernatants collected for analysis. Membrane damage was assessed by measuring absorbance at 260 nm and 280 nm using a UV–visible spectrophotometer to detect the release of nucleic acids and proteins, respectively. Increased absorbance values compared with the untreated control indicated leakage of intracellular materials and loss of membrane integrity, suggesting that the antibacterial activity of the extract may involve disruption of the bacterial cell membrane.[15]

Statistical Analysis

Experimental data were expressed as mean ± standard deviation (SD). Statistical comparisons between treatment groups were performed using one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons.

All analyses were conducted using SPSS version 26 and R version 4.3. Statistical significance was accepted at $p < 0.05$.

Results and Discussion

Isolation, identification and Test for Multi-Drug Resistance

A total of 10 smooth, transparent colonies of bacterial isolates with black centers which are non-lactose fermenters; typical cultural characteristics of *Salmonella Typhi* were recovered from 32 stool samples collected from patients attending Alheri Diagnostic laboratory Jalingo. The 10 isolates showed the following biochemical results: TSI showed alkaline slant/acid butt with H_2S production on TSI agar (K/A + H_2S), urease negative, citrate positive, indole negative, and lysine decarboxylation with H_2S production on LIA. These results are consistent with the Forbes *et al*., 2016 characteristics typical of S. *Typhi*.[16] The recovery of non-lactose fermenting colonies with black centers on selective media is consistent with the characteristic morphology of *Salmonella* species, particularly *S. typhi*, due to their ability to produce hydrogen sulfide (H_2S).[17] Five of the 10 isolates were confirmed to be multi-drug-resistant species following Kirby-Bauer disk diffusion sensitivity test (figure 1).

Figure 1: Antibacterial Susceptibility showing Multi Drug-Resistant Pattern

Serological test confirmed the presence of O9 somatic antigen, Vi capsular antigen, and phase 1 d flagellar antigens on 3 of the 5 bacterial isolates. The serological confirmation of the isolates based on the detection of O9 somatic antigen, Vi capsular antigen, and phase 1 d flagellar antigen further validated the identification of the isolates as *Salmonella enterica* serovar *typhi*. Agglutination occurring within one minute indicates strong antigen–antibody specificity and supports previous findings that serotyping remains an important confirmatory tool in typhoid diagnosis.[18]

Molecular identification confirmed the presence of both InvA and *tviA* genes in all the three (3) authenticating the isolates to be multi-drug-resistant *Salmonella typhi*. Molecular detection of both the *invA* and *tviA* genes in all three isolates affirmed the presence of conserved virulence marker used for the molecular identification of *Salmonella* species, whereas the *tviA* gene is associated with Vi antigen expression and virulence regulation.[19]

The detection of MDR *S. typhi* in this study is consistent with increasing reports of antimicrobial-resistant typhoid fever worldwide,

particularly in developing countries where indiscriminate antibiotic use and poor sanitation facilitate the emergence and spread of resistant strains. This finding highlights the urgent need for alternative therapeutic agents, including plant-derived antimicrobials.[20] The occurrence of MDR *S. typhi* observed in this study aligns with increasing global reports of antimicrobial-resistant typhoid fever, particularly in developing countries where antibiotic misuse and poor sanitation contribute to resistance emergence.[21] Resistance among *Salmonella* isolates has become a major public health concern because it limits treatment options and increases morbidity and mortality associated with typhoid fever.[22] The identification of MDR strains in this study therefore emphasizes the urgent need for alternative antimicrobial agents, including plant-derived compounds.

Plant Extracts Antibacterial Activity Against Salmonella Typhi

The plants crude extract antibacterial activity against *Salmonella typhi* was tested using the agar well diffusion method at different concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL and 12.5 mg/mL. The results shows that several extracts exhibited inhibitory activity against the test organism. The activities were concentration depended, as all extracts showed stronger activities at 100mg/mL concentration. As the concentration reduced to 50 mg/mL, the inhibitory activity across all extract decreased. The plants' activity became week at 25 mg/mL, at this concentration, all but Azadirachta indica showed no detectable inhibition. *Azadirachta indica* exhibited the strongest antibacterial activity against the tested isolate, with zones of inhibition of 19.6 ± 0.8 mm compared with other plants extracts (Table 1). At the lowest tested concentration (12.5 mg/mL), only *Azadirachta indica* showed a minimal effect, while the remaining plant extracts exhibited no detectable antibacterial activities. Table 1 also shows superior activity of *Azadirachta indica* over the other plants at all concentrations, while the other extracts showed moderate activity only at higher doses and became ineffective at lower concentrations A. indica was effective at all concentration. Statistical analysis using one-way ANOVA revealed a significant difference ($p < 0.05$) between the antibacterial activities of the different solvent extracts at different concentration. Ethanol extracts produced significantly larger inhibition zones compared with non-polar extracts.

Table 1: Antibacterial Activities of the Tested Medicinal Plants

Plant extract	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL
Azadirachta indica (Leaf)	19.6 ± 0.8	15 ± 0.4	8 ± 0.2	2 ± 0.2
Bambusa vulgaris (Leaves)	13 ± 0.4	6 ± 0.4	2 ± 0.2	0.0
Cymbopogon citratus (Leaves)	15 ± 0.2	11 ± 0.2	3 ± 0.4	0.0
Moringa oleifera (Leaves)	11 ± 0.8	7 ± 0.4	0.0	0.0
Carica papaya (Leaves)	12± 0.6	6 ± 0.2	0.0	0.0

Values represent mean ± SD

The antibacterial screening of the crude plant extracts demonstrated varying degrees of inhibitory activity against MDR *S. typhi*. The concentration-dependent antibacterial effect observed in this study agrees with previous reports that higher concentrations of plant extracts generally contain greater quantities of active phytochemicals capable of exerting stronger antimicrobial actions.[23] Among the plants investigated, *Azadirachta indica* exhibited the highest antibacterial activity, producing the largest zones of inhibition at all tested concentrations. This finding is consistent with earlier studies reporting the potent antibacterial activity of *A. indica* against Gram-negative pathogens including *Salmonella* species.[24] The superior activity of *A. indica* may be attributed to its rich phytochemical composition, including flavonoids, tannins, alkaloids, terpenoids, and phenolic compounds known for membrane-disrupting and enzyme-inhibitory properties.[25]

The reduction in antibacterial activity with decreasing extract concentration suggests that the bioactive compounds were less effective at lower doses. Interestingly, only *Azadirachta indica* retained inhibitory activity at 12.5 mg/mL, indicating stronger potency and a broader antibacterial spectrum than the other extracts. The significant differences observed between solvent extracts further revealed that ethanol was the most effective extraction solvent. Ethanol is known to extract a wider range of polar and moderately non-polar phytochemicals, including phenols, flavonoids, and alkaloids, which are often responsible for antimicrobial activities. The findings therefore support the suitability of ethanol for extracting bioactive compounds from medicinal plants.[26]

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

The MIC and MBC values for *Azadirachta indica* (the most active extracts) was determined to be 50mg/mL indicting the lowest concentration capable of inhibiting visible growth of the test organism. The MBC was determined to be 100mg/mL; the concentration that showed no growth after subculturing well with no visible growth for an additional 24 hours. At this concentration the plant extract showed complete bactericidal activity against multi-drug-resistant *S. typhi*. The MIC value of 50 mg/mL and MBC value of 100 mg/mL obtained for *Azadirachta indica* indicate that the extract possesses both bacteriostatic and bactericidal properties against MDR *S. typhi*. Similar MIC and MBC ranges have been reported for neem extracts against enteric bacteria.[27] The higher MBC value compared with MIC suggests that greater concentrations are required for complete bacterial killing than for growth inhibition, which is common for many plant-derived antimicrobials. The bactericidal effect observed at 100 mg/mL indicates that the extract may cause irreversible damage to bacterial cellular structures or metabolic pathways.

Fractionation and Identification of Bioactive Compounds

The ethanolic fraction of *Azadirachta indica* (the most active extracts showing significant antibacterial activity) was further fractionated for chemical analysis in order to separate and identify the bioactive constituents responsible for the observed effects against *Salmonella Typhi*.

Column Chromatography Fractions

Using silica gel column chromatography (60–120 mesh) the plant extract was separated based on polarity differences. Elution was

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carried out sequentially using solvents of increasing polarity, including 100% n-hexane, ethyl acetate, and ethanol. The pooled fractions antibacterial activity against MDR *Salmonella typhi* each was tested, and ethanolic extract showed greater activities compared to the ethyl acetate and n-hexane solvents suggesting that the bioactive compounds were more concentrated in the ethanolic fraction. (Table 2). Column chromatography fractionation revealed that the ethanolic fraction exhibited stronger antibacterial activity than the n-hexane and ethyl acetate fractions. This observation suggests that the major antibacterial compounds present in *A. indica* are more polar in nature. Similar findings have been reported where polar fractions of medicinal plants demonstrated greater antimicrobial activity due to higher concentrations of phenolic compounds and flavonoids.[28] The effectiveness of the ethanolic fraction justified its further chemical characterization using GC–MS and FTIR analyses.

Table 2: Zones of inhibition of *A. indica* extracts against *Salmonella Typhi*

Extract Type	Concentration (mg/mL)	Zone of Inhibition (mm)
Ethanol extract	100	19.6 ± 0.8
Ethyl acetate extract	100	10.3 ± 0.6
n-Hexane extract	100	8.4 ± 0.5
Ciprofloxacin (control)	5 µg/mL	28.7 ± 0.9
PBS (negative control)	—	0

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis of Bioactive Fractions

Figure 2 shows the GC–MS analysis of the sample, it revealed the presence of several bioactive phytochemical constituents, including fatty acids, aromatic hydrocarbons, esters, phenolic compounds, and ether derivatives. The identified compounds varied in retention time and peak area percentage, indicating differences in their abundance within the extract. The major chemical constituent identified was cis-13-Octadecenoic acid with a relative peak area of 19.63% at a retention time of 43.13 min. This compound is an unsaturated fatty acid known for important biological activities such as antimicrobial, anti-inflammatory, antioxidant, and membrane-stabilizing properties. Its high abundance suggests that it may contribute significantly to the biological activity of the extract. Another major component detected was n-Hexadecanoic acid (Palmitic acid) with a relative area of 17.05% at 39.12 min retention time. Palmitic acid is a saturated fatty acid widely reported in plant extracts and essential oils. It possesses antimicrobial, antioxidant, anti-inflammatory, and pesticidal activities. The high percentage of this compound indicates that it is one of the dominant metabolites in the sample and may contribute to the observed pharmacological effects. The analysis also identified Octadecanoic acid (Stearic acid) with a relative peak area of 6.99% at 42.68 min. Stearic acid is a long-chain fatty acid associated with antimicrobial and emulsifying properties. Its presence further confirms the richness of the extract in lipid-derived bioactive compounds. Another important constituent was trans-13-Octadecenoic acid**,** detected with a peak area of 3.74%**.** This unsaturated fatty acid has been associated with antimicrobial and antioxidant activities and may synergistically enhance the biological potential of the extract. Among the aromatic compounds identified, Naphthalene and its derivatives were notable. Naphthalene recorded a peak area of 2.54%**,** while 2-methylnaphthalene showed peak areas of 2.14% and 0.97% at different retention times. These aromatic hydrocarbons are often associated with antimicrobial and insecticidal activities. The monoterpene compound p-Cymene was also detected with a relative area of 1.69%**.** p-Cymene is a biologically active aromatic terpene commonly reported in medicinal plants and essential oils. It possesses antioxidant, antibacterial, anti-inflammatory, and antifungal activities, which may support the therapeutic potential of the extract. (Table 3).

Table 3: Major compounds identified by GC–MS Analysis of Bioactive Components

S/N	Compound Identified	Retention Time (min)	Peak Area (%)	Chemical Class	Reported Biological Activities
1	cis-13-Octadecenoic acid	43.13	19.63	Unsaturated fatty acid	Antimicrobial, antioxidant, anti-inflammatory, membrane-stabilizing
2	n-Hexadecanoic acid (Palmitic acid)	39.12	17.05	Saturated fatty acid	Antimicrobial, antioxidant, anti-inflammatory, pesticidal
3	Octadecanoic acid (Stearic acid)	42.68	6.99	Long-chain fatty acid	Antimicrobial, emulsifying properties
4	trans-13-Octadecenoic acid	—	3.74	Unsaturated fatty acid	Antimicrobial, antioxidant
5	Naphthalene	—	2.54	Aromatic hydrocarbon	Antimicrobial, insecticidal
6	2-Methylnaphthalene	—	2.14	Aromatic hydrocarbon	Antimicrobial, insecticidal
7	p-Cymene	—	1.69	Monoterpene	Antibacterial, antifungal, antioxidant, anti-inflammatory
8	2-Methylnaphthalene	—	0.97	Aromatic hydrocarbon	Antimicrobial, insecticidal
9	2,4-Di-tert-butylphenol	—	0.17	Phenolic compound	Antioxidant, antimicrobial

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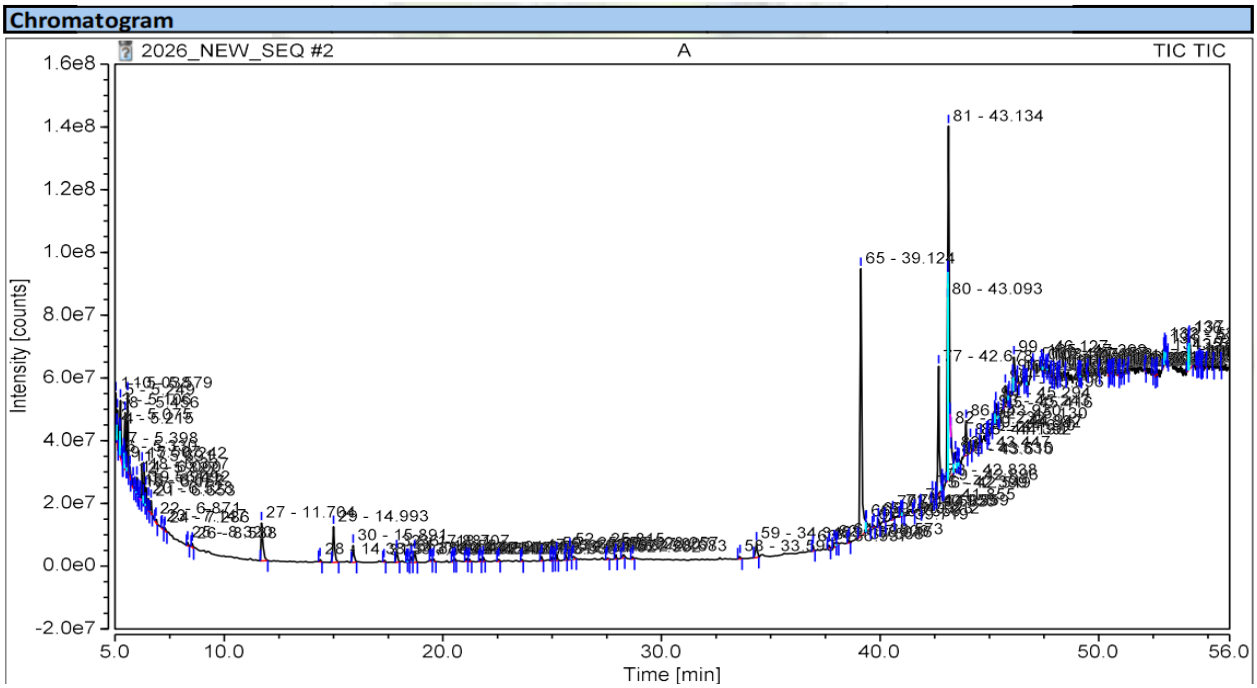


Figure 2: GCMS Analysis of *Azadirachta indica* for Bioactive Components Identification

Additionally, the presence of 2,4-Di-tert-butylphenol (0.17%) is noteworthy because this phenolic compound is widely recognized for strong antioxidant and antimicrobial properties. Even at low concentration, phenolic compounds can contribute significantly to bioactivity due to their radical-scavenging ability. Several ester compounds such as Dimethyl phthalate**,** Methyl 2,6,10-trimethyltridecanoate**,** and cyclic fatty acid esters were also identified. These compounds may contribute to membrane disruption and antimicrobial effects reported in plant-derived extracts. The chromatogram further revealed repeated peaks corresponding to Octaethylene glycol monododecyl ether and Heptaethylene glycol monododecyl ether across retention times between approximately 37 and 55 min. These compounds are surfactant-like ether derivatives and may originate from extraction solvents, laboratory contaminants, or sample preparation materials rather than natural phytochemicals. Therefore, they should be interpreted cautiously when discussing the natural chemical composition of the extract. The GC–MS profile demonstrated that the sample is rich in fatty acids and bioactive lipid compounds, particularly cis-13-octadecenoic acid, n-hexadecanoic acid, and octadecanoic acid. These compounds are known for antimicrobial, antioxidant, anti-inflammatory, and membrane-disrupting activities, suggesting that they may be responsible for the pharmacological and antimicrobial properties of the extract. The presence of terpenes, phenolic compounds, and aromatic hydrocarbons further supports the therapeutic potential of the sample.

GC–MS analysis revealed the presence of several bioactive phytochemicals, particularly fatty acids and aromatic compounds. The major constituent identified was cis-13-octadecenoic acid, followed by n-hexadecanoic acid and octadecanoic acid. Fatty acids are widely reported to possess antimicrobial activity through disruption of microbial membranes, inhibition of enzyme systems, and interference with nutrient uptake.[29] The high abundance of these fatty acids suggests that they may significantly contribute to the antibacterial activity observed in this study. Palmitic acid and stearic acid have also been reported to exhibit antibacterial and anti-inflammatory properties in medicinal plant extracts.[30]

The detection of p-cymene and 2,4-di-tert-butylphenol further supports the antimicrobial potential of the extract. p-Cymene is a monoterpene known for antibacterial, antioxidant, and anti-inflammatory activities, often acting through membrane destabilization.[31] Similarly, 2,4-di-tert-butylphenol has been identified in several medicinal plants and microbial metabolites with potent antioxidant and antimicrobial effects.[32] The aromatic hydrocarbons detected, including naphthalene derivatives, may also contribute synergistically to the observed antimicrobial activity. However, the repeated detection of octaethylene glycol monododecyl ether and related compounds may represent contaminants from solvents or laboratory materials, and therefore should be interpreted cautiously. Adams *et al*. 2022 in his review of antimicrobial activity of medicinal plants as a panacea for antibiotic resistance identify several bioactive compounds that exhibit strong antibacterial properties.[33]

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum presented shows the presence of several important absorption bands (Figure 3), indicating different functional groups in the sample. These functional groups suggest that the extract contains bioactive phytochemical compounds such as alcohols, phenols, amines, alkanes, carbonyl compounds, and aromatic compounds.

Table 4 shows the major functional group; the broad absorption bands observed between approximately 3900 and 3500 cm^{-1} are characteristic of O–H stretching vibrations, indicating the presence of hydroxyl-containing compounds such as phenols and alcohols. These compounds are commonly associated with antioxidant and antimicrobial activities in plant extracts. The absorption peak at 3205.61 cm^{-1} corresponds to N–H stretching vibrations, suggesting the presence of amines or amide-containing compounds, which may originate from proteins or alkaloids. The peaks observed between 2444 and 2233 cm^{-1} may be attributed to C≡N or C≡C stretching vibrations, indicating nitriles or alkynes. Peaks within the 2115–1993 cm^{-1} region further support the presence of unsaturated compounds and aromatic overtones.

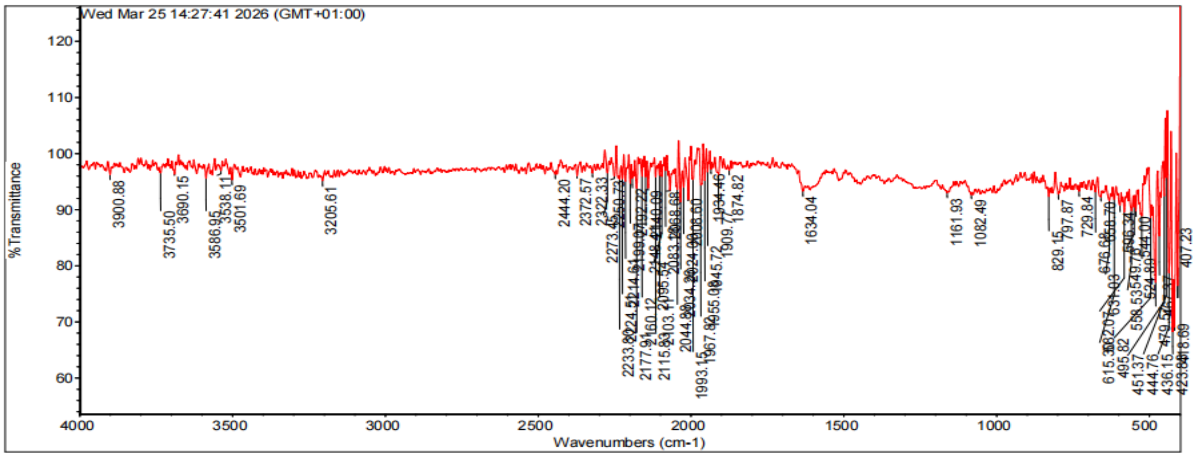
The strong band at 1634.04 cm^{-1} is characteristic of C=O stretching or C=C stretching vibrations. This suggests the presence of carbonyl-containing compounds such as ketones, aldehydes, esters, or aromatic structures. The peaks at 1161.93 cm^{-1} and 1002.49 cm^{-1} correspond to C–O and C–N stretching vibrations, respectively, indicating alcohols, ethers, esters, and amine-containing compounds. The absorption bands between 829 and 797 cm^{-1} are associated with aromatic C–H bending, confirming the presence of aromatic compounds. The lower frequency peaks between 697 and 407 cm^{-1} fall within the fingerprint region and may correspond to C–Cl and C–Br stretching vibrations.

The FTIR analysis shows that *Azadirachta indica* contains many biologically active functional groups, including: hydroxyl compounds (phenols and alcohols), amines and amides, carbonyl compounds, aromatic compounds, ether/ester groups, unsaturated hydrocarbons.

These functional groups are commonly associated with phytochemicals such as flavonoids, tannins, alkaloids, terpenoids, and phenolic compounds, which may contribute to the biological activities of the extract, including antimicrobial and antioxidant properties. The FTIR analysis further confirmed the presence of biologically active functional groups in the extract. The broad O–H stretching bands observed between 3900 and 3500 cm^{-1} indicate the presence of hydroxyl-containing compounds such as phenols and alcohols, which are known for antioxidant and antimicrobial properties.[34] The presence of N–H stretching vibrations suggests amines or alkaloidal compounds, while the strong absorption at 1634 cm^{-1} indicates carbonyl-containing compounds such as ketones, aldehydes, or flavonoids. These functional groups are commonly associated with phytochemicals capable of exerting antimicrobial effects through membrane disruption, enzyme inhibition, and oxidative stress induction.[35] The FTIR findings therefore complement the GC–MS results by confirming the chemical complexity and therapeutic potential of the extract.

Table 4: Fourier Transform Infrared Spectroscopy Analysis of *Azadirachta indica*

Peak (cm^{-1})	Possible Functional Group	Interpretation
3900.88 – 3501.08	O–H stretching	Indicates hydroxyl groups of alcohols and phenolic compounds
3735.50	Free O–H vibration	Suggests phenolic or alcoholic constituents
3205.61	N–H stretching	Presence of amines or amide compounds
2444.20 – 2233.78	C≡N / C≡C stretching	Indicates nitriles or alkynes
2115.82 – 1993.15	C≡C stretching / aromatic overtone	May indicate unsaturated compounds
1634.04	C=O or C=C stretching	Corresponds to carbonyl groups, alkenes, or aromatic rings
1161.93	C–O stretching	Indicates alcohols, ethers, or esters
1002.49	C–N stretching	Suggests amines or carbohydrate-related compounds
829.15 – 797.87	Aromatic C–H bending	Indicates substituted aromatic rings
697.06 – 615.88	C–Cl stretching	Presence of halogenated compounds
534.91 – 407.23	C–Br stretching	Suggests brominated compounds or fingerprint region absorptions



Wed Mar 25 14:29:14 2026 (GMT+01:00)
FIND PEAKS:
Spectrum: Wed Mar 25 14:27:41 2026 (GMT+01:00)
Region: 4000.00 400.00
Absolute threshold: 126.118
Sensitivity: 50
Peak list:

Position:	407.23	Intensity:	76.319
Position:	418.69	Intensity:	68.839
Position:	423.83	Intensity:	68.209
Position:	436.15	Intensity:	78.650
Position:	444.76	Intensity:	95.567
Position:	451.37	Intensity:	92.397
Position:	467.37	Intensity:	85.222

Figure 3: FTIR Spectrum of *Azadirachta indica* extract

Effects of Plant Extracts on Bacterial Cell Morphology

Scanning Electron Microscopy (SEM) analysis revealed significant structural alterations in *Salmonella typhi* cells treated with active plant extracts. Untreated control cells displayed smooth, intact rod-shaped morphology, whereas treated cells showed: Cell wall deformation, Membrane disruption, Cytoplasmic leakage and Cell shrinkage These observations indicate that the plant-derived compounds may exert antibacterial effects by damaging bacterial cell membrane integrity.

Effect of plant extract on cell Membrane

These results suggest that the plant-derived compounds may interfere with bacterial pathogenicity mechanisms. The UV spectrophotometric analysis at 260 nm and 280 nm was carried out to evaluate the extent of bacterial cell membrane damage and intracellular component leakage after exposure to the test extract. Absorbance at 260 nm indicates the release of nucleic acids (DNA and RNA), while absorbance at 280 nm reflects the leakage of proteins and other

aromatic amino acid-containing compounds. An increase in absorbance at these wavelengths generally suggests disruption of the bacterial cell membrane, leading to leakage of intracellular materials into the surrounding medium.

At 260 nm, the control group showed a gradual increase in absorbance from 1.655 at 0 hr to 1.799 at 6 hours, indicating minimal natural leakage over time. The bacterial cells treated with 50 mg/mL showed an initial increase at 2 hours (1.770) followed by a reduction at 4 hours (1.637) and 6 hours (1.624). This decrease may suggest limited membrane disruption or possible degradation and utilization of leaked nucleic acids over time. In contrast, treatment with 100 mg/mL maintained relatively higher absorbance values throughout the exposure period, with a peak at 4 hours (1.812), indicating greater leakage of nucleic acids and stronger membrane damage at the higher concentration.

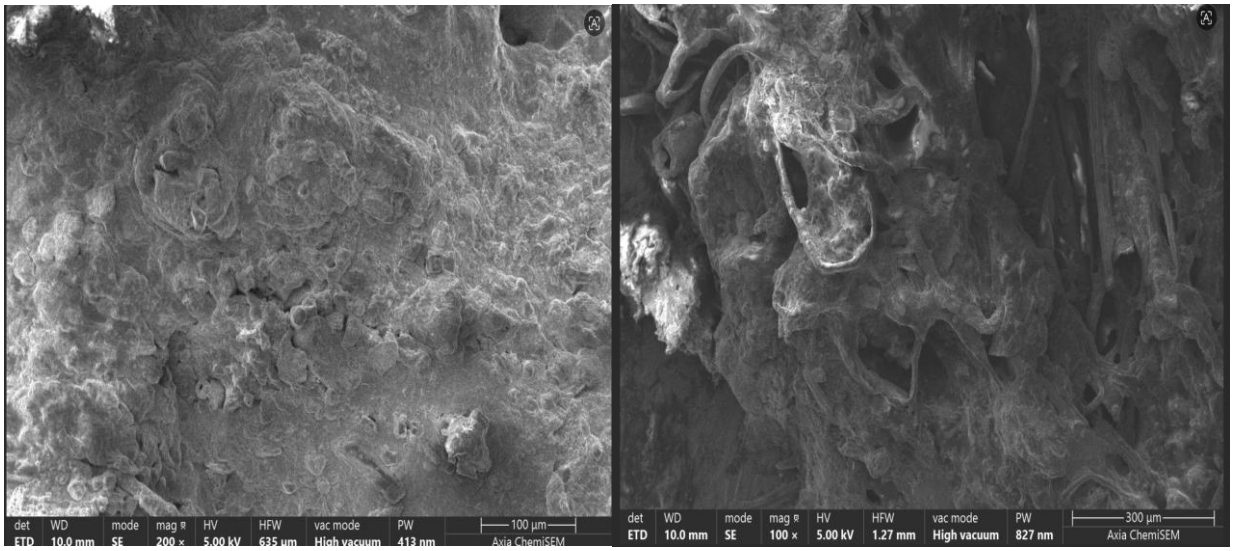
Similarly, at 280 nm, the control recorded consistently high absorbance values ranging from 2.123 to 2.294, representing baseline protein presence in the medium. The 50 mg/mL concentration showed a decline in absorbance from 1.897 at 0 hr to 1.659 at 4 hours, before increasing to 2.076 at 6 hrs. This pattern suggests delayed protein leakage or gradual membrane destabilization. The 100 mg/mL concentration produced comparatively higher absorbance values, especially at 4 hours (2.266) and 6 hours (2.233), indicating substantial release of proteins and intracellular macromolecules due to increased membrane permeability.

The results demonstrate that the extract affected bacterial cell membrane integrity in a concentration- and time-dependent manner. The higher concentration (100 mg/mL) caused greater leakage of nucleic acids and proteins, suggesting stronger antibacterial activity through membrane disruption. Increased absorbance at both wavelengths supports the hypothesis that the extract compromises the bacterial cell membrane, leading to leakage of intracellular contents and eventual cell death.

The SEM observations provided important insights into the mechanism of antibacterial action of the plant extract. Untreated *S. Typhi* cells maintained smooth and intact rod-shaped morphology, whereas treated cells exhibited membrane disruption, cytoplasmic leakage, shrinkage, and deformation. Similar morphological alterations have been reported in bacteria exposed to plant-derived antimicrobial compounds, indicating damage to the bacterial cell envelope[36]. Disruption of membrane integrity compromises selective permeability, resulting in leakage of intracellular constituents and eventual cell death.

The UV spectrophotometric analysis at 260 nm and 280 nm further supported the membrane-damaging effects of the extract. Increased absorbance at 260 nm indicates leakage of nucleic acids, whereas increased absorbance at 280 nm reflects protein leakage from damaged bacterial cells. The higher absorbance values observed at 100 mg/mL suggest more severe membrane disruption at higher extract concentrations. These findings agree with previous studies showing that phytochemicals such as phenolics, terpenoids, and fatty acids exert antibacterial effects by destabilizing bacterial membranes and increasing permeability.[37] The concentration- and time-dependent increase in membrane leakage observed in this study therefore confirms that the antibacterial mechanism of *Azadirachta indica* involves disruption of cell membrane integrity, leakage of intracellular materials, and eventual bacterial death.

The findings of this study demonstrate that *Azadirachta indica* possesses significant antibacterial activity against MDR *Salmonella Typhi*. The observed activity may be attributed to the synergistic effects of bioactive phytochemicals including fatty acids, phenolic compounds, and terpenes identified through GC–MS and FTIR analyses. The extract appears to exert its antibacterial effect primarily through membrane disruption and leakage of intracellular components. These findings support the ethnomedicinal use of *A. indica* and suggest its potential as a source of novel antibacterial agents for the management of drug-resistant typhoid infections.



(a) (b)

Figure 4: The SEM Micrograph of the isolates (a) The negative control isolates showing intact cell surface (b) The test isolates showing wrinkled cell surface

Conclusion

Azadirachta indica demonstrated the highest antibacterial activity against MDR *S. Typhi*. The antibacterial effect was concentration-dependent, with the ethanolic extract producing significantly larger zones of inhibition compared with other solvent extracts. The MIC and MBC values were 50 mg/mL and 100 mg/mL respectively, indicating strong inhibitory and killing effects against the test organism. GC–MS analysis identified several important bioactive compounds, particularly fatty acids such as cis-13-octadecenoic acid, n-hexadecenoic acid, and octadecanoic acid, alongside phenolic compounds, terpenes, and aromatic hydrocarbons. These compounds are known to possess antimicrobial, antioxidant, anti-inflammatory, and membrane-disrupting activities. FTIR analysis further confirmed the presence of biologically important functional groups including hydroxyl, carbonyl, amine, aromatic, and ether groups commonly associated with flavonoids, phenols, alkaloids, tannins, and terpenoids. Studies on mechanisms of action demonstrated that the antibacterial activity of *Azadirachta indica* may occur primarily through disruption of bacterial cell membrane integrity. SEM analysis revealed

morphological alterations in treated bacterial cells including membrane deformation, shrinkage, cytoplasmic leakage, and destruction of normal rod-shaped structures. Similarly, UV spectrophotometric analysis at 260 nm and 280 nm confirmed leakage of intracellular nucleic acids and proteins, indicating increased membrane permeability proving that *Azadirachta indica* possesses significant antibacterial activity against multidrug-resistant *Salmonella typhi*.

Conflicts Of Interest

The authors wish to declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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