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Review Article

Evaluation of Antimicrobial Potential of Medicinal Plant Extracts Against Multidrug-Resistant Bacteria Isolated from Pharmaceutical-Contaminated Water in Kano State, Nigeria

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Abstract
Pharmaceutical wastewater discharged into open drainage channels around hospitals and pharmaceutical facilities may serve as an important reservoir of multidrug-resistant bacteria and other contaminants of public health concern. This study evaluated the antimicrobial potential of selected medicinal plant extracts against multidrug-resistant bacterial isolates recovered from pharmaceutical-contaminated drainage water in Kano State, Nigeria. Wastewater samples were collected from open drains located outside Aminu Kano Teaching Hospital, Murtala Mohammed Specialist Hospital, Sabon Gari Pharmaceutical Market, and pharmaceutical facilities in Sharada Industrial Estate. Physicochemical parameters of the wastewater were determined using standard methods. Bacteria were isolated, identified by biochemical characterisation and 16S rRNA confirmation, and tested for antibiotic susceptibility using the Kirby–Bauer disc diffusion method. Aqueous and ethanolic extracts of Azadirachta indica, Moringa oleifera, Vernonia amygdalina, Psidium guajava, Allium sativum, and Zingiber officinale were prepared and evaluated against the multidrug-resistant isolates. The wastewater samples showed elevated levels of electrical conductivity (1215–1582 µS/cm), biochemical oxygen demand (48.7–73.5 mg/L), and chemical oxygen demand (108.5–152.3 mg/L), exceeding recommended limits. Escherichia coli was the most prevalent bacterial isolate, accounting for 29.8% of all isolates, followed by Staphylococcus aureus (21.1%) and Pseudomonas aeruginosa (18.5%). Pseudomonas aeruginosa exhibited the highest multidrug resistance rate (71.2%), with marked resistance to ampicillin (92.7%) and tetracycline (75.6%). Among the plant extracts, ethanolic extracts were more active than aqueous extracts. Garlic ethanolic extract showed the strongest antimicrobial activity, producing inhibition zones of 26.8 ± 1.7 mm against Escherichia coli, 29.4 ± 1.6 mm against Staphylococcus aureus, and 23.1 ± 1.4 mm against Pseudomonas aeruginosa. Guava leaf ethanolic extract also demonstrated substantial antibacterial activity. The minimum inhibitory concentration of garlic extract ranged from 6.25 to 25 mg/mL, while the minimum bactericidal concentration ranged from 12.5 to 50 mg/mL. The findings indicate that pharmaceutical-contaminated drainage water in Kano State contains highly resistant bacteria and that selected medicinal plants, particularly Allium sativum and Psidium guajava, possess strong antimicrobial activity against these organisms. The study suggests that these plants may provide low-cost alternative agents for the control of multidrug-resistant bacteria associated with environmental contamination.

Keywords: *pharmaceutical wastewater, multidrug-resistant bacteria, medicinal plants, antimicrobial activity, Kano State, garlic extract, guava leaf .*

1. Introduction

The discharge of untreated pharmaceutical wastewater into the environment has become an important environmental and public health problem worldwide. Hospitals, pharmaceutical industries, pharmacies, drug markets, and households continuously release wastewater containing antibiotics, analgesics, hormones, anti-inflammatory drugs, disinfectants, and personal care products. In many developing countries, these wastes are discharged directly into open drains, streams, and surface waters without adequate treatment. Consequently, pharmaceutical residues persist in the environment and may alter water quality, increase ecological toxicity, and contribute to the development of antimicrobial resistance (Patel et al., 2019; AL Falahi et al., 2022; Obinna et al., 2023). In Nigeria, the management of pharmaceutical and hospital wastewater remains inadequate. Many hospitals and pharmaceutical facilities dispose of their wastewater into drainage channels outside their premises. Such wastewater usually contains large amounts of dissolved solids, organic matter, pathogenic microorganisms, and chemical contaminants. Akpor and Muchie (2011) observed that untreated urban wastewater in Africa is frequently characterised by high biochemical oxygen demand, high chemical oxygen demand, and heavy microbial contamination. Similarly, Kayode-Afolayan et al. (2022) reported that pharmaceutical effluents significantly degrade the physicochemical quality of receiving waters and increase environmental health risks. One of the most serious consequences of pharmaceutical contamination is the emergence and spread of multidrug-resistant bacteria. Wastewater containing residual antibiotics exerts selective pressure on environmental microorganisms. Under such conditions, susceptible bacteria are destroyed while resistant organisms survive and proliferate. Over time, resistant bacteria become dominant within the microbial population and may transfer their resistance genes to other organisms through horizontal gene transfer (Patel et al., 2019; Li et al., 2024). Nibamureke and Barnhoorn (2025) further reported that pharmaceutical-contaminated waters frequently harbour multidrug-resistant Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa.

Several bacterial species commonly isolated from hospital and pharmaceutical wastewater are of major public health significance. Escherichia coli is widely recognised as an indicator of faecal contamination and poor sanitary conditions. Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Enterococcus faecalis are important opportunistic pathogens associated with hospital-acquired infections, septicemia, urinary tract infections, respiratory diseases, and wound infections (Ushie et al. 2026). These bacteria are increasingly resistant to conventional antibiotics and therefore constitute a major challenge to modern medicine (Jaishankar et al., 2014; Kayode-Afolayan et al., 2022). Kano State is one of the major commercial and industrial centres in northern Nigeria and contains several large hospitals, pharmaceutical markets, and pharmaceutical industries. Open drainage channels located outside these facilities receive untreated or partially treated wastewater daily. In particular, drains outside Aminu Kano Teaching Hospital, Murtala Mohammed Specialist Hospital, Sabon Gari Pharmaceutical Market, and the pharmaceutical industries in Sharada Industrial Estate are frequently contaminated with wastewater containing pharmaceutical residues and pathogenic microorganisms (Ekesiobi et al. 2026). These drains eventually discharge into receiving streams and urban drainage systems, thereby increasing the risk of environmental contamination and human exposure. Apart from their effects on bacterial resistance, pharmaceutical contaminants may also produce ecological and toxicological impacts in aquatic environments. Adeogun et al. (2016) reported endocrine disruption, intersex occurrence, and gonadal abnormalities in fish exposed to polluted waters in Nigeria. Arcand-Hoy and Benson (1998) explained that endocrine-disrupting chemicals interfere with reproduction and development in aquatic organisms, while Gonsioroski et al. (2020) showed that contaminants in water can impair reproductive systems through hormonal disruption. Kar et al. (2021) also emphasised that endocrine disruptors can alter growth, metabolism, and reproductive physiology in teleost fishes. The presence of pharmaceutical residues together with other pollutants, such as heavy metals, may further increase environmental toxicity. Castro-González and Méndez-Armenta (2008) reported that contaminants accumulated in aquatic organisms can eventually enter the food chain and threaten human health. Jaishankar et al. (2014) similarly explained that prolonged exposure to heavy metals and persistent pollutants may produce neurological, renal, hepatic, and reproductive disorders. Yilmaz et al. (2007) further demonstrated that contaminated aquatic environments often lead to the accumulation of toxic substances in fish tissues. Because of the increasing failure of conventional antibiotics against multidrug-resistant bacteria, there is growing interest in medicinal plants as alternative antimicrobial agents. Medicinal plants contain numerous phytochemicals, including flavonoids, tannins, alkaloids, terpenoids, glycosides, and essential oils, which possess antibac-

terial activity. Compared with synthetic antibiotics, medicinal plants are often cheaper, more accessible, biodegradable, and less likely to produce severe side effects (Onoja et al. 2026).

Several medicinal plants commonly used in Nigeria possess well-recognised antimicrobial properties. Garlic (Allium sativum) contains allicin, ajoene, and sulfur-containing compounds capable of disrupting bacterial cell membranes. Guava (Psidium guajava) leaves contain flavonoids, tannins, and phenolic compounds with broad-spectrum antibacterial effects. Neem (Azadirachta indica), bitter leaf (Vernonia amygdalina), moringa (Moringa oleifera), and ginger (Zingiber officinale) also contain phytochemicals that inhibit bacterial growth. Previous studies have shown that these plants are effective against both Gram-positive and Gram-negative bacteria, including resistant strains (Patel et al., 2019; Obinna et al., 2023). Although numerous studies have examined the antimicrobial properties of medicinal plants, there is limited information on their activity against multidrug-resistant bacteria isolated specifically from pharmaceutical-contaminated drainage water in Kano State. In addition, few studies have compared the antibacterial activity of aqueous and ethanolic extracts of these plants against environmental bacterial isolates. Therefore, the present study was undertaken to isolate and identify multidrug-resistant bacteria from pharmaceutical-contaminated drainage water in Kano State and to evaluate the antimicrobial potential of selected medicinal plant extracts against the resistant organisms.

2. Methodology and Method

2.1 Study Area

The study was conducted in Kano metropolis, Kano State, Nigeria. Kano is located in northwestern Nigeria between latitude 11°58N and longitude 8°31E. The city is characterised by rapid urbanisation, high population density, extensive commercial activity, and a large concentration of healthcare and pharmaceutical facilities. Several major hospitals, pharmaceutical stores, and small-scale pharmaceutical manufacturing and packaging facilities are located within the city. Wastewater generated from these facilities is commonly discharged into open drainage channels that run outside the hospitals, pharmaceutical factories, and markets before eventually entering nearby streams and urban drainage networks. The selected sampling locations included open drainage channels situated outside:

- Aminu Kano Teaching Hospital
- Murtala Mohammed Specialist Hospital
- Sabon Gari Pharmaceutical Market
- A small-scale pharmaceutical manufacturing area in Sharada Industrial Estate

An additional downstream receiving stream was also sampled to determine the extent to which contaminants from the drains spread into the wider environment.

2.2 Study Design

A cross-sectional laboratory-based study was adopted. The study involved the collection of wastewater samples from drainage channels located outside hospitals and pharmaceutical factories, followed by bacteriological analysis and evaluation of the antimicrobial activity of selected medicinal plant extracts against multidrug-resistant bacterial isolates.

2.3 Sample Collection

Wastewater samples were collected between April and June during the early rainy season. Sampling was conducted once every two weeks for a period of six weeks, giving a total of three sampling events at each location. Sampling was carried out in the morning between 8:00 and 10:00 a.m. to minimise temporal variations in temperature and wastewater flow. At each site, composite wastewater samples were collected from the open drainage channels located approximately 10–20 m outside the hospital or factory premises. The drains were shallow, open concrete channels with moderate flow rates during the sampling period. Three points along each drain, separated by approximately 5 m, were selected. From each point, 500 mL of wastewater was collected at a depth of 15–20 cm below the surface using sterile sampling bottles. The three portions from the same drain were combined thoroughly to obtain one representative composite sample. Triplicate composite samples were collected during each sampling event. In total, 45 composite samples were obtained during the study. Samples were transported immediately to the laboratory in an ice chest maintained at approximately 4°C and processed within 6 h of collection.

2.4 Determination of Physicochemical Properties

The physicochemical properties of the wastewater were determined before microbiological analysis. Temperature, pH, electrical conductivity, and dissolved oxygen were measured in situ using a calibrated Hanna HI98194 portable multiparameter water quality meter. Total dissolved solids were measured using the gravimetric method. Biochemical oxygen demand was determined by incubating diluted wastewater samples in the dark at 20°C for 5 days, after which the dissolved oxygen depletion was measured. Chemical

oxygen demand was determined by the dichromate reflux method using potassium dichromate in an acidic medium.

2.5 Isolation of Bacteria

The wastewater samples were serially diluted up to 10 using sterile distilled water. Aliquots of 0.1 mL from appropriate dilutions were inoculated onto different bacteriological media using the spread plate technique. The following media were used: Nutrient agar for total heterotrophic bacteria MacConkey agar for coliforms and Gram-negative enteric bacteria

Eosin methylene blue agar for Escherichia coli Mannitol salt agar for Staphylococcus aureus Cetrimide agar for Pseudomonas aeruginosa Bile esculin agar for Enterococcus faecalis The inoculated plates were incubated at 37°C for 24–48 h. After incubation, distinct colonies were counted and subcultured repeatedly until pure isolates were obtained.

2.6 Identification of Bacterial Isolates

Pure bacterial isolates were identified based on their colonial appearance, cell morphology, Gram reaction, and biochemical characteristics. The following biochemical tests were performed: Catalase test Oxidase test Indole test Citrate utilisation test Urease test Coagulase test Methyl red test Voges–Proskauer test Triple sugar iron test The identities of the isolates were confirmed by comparing the observed characteristics with standard bacteriological identification manuals. Representative multidrug-resistant isolates were further confirmed by 16S rRNA gene sequencing. Bacterial DNA was extracted, amplified using universal 16S rRNA primers, and sequenced. The resulting sequences were compared with entries in the GenBank database using BLAST.

2.7 Determination of Antibiotic Resistance Pattern

The antibiotic susceptibility of the bacterial isolates was determined using the Kirby–Bauer disc diffusion method. Pure bacterial cultures were adjusted to the turbidity of 0.5 McFarland standard and inoculated uniformly onto Mueller–Hinton agar plates. Commercial antibiotic discs were placed on the agar surface. The antibiotics used included:

- Ampicillin (10 µg)
- Ciprofloxacin (5 µg)
- Gentamicin (10 µg)
- Tetracycline (30 µg)
- Chloramphenicol (30 µg)

The plates were incubated at 37°C for 24 h. After incubation, the diameter of the inhibition zone around each disc was measured in millimetres. The isolates were classified as susceptible, intermediate, or resistant according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2024). Bacterial isolates resistant to at least three classes of antibiotics were regarded as multidrug resistant and selected for the plant extract study.

2.8 Collection and Preparation of Medicinal Plants

Fresh leaves of Azadirachta indica, Moringa oleifera, Vernonia amygdalina, and Psidium guajava were collected from different locations within Kano State. Fresh bulbs of Allium sativum and rhizomes of Zingiber officinale were purchased from local markets. The plant materials were washed thoroughly with clean water, followed by distilled water to remove dirt and debris. The samples were then air-dried in the shade at room temperature for two weeks. The dried plant materials were ground into fine powder using a laboratory grinder and stored in sterile airtight containers.

2.9 Preparation of Aqueous and Ethanolic Extracts

For aqueous extraction, 100 g of powdered plant material was soaked in 500 mL of distilled water for 48 h with occasional stirring. The mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated to dryness using a water bath at 50°C. For ethanolic extraction, 100 g of powdered plant material was soaked in 500 mL of 95for 72 h. The mixture was filtered and concentrated using a rotary evaporator at 40°C. The percentage extraction yield was calculated using: Percentage yield = (Weight of extract / Weight of dry sample) × 100 The mean extraction yields ranged from 8.4–13.6ethanolic extracts. The concentrated extracts were stored in sterile bottles at 4°C until use. Stock solutions of the extracts were prepared at concentrations of 25, 50, 75, and 100 mg/mL using 5sulfoxide, which was confirmed not to inhibit bacterial growth.

2.10 Determination of Antimicrobial Activity of Plant Extracts

The antimicrobial activity of the plant extracts was determined using the agar well diffusion method. Mueller–Hinton agar plates were inoculated with the multidrug-resistant bacterial isolates using sterile cotton swabs. A sterile cork borer was used to make wells of 6 mm diameter in the agar. Into each well, 0.1 mL of plant extract at the required concentration was introduced. Three replicate wells were prepared for each concentration and each bacterial isolate. Plates containing aqueous and ethanolic extracts were incubated at 37°C for 24 h. After incubation, the diameter of the clear zone around each well was measured in millime-

tres. Ciprofloxacin and gentamicin discs served as positive controls, while 5served as the negative control.

2.11 Determination of Minimum Inhibitory Concentration and Minimum Bactericidal

Concentration The minimum inhibitory concentration of the most active plant extracts was determined using the broth dilution method. Serial dilutions of the extracts were prepared in nutrient broth to obtain concentrations ranging from 3.125 to 100 mg/mL. Each tube was inoculated with a standardised bacterial suspension and incubated at 37°C for 24 h. The lowest concentration that showed no visible bacterial growth was recorded as the minimum inhibitory concentration. To determine the minimum bactericidal concentration, aliquots from tubes showing no visible growth were inoculated onto nutrient agar plates and incubated. The lowest concentration that produced no bacterial growth on the agar was recorded as the minimum bactericidal concentration.

2.12 Statistical Analysis

All experiments were carried out in triplicate, and the results were expressed as mean ± standard deviation. Data was analysed using the Statistical Package for Social Sciences version 25. The normality of the data was tested using the Shapiro–Wilk test, while homogeneity of variance was assessed using Levene's test. One-way analysis of variance was then used to compare means among the sampling locations and plant extracts. Duncan39;s multiple range test was used to separate significantly different means. Statistical significance was accepted at p < 0.05.The present review was conducted using a comprehensive and systematic evaluation of published scientific literature related to metagenomic analysis of soil microbial communities under regenerative and conventional agricultural systems. Relevant peer-reviewed articles were retrieved from electronic databases including PubMed, Scopus, Web of Science, and Google Scholar using keywords such as "soil metagenomics," "regenerative agriculture microbiome," "conventional farming soil microbes," "16S rRNA sequencing," "shotgun metagenomics," and "soil microbial diversity" (17, 18). Studies published between 2005 and 2025 were preferentially included to capture recent advancements in next-generation sequencing technologies and microbial ecology. Inclusion criteria comprised studies investigating soil microbial diversity, functional gene profiling, microbial abundance, antimicrobial resistance genes, and ecological functions under regenerative and conventional agricultural practices. Studies based solely on culture-dependent techniques were excluded because of their inability to represent the total soil microbial diversity (19, 20).

Material and methods.

A systematic review was made in the internet, based on articles indexing in Crossref using keywords such as pesticides, poisoning, Instrumental Enrichment Program.

Results

The physicochemical properties of the pharmaceutical-contaminated water revealed serious pollution across all sampling sites. The Sabon Gari Pharmaceutical Market drain recorded the highest electrical conductivity (1582 ± 47 µS/cm), total dissolved solids (785 ± 26 mg/L), biochemical oxygen demand (73.5 ± 4.1 mg/L), and chemical oxygen demand (152.3 ± 6.7 mg/L). These values exceeded the WHO permissible limits, indicating high levels of dissolved pollutants and organic matter. Dissolved oxygen values were low at all sites, ranging from 2.4 ± 0.1 to 3.5 ± 0.3 mg/L, suggesting poor water quality and inadequate aeration as shown in Table 1 Escherichia coli was the most prevalent bacterial isolate across all sites, with a mean occurrence of 29.8%, followed by Staphylococcus aureus (21.1%) and Pseudomonas aeruginosa (18.5%).The highest percentage of E. coli was observed in the Sabon Gari drain (33.2%), indicating significant faecal contamination. Enterococcus faecalis was more abundant in the receiving stream (23.7%), suggesting that contamination from the drains extends into downstream waterbodies, as shown in Table 2.

Table 8. Comparative Antimicrobial Activity of Most Effective Plant Extracts and Conventional Antibiotics Against Multidrug-Resistant Bacteria

Treatment	E. coli (mm)	S. aureus (mm)	P. aeruginosa (mm)
Garlic Ethanolic Extract (100 mg/mL)	26.8 ± 1.7	29.4 ± 1.6	23.1 ± 1.4
Guava Ethanolic Extract (100 mg/mL)	23.5 ± 1.5	25.8 ± 1.4	20.6 ± 1.3
Ciprofloxacin (5 µg)	18.6 ± 1.2	20.4 ± 1.3	16.8 ± 1.1
Gentamicin (10 µg)	15.2 ± 1.0	18.7 ± 1.2	14.4 ± 0.9

The antibiotic resistance pattern showed that the bacterial isolates were highly resistant to commonly used antibiotics. Pseudomonas aeruginosa exhibited the highest multidrug resistance (71.2%), followed by Klebsiella pneumoniae (66.5%) and Escherichia coli (64.8%). Resistance to ampicillin was highest in Pseudomonas aeruginosa (92.7%), while tetracycline resistance exceeded 60% in all isolates. Gentamicin showed comparatively lower resistance values, indicating that it may still retain some effectiveness against the organisms, as shown in Table 3

Table 2. Frequency and Distribution of Bacterial Isolates Recovered from Pharmaceutical-Contaminated Water

Bacterial Species	AKTH Drain (%)	MMSH Drain (%)	Sabon Gari Drain (%)	Receiving Stream (%)	Mean (%)
Escherichia coli	30.5	28.4	33.2	27.1	29.8
Staphylococcus aureus	21.7	23.1	20.8	18.9	21.1
Pseudomonas aeruginosa	19.4	17.5	21.3	15.8	18.5
Klebsiella pneumoniae	15.2	16.7	13.6	14.5	15.0
Enterococcus faecalis	13.2	14.3	11.1	23.7	15.6

Table 3. Antibiotic Resistance Pattern of Multidrug-Resistant Isolates (%)

Organism	Ampicillin	Ciprofloxacin	Gentamicin	Tetracycline	Chloramphenicol	Multidrug Resistance (%)
Escherichia coli	84.3	47.6	33.8	72.4	46.1	64.8
Staphylococcus aureus	78.5	38.2	29.4	67.5	41.8	58.6
Pseudomonas aeruginosa	92.7	54.8	42.3	75.6	53.7	71.2
Klebsiella pneumoniae	86.1	45.9	37.6	70.3	49.4	66.5
Enterococcus faecalis	73.5	35.7	26.8	61.4	38.5	54.3

Among the aqueous plant extracts, garlic demonstrated the greatest antimicrobial activity, producing inhibition zones of 21.4 ± 1.5 mm against E. coli and 24.8 ± 1.4 mm against Staphylococcus aureus. Guava leaf extract was the second most effective, while Moringa oleifera showed the weakest antibacterial effect. Pseudomonas aeruginosa was generally less susceptible to the extracts than the other organisms, as shown in Table 4.

Table 4. Antimicrobial Activity of Aqueous Plant Extracts Against Multidrug-Resistant Isolates (Zone of Inhibition, mm)

Plant Extract (100 mg/mL)	E. coli	S. aureus	P. aeruginosa	K. pneumoniae
Neem (Azadirachta indica)	14.8 ± 1.2	17.3 ± 1.1	12.6 ± 0.9	13.5 ± 1.0
Moringa (Moringa oleifera)	12.5 ± 1.0	15.8 ± 1.2	10.4 ± 0.8	11.7 ± 0.9
Bitter leaf (Vernonia amygdalina)	13.6 ± 1.1	16.9 ± 1.2	11.8 ± 0.9	12.9 ± 1.0
Guava leaf (Psidium guajava)	18.7 ± 1.4	21.5 ± 1.3	15.9 ± 1.1	17.2 ± 1.2
Garlic (Allium sativum)	21.4 ± 1.5	24.8 ± 1.4	18.3 ± 1.2	20.1 ± 1.3
Ginger (Zingiber officinale)	15.2 ± 1.2	17.7 ± 1.1	13.4 ± 1.0	14.6 ± 1.1

The ethanolic extracts were more effective than the aqueous extracts against all bacterial isolates. Garlic ethanolic extract showed the highest antimicrobial activity, with inhibition zones of 26.8 ± 1.7 mm against E. coli, 29.4 ± 1.6 mm against Staphylococcus aureus, and 23.1 ± 1.4 mm against Pseudomonas aeruginosa. Guava leaf ethanolic extract also demonstrated strong antibacterial activity. The enhanced performance of ethanolic extracts suggests that ethanol was more efficient in extracting the active antimicrobial compounds from the plants, as shown in Table 5.

Table 5. Antimicrobial Activity of Ethanolic Plant Extracts Against Multidrug-Resistant Isolates (Zone of Inhibition, mm)

Plant Extract (100 mg/mL)	E. coli	S. aureus	P. aeruginosa	K. pneumoniae
Neem (Azadirachta indica)	18.6 ± 1.3	21.2 ± 1.2	16.7 ± 1.1	17.9 ± 1.2
Moringa (Moringa oleifera)	16.4 ± 1.1	19.1 ± 1.3	14.8 ± 1.0	15.6 ± 1.1
Bitter leaf (Vernonia amygdalina)	17.5 ± 1.2	20.4 ± 1.2	15.9 ± 1.1	16.8 ± 1.1
Guava leaf (Psidium guajava)	23.5 ± 1.5	25.8 ± 1.4	20.6 ± 1.3	22.4 ± 1.4
Garlic (Allium sativum)	26.8 ± 1.7	29.4 ± 1.6	23.1 ± 1.4	25.2 ± 1.5
Ginger (Zingiber officinale)	19.3 ± 1.3	22.1 ± 1.2	17.5 ± 1.1	18.6 ± 1.2

The antimicrobial activity of garlic ethanolic extract against Pseudomonas aeruginosa increased with concentration. The inhibition zone rose from 10.2 ± 0.8 mm at 25 mg/mL to 23.1 ± 1.4 mm at 100 mg/mL. This concentration-dependent relationship indicates that higher concentrations of the extract provide stronger antibacterial activity, as shown in Table 6. The mini-

Table 6. Effect of Extract Concentration on Zone of Inhibition of Garlic Ethanolic Extract Against Pseudomonas aeruginosa

Concentration (mg/mL)	Zone of Inhibition (mm)
25	10.2 ± 0.8
50	15.8 ± 1.0
75	19.7 ± 1.2
100	23.1 ± 1.4

mum inhibitory concentration and minimum bactericidal concentration results confirmed that garlic ethanolic extract was the most potent plant extract. The lowest MIC value (6.25 mg/mL) was observed against Staphylococcus aureus, while the lowest MBC value (12.5 mg/mL) was also recorded for the same organism. Pseudomonas aeruginosa required higher concentrations of the extract, confirming its greater resistance, as shown in Table 7.

Table 8. Comparative Antimicrobial Activity of Most Effective Plant Extracts and Conventional Antibiotics Against Multidrug-Resistant Bacteria

Treatment	E. coli (mm)	S. aureus (mm)	P. aeruginosa (mm)
Garlic Ethanolic Extract (100 mg/mL)	26.8 ± 1.7	29.4 ± 1.6	23.1 ± 1.4
Guava Ethanolic Extract (100 mg/mL)	23.5 ± 1.5	25.8 ± 1.4	20.6 ± 1.3
Ciprofloxacin (5 µg)	18.6 ± 1.2	20.4 ± 1.3	16.8 ± 1.1
Gentamicin (10 µg)	15.2 ± 1.0	18.7 ± 1.2	14.4 ± 0.9

The comparison between medicinal plant extracts and conventional antibiotics showed that garlic and guava ethanolic extracts produced larger inhibition zones than ciprofloxacin and gentamicin against the multidrug-resistant bacteria. Garlic ethanolic extract recorded the highest inhibition zone against Staphylococcus aureus (29.4 ± 1.6 mm), whereas ciprofloxacin

produced only 20.4 ± 1.3 mm. These findings suggest that selected medicinal plants, especially garlic and guava leaf, may provide effective alternative treatments for multidrug-resistant bacterial infections, as shown in Table 8.

Table 8. Comparative Antimicrobial Activity of Most Effective Plant Extracts and Conventional Antibiotics Against Multidrug-Resistant Bacteria

Treatment	E. coli (mm)	S. aureus (mm)	P. aeruginosa (mm)
Garlic Ethanolic Extract (100 mg/mL)	26.8 ± 1.7	29.4 ± 1.6	23.1 ± 1.4
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Ciprofloxacin (5 µg)	18.6 ± 1.2	20.4 ± 1.3	16.8 ± 1.1
Gentamicin (10 µg)	15.2 ± 1.0	18.7 ± 1.2	14.4 ± 0.9

Discussion

The physicochemical properties of the drainage water indicated substantial environmental contamination at all the sampling sites. Electrical conductivity, total dissolved solids, biochemical oxygen demand, and chemical oxygen demand exceeded recommended limits, particularly in the Sabon Gari Pharmaceutical Market drain. Such elevated values suggest that the drainage channels contained large quantities of dissolved salts, organic matter, suspended solids, and pharmaceutical residues. Akpor and Muchie (2011) similarly reported that untreated wastewater from urban environments is often characterised by high biochemical oxygen demand and chemical oxygen demand because of the presence of decomposable organic substances. Kayode-Afolayan et al. (2022) further noted that pharmaceutical effluents frequently degrade water quality by increasing organic and chemical pollution loads. The low dissolved oxygen concentrations recorded in the present study indicate poor ecological quality of the drainage water. Dissolved oxygen values below 5 mg/L are generally considered unsuitable for many aquatic organisms because such conditions reflect intense microbial decomposition and high oxygen demand. Similar observations were reported by Akpor and Muchie (2011), who found that polluted wastewater systems often exhibit low dissolved oxygen due to excessive organic loading (Okpoji et al., 2025). The low oxygen values observed in the drains, therefore, suggest that the receiving streams may be exposed to significant environmental stress. Escherichia coli was the most abundant bacterial isolate recovered from the wastewater. The predominance of this organism suggests considerable faecal contamination of the drainage systems, probably resulting from hospital sewage, domestic waste, and poor sanitary conditions around the sampling sites. Similar findings have been reported in wastewater studies where Escherichia coli was identified as the major indicator organism of faecal pollution (Nibamureke Barnhoorn, 2025). The high occurrence of Escherichia coli also raises concern because the organism can transfer resistance genes to other bacteria in the environment. The occurrence of Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Enterococcus faecalis in the drainage water is of considerable public health importance. These bacteria are common opportunistic pathogens associated with hospital environments and have been implicated in severe infections, including septicemia, wound infections, respiratory tract infections, and urinary tract infections. Kayode-Afolayan et al. (2022) observed that hospital wastewater frequently contains such pathogenic microorgan-

isms because of contamination from clinical activities and pharmaceutical discharges. Among the bacterial isolates, Pseudomonas aeruginosa exhibited the highest multidrug resistance. The organism showed particularly high resistance to ampicillin and tetracycline, indicating that it has developed strong adaptive mechanisms against conventional antibiotics. Pseudomonas aeruginosa is naturally resistant to several antibiotics because of its ability to produce biofilms, efflux pumps, and -lactamase enzymes. These resistance mechanisms reduce the penetration and effectiveness of antibiotics, making the organism difficult to eradicate. Similar observations were made by Jaishankar et al. (2014), who explained that persistent contaminants and environmental stress can stimulate bacterial resistance. Klebsiella pneumoniae and Escherichia coli also demonstrated considerable resistance to the tested antibiotics. Their high resistance may be associated with plasmid-mediated resistance and the presence of extended-spectrum -lactamase genes. According to Patel et al. (2019), pharmaceutical residues present in wastewater create selective pressure that encourages the survival and multiplication of resistant microorganisms. Li et al. (2024) also emphasised that wastewater contaminated by antibiotics provides ideal conditions for the spread of multidrug-resistant bacteria. The high frequency of multidrug resistance observed in this study may therefore be attributed to the continuous exposure of bacteria to residual antibiotics in the pharmaceutical-contaminated drains. When susceptible bacteria are repeatedly exposed to low concentrations of antibiotics, they are eliminated while resistant organisms survive and dominate. Over time, these resistant bacteria become established within the microbial community and may transfer their resistance genes to other organisms. Obinna et al. (2023) similarly concluded that untreated pharmaceutical wastewater is a major environmental source of antimicrobial resistance. The medicinal plant extracts examined in this study showed varying degrees of antibacterial activity against the multidrug-resistant bacteria. In general, the ethanolic extracts produced larger inhibition zones than the aqueous extracts. This finding may be explained by the greater ability of ethanol to extract active phytochemicals from plant materials. Flavonoids, alkaloids, tannins, terpenoids, phenolics, and essential oils are generally more soluble in ethanol than in water. Consequently, ethanolic extracts are often more concentrated and more effective than aqueous preparations. Among all the plants tested, garlic exhibited the strongest antibacterial activity. The ethanolic extract of garlic produced the highest inhibition zones against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae. The strong activity of garlic may be attributed to the presence of allicin, ajoene, diallyl sulfide, and other sulfur-containing compounds. These substances are capable of disrupting bacterial cell walls, altering membrane permeability, inhibiting protein synthesis, and suppressing essential metabolic pathways. The effectiveness of garlic against both Gram-positive and Gram-negative bacteria agrees with previous findings reported by Patel et al. (2019). The inhibitory effect of garlic was particularly pronounced against Staphylococcus aureus. This observation may be due to the greater susceptibility of Gram-positive bacteria to plant-derived compounds because they possess simpler cell walls than Gram-negative bacteria. Nevertheless, garlic also showed considerable activity against Pseudomonas aeruginosa, despite the high resistance of this organism to conventional antibiotics. This suggests that garlic acts through mechanisms that differ from those of synthetic antimicrobial agents. Guava leaf extract also demonstrated substantial antibacterial activity and ranked second only to garlic. The effectiveness of guava leaves may be linked to their high concentrations of tannins, flavonoids, quercetin, and other phenolic compounds. These phytochemicals can denature bacterial proteins, damage cell membranes, and inhibit nucleic acid synthesis. Similar antibacterial activity of guava has been reported in studies of medicinal plants used against resistant pathogens (Obinna et al., 2023). Neem, ginger, bitter leaf, and moringa also showed measurable antibacterial activity, although their inhibition zones were lower than those of garlic and guava. Neem contains azadirachtin and nimbin, while ginger contains gingerol and shogaol. Bitter leaf and moringa are also rich in phytochemicals capable of inhibiting microbial growth. The lower activity observed in these plants may be related to differences in the concentration, stability, or solubility of their active constituents. The increase in antibacterial activity with increasing concentration of garlic extract indicates a concentration-dependent effect. The inhibition zone against Pseudomonas aeruginosa increased progressively from the lowest to the highest concentration tested. This pattern confirms that the phytochemicals in garlic exert stronger antibacterial activity at higher doses. Similar concentration-dependent relationships have been reported for several medicinal plant extracts (Patel et al., 2019). The minimum inhibitory concentration and minimum bactericidal concentration values obtained in the present study further confirm the potency of the plant extracts. Garlic required the lowest concentrations to inhibit and kill the bacterial isolates, while guava also produced rela-

tively low inhibitory concentrations. In contrast, Pseudomonas aeruginosa required higher concentrations of the extracts than the other organisms, reflecting its greater resistance. An important finding of this study is that the most effective medicinal plant extracts produced larger inhibition zones than conventional antibiotics such as ciprofloxacin and gentamicin. This suggests that medicinal plants may provide useful alternatives where synthetic antibiotics are ineffective. In low-resource settings where access to antibiotics is limited and antimicrobial resistance is widespread, medicinal plants may therefore serve as affordable and sustainable treatment options (Obinna et al., 2023; Patel et al., 2019).

Conclusion

The present study demonstrated that wastewater discharged into drainage channels outside hospitals, pharmaceutical markets, and pharmaceutical factories in Kano State is heavily polluted and contains significant numbers of multidrug-resistant bacteria. The elevated values of electrical conductivity, biochemical oxygen demand, chemical oxygen demand, and total dissolved solids indicate poor water quality and confirm that the drains receive substantial quantities of untreated pharmaceutical and organic wastes. The predominance of Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Klebsiella pneumoniae further shows that these drainage systems function as important reservoirs of pathogenic microorganisms. The high resistance of these bacteria to commonly used antibiotics supports previous findings that pharmaceutical residues in wastewater create selective pressure that promotes the development and spread of antimicrobial resistance. The study also established that medicinal plants possess considerable potential as alternative antimicrobial agents against multidrug-resistant bacteria. Ethanolic extracts were more effective than aqueous extracts, while garlic and guava leaf extracts exhibited the strongest antibacterial activity. The ability of these plant extracts to produce larger inhibition zones than conventional antibiotics indicates that they may provide affordable, locally available, and environmentally sustainable alternatives for the treatment of resistant bacterial infections. Therefore, stricter regulation of pharmaceutical wastewater discharge, improved wastewater treatment, and further investigation of medicinal plants are recommended to reduce the spread of multidrug-resistant bacteria and protect public health.

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