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Evaluation of the Antibacterial Potential of *Acacia nilotica* Leaf Extracts Against Clinical *Salmonella* Isolates from Murtala Muhammad Specialist Hospital, Kano, Nigeria

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Abstract

Background: *Salmonella* infection remains endemic in northern Nigeria, with rising antimicrobial resistance. While *Acacia nilotica* has been widely studied, research has focused mainly on bark and roots.
Aims: this study provides novel clinical evidence on the antibacterial activity of its leaf extracts against *Salmonella* isolates from patients in Kano.
Methods: Four hundred stool samples from patients screened for typhoid were enriched in Selenite F broth and cultured on selective media. Presumptive *Salmonella* isolates were identified phenotypically by morphology, Gram staining, and biochemical profiling. Leaves of *A. nilotica* were extracted with water and ethanol. Phytochemical screening was performed, and antibacterial activity was assessed by agar diffusion at graded concentrations. MIC and MBC were determined by broth dilution.
Result; Fourteen isolates (3.5%) were identified as *Salmonella* phenotpically. Phytochemical analysis revealed abundant tannins, alkaloids, saponins, steroids, and glycosides. Both extracts exhibited concentration-dependent inhibition against a subset of isolates, with ethanol showing higher activity (zones up to 20 mm at 50 mg/mL) than water. MICs ranged from 25–50 mg/mL, and MBCs were typically 50 mg/mL for susceptible isolates. Activity was substantially lower than ciprofloxacin.
Conclusion: *A. nilotica* leaves possess measurable but modest anti-*Salmonella* activity. While weaker than reports for bark and roots, the leaf-based approach offers sustainability and safety advantages. Further molecular identification is warranted, with tannins, alkaloids, and saponins prioritized for bioassay-guided isolation.

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INTRODUCTION

Enteric fever caused by *Salmonella enterica* serovar Typhi remains a major public health challenge in low- and middle-income countries, where inadequate sanitation, limited access to clean water, and diagnostic constraints sustain transmission (Crump et al., 2015). In Nigeria, typhoid fever continues to contribute substantially to outpatient visits and hospital admissions, with several studies from the north-western region reporting persistent circulation of *Salmonella* and increasing antimicrobial resistance to commonly used agents (Abdullahi et al., 2015; Obaro et al., 2015; Musa et al., 2025). The growing prevalence of multidrug-resistant *Salmonella* strains compromises empirical therapy, prolongs illness, and increases healthcare costs, reinforcing the urgency for complementary antimicrobial strategies that are locally accessible, affordable, and culturally acceptable.

The accelerating burden of antimicrobial-resistant *Salmonella enterica* serovar Typhi, including multidrug-resistant (MDR) and extensively drug-resistant (XDR) lineages, has markedly narrowed effective oral treatment options and increased the risk of therapeutic failure in endemic regions. Recent genomic and epidemiological studies document the rapid international spread of resistant

clones and the progressive loss of susceptibility to first-line and even reserve agents, underscoring a growing treatment crisis for enteric fever (Da-Silva et al., 2021; Tanmoy et al., 2024; Walker et al., 2023). In this context, there is renewed interest in medicinal plants as potential substitutes or adjuncts to conventional antibiotics. Contemporary reviews highlight that plant-derived secondary metabolites exert multi-target antibacterial actions such as membrane disruption, enzyme inhibition, anti-virulence effects, and interference with quorum sensing that may reduce selective pressure for single-mechanism resistance and provide locally accessible options where antibiotics are failing or unaffordable (Vaou et al., 2021; Angelini, 2024; Arip et al., 2022). Collectively, this evidence supports systematic evaluation of traditionally used plants as part of an integrated response to MDR/XDR *Salmonella* in resource-limited settings.

Medicinal plants have long constituted a major component of primary healthcare in sub-Saharan Africa, and phytochemicals derived from these plants are increasingly investigated as alternative or adjunct antimicrobial agents. Members of the Fabaceae family are particularly notable for their rich secondary metabolite profiles and ethnomedicinal relevance in northern Nigeria (Musa & Aji, 2025). Among these, *Acacia nilotica* (syn. *Vachellia nilotica*) is widely distributed across the savannah belt and has been traditionally employed in the management of gastrointestinal disorders, wound infections, and febrile illnesses. Phytochemical and pharmacological studies consistently demonstrate that *A. nilotica* contains high levels of bioactive compounds, including hydrolysable tannins, alkaloids, saponins, steroids, and glycosides, many of which exhibit broad-spectrum antibacterial activity (Hafez et al., 2024; Sadiq et al., 2015). Experimental evidence further indicates inhibitory effects of *A. nilotica* extracts against Gram-negative pathogens, including *Salmonella* spp., with proposed mechanisms involving disruption of cell membrane integrity, enzyme inhibition, metal ion chelation, and interference with microbial adhesion and biofilm formation (Chandra et al., 2017; Ekambaram et al., 2016; Sadiq et al., 2017).

Despite this growing body of evidence, most investigations have focused on bark, stem, and root extracts, which frequently exhibit stronger antibacterial activity than aerial parts due to their higher concentration of condensed and hydrolysable tannins and phenolic compounds (Sadiq et al., 2015, 2017). However, harvesting these tissues is ecologically destructive and can irreversibly damage or kill the plant, undermining sustainability and long-term availability. In contrast, leaves represent a renewable, non-destructive resource that can be repeatedly harvested with minimal environmental impact, making them more suitable for community-based use and scalable phytotherapeutic development (Hafez et al., 2024; Musa & Aji, 2025). From a public health perspective, leaf-based preparations are also potentially safer for oral application, as excessive intake of tannin-rich bark and root extracts has been associated with gastrointestinal irritation and reduced nutrient absorption (Ekambaram et al., 2016). Nonetheless, relatively few studies have evaluated the antibacterial efficacy of *A. nilotica* leaves specifically against clinically relevant human isolates of *Salmonella*, and data from hospital-based settings in northern Nigeria are particularly scarce.

Importantly, available evidence suggests that the antibacterial activity of *A. nilotica* leaves may be weaker than that of bark or roots, a difference attributed to lower tannin density, qualitative variation in phenolic composition, and reduced abundance of certain flavonoid derivatives in leaf tissues (Hafez et al., 2024; Sadiq et al., 2015). This does not negate their therapeutic relevance; rather, it highlights the need to define the realistic antimicrobial potential of leaves within a sustainable framework. In the context of increasing antimicrobial resistance, even modest inhibitory activity may offer value as an adjunct to conventional antibiotics, as a template for bioassay-guided isolation of active fractions, or as a foundation for combination therapies aimed at reducing resistance selection pressure (Negi, 2012; Upadhyay et al., 2014). Furthermore, systematic reviews of Fabaceae medicinal plants from northern Nigeria emphasize the importance of validating traditionally used plant parts under standardized laboratory conditions to bridge the gap between ethnomedicine and evidence-based phytotherapy (Musa & Aji, 2025).

Against this background, the present study investigated the antibacterial activity of aqueous and ethanolic leaf extracts of *A. nilotica* against *Salmonella* species isolated from patients attending Murtala Muhammad Specialist Hospital, Kano. By anchoring the work in a clinical setting and prioritizing a sustainable plant part, this study aims to provide locally relevant data on the anti-*Salmonella* potential of *A. nilotica* leaves, contribute to the growing evidence base for plant-derived

antimicrobials, and inform future efforts toward the development of safe, accessible adjunct therapies in regions where typhoid fever remains endemic.

METHOD

Ethical Approval

Ethical clearance for this prospective, laboratory-based study was obtained from the Kano State Ministry of Health, Health Service Management Board, Nigeria (Ref: MOH/off/797/T.I/49). Informed consent was obtained from all adult participants or guardians of minors prior to sample collection. Data confidentiality was maintained through anonymized sample coding.

Study Area

The study was conducted from June to November 2023 across two sites: the diagnostic microbiology laboratory of Murtala Muhammad Specialist Hospital (MMSH), a tertiary referral center in Kano, and the research microbiology laboratories at Bayero University Kano (BUK). Kano, the study location (12°00'N, 8°31'E), with a land area of approximately 499 km^2, is a typhoid-endemic region in northwestern Nigeria (Musa & Aji, 2025). Murtala Muhammad Specialist Hospital, Kano, Nigeria, is a major government-owned tertiary healthcare facility that serves as a referral center for Kano State and neighboring areas in north-western Nigeria. The hospital provides comprehensive clinical services, including the diagnosis and management of infectious diseases such as typhoid fever. Patients who are presented with febrile illness and gastrointestinal symptoms are routinely evaluated in the hospital, and stool and blood specimens are processed in the Microbiology Laboratory for bacteriological culture, identification, and antimicrobial susceptibility testing. Owing to its high patient turnover and the regular presentation of enteric infections, MMSH offers an appropriate clinical and laboratory setting for the isolation of *Salmonella* species from patients receiving treatment for typhoid.

Sample Collection

A total of 400 fresh stool samples were collected from patients attending Murtala Muhammad Specialist Hospital (MMSH), Kano, who presented with clinical features suggestive of enteric fever, including prolonged fever (>39°C), headache, malaise, and abdominal discomfort. The study population was purposively selected to include patients with a positive Widal test (single titre ≥1:160 for anti-O or anti-H antibodies), as this group represents individuals with a higher likelihood of *Salmonella* infection in routine clinical practice within endemic settings. This selection strategy was adopted to enhance the recovery of clinically relevant isolates while reflecting real-world diagnostic and treatment conditions in the study area.

All stool samples were collected aseptically in sterile, wide-mouthed, leak-proof containers and immediately maintained under cold chain conditions (4°C). Samples were transported to the Microbiology Research Laboratory, Bayero University Kano, within two hours of collection for prompt processing. Although Widal testing was used for initial clinical screening, all samples included in the study were subsequently confirmed by culture and biochemical characterization to minimize false-positive inclusion and ensure microbiological validity.

Isolation and Identification of *Salmonella*

Bacterial Enrichment, Isolation, and Phenotypic Identification

Approximately 1 gram of each stool sample was aseptically inoculated into 10 mL of sterile Selenite F Broth (Oxoid, UK) for selective enrichment of *Salmonella* spp. and incubated at 37°C for 18-24 hours under aerobic conditions (Boer et al., 2019). A loopful of the enriched broth was streaked on Salmonella-Shigella Agar and MacConkey Agar. Presumptive isolates were characterized by colony morphology, Gram staining, and biochemical tests including triple sugar iron (TSI), urease, indole, motility, and citrate utilization, following standard microbiological procedures.

Plant Material Collection and Authentication

Fresh, mature leaves of *Acacia nilotica* (L.) Willd. ex Del. were collected in July 2021 from Kano metropolis, Nigeria, during the peak vegetative season to ensure optimal phytochemical content. A

certified botanist at the Herbarium of the Department of Plant Biology, Bayero University Kano, performed formal taxonomic identification. A voucher specimen (Accession No: BUKHAN 0186) was deposited in the herbarium for future reference.

Plant Extraction

The collected leaves were washed, shade-dried at ambient temperature (25 ± 3°C) to constant weight, and pulverized. For aqueous extraction, 200 g of powder was cold-macerated in sterile distilled water (1:10 w/v) for 72 hours with agitation. The filtrate was lyophilized to obtain the dry crude extract (Yield: 18.2%). For ethanolic extraction, an equivalent amount of powder was macerated in 95% ethanol (1:10 w/v) under identical conditions. The ethanolic filtrate was concentrated using a rotary evaporator (40°C) and desiccated, yielding the dry extract (Yield: 21.5%). Both extracts were stored at -20°C in amber vials (Yusuf et al., 2026).

Phytochemical Screening

The qualitative phytochemical profiles of the aqueous and ethanolic leaf extracts were determined using standardized chemical assays (Harborne, 1998 ;Sofowora, 1993). Tests were conducted in triplicate to detect the presence of key secondary metabolite classes, including tannins, alkaloids, flavonoids, saponins, cardiac glycosides (S- and C-glycosides), steroids, reducing sugars, and volatile oils. Distinct colour changes, precipitates, or froth formation, as specified by each protocol, were recorded as positive indicators.

Antibacterial Susceptibility Testing

The antibacterial activity of the crude extracts was evaluated using the standardized agar well diffusion method, as described in the Clinical and Laboratory Standards Institute guidelines with adaptations for plant-derived compounds (CLSI, 2018). Briefly, a fresh bacterial suspension was adjusted to a 0.5 McFarland standard (~1.5 x 10^8 CFU/mL) in sterile saline. Stock solutions of each extract were prepared at 200 mg/mL in 10% dimethyl sulfoxide (DMSO) and serially diluted two-fold in Mueller-Hinton Broth to obtain final test concentrations of 50, 25, 12.5, and 6.25 mg/mL. Mueller-Hinton Agar plates were uniformly inoculated with the standardized inoculum. Wells (6 mm diameter) were bored, and 100µL of each extract concentration was dispensed. Control wells contained ciprofloxacin (5µg) as a positive control and 10% DMSO as a vehicle control. After a 30-minute pre-diffusion period at room temperature, plates were incubated aerobically at 37°C for 24 hours. The diameters of the resulting zones of inhibition (ZOI) were measured to the nearest millimeter using a digital caliper. All assays were conducted in triplicate, and the antibacterial activity was expressed as the mean ZOI ± standard deviation.

Determination of Minimum Inhibitory and Bactericidal Concentrations (MIC/MBC)

The MIC was determined using the broth macrodilution method in sterile glass test tubes (18 x 150 mm), performed in accordance with standardized guidelines for plant extracts with modifications (Eloff, 1998). A two-fold serial dilution of each stock extract (200 mg/mL) was prepared in Mueller-Hinton Broth (MHB), resulting in a final concentration range of 50 mg/mL to 0.78 mg/mL in a final volume of 5 mL per tube. Each tube was inoculated with the standardized bacterial suspension to achieve a final concentration of approximately 5 x 10^5 CFU/mL. Control tubes included: sterile MHB (sterility control), MHB with inoculum (growth control), and MHB with inoculum and ciprofloxacin (5µg, positive control). Tubes were incubated aerobically at 37°C for 24 hours. The MIC was visually determined as the lowest concentration of extract that completely inhibited visible turbidity (Musa et al., 2026). For MBC determination, 10 µL from each clear tube was sub-cultured onto fresh Mueller-Hinton Agar (MHA) plates and incubated at 37°C for 24 hours. The MBC was defined as the lowest extract concentration that resulted in no colony growth on the subculture, indicating ≥99.9% bactericidal activity. No inferential statistical comparison was performed due to the limited number of susceptible isolates (Musa et al., 2026).

RESULTS AND DISCUSSION

Results

Out of the 400 stool samples analyzed, 14 isolates (3.5%) were identified phenotypically as *Salmonella typhi* based on characteristic colony morphology on Salmonella–Shigella agar and MacConkey agar, Gram-negative rod morphology, and confirmatory biochemical reactions in TSI, urease, indole, motility, and citrate utilization tests. These isolates were subsequently coded (001–014) and used for antibacterial susceptibility testing against *Acacia nilotica* leaf extracts.

Qualitative phytochemical screening revealed that both aqueous and ethanolic extracts of *A. nilotica* leaves contained several bioactive secondary metabolites. Tannins, alkaloids, saponins, steroids, and glycosides were consistently detected in both extracts. Reducing sugars were detected only in the aqueous extract, while volatile oils were present exclusively in the ethanolic extract. Flavonoids were not detected in either extract.

Table 1. Phytochemical constituents of *A. nilotica* leaf extracts

Phytochemical	**Ethanolic Extract**	**Water Extract**
Tannins	+	+
Alkaloids	+	+
Flavonoids	–	–
Saponins	+	+
S-Glycosides	+	–
C-Glycosides	+	+
Steroids	+	+
Reducing Sugars	–	+
Volatile Oils	+	–

Key: + = Present; – = Absent (qualitative screening only; intensity not determined)

Antibacterial Activity of Aqueous Leaf Extract

The aqueous extract of *A. nilotica* leaves exhibited variable antibacterial activity across the 14 isolates. At the highest concentration (50 mg/mL), measurable zones of inhibition were observed in six isolates (001, 002, 003, 007, 014), with inhibition diameters ranging from 7–17 mm. At 25 mg/mL, activity was reduced and detected in only four isolates (001, 002, 014), while no inhibitory effect was observed at concentrations of 12.5 mg/mL and 6.25 mg/mL. Several isolates (004, 005, 008–013) showed complete resistance to the aqueous extract at all tested concentrations. In contrast, the positive control (ciprofloxacin, 5 µg/mL) consistently produced large inhibition zones (18–25 mm), whereas the negative control showed no activity.

Table 2. Antibacterial activity of aqueous extract of *A. nilotica* leaves against *Salmonella* isolates

Isolate Code	Zone of inhibition (mm, mean ± SD) at different extract concentrations					
	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	Ciprofloxacin (5 µg)	Negative Control
001	12 ± 0.0	8 ± 0.0	0 ± 0.0	0 ± 0.0	25 ± 0.0	0.0
002	9 ± 0.0	5 ± 0.0	0 ± 0.0	0 ± 0.0	22 ± 0.0	0.0
003	7 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	21 ± 0.0	0.0
004	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	19 ± 0.0	0.0

005	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	18 ± 0.0	0.0
007	17 ± 0.0	10 ± 0.0	0 ± 0.0	0 ± 0.0	24 ± 0.0	0.0
008	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	20 ± 0.0	0.0
009	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	18 ± 0.0	0.0
010	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	19 ± 0.0	0.0
011	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	19 ± 0.0	0.0
012	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	19 ± 0.0	0.0
013	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	22 ± 0.0	0.0
014	14 ± 0.0	8 ± 0.0	0 ± 0.0	0 ± 0.0	22 ± 0.0	0.0

Antibacterial Activity of Ethanolic Leaf Extract

The ethanolic extract demonstrated comparatively higher antibacterial activity than the aqueous extract. At 50 mg/mL, seven isolates (001, 002, 003, 007, 013, 014) exhibited inhibition zones ranging from 8–20 mm. Activity persisted at 25 mg/mL for several isolates (001, 002, 007, 014), whereas no inhibition was observed at 12.5 mg/mL and 6.25 mg/mL. Similar to the aqueous extract, multiple isolates remained completely non-susceptible to the ethanolic extract across all concentrations tested. Ciprofloxacin consistently produced large zones of inhibition (18–25 mm), while the negative control showed no activity.

Table 3. Antibacterial activity of ethanolic extract of *A. nilotica* leaves against *Salmonella* isolates

Isolate Code	Zone of inhibition (mm, mean ± SD) at different extract concentrations					
	50 mg/mL	25 mg/mL	12.5 mg/mL	6.25 mg/mL	Ciprofloxacin (5 µg)	Negative Control
001	16 ± 0.0	12 ± 0.0	0 ± 0.0	0 ± 0.0	25 ± 0.0	0.0
002	15 ± 0.0	9 ± 0.0	0 ± 0.0	0 ± 0.0	22 ± 0.0	0.0
003	8 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	21 ± 0.0	0.0
004	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	18 ± 0.0	0.0
005	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	19 ± 0.0	0.0
007	20 ± 0.0	16 ± 0.0	0 ± 0.0	0 ± 0.0	22 ± 0.0	0.0
009	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	18 ± 0.0	0.0
010	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	20 ± 0.0	0.0
011	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	20 ± 0.0	0.0
012	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	19 ± 0.0	0.0
013	19 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	21 ± 0.0	0.0
014	16 ± 0.0	10 ± 0.0	0 ± 0.0	0 ± 0.0	23 ± 0.0	0.0

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

The MIC and MBC values further demonstrated the moderate antibacterial potency of the extracts. For the aqueous extract, MIC values ranged from 25–50 mg/mL, with MBC generally at 50 mg/mL for susceptible isolates. For the ethanolic extract, MIC values were similarly between 25–50 mg/mL, while MBCs were predominantly 50 mg/mL. Several isolates showed no detectable bactericidal effect at the highest tested concentrations.

Table 4. MIC and MBC of *A. nilotica* leaf extracts against *Salmonella* isolates

Isolate Code	Water Extract MIC (mg/mL)	Water Extract MBC (mg/mL)	Ethanol Extract MIC (mg/mL)	Ethanol Extract MBC (mg/mL)
001	25	50	25	50

002	50	50	25	50
003	50	–	50	–
007	25	50	25	50
013	–	–	50	50
014	25	50	25	50

Key: – indicates no detectable activity at the tested concentrations.

Discussion

The present study demonstrates that *Acacia nilotica* leaf extracts exhibit measurable but modest antibacterial activity against clinical *Salmonella* isolates obtained from patients attending Murtala Muhammad Specialist Hospital, Kano. The recovery rate of *Salmonella* (3.5%) is comparable to hospital-based reports from northern Nigeria, where typhoid fever remains endemic but often underdiagnosed due to reliance on serology and limited culture capacity (Abdullahi et al., 2015). The susceptibility of all isolates to ciprofloxacin is consistent with continued reliance on fluoroquinolones as effective agents for enteric fever in many Nigerian settings, although emerging resistance has been documented elsewhere (Crump et al., 2015 ; Obaro et al., 2015). Against this background, the observed inhibitory activity of *A. nilotica* leaves supports the ethnopharmacological relevance of the plant while highlighting its role as a potential adjunct rather than a replacement for conventional therapy.

The phytochemical profile of the extracts dominated by tannins, alkaloids, saponins, steroids, and glycosides aligns with previous reports on *A. nilotica* (Hafez et al., 2024; Sadiq et al., 2015). These secondary metabolites are well recognized for their antimicrobial mechanisms, including protein precipitation by tannins, membrane permeabilization by saponins, and enzyme inhibition by alkaloids (Chandra et al., 2017; Ekambaram et al., 2016). The greater activity observed in the ethanolic extract compared with the aqueous preparation is consistent with earlier findings that ethanol more efficiently solubilizes moderately polar phenolics and alkaloid fractions responsible for antimicrobial action (Sadiq et al., 2015, 2017). Ethanol typically yields stronger antibacterial extracts than water because it solubilizes a broader spectrum of antimicrobial phytochemicals (including phenolics and other membrane-active constituents), and studies comparing paired ethanolic vs aqueous preparations consistently report lower MICs/higher inhibition for ethanolic extracts. (Gonelimali et al., 2018). However, the zones of inhibition (maximum 20 mm at 50 mg/mL) and MIC values (25–50 mg/mL) indicate only moderate potency against *Salmonella*, particularly when contrasted with the pronounced activity of ciprofloxacin.

When compared with previous investigations on *A. nilotica*, the antibacterial effects observed in this study are notably weaker than those reported for bark and root extracts. Sadiq et al. (2015) demonstrated substantially larger inhibition zones and lower MICs using bark and pod extracts against enteric pathogens, while a subsequent study reported strong activity of *A. nilotica* bark-derived compounds against multidrug-resistant *Salmonella* and *E. coli*, attributed to high concentrations of hydrolysable tannins and catechin derivatives (Sadiq et al., 2017). Similar patterns have been reported in other ethnopharmacological evaluations, where bark and roots consistently outperform leaves in antibacterial assays (Ekambaram et al., 2016; Hafez et al., 2024). The comparatively weak activity of leaf extracts in the present study can be mechanistically justified by the tissue-specific distribution of bioactive compounds in *A. nilotica*. Bark and roots function as protective and structural tissues and are known to accumulate higher levels of condensed and hydrolysable tannins, phenolic acids, and flavan-3-ols that exhibit strong protein-binding and membrane-disruptive effects on bacteria (Ekambaram et al., 2016; Hafez et al., 2024). Plant secondary metabolites are organ-dependent, and woody tissues (e.g., bark) are often relatively enriched in phenolic defenses compared with leaves, which can translate into weaker antibacterial potency of leaf extracts (Jang et al., 2018). Because flavonoids constitute a major antibacterial phytochemical class, their absence in the present extracts likely reduced phytochemical diversity and contributed to the limited inhibitory activity observed (Babii et al., 2018).

In addition, variability in susceptibility among the *Salmonella* isolates where several strains showed no inhibition even at the highest tested concentrations mirrors findings from other plant-based antimicrobial studies that report strain-dependent tolerance to phytochemicals (Negi, 2012; Upadhyay et al., 2014). Clinical isolates of *Salmonella* and other Gram-negative pathogens often show adaptive resistance phenotypes such as enhanced efflux pump activity and reduced membrane permeability that can decrease the efficacy of antibacterial compounds, including crude plant extracts (Reygaert, 2018). This heterogeneity likely contributed to the incomplete spectrum of activity observed and underscores the importance of testing medicinal plants against locally circulating clinical strains rather than standard laboratory organisms.

Despite the lower potency relative to bark and root extracts, the use of leaves in this study is scientifically and ethically defensible, as leaf harvesting is generally less destructive to individual plants and more compatible with sustainable medicinal plant use, whereas removal of roots or bark can significantly damage or kill plants and reduce their long-term availability (van Wyk & Prinsloo, 2018). From a public health and ethnopharmacological perspective, leaf-based remedies are therefore more suitable for long-term community use, particularly in regions where *A. nilotica* is widely utilized in traditional medicine. Although the extracts exhibited modest activity, plant-derived antimicrobials may reduce selective pressure on conventional antibiotics when used as adjuncts, potentially contributing to resistance mitigation strategies in endemic settings. This rationale is further supported by the systematic review of Fabaceae medicinal plants in northern Nigeria, which emphasizes the need to prioritize aerial plant parts to balance therapeutic benefit with environmental sustainability (Musa & Aji, 2025).

Implications

The findings of the study have important implications for both clinical practice and ethnopharmacological research in typhoid-endemic regions. The demonstration of antibacterial activity of *Acacia nilotica* leaf extracts against clinical *Salmonella* isolates highlights the potential of locally available medicinal plants as adjunct therapeutic options in the context of rising antimicrobial resistance. Although the activity observed was modest, the use of leaves as a renewable and non-destructive plant part supports sustainable utilization and aligns with public health strategies aimed at promoting accessible and environmentally responsible healthcare solutions. In addition, the variability in susceptibility among clinical isolates underscores the need for continued surveillance of antimicrobial resistance patterns and the integration of plant-based alternatives into broader antimicrobial stewardship.

Research Contribution

The study contributes to existing knowledge by providing novel hospital-based evidence on the antibacterial activity of *Acacia nilotica* leaf extracts against clinical *Salmonella* isolates in northern Nigeria. Unlike previous studies that mostly focused on bark and root extracts or laboratory strains, this work emphasizes the use of a sustainable plant part and clinically relevant bacterial isolates, thereby enhancing translational relevance. Furthermore, the study establishes baseline data on the phytochemical composition, antibacterial activity, and MIC/MBC profiles of *A. nilotica* leaf extracts, which can serve as a foundation for future bioassay-guided fractionation and drug development efforts.

Limitations

This study has several limitations that should be considered when interpreting the findings. First, identification of *Salmonella* isolates was based solely on phenotypic and biochemical methods; the absence of molecular confirmation may allow for misclassification within the *Salmonella* complex. Second, the study did not assess cytotoxicity, pharmacokinetics, or in vivo efficacy, which limits translational relevance for therapeutic application. In addition, the relatively small number of isolates that demonstrated susceptibility to the extracts may not fully capture the diversity of circulating *Salmonella* strains in the study setting. Future investigations incorporating molecular

identification, compound purification, toxicity profiling, and animal or clinical models are therefore warranted.

Suggestions

Further studies should incorporate molecular characterization to accurately characterize *Salmonella* isolates and minimize the risk of misclassification associated with phenotypic methods. Again, bioassay-guided fractionation is recommended to isolate and characterize the specific bioactive compounds, particularly tannins, alkaloids, and saponins, responsible for the observed antibacterial activity. Further research should also evaluate the cytotoxicity, pharmacokinetics, and in vivo efficacy of *Acacia nilotica* leaf extracts to determine their safety and therapeutic potential. Lastly, investigations into synergistic interactions between plant extracts and conventional antibiotics are encouraged, as such combinations may enhance antibacterial efficacy and contribute to strategies aimed at mitigating multi-drug resistance.

CONCLUSION

The present study provides novel clinical evidence of the antibacterial activity of *Acacia nilotica* leaf extracts against hospital-derived *Salmonella* isolates in northern Nigeria, demonstrating concentration-dependent effects within a real-world endemic setting. Although the activity was modest and consistently lower than that of ciprofloxacin, the findings confirm the intrinsic anti-*Salmonella* potential of the leaves and provide clinical context for their traditional use. The comparatively weaker activity relative to bark and root extracts reported in previous studies is likely attributable to lower concentrations of tannins and other phenolic bioactives in leaf tissues. Nevertheless, the use of leaves offers important advantages in terms of sustainability, accessibility, and ecological conservation. These results support further investigation of *A. nilotica* leaves through bioassay-guided fractionation, molecular characterization of target organisms, and evaluation of synergistic interactions with conventional antibiotics. Such efforts may contribute to the development of safe, locally sourced adjuncts for managing enteric infections in resource-limited settings.

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AUTHOR CONTRIBUTION STATEMENT

All authors contributed equally to the conception and design of the study, data collection, laboratory analysis, data interpretation, and manuscript preparation. All authors critically revised the manuscript for important intellectual content and approved the final version for publication.

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