

Antibacterial Activity of *Senna siamea* Leaf extracts against multidrug-resistant *Salmonella* spp. isolated from poultry sources

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Abstract

Antimicrobial resistance (AMR) in foodborne pathogens poses a critical global health challenge, particularly in low- and middle-income countries where poultry-associated zoonoses are prevalent. *Salmonella* spp., a leading cause of foodborne illness worldwide, increasingly exhibit multidrug resistance (MDR), undermining conventional therapeutic options and amplifying the risk of transmission across the poultry-human interface. Plant-derived antimicrobials represent a promising complementary strategy in addressing this crisis. This study evaluated the antimicrobial resistance profiles of poultry-derived MDR *Salmonella* isolates and investigated the antibacterial activity of solvent-fractionated leaf extracts of *Senna siamea*.

Ten confirmed *Salmonella* spp. isolates obtained from poultry sources were subjected to antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method in accordance with CLSI guidelines. Leaves of *S. siamea* were sequentially extracted using n-hexane, ethyl acetate, and ethanol. Antibacterial activity was assessed using agar well diffusion across graded concentrations (12.5–200 mg/mL). Qualitative phytochemical screening was performed using standard protocols.

All isolates exhibited multidrug resistance, with complete resistance (100%) to carbapenems and ceftazidime-clavulanic acid, while universal susceptibility was retained for amikacin, ampicillin-sulbactam, and piperacillin-tazobactam. Among the extracts, the ethanol fraction demonstrated the highest antibacterial activity, producing mean inhibition zones of 14.0 ± 1.2 mm at 200 mg/mL, followed by ethyl acetate (11.0 ± 1.2 mm) and n-hexane (9.0 ± 1.2 mm). Antibacterial efficacy correlated with solvent polarity and phytochemical richness, particularly flavonoids, alkaloids, tannins, and saponins.

Senna siamea leaf extracts, particularly polar fractions, exhibit significant in vitro activity against MDR *Salmonella* spp. These findings support the potential of phytochemical-rich plant extracts as adjunct or alternative antimicrobial agents and underscore the need for further mechanistic, toxicological, and in vivo investigations.

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1. Introduction

Antimicrobial resistance (AMR) represents one of the most pressing threats to global public health, compromising decades of progress in infectious disease control (Ekpunobi *et al.*, 2024; Chukwunwejim *et al.*, 2025). The World Health Organisation has identified AMR as a top ten global health challenge, with foodborne pathogens playing a pivotal role in the dissemination of resistant bacteria across human, animal, and environmental reservoirs (WHO, 2021; 2024). Among these pathogens, non-typhoidal *Salmonella* (NTS) remains a leading cause of bacterial gastroenteritis and invasive infections worldwide, accounting for hundreds of millions of illnesses annually (Lautan *et al.*, 2025).

Poultry farming is a critical component of food security, nutrition and economic livelihood in many developing countries, including Nigeria. However, the poultry industry is increasingly burdened by bacterial infections, especially those caused by *Salmonella* spp. Poultry production systems constitute a major reservoir for NTS, facilitating zoonotic transmission through contaminated meat, eggs, and environmental exposure (Lautan *et al.*, 2025). The intensive use of antimicrobials in poultry farming, often for growth promotion, prophylaxis, and metaphylaxis, has accelerated the selection and spread of multidrug-resistant *Salmonella* strains. These resistant strains are associated with increased morbidity, prolonged hospitalisation, treatment failure, and elevated mortality, particularly among children, the elderly, and immunocompromised individuals (Okoye *et al.*, 2025; Ekpunobi *et al.*, 2025a).

Of particular concern is the emergence of *Salmonella* strains resistant to critically important antimicrobials, including fluoroquinolones, extended-spectrum cephalosporins, and carbapenems. Resistance mechanisms in *Salmonella* are multifactorial, encompassing horizontal gene transfer mediated by mobile genetic elements, target site mutations, efflux pump overexpression, reduced outer membrane permeability, biofilm formation, and persistence phenotypes (Li *et al.*, 2015; Varela *et al.*, 2021). These mechanisms not only limit therapeutic options but also enhance bacterial fitness and transmissibility along the poultry–human continuum.

In response to the dwindling antibiotic pipeline and escalating resistance rates, there is renewed scientific interest in plant-derived antimicrobials (Ochieng *et al.*, 2025; Ekpunobi *et al.*, 2025b). Medicinal plants have historically served as reservoirs of bioactive compounds with diverse antimicrobial mechanisms, including membrane disruption, efflux pump inhibition, enzyme inactivation, and quorum-sensing interference. Phytochemicals may exert selective pressure distinct from conventional antibiotics, potentially reducing the likelihood of cross-resistance (Li *et al.*, 2015; Al-Sallami *et al.*, 2023).

Senna siamea (Fabaceae), a tropical medicinal plant widely distributed in Africa and Asia, has been traditionally employed in the treatment of gastrointestinal infections, fever, and inflammatory conditions. Phytochemical analyses have identified a broad spectrum of secondary metabolites in *S. siamea*, including flavonoids, alkaloids, tannins, saponins, and anthraquinones, many of which possess documented antimicrobial and resistance-modulating properties (Nas *et al.*, 2018; Sunmonu & Ekpunobi, 2023; Ogunniran *et al.*, 2024). However, systematic evaluation of solvent-partitioned *S. siamea* extracts against poultry-derived MDR *Salmonella* remains limited.

This study aims to evaluate the antibacterial activity of *Senna siamea* leaf extracts against poultry derived multi-drug resistance *Salmonella* isolates and to identify the phytochemical constituents responsible.

2. Materials and Methods

2.1. Study Design and Bacterial Isolates

A laboratory-based experimental study was conducted using ten confirmed *Salmonella* spp. isolates obtained from poultry sources. Isolates were authenticated by cultural characteristics on Salmonella–Shigella agar, Gram staining, and standard biochemical tests, including oxidase, catalase, indole, and citrate utilisation (Nas *et al.*, 2018).

2.2. Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, following Clinical and Laboratory Standards Institute (CLSI) guidelines. A panel of twelve antibiotics representing major therapeutic classes was used. Zone diameters were measured in millimetres and interpreted using CLSI breakpoints (CLSI, 2023).

2.3. Plant Material Collection and Authentication

Fresh leaves of *Senna siamea* were collected from Agulu, Nigeria, authenticated by a taxonomist, and assigned a voucher specimen. Leaves were washed, shade-dried, pulverised, and stored in airtight containers until extraction.

2.4. Extraction and Fractionation

Sequential Soxhlet extraction was performed using solvents of increasing polarity: n-hexane, ethyl acetate, and ethanol. Extracts were concentrated under reduced heat and stored at 4 °C until use.

2.5. Antibacterial Assay

Antibacterial activity was assessed using the agar well diffusion method. Extracts were tested at concentrations ranging from 12.5 to 200 mg/mL. Ciprofloxacin served as the positive control, while DMSO served as the negative control. All assays were performed in triplicate.

2.6. Phytochemical Screening

Qualitative phytochemical screening for alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and reducing sugars was conducted using standard chemical tests.

2.7. Data Analysis

Results were expressed as mean $\pm$ standard deviation. Descriptive analysis was used to compare antibacterial activity across extracts and concentrations.

3. Results

3.1. Antimicrobial Resistance Profile of *Salmonella* Isolates

All ten *Salmonella* isolates demonstrated multidrug resistance. Complete resistance was observed against meropenem, ertapenem, and ceftazidime–clavulanic acid. Intermediate resistance was variably observed for fluoroquinolones and cephalosporins, whereas universal susceptibility was retained for amikacin, ampicillin–sulbactam, and piperacillin–tazobactam (Table 1).

Table 1 Antibiotic susceptibility profiles of *Salmonella* isolates

Antibiotics	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8	Isolate 9	Isolate 10
CAZ	R	S	R	R	S	R	R	I	I	R
TZP	S	S	S	S	S	R	I	S	S	S
CXM	R	R	R	R	R	R	R	R	I	R
ETP	R	R	R	R	R	R	R	R	R	R
NOR	R	R	R	R	R	S	I	R	R	S
AMS	R	R	S	I	S	R	I	I	S	S
PGF	R	R	R	R	R	R	R	R	R	R
OFX	R	R	S	R	R	R	S	R	R	R
AK	S	S	S	S	S	I	S	S	S	S
CAL	R	R	R	R	R	R	R	R	R	R
ATM	I	R	R	R	R	R	R	R	R	R
MRP	R	R	R	R	R	R	R	R	R	R

CAZ: Ceftazidime; TZP: Piperacillin-tazobactam; CXM: Cefuroxime; ETP: Ertapenem; NOR: Norfloxacin; AMS: Ampicillin-sulbactam; PGF: Pefloxacin; OFX: Ofloxacin; AK: Amikacin; CAL: Ceftazidime- clavulanic acid, ATM: Aztreonam, MRP: Meropenem

3.2. Antibacterial Activity of *Senna siamea* Extracts

Antibacterial activity increased with extract concentration across all solvent fractions. The ethanol extract exhibited the strongest activity, achieving mean inhibition zones of 14.0 ± 1.2 mm at 200 mg/mL. Ethyl acetate and n-hexane extracts produced lower but measurable inhibition zones, indicating reduced efficacy with decreasing solvent polarity (Table 2 - 4).

Table 2 Inhibition zone diameter of N-hexane extract of *Senna siamea* against resistant *Salmonella* species

Conc.(mg/ml)	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8	Isolate 9	Isolate 10
200	0±0	0±0	9±0.7	0±0	0±0	0±0	0±0	8±1.2	0±0	0±0
100	0±0	0±0	8±0	0±0	0±0	0±0	0±0	6±0.7	0±0	0±0
50	0±0	0±0	8±0	0±0	0±0	0±0	0±0	4±1.2	0±0	0±0
25	0±0	0±0	7.5±0.7	0±0	0±0	0±0	0±0	0±0	0±0	0±0
12.5	0±0	0±0	7±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
Positive Control	0±0	0±0	23±0.7	7±0.7	9±0.7	13±0	20±0.7	0±0	9±0.7	12±0.7

Table 3 Inhibition zone diameter of Ethyl acetate extract of *Senna siamea* against resistant *Salmonella* species

Conc.(mg/ml)	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8	Isolate 9	Isolate 10
200	0±0	7±0.7	8±1.2	5±0	0±0	0±0	11±1.2	0±0	9±0.7	6±0.7
100	0±0	6±1.2	7.8±1.2	0±0	0±0	0±0	9±0.7	0±0	8±0	5±0.7
50	0±0	0±0	7±0.7	0±0	0±0	0±0	8.5±0.7	0±0	6±0.7	0±0
25	0±0	0±0	5±0.7	0±0	0±0	0±0	8±0.7	0±0	6±0.7	0±0
12.5	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	4±0	0±0
Positive Control	0±0	0±0	23±0.7	7±0.7	9±0.7	13±0	20±0.7	0±0	9±0.7	12±0.7

Table 4 Inhibition zone diameter of the ethanol extract of *Senna siamea* against resistant *Salmonella* species

Conc.(mg/ml)	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7	Isolate 8	Isolate 9	Isolate 10
200	0±0	0±0	7±1.2	11±0.7	0±0	14±1.2	9±0.7	7±0.7	7±1.2	0±0
100	0±0	0±0	5±0.7	9±0.7	0±0	10±1.2	7±0.7	5±0.7	2±0.7	0±0
50	0±0	0±0	4.5±0.7	8.5±0.7	0±0	0±0	6±1.2	0±0	5±0.7	0±0
25	0±0	0±0	4±0	8±0.7	0±0	0±0	5±1.2	0±0	0±0	0±0
12.5	0±0	0±0	3±0	6±0	0±0	0±0	0±0	0±0	0±0	0±0
Positive Control	0±0	0±0	23±0.7	7±0.7	9±0.7	13±0	20±0.7	0±0	9±0.7	12±0.7

3.3. Phytochemical Composition

Phytochemical screening revealed a higher abundance of flavonoids, tannins, saponins, and alkaloids in the ethanol extract compared with less polar fractions, aligning with observed antibacterial potency.

Table 5 Phytochemical constituents of *Senna siamea* leaves

Phytochemicals	Test	Ethanol extract	Ethyl acetate extract	N-hexane extract
Alkaloids	Mayer's	-	+	-
	Hager's	-	+	-
Flavonoids	Lead acetate test	+	+	-
	Alkaline reagent test	+	+	-
Reducing sugars	Benedict's test	-	-	-
	Fehling's test	-	-	-
Saponins	Frothing test	+	+	-
Tannins	Ferric Chloride test	+	+	-
Steroids	Salkowski test	+	+	+
Triterpenoids	Salkowski test	+	+	+
Glycosides	General test	-	-	-

4. Discussion

The present study provides compelling evidence that poultry-derived *Salmonella* spp. circulating within local production systems exhibit extensive multidrug resistance (MDR), while simultaneously demonstrating susceptibility to bioactive phytochemicals extracted from *Senna siamea*. These findings are highly relevant within the context of the global antimicrobial resistance (AMR) crisis and align closely with the One Health framework, which recognises the interconnectedness of human, animal, and environmental health (WHO, FAO, & WOA, 2017; Robinson *et al.*, 2016).

The universal multidrug resistance observed among the *Salmonella* isolates in this study underscores the escalating public health risk posed by poultry-associated zoonotic pathogens. Poultry production systems are well-established reservoirs for non-typhoidal *Salmonella* (NTS), facilitating silent colonisation of birds and subsequent transmission to humans through contaminated meat, eggs, and environmental exposure (EFSA & ECDC, 2023; Foley *et al.*, 2011). The detection of resistance across multiple antimicrobial classes, including cephalosporins, fluoroquinolones, monobactams, and carbapenems, reflects sustained selective pressure likely driven by extensive antimicrobial use in poultry husbandry (Van Boeckel *et al.*, 2015).

Particularly alarming is the complete resistance observed against carbapenems and β -lactam/ β -lactamase inhibitor combinations such as ceftazidime–clavulanic acid. Carbapenems are classified as critically important antimicrobials for human medicine, and resistance in zoonotic *Salmonella* suggests the possible circulation of high-risk clones or plasmid-mediated carbapenemase determinants within the food chain (WHO, 2019; Oghonyon *et al.*, 2025). Even in the absence of molecular characterisation, the phenotypic resistance patterns observed are consistent with global reports describing the spread of extended-spectrum β -lactamases (ESBLs), AmpC enzymes, and carbapenem resistance genes via mobile genetic elements (MGEs) in poultry-associated *Salmonella* (Carattoli, 2013; Partridge *et al.*, 2018).

The antibacterial activity exhibited by *Senna siamea* leaf extracts against MDR *Salmonella* spp. supports the growing body of evidence that medicinal plants represent valuable reservoirs of antimicrobial and resistance-modulating compounds (Cowan, 1999; Gibbons, 2008). Unlike conventional antibiotics, which often target single bacterial pathways, phytochemicals exert pleiotropic effects that can simultaneously disrupt multiple cellular processes, thereby reducing the likelihood of rapid resistance emergence (Hemaiswarya *et al.*, 2008).

The concentration-dependent inhibition observed across all extracts confirms true antibacterial activity rather than incidental growth suppression. Importantly, the differential efficacy among solvent fractions highlights the critical role

of extraction polarity in isolating bioactive compounds. The ethanol extract consistently demonstrated superior antibacterial activity, followed by ethyl acetate and n-hexane fractions, establishing a clear polarity–activity relationship.

The enhanced activity of the ethanol extract can be attributed to its ability to solubilise a broad spectrum of polar and semi-polar phytochemicals, including flavonoids, tannins, saponins, and alkaloids (Eloff, 1998; Harborne, 1998). These compounds were largely absent or minimally represented in the non-polar n-hexane fraction, which exhibited the weakest antibacterial effects. This observation aligns with the structural characteristics of Gram-negative bacteria such as *Salmonella*, whose outer membrane, rich in lipopolysaccharides (LPS), acts as a permeability barrier to hydrophobic compounds.

The observed inhibitory effects of *S. siamea* extracts against MDR *Salmonella* may be mechanistically linked to interference with established resistance pathways. Flavonoids and tannins have been shown to disrupt bacterial membrane integrity, increasing permeability and facilitating intracellular accumulation of antimicrobial agents (Cushnie & Lamb, 2011; Kumar & Pandey, 2013).

Additionally, several plant-derived polyphenols have been reported to inhibit efflux pump systems such as AcrAB–TolC, which play a central role in multidrug resistance in *Salmonella* (Varela *et al.*, 2023; Al-Sallami *et al.*, 2023). Overexpression of these efflux pumps reduces intracellular antibiotic concentrations and contributes to fluoroquinolone and β -lactam resistance (Li *et al.*, 2015). By attenuating efflux activity, phytochemicals may restore bacterial susceptibility or enhance the efficacy of existing antibiotics.

Tannins and alkaloids may also interact with resistance determinants encoded on mobile genetic elements. Through protein precipitation, metal chelation, or interference with plasmid replication and conjugation, these compounds could indirectly limit horizontal gene transfer (HGT), a key driver of resistance dissemination in poultry-associated *Salmonella* populations (Smillie *et al.*, 2010; Varela *et al.*, 2021).

From a One Health perspective, the findings of this study have significant implications for antimicrobial stewardship in poultry production systems. The integration of phytochemical-based interventions, either as feed additives, prophylactic agents, or therapeutic adjuncts, could reduce dependence on conventional antibiotics and mitigate selection pressure for resistance (FAO, 2016). Such strategies may be particularly valuable in low- and middle-income countries, where regulatory oversight of veterinary antimicrobial use is often limited (Grace, 2015).

Moreover, the use of locally available medicinal plants such as *Senna siamea* aligns with sustainable and culturally relevant approaches to disease management. By targeting MDR *Salmonella* at the animal production level, phytochemical interventions could indirectly reduce the burden of resistant infections in humans and limit environmental dissemination of resistance genes (Robinson *et al.*, 2016).

The heterogeneity observed in isolate-level susceptibility to *S. siamea* extracts reflects underlying genetic and phenotypic diversity among the *Salmonella* strains. Differences in outer membrane composition, efflux pump expression, stress response pathways, and plasmid content may influence individual isolate responses to phytochemicals (Bossé *et al.*, 2017). This variability also highlights the need for future studies to integrate genomic and transcriptomic analyses, which would allow direct correlation of resistance determinants with phytochemical susceptibility profiles (Didelot *et al.*, 2012).

5. Conclusion

This study demonstrated that ethanol, ethyl acetate, and n-hexane extracts of *Senna siamea* possess inhibitory activity against multidrug-resistant *Salmonella* species, with the ethanol extract exhibiting the most pronounced antibacterial effect. This finding suggests that a substantial proportion of the plant's bioactive phytochemical constituents are preferentially soluble in ethanol relative to the other solvents. The appreciable zones of inhibition observed indicate that *S. siamea* contains potent antimicrobial compounds with promising therapeutic potential against antibiotic-resistant *Salmonella*, which continue to pose significant challenges in clinical management. Moreover, the variation in antibacterial activity across solvent extracts highlights the pivotal influence of solvent polarity on phytochemical extraction efficiency and antimicrobial efficacy. Collectively, these results provide compelling support for the exploration of medicinal plants as alternative or complementary approaches to combating antimicrobial resistance and underscore the need for further isolation, characterisation, and clinical evaluation of the active constituents of *Senna siamea*.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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