

**ANTIBACTERIAL ACTIVITY OF *Cassia occidentalis* WHOLE PLANT EXTRACT AGAINST MDR-*Salmonella* SPECIES**

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**ABSTRACT**

This study investigated the antibacterial activity of *Cassia occidentalis* (Coffee Senna) whole plant extracts against multidrug-resistant (MDR) *Salmonella* species isolates obtained from Ahmadu Bello University Teaching Hospital (ABUTH), Zaria. The isolates were subjected to gram staining and biochemical screening, antibiotic susceptibility testing was conducted on Mueller Hinton agar and the AMR status of the isolates was established. Phytoconstituents screening of the ethanolic and aqueous extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds, with steroids detected only in the ethanolic extract. Antibacterial assays conducted using the agar well diffusion method showed concentration-dependent inhibition, with the ethanolic extract exhibiting stronger activity than the aqueous extract. The ethanolic extract produced zones of inhibition up to 40 mm at 300 mg/mL, while the aqueous extract reached 34 mm at the same concentration. Minimum inhibitory concentration (MIC) values ranged from 9.38–18.75 mg/mL for the ethanolic extract and 18.75–37.5 mg/mL for the aqueous extract. Minimum bactericidal concentration (MBC) values ranged from 37.5–75 mg/mL. These results confirm the bacteriostatic and bactericidal effects of *C. occidentalis*, highlighting its potential as a source of novel antimicrobial agents. The findings support its ethnomedicinal use and suggest its possible application in developing alternative therapies for managing drug-resistant *Salmonella* infections.

**Keyword:** *Cassia occidentalis*, MDR-*Salmonella* species, Phytoconstituents, Whole plant

**INTRODUCTION**

*Salmonella* species are Gram-negative, rod shaped bacteria belonging to the family Enterobacteriaceae. This bacteria causes common infections such as salmonellosis and typhoid fever, especially in developing countries where sanitation is inadequate and access to clean water is limited (Ibikunle *et al.*, 2020).

The World Health Organization (WHO) estimates that there are between 11 and 20 million cases of typhoid fever each year, leading to approximately 128,000 to 161,000 fatalities globally (WHO, 2024). Typhoid fever is an infectious disease that constitutes a considerable risk to the health and well-being of people in many African countries. Though the disease menace has been eradicated in many developed nations, the poor health situations mediated by religious crises, natural disasters, terrorism, and corruption in African countries such as Nigeria have led to it causing high mortality among the human population in this region (Oyededeji *et al.*, 2024). In Nigeria, typhoid fever remains a public health concern with incidence posing substantial challenges to health care system (Abduljalil *et al.*, 2024).

The emergence of multi-drug-resistant (MDR) *Salmonella* species resistant to conventional antibiotics has further complicated treatment options, necessitating the exploration of alternative therapeutic strategies, including the use of natural antimicrobial agents (Aisheikh *et al.*, 2020). Multidrug-resistant strains of

*Salmonella* species create significant obstacles in effectively treating salmonellosis, leading to higher rates of illness and related complications (Stanislaw *et al.*, 2022).

Historically, plants have been employed in traditional medicine to combat infections. The diverse flora of Africa and Nigeria in particular, offers a substantial variety of aromatic, food, and medicinal plants (Lawal *et al.*, 2022). Several published studies have suggested the medicinal effectiveness of plants collected from different Nigerian states in typhoid fever treatment (Chukwodozie & Ezeonu, 2022). Studies indicated that these medicinal plants are vital to the African pharmacopeia. The World Health Organization estimates that nearly 80 % of the population, especially those in the rural areas of Africa, Asia, and Latin America, rely on traditional medicine for their primary healthcare needs which frequently utilizes active compounds derived from plants to treat numerous health conditions (Li *et al.*, 2020).

*Cassia occidentalis* is a slender, unarmed biennial plant that typically grows to a height of 5 to 8 meters having a distinct fetid odor and it belongs to the *Caesalpiniaceae* family (Arvind *et al.*, 2024). This erect biennial herb is frequently found along roadsides, ditches, and dumpsites in northern Nigeria. It is known locally as Akidi agbadain Igbo, Abo rere in Yoruba,

and rai dore in Hausa, while in English, it is referred to as Coffee Senna (Bagega *et al.*, 2018).

Studies show that extracts from various parts of *Cassia occidentalis* possess different levels of antibacterial activity, especially against MDR strains of *Salmonella* species. Different parts of this plant have demonstrated promising antibacterial activity at various concentrations. The leaf extracts of *Cassia occidentalis* demonstrated antimicrobial effects against *Salmonella* species (Otorokpa *et al.*, 2018). This study assessed the antibacterial activity of *C. occidentalis* whole plant extract (at varying concentrations) against MDR-*Salmonella* species.

## MATERIALS AND METHODS

### Plant Collection and Identification

The whole plant of *Cassia occidentalis* (leaves, stem bark, root, flowers, and seeds) were collected from a road side area behind Dan Fodio Hostel in Ahmadu Bello University, Zaria. The plants were submitted to the Herbarium at the Department of Botany, Ahmadu Bello University, Kaduna State, for identification and authentication. A voucher number (ABUH01681) was assigned to the plant.

### Plant Processing

The plant sample was transported to the Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, for processing and extraction. The sample was thoroughly washed with distilled water to remove debris. All plant parts were dried at room temperature for 20 days. The dried material was ground using a mortar and pestle and sieved through a 0.5 mm mesh. The resulting powder was stored in aluminum foil at room temperature.

### Ethanolic Extraction

Extraction procedure and processing were done following the method of Abduljalil *et al.* (2025). 25 g of the powder of the whole plant of *C. occidentalis* weighed and mixed with 250 mL of ethanol. The solution was filtered using Whatman (No 1) filter paper. The ethanol was then allowed to evaporate using a water bath and the resulting extract obtained was stored in a glass container until further processing.

### Aqueous Extraction

Extraction procedure and processing were done following the method of Abduljalil *et al.* (2025) Twenty-five grams of the powder of the whole plant of *C. occidentalis* weighed and mixed with 250 mL of ethanol. The solution was filtered using Whatman (No 1) filter paper. The water was then allowed to allowed to evaporate by heating it from on water bath and the resulting extract obtained was stored in a glass container until further processing

### Plant Screening of Extract (Qualitative Test)

The qualitative Phytoconstituents screening of both the ethanol and the aqueous extracts was carried out at the Department of Pharmacognosy, at the Faculty of

Pharmaceutical Sciences, Ahmadu Bello University, Zaria, using standard methods as described by Bagega *et al.* (2018).

Testing for glycosides conducted using Keller-Kiliani test; frothing test was adopted to test for saponins; alkaloids were tested using Dragendoff's test; testing for tannins was done using Ferric chloride test; flavanoids were tested using Shinoda test; Borntrager's test was adopted to test for anthraquinones. Also, the presence of Steroids was tested using Salkowski's test, and presence of phenols was detected by dropping a few drops of lead acetate solution on the extract for the presence or absence of yellow-colored precipitate.

### Culture Media Preparation

The media were prepared according to the manufacturer's (AVONCHEM limited, Wellington House waterloo, west Macclesfield Cheshire, England) instruction.

### Collection of Isolates and Identification

A total of four presumed MDR clinical isolates of *Salmonella* species were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH), Shika, Zaria, and were transported to the department of Microbiology, Ahmadu Bello University where they were sub cultured onto *Salmonella-Shigella* agar (SSA).

### Gram Staining

Gram staining was carried by picking a colony and making a smear of the overnight bacterial culture on a clean glass slide, heat-fixed, and stained with crystal violet for one minute. Lugol's iodine was then applied as a mordant, followed by decolorization with 95 % ethanol for 30 seconds. Finally, neutral red was used as the counter-stain for 60 seconds. The slides were examined under the microscope using oil immersion ( $\times 100$  objective lens) to observe the Gram reaction and cell morphology.

### Biochemical Identification of Isolates

The bacterial isolates were subjected to a series of biochemical tests including Citrate Utilization Test Triple Sugar Iron Test, Indole, Catalase, Motility, Methyl Red and Voges Proskauer. Each isolate was inoculated into the respective media and incubated under standard conditions as describe by Ibikunle *et al.* (2020).

### Standardization of the Inoculum

Colonies of the four clinical isolates were picked and suspended in 5 mL of sterile normal saline, and the turbidity of the suspension was compared to that of a 0.5 McFarland standard to give an equivalent of  $1.5 \times 10^8$  CFU/mL.

### Antibiotic Susceptibility Testing (Kirby-Bauer Method)

A panel of Gram-negative antibiotic discs was used to determine the antibiotic susceptibility pattern and to

establish the multi-drug resistances status of the isolates using the disc diffusion method.

The standardized isolates were picked using a sterile swab stick and were lawn onto the surface of a sterile plate of freshly prepared Muller-Hinton agar, there after the antibiotic discs were picked and placed on the surface using sterile forceps. The plates were allowed to stay for 1 h before incubating at 37 °C for 24 h. After the incubation period, the clear zones of inhibition were measured using a millimeter ruler, and the results were compared to those of Clinical and Laboratory Standards Institute (CLSI) version 2024.

#### Preparation of Concentrations of Plant Extract

Three grams (3 g) were weighed and poured into separate conical flasks, both ethanol and aqueous extracts were added to 10 ml of dimethyl sulfoxide (DMSO) to give a concentration of 300 mg/ml. Other concentrations, 150, 75, and 37.5 mg/ml, were prepared by the double dilution method. In this procedure, 2 mL of content in the stock concentration (containing 300 mg/ml) were added to 2 ml of sterile distilled water in sterile test tubes to give a 150 mg/ml concentration. Two ml of this 150 mg/ml were added to another 2 ml distilled water to give 75 mg/ml until 37.5 mg/ml concentration were achieved respectively (Jacinta *et al.*, 2018).

#### Determination of the Antibacterial Activity of the Plant Extract

The antibacterial activity of the various concentrations of both ethanol and aqueous extracts was determined by the agar well diffusion technique. Plates of Mueller-Hinton agar were prepared according to the manufacturer's instructions. Sterile swab sticks were used to lawn the standardized inoculum on the surface of the Muller-Hinton agar. Thereafter, a sterile Cork borer (of 6 mm in diameter) was used to dig wells in the agar, in which 0.1 ml of the different concentrations of the extract was placed, respectively. The plates were incubated at 37 °C for 24 h (Adenuga, *et al.*, 2024).

#### Determination of the Minimum Inhibitory Concentration (MIC)

Broth tube dilution method was used to find the MIC. Two-fold serial dilution of the extract in the broth was made from the stock concentration of (75 mg/ml) of the extract to obtain 37.5, 18, 75, and 9.38 mg/ml, respectively. Thereafter, 0.1 ml each of the standardized inocula of the *Salmonella* species was then inoculated

into the different concentrations of the extracts in the broth, respectively. The tubes were then incubated at 37 °C for 24 h and observed for turbidity. The lowest concentration which showed no turbidity in the test tube was recorded as the MIC as described in the study conducted by Bello *et al.* (2023).

#### Determination of the Minimum Bactericidal Concentration (MBC)

Sterile Muller-Hinton agar plates were inoculated with samples from each test tube showing visible growth from the MIC plates. The plates were incubated at 37 °C for 24 h. The minimum bactericidal concentrations were determined from the MIC tubes that showed no growth. A sterile wire loop was used to pick a loopful from the broth tube that showed no growth and subculture on a fresh plate of Nutrient agar. The plates were incubated at 37°C for 24 h. The lowest concentrations that show no visible growth were recorded as the MBC as described by (Bello *et al.*, 2023).

### RESULTS AND DISCUSSION

The Phytoconstituents screening of the aqueous and ethanolic extracts of *Cassia occidentalis* whole plant revealed the presence of various bioactive compounds as summarized in Table 1. All the four clinical isolates were confirmed as *Salmonella* species based on the macroscopic observation, cell morphology, gram reaction and biochemical tests conducted (Table 2). The antibiotic susceptibility testing revealed that all four *Salmonella* species exhibited multi-drug resistance (resistance to  $\geq 3$  classes of antibiotics) as detailed in Table 3.

**Table 1: Phytoconstituents constituents of ethanolic and aqueous extracts of *C. occidentalis* whole plant**

Phytoconstituents	Extracts	
	Ethanol	Aqueous
Flavonoids	+	+
Glycosides	+	+
Saponins	+	+
Alkaloids	+	+
Phenols	+	+
Tannins	+	+
Steroids	+	-
Anthraquinones	-	-

+ = Present; - = Absent

**Table 2: Gram's reaction and biochemical characteristics of clinical isolates of *Salmonella* species**

I/C	Gram's reaction	Biochemical test							Inferences	
		TSI	Catalase	Motility	Citrate	Indole	Urease	MR VP		
I	Gram-rod	K/A g +H <sub>2</sub> S	+	+	+	+	+	+	-	<i>Salmonella</i> spp
II	Gram-rod	K/A g +H <sub>2</sub> S	+	+	+	-	+	+	-	<i>Salmonella</i> spp
III	Gram-rod	K/A g +H <sub>2</sub> S	-	+	+	-	+	+	-	<i>Salmonella</i> spp
IV	Gram-rod	K/A g +H <sub>2</sub> S	+	+	+	-	+	+	-	<i>Salmonella</i> spp

A=Acid, K= Alkaline, +=Positive, - = Negative, MR= Methyl reds, TSI=Triple sugar iron test, I/C=Isolate code

**Table 3: Antibiotic susceptibility profile of clinical isolates *Salmonella* species collected from ABUTH, Zaria**

Antibiotics	Antibiotic susceptibility of the clinical isolates (zones of growth inhibition (mm))			
	Isolate I	Isolate II	Isolate III	Isolate IV
Ciprofloxacin	27 (I)	29 (I)	26 (I)	30 (S)
Azithromycin	22 (S)	23 (S)	21 (S)	11 (R)
Gentamicin	18 (S)	18 (S)	12 (R)	20 (S)
Augmentin	22 (S)	15 (R)	16 (R)	18 (I)
Pefloxacin	21 (R)	23(R)	20 (R)	21 (R)
Levofloxacin	29 (I)	28(I)	29 (I)	22 (I)
Ofloxacin	26 (I)	25(I)	26 (I)	26 (I)
Amoxicillin	15 (R)	10(R)	16 (R)	6 (R)
Sulfamethoxazole	28 (S)	21(R)	20 (R)	23 (R)
Cephalosporine	17 (R)	16 (R)	14 (R)	16 (R)

I = Intermediate, S= Susceptible, R = Resistance

The antibacterial activity of both ethanolic and aqueous extracts of *C. occidentalis* against the MDR *Salmonella* isolates was determined by the agar well diffusion method. The results are presented in Table 4. The minimum inhibitory concentrations and minimum bactericidal concentrations values for the plant extracts against the MDR *Salmonella* isolates are presented in Table 5.

**Table 4: Antibacterial activity of ethanolic extract of *C. occidentalis* whole plant against clinical isolates of *Salmonella* species from ABUTH, Zaria**

Concentrations (mg/ml)	Diameter zone of inhibition (mm)			
	Isolate I	Isolate II	Isolate III	Isolate IV
300	29	37	38	40
150	25	36	30	35
75	22	30	30	33
37.5	21	28	28	31

**Table 5: Antibacterial activity of aqueous extract of *C. occidentalis* whole plant against clinical isolates of *Salmonella* species from ABUTH, Zaria**

Concentrations (mg/ml)	Diameter zone of inhibition (mm)			
	Isolate I	Isolate II	Isolate III	Isolate IV
300	25	34	31	26
150	23	30	30	31
75	21	23	27	30
37.5	18	21	23	25

**Table 6: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanolic and aqueous extracts of *C. occidentalis* against MDR-Salmonella species from ABUTH, Zaria**

Isolates	Ethanolic extraction		Aqueous extract	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Isolate I	18.75	75	18.75	75
Isolate II	9.38	75	37.5	75
Isolate III	9.38	37.5	18.75	75
Isolate IV	18.75	75	18.75	75

The Phytoconstituents analysis of both the ethanolic and aqueous extracts of the whole plant of *Cassia occidentalis* revealed a variety of antibacterial compounds including saponins, flavonoids, alkaloid, phenols, tannins and glycosides. Steroids were detected only in the aqueous extract only while anthraquinones were not detected in both extracts. This finding agrees with the work of Imon *et al.* (2023) where the presence of various phytoconstituents including flavonoids, tannins, terpenoids, and saponins were detected, this agreement between the previous findings and the current one suggests a relatively stable phytoconstituents in *C. occidentalis* which further validates its potential use as a source of bioactive compounds. The ethanolic extract revealed more bioactive constituents, including steroids due to non-polarity which enabled it to extract more of the compounds than the aqueous. A study conducted by Adamu *et al.* (2018); Kumar and Na'ala (2022) expressed that ethanol exhibits greater efficiency in extracting a wider array of Phytoconstituents compared to water.

The antibiotic susceptibility pattern observed in this study confirmed the multidrug-resistant (MDR) characteristics of the clinical isolates, with marked resistance detected against frequently prescribed antibiotics, including Amoxicillin, Augmentin, and Pefloxacin. This resistance trend underscores the growing global burden of MDR *Salmonella* species, which has been widely linked to the indiscriminate and excessive use of antimicrobial agents in clinical practice, livestock production, and agricultural settings. Recent studies have highlighted that the persistent misuse and overreliance on antibiotics significantly contribute to the emergence, dissemination, and persistence of resistant bacterial strains across both human and environmental reservoirs (Lee *et al.*, 2021; Gharbi *et al.*, 2023). All four *Salmonella* isolates investigated in the present study were confirmed to be multidrug resistant (MDR). Although the number of isolates examined was limited, the finding is consistent with previous reports from Nigeria that documented the emergence and persistence of MDR *Salmonella* strains. Oyedum *et al.* (2016), in a retrospective study of *Salmonella typhi* isolates from different regions of Nigeria, reported widespread resistance to several first-line antimicrobial agents and highlighted the increasing burden of multidrug resistance among *Salmonella* isolates. Similarly, Musa *et al.* (2025) reported that 75.9 % of *Salmonella typhi* isolates recovered in Niger State exhibited multidrug resistance, indicating that resistant strains remain widely distributed within the country. The agreement between the present findings and these previous studies suggests that multidrug resistance among *Salmonella* species continues to be a significant public health concern in Nigeria (Alvarez *et al.*, 2024). The significant antibacterial activity exhibited by both extracts, particularly the ethanolic extract, against multidrug-resistant (MDR) isolates underscores the

therapeutic potential of *C. occidentalis* whole plant as a promising source of novel antibacterial agents. The concentration-dependent increase in antibacterial activity observed in this study suggests the presence of potent bioactive phytoconstituents responsible for the inhibitory effects. The superior efficacy demonstrated by the ethanolic extract may be attributed to its higher extraction yield and broader spectrum of phytochemical constituent. The antibacterial activity observed may be associated with the synergistic actions of these phytoconstituents. Flavonoids and phenolic compounds have been reported to exert antimicrobial effects through disruption of bacterial cell membranes, inhibition of nucleic acid synthesis, and interference with essential metabolic processes. Similarly, tannins are known to inhibit microbial growth through protein precipitation and enzyme inactivation, while alkaloids possess the ability to interfere with cellular metabolism and DNA replication. Saponins and terpenoids have also been reported to enhance membrane permeability, resulting in leakage of intracellular constituents and subsequent bacterial cell death. The presence of these bioactive compounds in the extracts may therefore account for the pronounced antibacterial activity observed against the MDR isolates. The findings of the present study are consistent with those of Shina *et al.* (2012), who reported that ethanolic extracts of *C. occidentalis* containing tannins, saponins, terpenoids, and anthraquinones exhibited significant antibacterial activity against several pathogenic bacteria, including *Salmonella typhi*. Likewise, Chukwujekwu *et al.* (2006) isolated emodin, an anthraquinone compound from *C. occidentalis*, and demonstrated notable antibacterial activity, thereby confirming that the plant contains potent antimicrobial principles. The greater activity of the ethanolic extract observed in the present study further supports previous reports that organic solvents, particularly ethanol, are more effective in extracting a wider range of bioactive compounds than aqueous solvents. Collectively, these findings suggest that the antibacterial activity of *C. occidentalis* is attributable to its rich phytochemical composition and support its potential application as a source of alternative therapeutic agents against multidrug-resistant bacteria pathogens.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays further substantiated the antibacterial potential of *Cassia occidentalis* extracts against the multidrug-resistant (MDR) *Salmonella* isolates. The MIC values ranged from 9.38 to 75 mg/mL, while the MBC values ranged from 37.5 to 75 mg/mL, indicating that the extracts possessed both growth-inhibitory and bactericidal properties. These findings demonstrate the effectiveness of the plant extracts in suppressing and eliminating MDR bacterial pathogens at varying concentrations.

The results obtained in the present study are consistent with those reported by Bagega *et al.* (2018), who demonstrated significant antibacterial activity of *C. occidentalis* leaf extract against *Salmonella*

*typhimurium*, with MIC and MBC values of 30 and 60 mg/mL, respectively. Similarly, Adamu *et al.* (2018) reported appreciable antibacterial activity of ethanolic, aqueous, and methanolic extracts of *C. occidentalis* against clinical isolates of *Salmonella typhi*, *Salmonella paratyphi* A, and *Salmonella paratyphi* B, with MIC values ranging from 62.5 to 125 µg/mL and MBC values ranging from 125 to 500 µg/mL. Furthermore, antibacterial activity of *C. occidentalis* against *Salmonella* species has been documented in earlier studies, which attributed the inhibitory effects to the presence of bioactive secondary metabolites.

The bactericidal activity observed in this study may be attributed to the synergistic effects of the phytochemical constituents detected in the extracts. These compounds have been reported to exert antimicrobial effects through membrane disruption, inhibition of essential bacterial enzymes, interference with nucleic acid synthesis, and alteration of cellular metabolic pathways. Consequently, the favourable MIC and MBC values obtained in this study support the potential application of *C. occidentalis* as a source of bioactive compounds for the development of alternative therapeutic agents against multidrug-resistant *Salmonella* infections (Arvind *et al.*, 2024).

## CONCLUSION

This study demonstrates that *Cassia occidentalis* whole plant possesses notable antibacterial activity against multidrug-resistant (MDR) *Salmonella* isolates. The confirmed presence of key phytoconstituents, including flavonoids, tannins, saponins, alkaloids, phenols, and glycosides, provides a strong phytochemical basis for the observed antimicrobial effects. The absence of anthraquinones in both extracts and the selective presence of steroids in the aqueous extract further highlight variations in solvent extraction efficiency, with ethanol exhibiting greater capacity to extract a broader spectrum of bioactive compounds.

The MDR profile of all clinical isolates underscores the persistent global challenge of antimicrobial resistance, particularly among *Salmonella* species, and reflects patterns reported in previous studies. The antibacterial activity demonstrated by the extracts, supported by concentration-dependent inhibition and favourable MIC and MBC values, confirms the bacteriostatic and bactericidal potential of the plant. The consistency of these findings with earlier reports further validates the antimicrobial relevance of *C. occidentalis*.

Overall, the results support the ethnomedicinal use of *C. occidentalis* and highlight its potential as a promising source of natural antimicrobial agents. However, further studies involving purification, characterization of active compounds, and in vivo evaluation are recommended to fully elucidate its therapeutic potential and safety profile.

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