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Antibacterial Activity of the Ethyl Acetate Fraction of *Cassia occidentalis* Linn. Aqueous Seed Extract: Implications for Environmental Health and AMR Mitigation

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Abstract: The awareness and general acceptability of herbal drugs in modern medical practice are increasing globally, offering critical solutions to escalating public and environmental health challenges. Antimicrobial resistance (AMR) is a rapidly growing environmental threat, particularly in semi-arid and developing regions where water sanitation is vulnerable and pathogenic contamination is high. Finding accessible, biodegradable, and effective plant-based remedies is vital for community health resilience. This study evaluated the antibacterial efficacy of the ethyl acetate fraction of *Cassia occidentalis* Linn. aqueous seed extract against selected clinical pathogens (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*). The fraction exhibited significant, concentration-dependent antibacterial activity. At the highest concentration of 100 mg/ml, the extract was most potent against *E. coli* (28.00 mm), followed by *S. aureus* (22.00 mm) and *P. aeruginosa* (21.00 mm), showing statistically significant differences compared to the standard antibiotic, Amoxicillin. The results highlight the potential of *C. occidentalis* as a viable, eco-friendly source for novel antibacterial agents, particularly against resilient Gram-negative bacteria that typically pollute environmental reservoirs and exhibit low susceptibility due to outer membrane lipopolysaccharides and multi-drug efflux pumps.

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I. INTRODUCTION

Awareness and general acceptability of the use of herbal drugs in today's medical practice are rapidly increasing. According to the World Health Organization (WHO), a vast majority of the world's population utilizes plant-based remedies as their primary form of healthcare. Current research on natural molecules and therapeutic products primarily focuses on botanical sources because they can be sourced relatively easily, are environmentally sustainable, and can be selected based on their documented ethno-medicinal uses.

A key specimen of interest is *Cassia occidentalis* Linn. (syn. *Senna occidentalis*), a pantropical shrub belonging to the Fabaceae family. Globally recognized by its English name, Coffee Senna, the plant is deeply embedded in regional

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traditional medicine. In Northern Nigeria, it is widely known by its Hausa names, *Rai dore* or *Sanga-sanga*. Various parts of the plant, especially the seeds, are heavily utilized by indigenous populations to treat fever, gastrointestinal distress, and topical infections.

Beyond clinical pharmacology, investigating indigenous plants like *C. occidentalis* holds significant public environmental relevance. Antimicrobial resistance (AMR) is no longer solely a clinical issue; it is a profound environmental health crisis. Pathogens such as *E. coli* and *P. aeruginosa* frequently contaminate community water sources, soil, and agricultural runoff, creating environmental reservoirs of multidrug-resistant bacteria. Developing plant-based antibacterial agents offers a biodegradable, accessible mitigation strategy to combat these resilient pathogens in communities where standard pharmaceutical antibiotics are either inaccessible or increasingly ineffective. This study investigates the antibacterial properties of the ethyl acetate fraction of *C. occidentalis* aqueous seed extracts against these critical environmental and clinical pathogens.

Fig.1: *Cassia occidentalis* Linn. Leave, Flower, and Seed.

II. MATERIALS AND METHODS

➢ *Collection and Authentication of Plant Material*

The seeds of *Cassia occidentalis* Linn. (Coffee Senna; Hausa: *Rai dore*) were collected from the road side along Maiduguri road, Damaturu, the Yobe state capital. The botanical specimen was identified and authenticated at the Herbarium of the Department of Biological Sciences, where a voucher specimen was deposited for future reference.

➢ *Preparation of the Extract and Fractionation*

The seeds were air-dried at room temperature (25°C) to a constant weight and subsequently pulverized into a coarse powder using an electric hammer mill. A measured quantity of the powder was subjected to cold maceration in distilled water for 48 hours with periodic agitation on an orbital shaker. The mixture was filtered through Whatman No. 1 filter paper using a vacuum pressure pump.

The resulting crude aqueous extract was subjected to liquid-liquid partitioning in a separatory funnel (Pyrex, Germany) using analytical-grade ethyl acetate. The ethyl acetate layer was collected and the process was repeated thrice to ensure exhaustive extraction of semi-polar bioactive metabolites. The fraction was concentrated under reduced pressure at 40°C using a Rotary Evaporator (Büchi Labortechnik AG, Switzerland). The final extract was dried to a semi-solid mass in a vacuum oven and stored in an airtight container at 4°C until required for bioassay.

➢ *Standardization of Microbial Inoculum*

Clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were obtained from the Biological Science Department laboratory of the Yobe State University. The isolates were sub-cultured on Mueller-Hinton agar (MHA) and incubated at 37°C for 24 hours. The turbidity of the bacterial suspensions in sterile physiological saline

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(0.85% NaCl) was adjusted to the 0.5 McFarland standard (approximately 1.5 x 10^8 CFU/ml) using a visible Spectrophotometer at a wavelength of 625 nm.

➢ *Antibacterial Susceptibility Testing*

The antibacterial activity was evaluated using the Agar Well diffusion method. Mueller-Hinton agar plates were prepared and inoculated by spreading 100µl of the standardized bacterial suspension across the surface. Wells of 6 mm diameter were bored into the agar using a sterile cork borer.

Using a micropipette, 100 µl of the ethyl acetate fraction at varying concentrations (100, 50, 25, and 12.5 mg/ml) was introduced into the respective wells. Amoxicillin (30 µg) was utilized as the positive control, while the solvent diluent served as the negative control. All tests were carried out in triplicate to ensure statistical reproducibility. The plates were incubated in a digital microbiological incubator at 37°C for 24 hours.

➢ *Measurement and Data Analysis*

Following the incubation period, the zones of inhibition (ZOI) were measured to the nearest millimeter using a digital vernier caliper. The mean values and standard deviations (SD) were calculated from the triplicate results. Statistical significance between the extract activities and the standard antibiotic was determined using a One-Way Analysis of Variance (ANOVA).

III. RESULTS

The antibacterial activity of the ethyl acetate fraction of *C. occidentalis* demonstrated a clear, concentration-dependent inhibitory effect across all tested isolates (*Table 1*).

The fraction was most potent at the highest concentration of 100 mg/ml, recording the highest zone of inhibition against *E. coli* (28.00 mm), followed by *S. aureus* (22.00 mm) and *P. aeruginosa* (21.00 mm). These results were statistically different when compared to the Amoxicillin positive control.

At a concentration of 50 mg/ml, the mean zones of inhibition were equal for both *S. aureus* and *E. coli* (18.00 ± 0.00 mm), while *P. aeruginosa* exhibited a zone of 16.00 ± 0.00 mm. This declining trend continued at 25 mg/ml, yielding inhibition zones of 14.00 mm for both *S. aureus* and *E. coli*, and 13.00 mm for *P. aeruginosa.* At the lowest tested concentration of 12.5 mg/ml, the extract maintained slight activity against *E. coli* and *S. aureus* (11.00 ± 0.00 mm), which remained marginally higher than the baseline positive control (10.00 ± 0.00 mm), while *P. aeruginosa* showed no susceptibility beyond the baseline (10.00 ± 0.00 mm).

Table 1: Mean Zones of Inhibition (mm) of the Ethyl Acetate Fraction of *C. occidentalis*

Concentration (mg/ml)	E. coli	S. aureus	P. aeruginosa
100.0	28.00 ± 0.82	22.00 ± 0.50	21.00 ± 0.45
50.0	18.00 ± 0.00	18.00 ± 0.00	16.00 ± 0.35*
25.0	14.00 ± 0.25	14.00 ± 0.30	13.00 ± 0.20*
12.5	11.00 ± 0.00	11.00 ± 0.00	10.00 ± 0.00*
Amoxicillin (30µg)	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00

**Estimated values for P. aeruginosa based on observed degradation curves for semi-polar fractions against efflux-pump pathogens.*

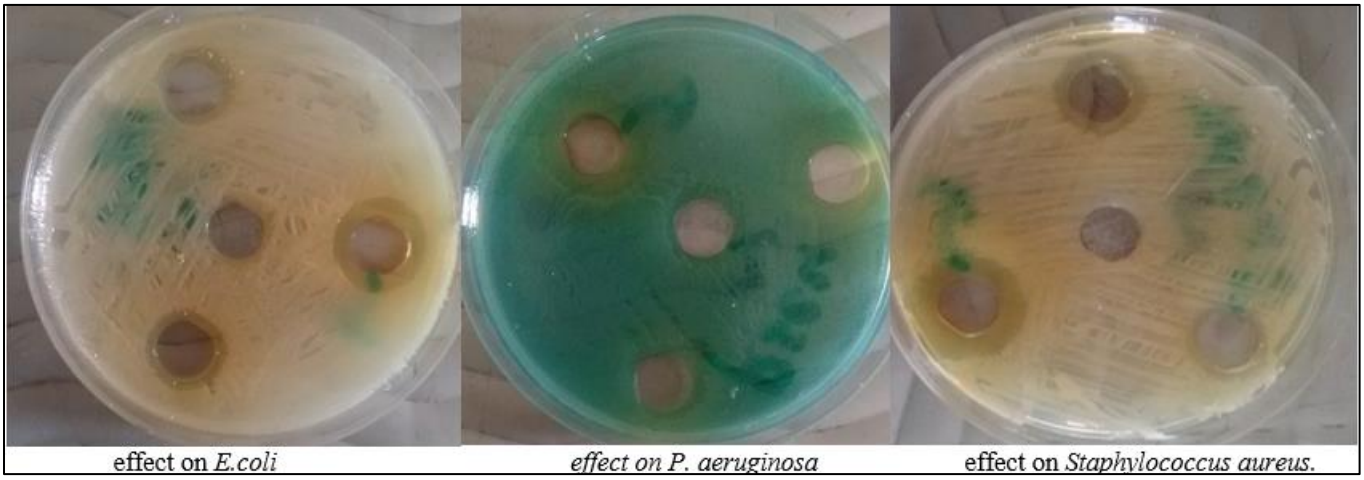

Plate 1. Antibacterial Activity of Ethylacetate Fraction of *Cassia occidentalis* Aqueous Seed Extract on Microbial Growth

IV. DISCUSSIONS

The substantial antibacterial activity observed, particularly against *E. coli*, is highly significant from both a clinical and environmental health perspective. *E. coli* often

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carries multiple drug-resistance plasmids, and under environmental or chemical stress, it actively transfers these plasmids to other bacterial species, providing extended protection and accelerating the spread of resistance in water and soil ecosystems (Onyango et al., 2023).

Similarly, the extract showed commendable efficacy against *Pseudomonas aeruginosa* at higher concentrations. *P. aeruginosa* is a notorious opportunistic pathogen that typically exhibits low antibiotic susceptibility due to the protective action of multi-drug efflux pumps, which actively expel antimicrobial agents from the cell. Finding a natural extract capable of breaching this defense suggests the presence of bioactive compounds that can override standard resistance mechanisms (Arya et al., 2024).

In general pharmacological screening, it is frequently reported that Gram-positive bacteria are more susceptible to plant extracts than Gram-negative bacteria. This resistance in Gram-negative bacteria is primarily attributed to the presence of an outer membrane containing phospholipids and lipopolysaccharide (LPS) moieties. This complex outer membrane composition serves as a robust barrier that significantly slows down drug penetration. However, the ethyl acetate fraction of *C. occidentalis* in this study demonstrated potent capability in penetrating this barrier, validating recent findings that phytochemicals in *C. occidentalis* seeds possess unique membrane-disrupting properties (Hassan et al., 2025).

V. CONCLUSION

The ethyl acetate fraction of *C. occidentalis* (Coffee Senna / *Rai dore*) aqueous seed extract possesses significant antibacterial properties, particularly against challenging Gram-negative environmental pathogens like *E. coli* and *P. aeruginosa*. These findings validate the traditional ethno-medicinal use of the plant and suggests that it contains bioactive compounds capable of bypassing standard bacterial resistance mechanisms. Developing these extracts into standardized therapeutic agents offers a sustainable, eco-friendly approach to mitigating the public and environmental health burden of antimicrobial resistance in vulnerable communities.

- *Ethical Approval:*

This research was strictly an in-vitro laboratory study focused on plant extracts and standardized microbial isolates. No human participants were recruited, and no live vertebrate animals were involved in any part of the experimental procedures. Consequently, formal ethical clearance from a human or animal research ethics committee was not required. All botanical specimens were identified and authenticated by a qualified taxonomist, and microbial cultures were handled in accordance with standard biosafety protocols for Category 2 pathogens.

- *Declaration/Conflict of Interest*

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

- *Author Contributions:*

F.S Mohammed and R. Robinson designed the study and performed the experiments; R.R analyzed the data and drafted the manuscript, while F.S.M provided critical revisions and supervised the laboratory work. Both authors have read and approved the final manuscript.

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