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Antibacterial Evaluation Of Alchornea Cordifolia Fractionated Leaf Extracts On Salmonella Typhimurium- Induced Gastroenteritis In Wistar Rats

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Abstract: ***Gastroenteritis, primarily presenting as diarrhea, remains a major public health challenge responsible for over five million child deaths annually. In this study, the antibacterial efficacy and safety profile of a hydro-ethanolic leaf extract of Alchornea cordifolia in a Wistar rat model of Salmonella Typhimurium–induced gastroenteritis, was investigated. Thirty female Wistar rats were randomized into six groups (A–F). All but the negative control (group F) were inoculated with S. Typhimurium. Groups A–D received oral standardized doses of 4.4, 60, 301 and 20.4 mg/kg body weight of n-hexane, aqueous, n-butanol and ethylacetate fractions of A. cordifolia leaf extract, respectively. All extract-treated groups exhibited progressive, dose-dependent reductions in faecal bacterial counts compared to controls. By day 2, the ethylacetate fraction group reduced counts from 4.50 to 3.42 $\log_{10}$ CFU/mL; by day 3, the aqueous fraction group reached 3.32 $\log_{10}$ CFU/mL (from 4.40; and by the final day, the ethylacetate group fell to 3.20 $\log_{10}$ CFU/mL (from 3.31). Further phytochemical isolation, synergistic studies with reduced antibiotic doses, and long-term safety assessments are warranted to translate these promising findings into clinical applications.***

Keywords: ***Antibacterial Agents, Salmonella Typhimurium, Diarrhea, Gastroenteritis, Plant Extracts, Phytochemicals, Phytomedicine***

I. INTRODUCTION

Diarrhea, cramps, nausea, vomiting, and fever are symptoms of gastroenteritis, an intestinal infection caused by bacteria, viruses, and other microorganisms. It is also brought on by poisons, or toxins, the majority of which come from microbes (Barlow et al., 2020). Gastroenteritis, commonly presenting as diarrhea, is recognized as the second leading cause of death, particularly in low-income regions of developing countries. The condition is characterized by abnormally high frequency, volume, or fluidity of stools, which leads to dehydration and loss of salt and body fluids. Contaminated food and water, which might result from unhygienic activities before or after using the lavatory, are the main causes of diarrhea (Badria & Zidan, 2021).

Antibiotic resistance refers to a bacterium's ability to survive exposure to an antibiotic that would normally kill it or inhibit its growth. This resistance can develop through spontaneous or induced genetic mutations. Additionally, bacteria can exchange resistance genes via mechanisms such as conjugation, transduction, or transformation. As a result, genes conferring antibiotic resistance can spread rapidly

among bacterial populations. Evolutionary pressures, particularly antibiotic exposure, select for bacteria carrying resistance genes, promoting their survival and proliferation (Hawkey & Jones, 2019).

Alchornea cordifolia, a well-known medicinal plant from the Euphorbiaceae family, is widely used in African traditional medicine to treat a range of inflammatory, bacterial, fungal, and parasitic conditions (Lamikanra *et al*., 2019). The leaves are commonly applied topically to promote the healing of wounds, cuts, sores, and ulcers. Traditionally, the plant is also employed to relieve symptoms such as coughs, colds, and headaches, and is used to manage asthma attacks and prevent miscarriage (Adeshina *et al*., 2021). Pharmacological research has confirmed the plant's broad biological activities, including antidiabetic, hepatoprotective, antiviral, anti-inflammatory, and antidiarrheal effects (Adeshina *et al*. 2019; Manga *et al*., 2020).

II. MATERIALS AND METHODS

Leaves of *A. cordifolia* were collected from University of Ilorin school gate and identified at the University of Ilorin herbarium. The type specimen was deposited for documentation and indexed with a herbarium code/voucher number UILH/001/1798/2024

Ethical permit for the use of laboratory animal (Wistar rats) was processed and collected from Landmark University Omu-aran, Kwara State from the committee on ethical permit with reference no LMUIREC/ACSC/028/2023

The fresh leaves collected were air-dried for three weeks and pulverised using a grinder (Mikachi Mk-198). The extracting solvent was hydroethanol. Nine hundred grams of the pulverized leaf was soaked in 2.25 litres of ethanol and 2.25 litres of distilled water as solvent for 48 hours and stirred intermittently using the cold maceration extraction method. The filtrate was collected and a rotary evaporator was used to expel the solvents so that the extract appeared pasty. The paste was freeze-dried, stored in an air-tight glass specimen tube and kept in the refrigerator at 4 °C pending when it was used for experiment (Owoseni, 2020).

The Crude extract from plant leaf was subjected to liquid-liquid partitioning by a separating funnel. Solvents such as n-hexane, n-butanol, ethyl acetate and water were used. They were carefully chosen based on their polarity (Trujillo-correa et al., 2021). The resulting fractions were tested against the test *S. Typhimurium* using the antibiotic susceptibility test. (Ighodaro et al., 2021)

Wistar rats with average weight of 100g were randomly placed in 6 groups. Each group contains 5 rats. When diarrhea was induced, the treatment continued for 5 days. During treatment, the microbial load of the bacteria in the rats fecal pellet were evaluated at intervals of 8 hours for 5 days. 1 mL/kg body weight parasite volume was given to each rat to induce infection as compared with the MacFarland standard of turbidity.

The Minimum Inhibitory Concentration (MIC) of the hydro-ethanolic leaf extract of *Alchornea cordifolia* against *Salmonella Typhimurium* was determined using a two-step serial dilution method. Test tubes containing 9 mL of sterile nutrient broth were supplemented with 1 mL of the extract at various concentrations (0.1, 0.2, and 0.4 mg/mL). Each tube was inoculated with a standardized culture of *S. Typhimurium* and incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration of the extract at which no visible microbial growth (turbidity) was observed, indicating effective inhibition of bacterial proliferation (N.C.F.C.L.S., 2022).

To determine the Minimum Bactericidal Concentration (MBC), 10 µL samples were taken from the MIC test tubes that exhibited no visible turbidity. These samples were subcultured onto Deoxycholate Citrate Agar (DCA) plates using the pour plate method and incubated at 37°C for 24 hours in a dry-air incubator. The MBC was defined as the lowest concentration of the extract at which there was a complete absence or a significant reduction in bacterial colony formation. This outcome indicates the extract's bactericidal potential (N.C.F.C.L.S., 2022).

III. RESULTS

From this study the results of the phytochemical analysis from the hydroethanolic leaf extract of *Alchornea cordifolia* include phytochemicals such as saponins, tannins, phenols, flavonoids, coumarins, steroids, triterpenes, terpenoids, and glycosides. Quantitative analysis revealed flavonoids as the most abundant compound (81.88 mg/100 g), followed by phenolics (56.35 mg/100 g). Others detected were Triterpenes (15.87 mg/100 g), steroids (15.45 mg/100 g), tannins (4.77 mg/100 g), terpenoids (4.18 mg/100 g), Coumarins (3.82 mg/100 g), glycosides (0.21 mg/100 g) and saponins (0.01 mg/100 g). Notably, anthocyanins, phlobatannins, alkaloids, and amino acids were absent (Table 1).

Phytochemicals present	**Qualitative**	**Quantitative representation in (mg/100g)**
Flavanoid	+	81.88±0.22
Phenolics	+	56.35±0.18
Triterpenes	+	15.87±0.05
Steroids	+	15.45±0.00
Tannin	+	4.77±0.00
Terpenoids	+	4.18±0.00
Coumarin	+	3.82±0.00
Glycosides	+	0.21±0.01
Saponin	+	0.01±0.00
Anthocyanin	-	------
Phlobatanin	-	
Alkaloids	-	
Amino acid	-	

Table 1: Qualitative and quantitative analysis of phytochemicals in Alchornea cordifolia leaf extracts

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In vitro antimicrobial assays were performed on various fractions of *A. cordifolia* leaf extracts. The results showed differing zones of inhibition against the test microorganisms. The ethyl acetate and aqueous fractions, along with ciprofloxacin (used as the positive control), demonstrated the largest zones of inhibitions of (36,33 and 34mm) respectively. In contrast, the n-butanol and n-hexane fractions exhibited comparatively smaller zones of (24 and 20mm) respectively as illustrated in Table 2.

Furthermore, bacterial load counts from fecal samples were monitored throughout the treatment period. On days one and two, the ethyl acetate fraction produced the lowest bacterial counts with a $\log_{10}$ reduction from 4.50 to 3.42 $\log_{10}$ CFU/ml on the third day of treatment. By day three, the aqueous fraction recorded the least bacterial load with a $\log_{10}$ reduction from 4.40-3.32 $\log_{10}$ CFU/ml on the fourth day of treatment. On day four, ciprofloxacin treatment resulted in the lowest bacterial count with a reduction from 4.45 to 3.30 $\log_{10}$ CFU/ml. On the final day, the ethyl acetate fraction again showed the lowest microbial load from 3.31 to 3.20 $\log_{10}$ CFU/ml. These findings are presented in Table 3 and Table 4 respectively.

Sample	Zone of inhibition in millimeter	Zone of inhibition in millimeter	Zone of inhibition in millimeter	Zone of inhibition in millimeter	Average
EthylA	36	35	37	36	36
Aqueous	33	32	34	33	33
N-but	24	23	25	24	24
CIPR	34	33	35	34	34
N-hex	20	19	21	20	20

KEY
EthylA= Ethylacetate
Aqueous= Aqueous
N-but=N-butanol
CIPR= Ciprofloxacin
N-hex= N-hexane

Table 2: Zone of inhibition of fractionated leaf extracts of Alchornea cordifolia against Salmonella Typhimurium

Total viable count on rat stool sample in CFU/ml

Sample	Day 1	Day 2	Day 3	Day 4	Day 5
PC	6.0×10^4	8.0×10^4	9.0×10^4	9.2×10^4	9.5×10^4
EthylA	6.0×10^4	5.0×10^4	4.2×10^3	3.1×10^3	2.0×10^3
Aqueous	6.2×10^4	5.9×10^4	4.0×10^4	3.2×10^3	2.8×10^3
N-but	8.1×10^4	5.8×10^4	4.3×10^4	3.3×10^4	2.6×10^4
CIPR	8.6×10^4	6.0×10^4	4.5×10^4	3.0×10^3	2.5×10^3
N-hex	8.0×10^4	6.2×10^4	4.8×10^4	3.9×10^4	3.0×10^4

KEY
EthylA= Ethylacetate
Aqueous= Aqueous
N-but=N-butanol
CIPR= Ciprofloxacin
N-hex= N-hexane

Table 3: In vitro antibacterial activity of the fractionated extracts of Alchornea cordifolia

Sample	Day 1	Day 2	Day 3	Day 4	Day 5
PC	4.60	4.80	4.90	4.92	4.95
EthylA	4.60	4.50	3.42	3.31	3.20
Aqueous	4.62	4.59	4.40	3.32	3.28
N-but	4.81	4.58	4.43	4.33	4.26
CIPR	4.86	4.60	4.45	3.30	3.25
N-but	4.80	4.62	4.48	4.39	4.30

KEY
EthylA= Ethylacetate
Aqueous= Aqueous
N-but=N-butanol
CIPR= Ciprofloxacin
N-hex= N-hexane

Table 4: Total viable count on rat stool sample when administered with fractions in $\log_{10}$ CFU/ml (In-vivo)

IV. DISCUSSION

The in-vitro antimicrobial assessment suggests that the ethyl acetate and aqueous fractions possess significant antibacterial potential, comparable to that of ciprofloxacin. The superior activity of the ethyl acetate fraction may stem from the solvent's ability to extract medium-polarity phytochemicals including flavonoids, alkaloids, phenolics, and terpenoids known for their antimicrobial mechanisms, such as cell wall disruption, inhibition of protein synthesis, and interference with nucleic acid replication. These bioactive constituents likely act synergistically to enhance antibacterial potency. These results are in agreement with those of Ishaq *et al*. (2022), who evaluated the antibacterial activity of *A. cordifolia* against extended-spectrum β-lactamase (ESBL)-producing multidrug-resistant *Klebsiella* spp. and *Escherichia coli*. Using both agar diffusion and dilution methods, they found that ethyl acetate and ethanol extracts of *A. cordifolia* showed substantial inhibition, with ethanol extract zones measuring up to 17 mm and MIC values ranging from 2.5–20 mg/ml. The similarity in findings underscores the potential of *A. cordifolia* as an effective source of natural antibacterial compounds, especially against resistant strains (Ishaq *et al*., 2022).

The therapeutic efficacy of the fractionated extracts *in vivo*, fecal bacterial load was monitored in Salmonella Typhimurium-infected Wistar rats over a five-day treatment period. The groups were treated with each of the four fractions ethyl acetate, *n*-butanol, *n*-hexane, and aqueous as well as ciprofloxacin (positive control) and an untreated infected group (negative control). On day one, all groups exhibited comparable baseline fecal bacterial loads. By day five, notable reductions were observed in the treated groups: Ethyl acetate fraction: reduced from 4.60 to 4.20 $\log_{10}$ CFU/ml. Ciprofloxacin: reduced from 4.86 to 4.25 $\log_{10}$ CFU/ml. *n*-butanol fraction: reduced from 4.81 to 4.26 $\log_{10}$ CFU/ml. Aqueous fraction: reduced from 4.62 to 4.28 $\log_{10}$ CFU/ml. *n*-hexane fraction: reduced from 4.80 to 4.30 $\log_{10}$ CFU/ml. Untreated control group: increased from 4.60 to 4.95 $\log_{10}$ CFU/ml These results demonstrate that all fractions, particularly the ethyl acetate fraction, significantly suppressed bacterial proliferation. The ethyl acetate fraction outperformed ciprofloxacin in terms of reduction magnitude and consistency, reinforcing its potential as an effective treatment against *Salmonella*-induced gastroenteritis. The observed antibacterial effects may be attributed to bioactive phytochemicals enriched in the ethyl acetate fraction, which could exert bacteriostatic or bactericidal effects and possibly modulate host immune responses to facilitate pathogen clearance. These findings are supported by Igbenughu (2021), who investigated the *in vivo* antibacterial activity of a 50% aqueous ethanol extract of *A. cordifolia* against multidrug-

resistant *Staphylococcus aureus* in mice. The study reported dose-dependent improvements in survival and bacterial clearance at extract doses ranging from 25 to 200 mg/kg. The outcomes highlight *A. cordifolia*'s broad-spectrum antimicrobial potential and support its use as a natural therapeutic option for treating infections caused by resistant bacterial pathogens such as S. Typhimurium and *S. aureus* (Igbenughu, 2021)

Together, these results highlight the anti-inflammatory potential of *A. cordifolia* extract fractions and their capacity to modulate immune responses during infection, supporting their therapeutic application in inflammatory and infectious conditions.

V. CONCLUSION

The hydroethanolic extract of *Alchornea cordifolia* leaves is rich in bioactive phytochemicals including saponins, flavonoids, tannins, phenolics, coumarins, steroids, triterpenes, terpenoids, and glycosides that collectively contribute to its significant antimicrobial and anti-inflammatory properties. Collectively, these findings affirm *Alchornea cordifolia* as a promising candidate for the development of novel antimicrobial and anti-inflammatory therapies.

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