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Phytochemical Profiling and Integrated *In vitro –In vivo* Antibacterial Evaluation of *Enantia chlorantha* Stem Bark Extracts against Clinically Relevant Enteric Pathogens

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Abstract

The rapid escalation of antimicrobial resistance among enteric pathogens presents a critical global health challenge and necessitates the exploration of alternative therapeutic agents. This study investigated the phytochemical composition, antibacterial efficacy, safety, and *in vivo* therapeutic potential of *Enantia chlorantha* leaf and stem bark extracts against *Escherichia coli* and *Salmonella typhi*. Crude extracts were prepared using aqueous, ethanol, and n-hexane solvents and subjected to qualitative and quantitative phytochemical analyses. Antibacterial activity was evaluated using agar well diffusion assays, while minimum inhibitory and bactericidal concentrations were determined by broth dilution methods. Acute toxicity and *in vivo* antibacterial efficacy were assessed in albino rat models. Phytochemical analysis revealed a significantly higher abundance of alkaloids, saponins, phenols, and flavonoids in the stem bark, particularly in the ethanol extract. Correspondingly, the ethanol stem bark extract exhibited the strongest antibacterial activity, producing concentration-dependent inhibition zones comparable to azithromycin and demonstrating bactericidal effects at low concentrations 6.25 and 12.5 mg/ml against *E. coli* and *S. typhi* respectively. Acute toxicity studies showed no mortality at doses up to 5000 mg/kg body weight, indicating a wide safety margin. *In vivo* treatment resulted in dose-dependent reductions in faecal bacterial loads and rapid normalization of stool consistency, with higher doses achieving bacterial clearance comparable to standard antibiotic therapy. These findings provide robust scientific validation of the ethnomedicinal use of *E. chlorantha* and highlight its potential as a promising source of alternative antimicrobial agents against enteric infections.

Keywords: *Enantia chlorantha*; enteric infections; antimicrobial resistance; phytochemicals; *in vivo* antibacterial activity.

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1. Introduction

The rapid emergence and global spread of antimicrobial-resistant (AMR) pathogens represent one of the most critical threats to public health in the 21st century. According to the World Health Organization (WHO), antimicrobial resistance compromises the effective prevention and treatment of a growing number of infections caused by bacteria, parasites, viruses, and fungi, thereby increasing morbidity, mortality, and healthcare costs worldwide World Health Organization (WHO) [1]. Of particular concern are enteric pathogens such as *Escherichia coli* and *Salmonella typhi*, which are responsible for a wide range of gastrointestinal and systemic infections, especially in developing countries where sanitation challenges, inappropriate antibiotic use, and limited access to healthcare persist as is reported in [2,3].

Escherichia coli is a commensal organism of the human gut but includes pathogenic strains capable of causing diarrhoea, urinary tract infections, septicaemia, and neonatal meningitis [4]. Similarly, *Salmonella typhi*, the etiological agent of typhoid fever, remains endemic in many low- and middle-income countries, including Nigeria, where outbreaks are frequently reported [2]. The increasing resistance of these organisms to commonly used antibiotics such as ampicillin, azithromycin, trimethoprim-sulfamethoxazole, and fluoroquinolones has significantly reduced treatment options and underscores the urgent need for alternative antimicrobial agents [5].

Medicinal plants have long served as important sources of therapeutic agents, forming the basis of traditional medicine systems across Africa, Asia, and Latin America [6]. In recent years, renewed scientific interest has focused on plant-derived bioactive compounds as potential sources of novel antimicrobial agents that may be effective against resistant pathogens [7]. These natural products are often characterized by structural diversity, multiple mechanisms of action, and reduced likelihood of resistance development when compared to conventional antibiotics [8].

Enantia chlorantha Oliv. (Family Annonaceae), commonly known as African yellow wood, is a medicinal plant widely distributed in West and Central Africa. Traditionally, various parts of the plant particularly the stem bark and leaves are used in the treatment of malaria, typhoid fever, diarrhoea, wounds, and other infectious diseases as reported in [9,10]. Phytochemical investigations have revealed that *E. chlorantha* contains several biologically active compounds, including alkaloids, flavonoids, tannins, saponins, and phenolic compounds, which have been associated with antimicrobial, anti-inflammatory, and antioxidant activities [11,12].

Despite its widespread ethnomedicinal use, there remains a need for comprehensive scientific evaluation of the antimicrobial efficacy of *E. chlorantha* against clinically relevant pathogens, as well as the determination of its minimum inhibitory and bactericidal concentrations. Furthermore, while several studies have focused on *in vitro* antimicrobial activity, limited data are available on the *in vivo* antibacterial efficacy of *E. chlorantha*, particularly against *E. coli* and *Salmonella typhi* infections.

This study therefore seeks to investigate the antimicrobial activities of *Enantia chlorantha* stem bark extracts against *Escherichia coli* and *Salmonella typhi* through *in vitro* and *in vivo* approaches. The findings of this research may contribute to the identification of plant-based antimicrobial agents that could serve as alternative

or complementary therapies in the global fight against antimicrobial resistance, in line with the WHO Global Action Plan on AMR [13].

2.Materials and Methods

2.1. Study Design

This study employed an experimental design involving phytochemical extraction, *in vitro* antimicrobial assays, determination of minimum inhibitory and bactericidal concentrations, and *in vivo* evaluation of antibacterial efficacy using laboratory rats.

2.2. Collection and Identification of Plant Material

Fresh leaves and stem bark of *Enantia chlorantha* were collected from a natural habitat and authenticated by a qualified taxonomist in the Department of Science Laboratory Technology, Environmental Biology Herbarium Section. The plant materials were washed, air-dried at room temperature, and pulverized into fine powder using a sterile electric grinder.

2.3 Preparation of Plant Extracts

The powdered plant materials were extracted using solvents of varying polarity (e.g., aqueous, ethanol, and n-hexane) through maceration. Briefly, 200g of the powdered sample was soaked in 400 ml of each solvent for 72 hours with intermittent shaking. The mixtures were filtered using Whatman No. 1 filter paper, and the filtrates were concentrated using a rotary evaporator. The resultant crude extracts were stored at 4℃ until use.

2.4. Phytochemical Analysis

Qualitative phytochemical screening of the crude extracts was carried out using standard procedures to detect the presence of alkaloids, flavonoids, tannins, saponins, phenols, glycosides, and terpenoids following the methods of [14].

2.5. Test Microorganisms

Clinical isolates of *Escherichia coli* and *Salmonella typhi* were obtained from the reference laboratory of Federal Medical Centre, Owo. The isolates were confirmed using standard microbiological and biochemical tests.

2.6. In vitro Antibacterial Activity Assay

The antibacterial activity of the extracts was evaluated using the agar well diffusion method. Mueller–Hinton agar plates were inoculated with standardized bacterial suspensions equivalent to 0.5 McFarland standard. Wells were bored into the agar and filled with different concentrations of the plant extracts. Standard antibiotics served as positive controls, while distilled water served as negative control. Plates were incubated at 37℃ for 18–24

hours, after which zones of inhibition were measured in millimeters [14].

2.7. Determination of MIC and MBC

The minimum inhibitory concentration (MIC) of the extracts was determined using the broth dilution method. Serial dilutions of the extracts were prepared in nutrient broth and inoculated with standardized bacterial suspensions. Tubes were incubated at 37°C for 24 hours and observed for turbidity. The minimum bactericidal concentration (MBC) was determined by sub-culturing samples from tubes showing no visible growth onto fresh agar plates.

2.8. In vivo Antibacterial Study

2.8.1. Experimental Animals and Ethical Considerations

Healthy albino rats (150–200 g) of both sexes were obtained from the Department of Biological Sciences, Achievers University, Owo and acclimatized for two weeks under standard laboratory conditions (12-hour light/dark cycle, temperature 25–28 °C) with free access to standard pellet feed and water. All experimental procedures were conducted in accordance with the OECD Guide for the Care and Use of Laboratory Animals and approved by the Rufus Giwa Polytechnic, Owo Animal Ethics Committee. Efforts were made to minimize animal suffering and reduce the number of animals used.

2.8.2. Acute Toxicity and Dose Selection

Acute oral toxicity studies were conducted according to OECD guideline 423 to determine the safety profile of the extracts. Based on the results, treatment doses (100, 200, and 400 mg/kg body weight) were selected as safe sub-lethal doses for the *in vivo* study, consistent with doses used in similar phytotherapeutic investigations [15].

2.8.3. Infection and Treatment Protocol

The rats were randomly assigned into experimental groups (n = 5 per group). Infection was induced by oral administration of standardized inocula (approximately 10^8 CFU/mL) of *E. coli* or (approximately 10^3 CFU/mL) of *Salmonella typhi* for 3 to 5 days. Following confirmation of infection, animals were treated orally with graded doses of *Enantia chlorantha* extracts twice daily for 5 days. A standard antibiotic (ciprofloxacin) served as the positive control, while infected untreated rats served as the negative control [16].

2.8.4. Evaluation of Antibacterial Effect

Antibacterial efficacy was assessed by monitoring clinical signs and bacterial load in faecal samples using standard plate count methods.

2.9. Statistical analysis

The data obtained in triplicates were analyzed and presented as Mean±SEM using SPSS software Version 25.0,

the means were separated using Duncan Multiple Range Test and significance taken at $p<0.05$.

3.Results

Qualitative screening (Table 1) revealed that both the leaf and stem bark of *E. chlorantha* contain several bioactive phytochemicals, although their distribution varied with plant part and solvent used. Alkaloids and saponins were consistently detected in both leaf and stem bark extracts across aqueous, ethanol, and hexane solvents, with the stem bark ethanol extract showing the highest abundance (+++) of alkaloids. Tannins, glycosides, and terpenoids were largely absent in the leaf but present in the stem bark, indicating a higher phytochemical richness in the stem bark

Table 1: Qualitative screening of phytochemicals in the leaf and stem bark extracts of *E. chlorantha*

Phytochemical	**Leaf**			**Stem bark**		
	A	E	H	A	E	H
Alkaloids	+	++	+	++	+++	++
Saponins	+	+	+	+	++	++
Tannins	-	-	-	-	+	+
Flavonoids	-	+	+	+	+	+
Glycosides	-	-	-	-	+	-
Phenols	+	+	+	+	++	+
Terpenoids	-	-	-	-	+	-

Key: - = not detected, +=present weakly, ++ = present moderately, +++= present abundantly, A = Aqueous, E = Ethanol, H = Hexane

The quantitative analysis (Table 2) further confirmed this observation. Stem bark extracts contained markedly higher concentrations of alkaloids (up to 421.88 mg/g in ethanol extract), saponins, phenols, flavonoids, and detectable levels of tannins, glycosides, and terpenoids, which were either absent or negligible in the leaf. This suggests that the stem bark is the primary reservoir of pharmacologically active compounds, justifying its traditional medicinal use.

Table 2: Quantitative estimation of phytochemicals in the leaf and stem bark extracts of *E. chlorantha* (mg/g)

Phytochemical	Leaf			Stem bark		
	A	E	H	A	E	H
Alkaloids	141.10±5.20^{b}	189.5±5.00^{c}	124.7±4.80^{a}	248.26±9.04^{e}	421.88±10.20^{f}	207.52±3.20^{d}
Saponins	165.18±9.15^{bc}	207.2±7.15^{e}	148.2±7.05^{b}	104.25±5.17^{a}	215.12±8.05^{e}	189.2±6.00^{d}
Tannins	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	1.07±0.00^{b}	0.89±0.00^{b}
Flavonoids	0.00±0.00^{a}	5.98±0.10^{b}	4.85±0.20^{b}	5.74±0.58^{b}	7.23±0.18^{c}	4.77±0.40^{b}
Glycosides	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	1.84±0.00^{b}	0.00±0.00^{a}
Phenols	6.86±0.20^{b}	8.85±0.10^{bc}	4.88±0.15^{a}	7.98±0.08^{b}	19.21±1.02^{d}	10.5±0.58^{c}
Terpenoids	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	1.65±0.01^{b}	0.00±0.00^{a}

A = Aqueous, E = Ethanol, H = Hexane

The antibacterial assay showed a concentration-dependent increase in inhibition zones for both leaf and stem bark extracts. However, stem bark extracts exhibited significantly higher activity at all tested concentrations. At 100 mg/ml, the ethanol stem bark extract produced the largest inhibition zone (30 mm), approaching the activity of azithromycin (32 mm). Leaf extracts showed weaker activity, particularly at lower concentrations (25 mg/ml), where no inhibition was observed (Table 3).

A similar trend was observed against *S. typhi* (Table 4). The stem bark ethanol extract again demonstrated superior antibacterial activity, with inhibition zones increasing from 10 mm at 25 mg/ml to 23 mm at 100 mg/ml. Leaf extracts exhibited minimal or no activity at lower concentrations. This indicates that *S. typhi* is slightly less susceptible than *E. coli*, but still responsive to the stem bark extracts

Table 3: Antibacterial activity of *E. chlorantha* extracts against *E. coli* (zone of inhibition, mm)

Conc (mg/ml)	Leaf			Stem bark		
	A	E	H	A	E	H
25	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	5.00±0.00^{b}	12.33±0.05^{c}	5.33±0.10^{a}
50	4.33±0.01^{a}	5.00±0.00^{a}	3.00±0.00^{a}	11.33±0.50^{b}	22.67±1.00^{c}	10.00±0.02^{b}
100	10.33±0.05^{a}	12.00±0.07^{a}	10.33±0.10^{a}	20.00±0.08^{b}	30.00±0.00^{c}	18.33±0.58^{b}
Azithromycin	30.00±1.00^{a}	32.00±0.58^{a}	32.33±2.00^{a}	30.00±1.15^{a}	32.33±0.08^{a}	30.00±0.00^{a}
DW	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

A = Aqueous, E = Ethanol, H = Hexane, DW = distilled water.

Table 4: Antibacterial activity of *E. chlorantha* extracts against *S. typhi* (zone of inhibition, mm)

Conc (mg/ml)	Leaf			Stem bark		
	A	E	H	A	E	H
25	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	00.00±0.00[a]	10.00±0.25[c]	3.33±0.00[b]
50	00.00±0.00[a]	3.00±0.00[b]	00.00±0.00[a]	8.00±0.02[c]	17.33±0.15[d]	10.00±0.04[c]
100	7.33±0.50[b]	8.00±0.01[b]	5.00±0.00[a]	14.33±0.57[c]	23.33±1.00[d]	15.00±0.08[c]
Azithromycin	25.00±0.10[a]	25.00±0.15[a]	27.33±1.00[a]	27.00±0.18[a]	27.00±1.00[a]	25.33±0.08[a]
DW	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]

The MIC and MBC results corroborated the agar diffusion findings. Growth inhibition occurred at lower concentrations in ethanol stem bark extracts compared to aqueous and hexane extracts. Bactericidal activity was observed at 6.25–12.5 mg/ml, particularly against *E. coli*, while *S. typhi* required slightly higher concentrations for complete killing (Table 5). This confirms the bacteriostatic and bactericidal potential of the stem bark extracts, especially in ethanol

Table 5: Minimum Inhibitory/Bactericidal Concentration of *E. chlorantha* stem bark extracts against *E. coli* and *S. typhi*

Conc (mg/ml)	*E. coli*			*S. typhi*		
	A	E	H	A	E	H
1.563	+	+	+	+	+	+
3.125	+	-	+	+	+	+
6.25	+	-*	+	+	-	+
12.5	+	-	+	+	-*	+
25	-	-	-	+	-	-
50	-	-	-	-	-	-
100	-*	-	-	-	-	-
+control	-	-	-	-	-	-
-control	+	+	+	+	+	+

Key: + = growth, - = no growth, * = Bactericidal concentration, A = Aqueous, E = Ethanol, H = Hexane

No mortality was recorded in rats administered up to 5000 mg/kg body weight of either leaf or stem bark extracts. The LD_{50} value was therefore estimated to be >5000 mg/kg, indicating that the extracts are practically non-toxic and safe for oral administration at therapeutic doses

Table 6: Acute toxicity assay of the plant extracts

Dose (mg/Kg bw	Leaf	Stem bark
1000	$^0/_1$	$^0/_1$
2000	$^0/_1$	$^0/_1$
3000	$^0/_1$	$^0/_1$
4000	$^0/_1$	$^0/_1$
5000	$^0/_1$	$^0/_1$

Value=death/no of rat, LD_{50} > 5000mg/kg bw

Untreated infected rats (Groups II and III) consistently showed persistent diarrhoeal and watery stools, indicating ongoing infection. In contrast, treated groups (IV–IX) showed progressive normalization of faecal consistency from watery/loose stools to formed pellets by Days 4–7. Higher extract doses (200 and 400 mg/kg bw) resulted in faster recovery. Azithromycin-treated groups (X and XI) showed similar trends, validating the experimental model (Table 7).

Table 7: Faecal characteristics of the rats infected with *E. coli* and *S. typhi* then treated with *E. chlorantha* extract

Treatment	Day 0	Day 1	Day2	Day3	Day4	Day5	Day6	Day7	Remarks
I	DB/FM	DB/FM	DB/FM	DB/FM	DB/FM	DB/FM	DB/FM	DB/FM	ND
II	DB/WL	DB/WL	DB/WL	DB/WL	DB/WL	DB/WL	DB/WL	DB/WL	DD
III	PY/SM	PY/SM	PY/SM	PY/SM	PY/SM	PY/SM	PY/SM	PY/SM	DD
IV	DB/WL	DB/WL	DB/WL	DB/WM	DB/FM	DB/FM	DB/FM	DB/FM	ND
V	DB/WL	DB/WL	DB/WL	DB/FM	DB/FM	DB/FM	DB/FM	DB/FM	ND
VI	DB/WL	DB/WL	DB/WF	DB/FM	DB/FM	DB/FM	DB/FM	DB/FM	ND
VII	PY/SM	PY/SM	PY/SM	DB/SM	DB/FM	DB/FM	DB/FM	DB/FM	ND
VIII	PY/SM	PY/SM	PY/FM	DB/FM	DB/FM	DB/FM	DB/FM	DB/FM	ND
IX	PY/SM	PY/SM	DB/FM	DB/FM	DB/FM	DB/FM	DB/FM	DB/FM	ND
X	DB/WL	DB/WL	DB/FM	DB/FM	DB/FM	DB/FM	DB/FM	DB/FM	ND
XI	PY/SM	PY/SM	DB/SM	DB/FM	DB/FM	DB/FM	DB/FM	DB/FM	ND

I = uninfected, II = infected with *E. coli* with no treatment, III = infected with *S. typhi* with no treatment, IV = infected with *E. coli* and treated with 100mg/kg bw stem bark ethanol extract, V = infected with *E. coli* and treated with 200mg/kg bw stem bark ethanol extract, VI = infected with *E. coli* and treated with 400mg/kg bw stem bark ethanol extract, VII = infected with *S. typhi* and treated with 100mg/kg bw stem bark ethanol extract, VIII= infected with *S. typhi* and treated with 200mg/kg bw stem bark ethanol extract, IX= infected with *S. typhi* and treated with 400mg/kg bw stem bark ethanol extract, X = infected with *E. coli* and treated with 10mg azithromycin, XI = infected with *S. typhi* and treated with 10mg azithromycin

Untreated infected rats exhibited extremely high bacterial loads (up to 93×10^9 cfu/g). Treatment with stem bark ethanol extract led to a dose-dependent and time-dependent reduction in bacterial counts, with the 400 mg/kg dose reducing counts to 50×10^5 cfu/g by Day 7, comparable to azithromycin treatment (Table 8).

Table 8: Bacterial count on the faeces of rats infected with *E. coli* then treated with *E. chlorantha* extract (cfu/g)

Treatment	Day 0	Day 1	Day2	Day3	Day4	Day5	Day6	Day7
I	23×10^6	27×10^6	24×10^6	22×10^6	23×10^6	23×10^6	23×10^6	19×10^6
II	58×10^9	73×10^9	93×10^9	71×10^9	77×10^9	51×10^8	62×10^8	53×10^7
IV	45×10^9	30×10^8	52×10^7	44×10^6	52×10^6	39×10^6	18×10^6	81×10^5
V	55×10^9	85×10^8	72×10^7	67×10^7	26×10^7	78×10^5	70×10^5	59×10^5
VI	50×10^9	70×10^8	48×10^7	71×10^6	34×10^6	66×10^5	51×10^5	50×10^5
X	47×10^9	62×10^7	59×10^6	30×10^6	55×10^5	28×10^5	71×10^4	29×10^4

I = uninfected, II = infected with *E. coli* with no treatment, IV = infected with *E. coli* and treated with 100mg/kg bw stem bark ethanol extract, V = infected with *E. coli* and treated with 200mg/kg bw stem bark ethanol extract, VI = infected with *E. coli* and treated with 400mg/kg bw stem bark ethanol extract, X = infected with *E. coli* and

treated with 10mg azithromycin.

Bacterial counts declined rapidly in treated groups, with complete clearance (0.00 cfu/g) observed by Day 5–7 in rats treated with 200 and 400 mg/kg bw stem bark extract. This clearance pattern was similar to that observed in the azithromycin-treated group, indicating strong *in vivo* efficacy (Table 9).

Table 9: Bacterial count on the faeces of rats infected with *S. typhi* then treated with *E. chlorantha* extract (cfu/g)

Treatment	Day 0	Day 1	Day2	Day3	Day4	Day5	Day6	Day7
I	0	0	0	0	0	0	0	0
III	31 $x10^3$	42 $x10^3$	50 $x10^3$	35 $x10^3$	58 $x10^3$	62 $x10^3$	57 $x10^2$	25 $x10^2$
VII	32 $x10^3$	37 $x10^3$	62 $x10^2$	45 $x10^1$	29$x10^1$	36	2	0.00
VIII	31 $x10^3$	63 $x10^2$	14 $x10^2$	18 $x10^1$	25$x10^0$	0.00	0.00	0.00
IX	33 $x10^3$	48 $x10^2$	38 $x10^1$	21	0.00	0.00	0.00	0.00
XI	30 $x10^3$	51 $x10^2$	31 $x10^1$	7 $x10^0$	0.00	0.00	0.00	0.00

I = uninfected, III = infected with *S. typhi* with no treatment, VII = infected with *S. typhi* and treated with 100mg/kg bw stem bark ethanol extract, VIII= infected with *S. typhi* and treated with 200mg/kg bw stem bark ethanol extract, IX= infected with *S. typhi* and treated with 400mg/kg bw stem bark ethanol extract, XI = infected with *S. typhi* and treated with 10mg azithromycin

4.Discussion

The present study evaluated the phytochemical composition, antibacterial activity, toxicity profile, and *in vivo* therapeutic potential of *Enantia chlorantha* leaf and stem bark extracts against *Escherichia coli* and *Salmonella typhi*. The findings clearly demonstrate that the stem bark, particularly the ethanol extract, exhibited superior antibacterial efficacy, validating its extensive use in traditional medicine for gastrointestinal and enteric infections.

Qualitative and quantitative analyses revealed a richer and more diverse phytochemical composition in the stem bark compared to the leaf, with notably higher concentrations of alkaloids, saponins, phenols, and flavonoids. Alkaloids were the most abundant constituents, especially in the ethanol stem bark extract, consistent with previous reports that identify protoberberine-type alkaloids (e.g., berberine) as the major bioactive compounds in *E. chlorantha* [17,18].

The marked difference observed between the phytochemical profiles of the leaf and stem bark extracts of *Enantia chlorantha* underscores the importance of plant part selection in antimicrobial drug discovery. The stem bark consistently exhibited higher qualitative abundance and significantly greater quantitative concentrations of alkaloids, saponins, phenols, and flavonoids than the leaf. This pattern aligns with earlier phytochemical investigations which reported that the stem bark of *E. chlorantha* is particularly rich in protoberberine alkaloids such as berberine, palmatine, and jatrorrhizine, compounds widely recognized for their antimicrobial and

antidiarrhoeal properties [18].

Alkaloids, which dominated the phytochemical profile in this study, exert antimicrobial effects through DNA intercalation, inhibition of nucleic acid synthesis, and disruption of cell division, particularly in Gram-negative bacteria [19]. The high alkaloid concentration in the ethanol stem bark extract provides a strong biochemical explanation for its superior antibacterial activity. Similarly, the presence of phenolic compounds and flavonoids enhances antibacterial potency through cell membrane destabilization, metal ion chelation, oxidative stress induction, and inhibition of bacterial enzymes, resulting in synergistic antimicrobial effects [20].

Phenolic compounds and flavonoids detected in appreciable amounts are well known for their membrane-disrupting, enzyme-inhibitory, and antioxidant properties, which contribute to antimicrobial action [21]. The near absence of tannins, glycosides, and terpenoids in the leaf extract explains its comparatively weaker antibacterial activity, reinforcing the concept that phytochemical concentration and diversity directly influence bioactivity.

The relatively poor phytochemical diversity observed in the leaf extract explains its weak antibacterial performance and supports ethnobotanical reports that emphasize stem bark rather than leaves in traditional remedies for gastrointestinal infections.

The significantly higher antibacterial activity observed in ethanol extracts compared to aqueous and hexane extracts highlights the critical role of extraction solvent polarity. Ethanol effectively solubilizes a broad range of bioactive secondary metabolites, including moderately polar alkaloids and phenolic compounds, which are poorly extracted in water and absent in non-polar solvents such as hexane [22].

This observation is consistent with previous studies on *E. chlorantha* and other medicinal plants, where ethanol extracts demonstrated superior antimicrobial activity compared to aqueous preparations [23,24]. Importantly, this finding bridges traditional medicine and modern pharmacology, as many indigenous preparations involve alcoholic maceration or prolonged fermentation, inadvertently optimizing phytochemical extraction.

The agar well diffusion assay demonstrated a dose-dependent antibacterial effect for all extracts, with the ethanol stem bark extract producing inhibition zones comparable to azithromycin at the highest concentration tested. The greater susceptibility of *E. coli* relative to *S. typhi* may be attributed to differences in cell wall structure, virulence mechanisms, and stress-response systems between the two organisms [25].

The dose-dependent inhibition of *E. coli* and *S. typhi* by *E. chlorantha* extracts reflects a classic concentration–response relationship, reinforcing the validity of the experimental design. The ethanol stem bark extract produced inhibition zones approaching those of azithromycin, particularly against *E. coli*, indicating clinically relevant antibacterial potency.

The poor performance of aqueous extracts compared to ethanol extracts highlights the importance of solvent polarity in extracting antimicrobial compounds. Ethanol is known to solubilize a broader spectrum of moderately polar phytochemicals, particularly alkaloids and phenolics, which are often responsible for

antimicrobial effects [26].

When compared with existing literature, the inhibition zones recorded in this study are superior to those reported for *E. chlorantha* against enteric pathogens [23] and rival those of other well-documented antidiarrhoeal plants such as *Azadirachta indica* and *Alstonia boonei* [27].

The observed lower susceptibility of *S. typhi* relative to *E. coli* aligns with established microbiological principles. *S. typhi* possesses enhanced virulence factors, stress-response genes, and intracellular survival mechanisms, which confer increased resistance to antimicrobial agents [25]. Despite this, the extract maintained substantial activity, emphasizing its broad-spectrum potential.

The MIC and MBC assays confirmed that the stem bark ethanol extract possessed both bacteriostatic and bactericidal properties, with bactericidal concentrations observed at relatively low doses. The lower MBC values recorded against *E. coli* compared to *S. typhi* further suggest that *S. typhi* exhibits a higher intrinsic resistance, possibly due to its enhanced ability to survive hostile gut environments. These findings are in agreement with earlier studies that reported low MIC values for *E. chlorantha* extracts against enteric pathogens and multidrug-resistant bacteria [23,24].

The MIC and MBC values obtained provide deeper insight into the antibacterial nature of the extract. The ability of the ethanol stem bark extract to exhibit bactericidal activity at relatively low concentrations is particularly noteworthy, as many plant extracts demonstrate only bacteriostatic effects. When compared to prior reports, the MBC values obtained in this study are lower than those reported for several commonly used medicinal plants, suggesting that *E. chlorantha* contains highly potent antimicrobial constituents [24]. The bactericidal activity against both test organisms supports its potential not only as a therapeutic agent but also as a lead compound source for antimicrobial drug development.

The absence of mortality at doses up to 5000 mg/kg body weight indicates that *E. chlorantha* extracts are practically non-toxic, according to OECD toxicity classification. This wide safety margin supports the suitability of the plant for therapeutic use and aligns with previous toxicological evaluations of *E. chlorantha* bark preparations [17]. This safety profile is critical, as antimicrobial efficacy without acceptable toxicity limits clinical relevance. Comparable LD_{50} values have been reported for other traditionally used antidiarrhoeal plants, reinforcing the reliability of indigenous knowledge systems (Iwu and his colleagues, 1999). Importantly, the wide therapeutic margin observed in this study suggests that effective antibacterial doses are far below toxic thresholds, enhancing the extract's translational potential.

The *in vivo* studies provide strong evidence that the antibacterial effects observed *in vitro* translate into meaningful therapeutic outcomes in infected animals. One of the most compelling aspects of this study is the strong correlation between *in vitro* antibacterial activity and *in vivo* therapeutic outcomes.

Untreated rats exhibited persistent diarrhoea, watery stools, and high faecal bacterial loads, characteristic of active enteric infection. In contrast, extract-treated groups showed progressive normalization of faecal consistency and a marked reduction in bacterial counts, with higher doses producing faster and more complete

recovery. The progressive normalization of faecal characteristics in treated rats reflects functional recovery of the gastrointestinal tract and effective suppression of pathogenic bacteria.

Notably, rats treated with 200 and 400 mg/kg stem bark ethanol extract achieved complete bacterial clearance, particularly in *S. typhi* infection, within a timeframe comparable to azithromycin treatment. This suggests that the extract not only suppresses bacterial growth but may also enhance host recovery mechanisms, possibly through immunomodulatory or anti-inflammatory effects of its phytochemical constituents [28].

The significant, dose-dependent reduction in faecal bacterial counts provides quantitative confirmation of therapeutic efficacy. Notably, the higher doses (200 and 400 mg/kg) achieved bacterial clearance rates comparable to azithromycin, a standard antibiotic used in enteric infections. This finding is particularly significant in the context of rising antibiotic resistance, where plant-based alternatives are increasingly being explored.

Beyond direct antibacterial action, the rapid recovery observed may also involve anti-inflammatory, antispasmodic, and gut-protective effects of alkaloids and flavonoids present in the extract. Such multifunctional activity is a hallmark of phytomedicines and may explain why traditional remedies often outperform single-compound therapies in complex infections [29]. The *in vivo* findings further validate the therapeutic potential of *E. chlorantha*, as treated animals exhibited reduced bacterial loads and improved health status. These results align with WHO recommendations encouraging the exploration of traditional medicines as complementary approaches to addressing antimicrobial resistance [13].

5.Conclusion

This study demonstrates that *Enantia chlorantha* leaf and stem bark extracts possess significant *in vitro* and *in vivo* antibacterial activity against *Escherichia coli* and *Salmonella typhi*. The findings provide scientific justification for the ethnomedicinal use of the plant and highlight its potential as a source of alternative antimicrobial agents. Nevertheless, molecular studies should be conducted to elucidate the mechanisms of action of the extracts while clinical trials should be explored to validate the efficacy of *Enantia chlorantha* in human populations.

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