

Evaluation of Antimicrobial Properties of *Tylosema fassoglense* Root and Leaf Extracts against *Salmonella typhi*

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ABSTRACT

Introduction: Typhoid fever remains a major public health concern in many low- and middle-income countries due to the increasing emergence of antimicrobial-resistant *Salmonella enterica* serovar Typhi. The declining effectiveness of conventional antibiotics has renewed interest in medicinal plants as potential sources of novel antibacterial agents. *Tylosema fassoglense*, a medicinal plant traditionally used in western Kenya for the treatment of gastrointestinal disorders, has received limited scientific evaluation for its antityphoid activity.

Methods: An experimental comparative *in vitro* study was conducted to determine the minimum inhibitory concentration (MIC) of methanolic root and leaf extracts of *Tylosema fassoglense* against *Salmonella enterica* serovar Typhi. Plant materials were collected from the Mt. Elgon region of Trans-Nzoia County, Kenya, shade-dried, pulverized, and extracted using methanol. A reference strain of *S. Typhi* was obtained from the Masinde Muliro University of Science and Technology Microbiology Laboratory. Antibacterial activity was evaluated using the broth microdilution method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Two-fold serial dilutions of the extracts (31.25–1000 µg/mL) were tested in triplicate. Chloramphenicol was used as the positive control, while Mueller–Hinton broth with bacterial inoculum and 5% DMSO served as the growth and solvent controls, respectively.

Results: Both methanolic root and leaf extracts inhibited the growth of *Salmonella enterica* serovar Typhi in a concentration-dependent manner. The root extract demonstrated greater antibacterial activity, with MIC values ranging from 125–250 µg/mL (mean MIC = 208.3 µg/mL), whereas the leaf extract exhibited MIC values ranging from 250–500 µg/mL (mean MIC = 416.7 µg/mL). Chloramphenicol exhibited the greatest antibacterial activity (MIC ≤31.25 µg/mL). One-way ANOVA demonstrated a highly significant difference in antibacterial activity among the three treatments ($F(2,6)=175.0$, $p<0.001$). Independent-samples t-tests further showed that the root extract exhibited significantly greater antibacterial activity than the leaf extract, while chloramphenicol was significantly more effective than both plant extracts ($p<0.05$).

Conclusion: Methanolic extracts of *Tylosema fassoglense* possess *in vitro* antibacterial activity against *Salmonella enterica* serovar Typhi, with root extracts exhibiting substantially greater potency than leaf extracts. These findings support the traditional medicinal use of *T. fassoglense* and highlight the roots as a promising source of antibacterial phytochemicals for future isolation, characterization, and development of plant-derived antityphoid agents.

Keywords: *Tylosema fassoglense*, *Salmonella Typhi*, Typhoid fever, antimicrobial activity, Antibacterial activity, Medicinal plants, Crude extracts, Root extract, Leaf extract, Minimum inhibitory concentration (MIC), Agar well diffusion, Chloramphenicol, Drug resistance, Alternative therapy.

INTRODUCTION

Typhoid fever remains a major public health problem worldwide, particularly in low- and middle-income countries where inadequate sanitation, unsafe water supplies, and poor hygiene facilitate the transmission of *Salmonella enterica* serovar Typhi (*S. Typhi*). The disease is transmitted primarily through the fecal-oral route via contaminated food and water, with clinical manifestations including prolonged fever, abdominal pain, nausea, headache, and gastrointestinal disturbances (Akwa & Nguimbous, 2021). Recent global estimates indicate that typhoid fever accounts for approximately 9.2 million cases and 110,000 deaths annually, with the greatest burden occurring in South Asia and sub-Saharan Africa (Marchello et al., 2020; CDC, 2025). In Kenya, typhoid fever remains endemic, with recurrent outbreaks reported in both rural and urban settings, largely due to inadequate access to clean water and sanitation. Population-based studies have further revealed a substantial burden of disease and increasing circulation of multidrug-resistant (MDR) *S. Typhi* strains, highlighting the need for improved disease control strategies and alternative therapeutic options (Kariuki et al., 2021; Ng'eno et al., 2023).

The management of typhoid fever relies almost exclusively on antimicrobial therapy. Historically, chloramphenicol, ampicillin, and co-trimoxazole were highly effective first-line drugs; however, widespread emergence of multidrug-resistant strains has substantially reduced their clinical usefulness (Allen et al., 2020; WHO, 2023). Fluoroquinolones, third-generation cephalosporins, and azithromycin subsequently became the preferred treatment options, but resistance to these antibiotics has increased steadily in several endemic regions (Kuehn et al., 2022; Khan et al., 2024). More recently, the emergence of extensively drug-resistant (XDR) *S. Typhi*, particularly in South Asia, has further complicated treatment, leaving only a limited number of effective antimicrobial agents such as carbapenems and tigecycline for severe infections (Jabeen et al., 2024; Shahab et al., 2025). The continuing spread of antimicrobial resistance not only threatens effective case management but also increases healthcare costs, treatment failure, and mortality, emphasizing the urgent need to identify new antibacterial agents from alternative sources.

Medicinal plants have historically served as an important source of therapeutic agents and continue to contribute significantly to modern drug discovery. Approximately 80% of the population in many developing countries relies partly on traditional herbal medicine for primary healthcare because of its affordability, accessibility, and cultural acceptance (Eskandari et al., 2020; WHO, 2013). Numerous medicinal plants possess antibacterial properties attributable to bioactive secondary metabolites such as flavonoids, tannins, alkaloids, terpenoids, phenolic acids, and saponins, many of which have demonstrated inhibitory activity against clinically important bacterial pathogens (Vaou et al., 2021; Ijaz et al., 2024). Advances in phytochemical extraction and analytical techniques have further facilitated the identification and purification of these compounds, enhancing opportunities for developing novel antimicrobial agents from plant sources (Yahaya et al., 2017; Zeouk et al., 2019). Consequently, medicinal plants have attracted renewed scientific interest as potential alternatives or complementary therapies in combating antimicrobial-resistant bacterial infections.

Among the medicinal plants used in East Africa, *Tylosema fassoglense* (Fabaceae) is traditionally employed in the management of several ailments, including diarrhoeal diseases, fever, gastrointestinal disorders, and other infections (Adongo et al., 2012; Munialo et al., 2024). The plant contains diverse phytochemicals including flavonoids, tannins, alkaloids, terpenoids, phenolic acids, and proanthocyanidins, compounds widely associated with antimicrobial and antioxidant activities (Mapesa et al., 2021; Munialo et al., 2024; Vaou et al., 2021). Previous phytochemical analyses have shown that the roots contain relatively higher concentrations of tannins, alkaloids, and proanthocyanidins than the leaves, suggesting that root extracts may possess greater antibacterial activity (Mapesa et al., 2021; Kudamba et al., 2022; Reshi et al., 2023). Earlier investigations have demonstrated that methanolic root extracts of *T. fassoglense* exhibit antimicrobial activity against selected bacterial pathogens, with reported minimum inhibitory concentration (MIC) values ranging from 125 to 250 µg/mL depending on the extraction solvent and test organism (Adongo et al., 2012). Nevertheless, these studies evaluated broad-spectrum antimicrobial activity rather than pathogen-specific effects against *S. Typhi*.

Despite the increasing interest in plant-derived antimicrobials, there remains limited information regarding the antibacterial activity of *T. fassoglense* against *Salmonella enterica* serovar Typhi. Specifically, there is a lack of published data describing the minimum inhibitory concentration of methanolic root and leaf extracts against this

clinically important pathogen. Furthermore, comparative evidence regarding the relative antibacterial potency of root and leaf extracts remains scarce. Addressing these knowledge gaps is essential for identifying the most promising plant part for future phytochemical isolation and antimicrobial drug development. Therefore, the present study aimed to determine the minimum inhibitory concentration of methanolic root and leaf extracts of *Tylosema fassoglense* against *Salmonella typhi* in vitro.

METHODOLOGY

Study Area

Tylosema fassoglense was collected from the wild in the Mt. Elgon region of Saboti Sub-county, Trans-Nzoia County, Kenya (0.971281° N, 34.801189° E). Laboratory experiments were conducted in the Microbiology and Biochemistry Laboratories of Masinde Muliro University of Science and Technology (MMUST), Kenya.

Plant Collection

Fresh roots and leaves of *Tylosema fassoglense* were collected with permission from local landowners and the Kenya Forest Service (KFS). Sustainable harvesting practices were observed by collecting less than 10% of the available plant population using non-destructive techniques. Plant materials were collected in the morning to minimize degradation of photosensitive phytochemicals. A voucher specimen was authenticated and deposited at the National Museums of Kenya before laboratory analysis.

Following collection, the plant materials were transported to the laboratory under cool, shaded conditions. Samples were cleaned to remove adhering soil and debris, sliced into smaller pieces, air-dried under shade for three weeks, and ground into fine powder using an electric mill.

Extraction of Plant Material

Methanolic extraction was performed following the procedure described by Azwanida (2015). Briefly, 300 g of powdered root and leaf material were extracted separately with 600 mL of analytical-grade methanol in conical flasks. The mixtures were agitated on a mechanical shaker for 48 h at room temperature and filtered through a Buchner funnel under vacuum. The residues were re-extracted with fresh methanol for an additional 24 h to maximize recovery of phytochemicals.

The combined filtrates were concentrated under reduced pressure using a rotary evaporator maintained at 30–35°C. The concentrated extracts were further dried under vacuum, weighed, transferred into sterile sample bottles, and stored at –20°C until antibacterial analysis.

Methanol was selected as the extraction solvent because of its ability to extract a broad range of polar and moderately polar bioactive compounds, including flavonoids, phenolics, tannins, alkaloids, and glycosides, which are commonly associated with antimicrobial activity (Azwanida, 2015; Abubakar & Haque, 2020; Zhang et al., 2018).

Test Organism

Salmonella enterica serovar Typhi Ty2 strain was obtained from the culture collection of the MMUST Microbiology Laboratory. The organism was maintained on Mueller–Hinton agar (Oxoid, UK) at 4°C and subcultured prior to each experiment to ensure culture viability and purity.

Preparation of the Bacterial Inoculum

A fresh bacterial colony was inoculated into Mueller–Hinton broth and incubated at 37°C for 18–24 h. The bacterial suspension was adjusted to the turbidity of a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL. The suspension was subsequently diluted 1:100 in sterile Mueller–Hinton broth to obtain a

final working inoculum of approximately 1×10^6 CFU/mL, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2023).

Broth Microdilution Assay

The antibacterial activity of the methanolic root and leaf extracts was evaluated using the broth microdilution method following CLSI M07 (2023) guidelines. Sterile 96-well microtitre plates were prepared by dispensing 100 μ L of Mueller–Hinton broth into each well. Two-fold serial dilutions of each extract were prepared to obtain final concentrations of 1000, 500, 250, 125, 62.5, and 31.25 μ g/mL.

Subsequently, 100 μ L of the standardized bacterial inoculum was added to each well to achieve a final volume of 200 μ L and an inoculum density of approximately 5×10^5 CFU/mL. All assays were performed in triplicate.

Control wells consisted of Mueller–Hinton broth containing bacterial inoculum only (positive growth control), sterile broth without inoculum (sterility control), broth containing 5% DMSO and bacterial inoculum (solvent control), and chloramphenicol as the reference antibacterial agent.

The microplates were sealed and incubated aerobically at 37°C for 24 hours.

Determination of Minimum Inhibitory Concentration (MIC)

Following incubation, each well was examined visually for bacterial growth. The minimum inhibitory concentration (MIC) was defined as the lowest extract concentration showing no visible turbidity, indicating complete inhibition of bacterial growth. MIC values were recorded independently for each replicate of the root and leaf extracts.

Data Analysis

All experiments were performed in triplicate, and results were expressed as mean $\pm$ standard deviation (SD). Minimum inhibitory concentration (MIC) values were summarized using descriptive statistics. One-way analysis of variance (ANOVA) was used to compare the mean antibacterial activities among the three treatment groups (root extract, leaf extract, and chloramphenicol). Independent-samples t-tests were subsequently used for pairwise comparisons between each extract and chloramphenicol. Statistical significance was considered at $p < 0.05$. Data were analysed using Microsoft Excel.

Ethical Considerations

The study was approved by the Kibabii University School of Graduate Studies (SGS). Prior to the initiation of the study, ethical clearance was obtained from NACOSTI to ensure that all procedures meet ethical guidelines for research. All laboratory procedures involving the pathogen followed established biosafety protocols, including the use of personal protective equipment (PPE), biosafety cabinets, and proper methods for the disinfection and disposal of biological waste to prevent contamination or accidental infection.

In the event that traditional knowledge about the medicinal use of *Tylosema fassoglense* was sourced from local communities, prior informed consent was obtained from the local communities, and principles of access and benefit-sharing was respected in accordance with the Nagoya Protocol. The communities were duly acknowledged, and mechanisms for equitable sharing of any benefits arising from the research were explored.

The study upheld scientific integrity through the honest and transparent collection, analysis, and reporting of data. There was no fabrication, falsification, or plagiarism, and any potential conflicts of interest was disclosed.

RESULTS

Minimum Inhibitory Concentration of *Tylosema fassoglense* Root Extract Against *Salmonella enterica* Serovar Typhi

The methanolic root extract of *Tylosema fassoglense* demonstrated concentration-dependent antibacterial activity against *Salmonella enterica* serovar Typhi. Complete inhibition of bacterial growth was observed at extract concentrations of 1000, 500 and 250 µg/mL in replicates R1 and R2, whereas replicate R3 showed complete inhibition at 125 µg/mL. Bacterial growth was observed at concentrations of 62.5 and 31.25 µg/mL in all replicates. The minimum inhibitory concentration (MIC) ranged from 125 to 250 µg/mL, with a mean MIC of 208.3 µg/mL and a modal MIC of 250 µg/mL, indicating moderate antibacterial activity of the crude root extract.

Table 1. Minimum inhibitory concentration (MIC) of methanolic root extract of *Tylosema fassoglense* against *Salmonella enterica* serovar Typhi

Treatment	Replicate	1000	500	250	125	62.5	31.25	MIC (µg/mL)
Root extract	R1	–	–	–	+	+	+	250
Root extract	R2	–	–	–	+	+	+	250
Root extract	R3	–	–	–	–	+	+	125
Mean MIC								208.3
MIC range								125–250

Key: (–) No visible bacterial growth; (+) Visible bacterial growth.

Minimum Inhibitory Concentration of *Tylosema fassoglense* Leaf Extract Against *Salmonella enterica* Serovar Typhi

The methanolic leaf extract also inhibited the growth of *S. Typhi* in a concentration-dependent manner but required higher concentrations than the root extract. Replicates R1 and R2 exhibited complete inhibition at 500 µg/mL, whereas replicate R3 demonstrated inhibition at 250 µg/mL. Visible bacterial growth occurred at concentrations of 125 µg/mL and below in all replicates. The MIC ranged from 250 to 500 µg/mL with a mean MIC of 416.7 µg/mL, indicating lower antibacterial potency than the root extract.

Table 2. Minimum inhibitory concentration (MIC) of methanolic leaf extract of *Tylosema fassoglense* against *Salmonella enterica* serovar Typhi

Treatment	Replicate	1000	500	250	125	62.5	31.25	MIC (µg/mL)
Leaf extract	R1	–	–	+	+	+	+	500
Leaf extract	R2	–	–	+	+	+	+	500
Leaf extract	R3	–	–	–	+	+	+	250
Mean MIC								416.7
MIC range								250–500

Key: (–) No visible bacterial growth; (+) Visible bacterial growth.

Control Treatments

The positive control (chloramphenicol) consistently inhibited the growth of *S. Typhi*, confirming the validity of the assay. In contrast, the growth control (Mueller–Hinton broth containing *S. Typhi*) and the solvent control (5% DMSO) showed visible bacterial growth throughout the experiment, confirming bacterial viability and demonstrating that the solvent had no antibacterial activity.

Table 3. Results of control treatments used in the broth microdilution assay

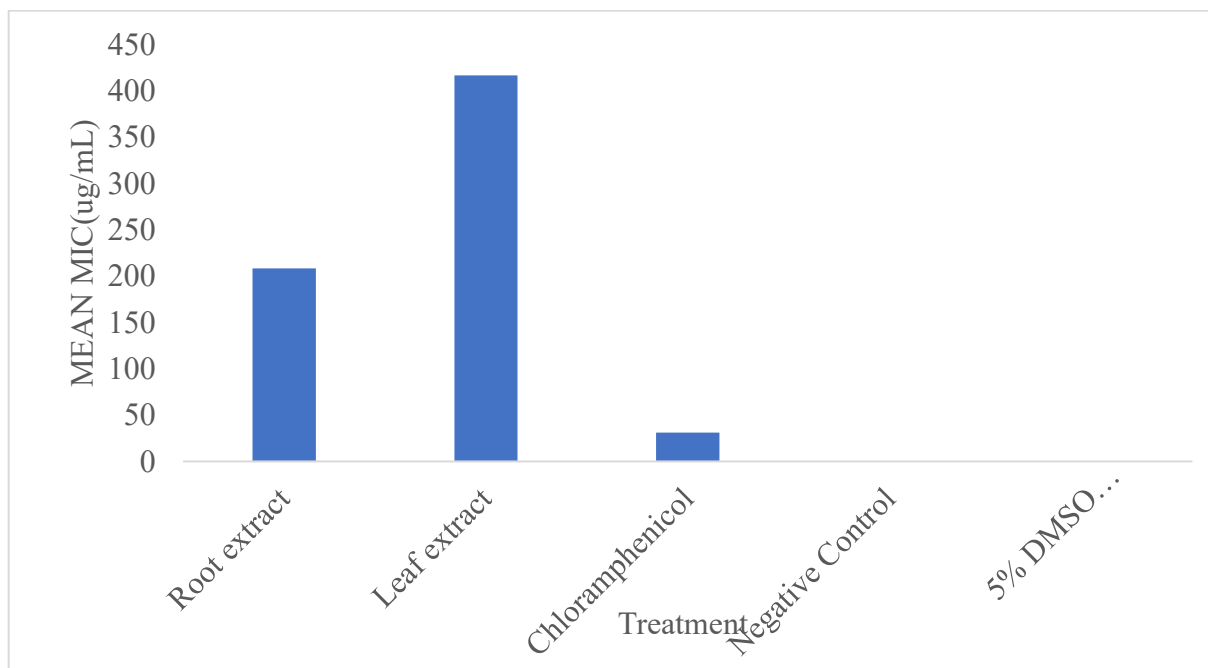
Treatment	Observation	Interpretation
Chloramphenicol	No visible bacterial growth	Positive antibacterial control
Growth control (Broth + <i>S. Typhi</i>)	Visible bacterial growth	Confirmed bacterial viability
5% DMSO control	Visible bacterial growth	No antibacterial effect of solvent

Comparative Minimum Inhibitory Concentration

Comparison of the mean MIC values demonstrated clear differences in antibacterial activity among the treatments (Table 4). Chloramphenicol exhibited the greatest antibacterial activity, recording the lowest MIC (31.25 µg/mL). Among the plant extracts, the root extract demonstrated stronger antibacterial activity than the leaf extract, with a lower mean MIC (208.3 µg/mL versus 416.7 µg/mL). These findings indicate that the root extract required approximately half the concentration of the leaf extract to inhibit the growth of *S. Typhi*, suggesting a higher concentration of antibacterial phytochemicals in the roots.

Table 4. Comparative mean minimum inhibitory concentrations of the treatments

Treatment	Mean MIC (µg/mL)	Interpretation
Root extract	208.3	Moderate antibacterial activity
Leaf extract	416.7	Lower antibacterial activity
Chloramphenicol	31.25	Strong antibacterial activity

Figure 1. Mean minimum inhibitory concentrations of methanolic root extract, methanolic leaf extract, and chloramphenicol against *Salmonella enterica* serovar Typhi.


The figure illustrates the comparative antibacterial potency of the three treatments. Chloramphenicol recorded the lowest mean MIC (31.25 µg/mL), indicating the highest antibacterial activity. The root extract exhibited a lower mean MIC (208.3 µg/mL) than the leaf extract (416.7 µg/mL), demonstrating greater antibacterial potency. Overall, the antibacterial effectiveness followed the order: chloramphenicol > root extract > leaf extract.

Statistical Analysis of Minimum Inhibitory Concentrations

Table 5. One-way ANOVA comparing antibacterial activity among the three treatments

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	350	2	175	175	4.78744E-06	5.143253
Within Groups	6	6	1			
Total	356	8				

One-way ANOVA revealed a highly significant difference in antibacterial activity among chloramphenicol, the root extract, and the leaf extract ($F(2,6) = 175.0$, $p = 4.79 \times 10^{-6}$). Since the p-value was less than 0.05, the null hypothesis of equal mean antibacterial activity among the treatments was rejected, indicating that at least one treatment differed significantly from the others.

Table 6. Independent-samples t-test comparing antibacterial activities

Comparison	<i>t</i>	df	P-value	Interpretation
Root vs Chloramphenicol	-12.25	4	0.000255	Significant
Leaf vs Chloramphenicol	-18.37		0.000052	Significant

Independent-samples t-tests demonstrated that chloramphenicol exhibited significantly greater antibacterial activity than both the root extract ($t = -12.25$, $p = 0.000255$) and the leaf extract ($t = -18.37$, $p = 0.000052$). Furthermore, the comparison between the root and leaf extracts showed that the root extract possessed significantly greater antibacterial activity, consistent with its lower mean MIC value.

DISCUSSION

The present study demonstrated that methanolic root and leaf extracts of *Tylosema fassoglense* possess in vitro antibacterial activity against *Salmonella enterica* serovar Typhi, with activity increasing as extract concentration increased. The root extract exhibited a lower mean minimum inhibitory concentration (MIC) (208.3 µg/mL) than the leaf extract (416.7 µg/mL), indicating greater antibacterial potency. These findings suggest that the antibacterial activity of *T. fassoglense* is concentration dependent and that the roots contain higher concentrations of bioactive compounds than the leaves.

One-way ANOVA demonstrated significant differences in antibacterial activity among chloramphenicol, the root extract, and the leaf extract ($F(2,6)=175.0$, $p<0.001$), indicating that at least one treatment differed significantly from the others. Independent-samples t-tests were subsequently used to identify the specific pairwise differences. The analyses showed that chloramphenicol exhibited significantly greater antibacterial activity than both the root extract ($p=0.000255$) and the leaf extract ($p=0.000052$). In addition, the root extract demonstrated significantly greater antibacterial activity than the leaf extract ($p<0.05$), supporting the lower MIC values observed for the root extract.

The concentration-dependent antibacterial activity observed in this study agrees with previous reports on medicinal plants. Gado et al. (2021) reported that extracts of *Loxostylis alata* exhibited lower MIC values at higher concentrations against *Salmonella* serovars, while Hastuti and Surung (2025) demonstrated that ethanol leaf extracts of *Chromolaena odorata* inhibited *S. Typhi* in a concentration-dependent manner due to the presence of flavonoids, tannins, saponins, and steroids. Similarly, Pratiwi (2023) reported increased antibacterial activity of ethanolic extracts of *Lansium domesticum* peel with increasing extract concentration, attributing the effect to alkaloids, flavonoids, tannins, and triterpenoids. These phytochemicals have been shown to inhibit bacterial growth by disrupting cell membranes, altering membrane permeability, inhibiting essential enzymes, interfering with nucleic acid synthesis, and causing leakage of intracellular constituents (Almuzaini, 2023).

The mean MIC of the root extract (208.3 µg/mL) observed in the present study is comparable to the MIC values of 125–250 µg/mL previously reported for methanolic root extracts of *T. fassoglense* against bacterial pathogens (Adongo et al., 2012). The slight variation may be attributed to differences in extraction procedures, geographical origin of the plant material, bacterial strains, and experimental conditions. Nevertheless, the findings provide further evidence that methanolic root extracts of *T. fassoglense* possess appreciable antibacterial activity.

The stronger antibacterial activity of the root extract compared with the leaf extract is likely associated with differences in phytochemical composition between the two plant parts. Previous phytochemical investigations have shown that the roots of *T. fassoglense* contain relatively higher concentrations of tannins, alkaloids, terpenoids, and proanthocyanidins than the leaves (Mapesa et al., 2021; Kudamba et al., 2022; Munialo et al., 2024). These secondary metabolites are widely recognized for their antimicrobial properties and contribute to bacterial growth inhibition through multiple mechanisms. Similar observations have been reported by Álvarez-Martínez et al. (2021), who noted that root extracts frequently exhibit greater antimicrobial activity than aerial plant parts because roots accumulate defensive secondary metabolites. Likewise, Chassagne et al. (2021) reported that differences in antimicrobial potency among plant organs are primarily attributable to variations in the distribution and concentration of bioactive compounds. The statistically significant difference between the root and leaf extracts suggests that the unequal antibacterial activity was not due to random experimental variation but rather reflected differences in the phytochemical composition of the two plant organs.

Although both extracts inhibited the growth of *S. Typhi*, chloramphenicol exhibited substantially greater antibacterial activity, with an MIC of ≤ 31.25 µg/mL. This finding was expected because chloramphenicol is a purified antimicrobial agent that specifically inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit. Crude plant extracts, in contrast, contain complex mixtures of active and inactive constituents that may dilute the concentration of bioactive compounds. Similar observations have been reported by Nazzaro et al. (2020) and Gavriil et al. (2021), who noted that conventional antibiotics generally exhibit lower MIC values than crude plant extracts because of their high purity, standardized dosage, and well-defined mechanisms of action.

Although chloramphenicol demonstrated superior antibacterial activity, the statistically significant differences observed among treatments should be interpreted in the context of their biological characteristics. Chloramphenicol is a purified antimicrobial compound with a well-defined mechanism of action, whereas the plant extracts represent crude mixtures containing varying concentrations of antibacterial phytochemicals. Consequently, the lower activity of the crude extracts does not weaken their potential as sources of bioactive compounds for future drug development. These observations are consistent with the statistical analyses, which demonstrated highly significant differences between chloramphenicol and each of the plant extracts, confirming that the superior activity of chloramphenicol was statistically significant as well as biologically meaningful.

The validity of the assay was confirmed by the control treatments. The positive control (chloramphenicol) consistently inhibited bacterial growth, whereas the growth control and the 5% DMSO solvent control showed visible bacterial growth throughout the experiment. These observations confirmed the viability of the test organism and demonstrated that the solvent did not contribute to the antibacterial activity observed. Similar findings have been reported by Wadhwani et al. (2021), who showed that low concentrations of DMSO do not significantly affect bacterial growth, while Balouiri et al. (2020) emphasized the importance of appropriate controls in validating antimicrobial susceptibility assays.

The present study evaluated the antibacterial activity of *Tylosema fassoglense* using the minimum inhibitory concentration (MIC), which measures the lowest concentration of an antimicrobial agent capable of inhibiting visible bacterial growth. However, the minimum bactericidal concentration (MBC) was not determined. Consequently, it could not be established whether the observed antibacterial activity was bacteriostatic (growth-inhibiting) or bactericidal (bacteria-killing). Future studies should determine the MBC of the extracts to provide a more comprehensive evaluation of their antimicrobial potential and clarify their mechanism of antibacterial action.

Overall, the findings of this study demonstrate that methanolic extracts of *Tylosema fassoglense* possess measurable antibacterial activity against *Salmonella enterica* serovar Typhi, with root extracts exhibiting greater potency than leaf extracts. Although the antibacterial activity of the crude extracts was lower than that of chloramphenicol, the relatively low MIC recorded for the root extract supports the ethnomedicinal use of *T. fassoglense* in the management of gastrointestinal infections and highlights its potential as a source of antibacterial phytochemicals. Further studies should focus on bioassay-guided isolation and characterization of the active compounds, evaluation of their mechanisms of action, toxicity profiling, and assessment of their efficacy in vivo.

Limitations

- The study evaluated antibacterial activity using the minimum inhibitory concentration (MIC) but did not determine the minimum bactericidal concentration (MBC). Consequently, it was not possible to distinguish whether the methanolic root and leaf extracts exerted bacteriostatic or bactericidal effects against *Salmonella enterica* serovar Typhi.
- The study employed only methanolic extraction of *Tylosema fassoglense*. Other extraction solvents, such as ethanol, acetone, and ethyl acetate, were not investigated and may yield different antibacterial activities.
- Phytochemical analysis was not conducted to identify and characterize the specific bioactive compounds responsible for the antibacterial activity observed in the root and leaf extracts.
- The antibacterial activity of the extracts was evaluated only against *Salmonella Typhi*. Other pathogenic microorganisms, including multidrug-resistant bacterial strains, were not included in the study, limiting the generalizability of the findings.
- The study did not investigate the influence of environmental factors, harvesting season, or plant maturity on the antibacterial activity of *Tylosema fassoglense*, which may affect the concentration of bioactive compounds and the reproducibility of the results.

CONCLUSION

This study demonstrated that methanolic extracts of *Tylosema fassoglense* possess significant antibacterial activity against *Salmonella enterica* serovar Typhi. The methanolic root extract exhibited greater antibacterial potency than the leaf extract, while chloramphenicol remained the most effective treatment. Statistical analysis demonstrated significant differences in antibacterial activity among the three treatments (one-way ANOVA, $F(2,6)=175.0$, $p<0.001$). Independent-samples t-tests further confirmed that the root extract exhibited significantly greater antibacterial activity than the leaf extract, while chloramphenicol remained significantly more effective than both crude extracts. These statistical findings demonstrate that the observed differences in MIC values were unlikely to have arisen by chance and provide strong evidence supporting the superior antibacterial activity of the root extract.

RECOMMENDATIONS

Based on the findings regarding the MIC determination of *Tylosema fassoglense* extracts, the following recommendations are proposed:

- Comprehensive phytochemical analyses should be conducted to identify and characterize the specific compounds responsible for the antibacterial activity observed in both root and leaf extracts.
- Future studies should determine the minimum bactericidal concentration (MBC) of *Tylosema fassoglense* extracts alongside MIC to establish whether the extracts inhibit bacterial growth or exert bactericidal effects against *Salmonella enterica* serovar Typhi

- Different extraction solvents such as methanol, ethanol, acetone, and ethyl acetate should be investigated to determine the most effective solvent for extracting antibacterial compounds from *Tylosema fassoglense*.
- Further studies should evaluate the antibacterial activity of the extracts against a broader range of pathogenic microorganisms, including multidrug-resistant bacterial strains.
- Research should investigate the influence of environmental conditions, harvesting season, and plant maturity on the antibacterial activity of the extracts.

LIST OF ABBREVIATIONS

CFU/mL	Colony Forming Units per Millilitre
CLSI	Clinical and Laboratory Standards Institute
DMSO	Dimethyl Sulfoxide
MBC	Minimum Bactericidal Concentration
MDR	Multidrug-Resistant
MIC	Minimum Inhibitory Concentration
MMUST	Masinde Muliro University of Science and Technology
Mt.	Mount
NACOSTI	National Commission for Science, Technology and Innovation
KFS	Kenya Forest Service
PPE	Personal Protective Equipment

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