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Evaluation of Antibacterial Potential of Ethanol Extract from Kirinyuh (*Chromolaena odorata* L.) Leaves Against *Salmonella typhi*

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Abstract
Kirinyuh leaves (*Chromolaena odorata* L.) are known to possess antibacterial activity against *Salmonella typhi*, the causative agent of typhoid fever. The leaves contain secondary metabolites such as flavonoids, saponins, tannins, and steroids, which contribute to their inhibitory effect on bacterial growth. This study evaluated the antibacterial efficacy of ethanol extract of *C. odorata* leaves at concentrations of 50%, 75%, and 100% using the Total Plate Count (TPC) method. Five treatment groups were tested: ciprofloxacin (positive control), distilled water (negative control), and kirinyuh extract at three concentrations. The results revealed five distinct statistical subsets, with significant differences observed between treatments in different subsets. Mean bacterial counts (CFU/mL) in the negative and positive controls were 138.3 and 24.7, respectively. The extract demonstrated a significant concentration-dependent inhibitory effect: the mean bacterial counts were 96.7 CFU/mL at 50%, 75.7 CFU/mL at 75%, and 45.0 CFU/mL at 100% concentration. These findings demonstrate that the ethanol extract of kirinyuh leaves effectively inhibits *Salmonella typhi* growth, with the degree of inhibition varying according to extract concentration.

INTRODUCTION

Typhoid fever continues to represent a significant public health burden in numerous developing nations, with Indonesia being a notable example of an endemic region. The disease is caused by *Salmonella typhi*, a Gram-negative bacterium transmitted mainly through contaminated food and water. Despite the availability of antibiotics, typhoid fever continues to affect 600,000–1,500,000 people annually in Indonesia, with incidence rates of 350–810 cases per 100,000 population (Levani & Prastya, 2020). Deficiencies in sanitation, coupled with limited accessibility to secure water sources and insufficient hygiene standards, are recognized as primary etiological factors. (Listyorini Asyahra *et al.,* 2022). Recent studies have also reported increasing antibiotic resistance in *S. typhi*, which complicates treatment and poses a serious public health challenge (Britto *et al.,* 2018). The increasing antimicrobial resistance of *S. typhi* underscores the urgent need to identify alternative antibacterial agents, including flavonoid-rich plant extracts that have demonstrated antibacterial activity and potential synergy with conventional antibiotics (Britto *et al.,* 2018; Górniak *et al.,* 2019; Zhang *et al.,* 2025).

In recent years, medicinal plants have attracted increasing attention as alternative therapies for infectious diseases. *Chromolaena odorata* L. (kirinyuh), a tropical plant widely

distributed in Asia, has been reported to exhibit antimicrobial activity. Several studies have reported the antibacterial activity and wound-healing potential of *C. odorata* (Li *et al.,* 2024), as well as evidence of its antibacterial effects against a wide range of pathogens (Bishoyi *et al.,* 2024; Phetburom *et al.,* 2025). Previous research demonstrated that the juice extract of *C. odorata* leaves showed inhibitory effects against *Candida albicans* and *Pseudomonas aeruginosa*, supporting its potential role as a natural antimicrobial agent (Ernawati & Jannah, 2021). Phytochemical analyses have identified the presence of flavonoids, tannins, saponins, and steroids, compounds that are known to contribute to the antibacterial potential of medicinal plants (Shrestha *et al.,* 2024). Recent investigations have highlighted the antimicrobial properties of *Chromolaena odorata*. Ethanolic and methanolic leaf extracts of *C. odorata* exhibited strong inhibitory effects against *Staphylococcus aureus* and several other Gram-positive bacteria (Hanphanphoom & Krajangsang, 2016). Further investigation confirmed that ethanolic extracts derived from *C. odorata* possess antimicrobial activity across a wide spectrum of bacteria (both Gram-positive and Gram-negative), in addition to their antioxidant capacity (Bunkaew *et al.,* 2025).

However, although several studies have evaluated the antibacterial properties of *C. odorata*, there is still limited research specifically addressing its effect on *S. typhi*, particularly in quantifying bacterial colony reduction using the Total Plate Count (TPC) method. This gap highlights the need to explore the potential of *C. odorata* as an alternative treatment for typhoid fever in regions with high prevalence and limited access to effective antibiotics.

The current study was thus designed to determine the antimicrobial potential of the ethanolic fraction of *C. odorata* foliage toward *S. typhi* via TPC method, aiming to provide empirical evidence for its proposed role as a biological antibacterial agent. Ethanol was selected as the extraction solvent due to its high polarity, which facilitates the efficient extraction of a broad spectrum of secondary metabolites, including flavonoids, tannins, and saponins, which are commonly associated with robust antibacterial properties. Furthermore, ethanol is a safe, food-grade solvent that ensures the extract's suitability for potential medicinal applications.

RESEARCH METHODS

Type of Research

The methodology utilized in this investigation was an experimental laboratory design, aimed at quantifying the antimicrobial action of the *C. odorata* leaf ethanol extract against the reference strain of *Salmonella typhi* (ATCC 14028) through the application of the TPC method.

This study was conducted in February 2025 at the Pharmacy Laboratory, Faculty of Health Sciences, Citra Bangsa University, Kupang. The leaves of *Chromolaena odorata* L. were collected on March 17, 2025, from Kelurahan Baumata, Taebenu District, Kupang City, East Nusa Tenggara. The geographical coordinates of the sampling location were [INSERT LATITUDE AND LONGITUDE COORDINATES HERE, e.g., 10^0 10’ S and 123^0 35’ E]. The plant material was taxonomically authenticated by the Herbarium Jatinangor, Laboratory of Plant Taxonomy, Department of Biology, FMIPA UNPAD. A voucher specimen has been deposited at the aforementioned institution under the accession number No. 116/HB.03.2025. This voucher

confirms the identity of the plant used in this study as *Chromolaena odorata* (L.) R.M. King & H.Rob.

Tools and Materials.

The main tools used were a moisture balance (BEL), water bath (HH-6), rotary evaporator (SH-DEA, RE-2000), incubator (DNP-9052), autoclave (GEA, Type YX-24LM), laminar air flow (IBS-V800), micropipette, vortex mixer, colony counter (I8-ONE, Type BCC 116), and standard glassware. The materials included Kirinyuh simplicia, 70% ethanol, Nutrient Agar (OXOID), Mueller Hinton Agar (MHA), ciprofloxacin, Gram staining reagents, and the test bacterium *Salmonella typhi* (TACC 14028).

Research Design

The experimental framework for this investigation adhered to a completely randomized design with 5 treatment groups. Each treatment was replicated three times. The treatment groups were as follows:

P0: No treatment (negative control, aquadest)
P1: Given ethanol extract of *C. odorata* leaves at 50%
P2: Given ethanol extract of *C. odorata* leaves at 75%
P3: Given ethanol extract of *C. odorata* leaves at 100%
P4: Positive control (ciprofloxacin)

Procedures

All glassware and culture media were sterilized using an autoclave at 121 °C for 15 minutes, while inoculating loops were flamed directly. NA medium was prepared to rejuvenate bacterial cultures, and MHA medium was used for antibacterial testing. Pure colonies of *S. typhi* obtained from the Microbiology Laboratory of Widya Mandira Catholic University, Kupang, were rejuvenated on NA slants and incubated at 37 °C for 24 hours. Gram staining was performed to confirm bacterial morphology.

A bacterial suspension was prepared in 0.9% NaCl solution and adjusted to 0.5 McFarland standard using a turbidity standard prepared from 1% H_2SO_4 and 1% $BaCl_2$ (Pinta *et al.,* 2017). Antibacterial activity was tested using the Total Plate Count (TPC) assay. Extract concentrations of 50%, 75%, and 100% were prepared, with ciprofloxacin serving as the positive control and sterile aquadest as the negative control. Each sample was inoculated with 100 µL of *S. typhi* suspension (10^{-3} dilution), homogenized with a vortex mixer, and spread onto MHA plates. The plates were incubated at 37 °C for 24 hours, after which bacterial colonies were observed and counted using a colony counter.

Data Analysis

The number of bacterial colonies was recorded after 24 hours of incubation. To determine the presence of a meaningful difference (significant inhibitory effect) caused by the *C. odorata* ethanol extract on *S. typhi*, the resulting data were subjected to a one-way ANOVA at the $P< 0.05$ level of significance.

RESULTS AND DISCUSSION

Assessment of the antibacterial action of the ethanol extract derived from *C. odorata* leaves toward *S. typhi* was conducted by employing the Total Plate Count (TPC) quantification method. The results are presented in Table 1, Figure 1 and Figure 2.

Table 1. Average number of *Salmonella typhi* colonies after treatment with ethanol extract of *C. odorata* leaves

Treatment Group	Replication			Mean (CFU/mL)
	1	2	3	
Negative control (aquadest)	133	139	143	138.3±5.03
Positive control (ciprofloxacin)	22	28	24	24.7±3.06
Test group 1 (50%)	93	97	100	96.7±3.51
Test group 2 (75%)	76	79	72	75.7±3.51
Test group 3 (100%)	44	48	43	45.0±2.65

Note: Data are expressed as mean colony counts (CFU/mL). Different treatments showed significant differences ($p < 0.05$) (Table 2).

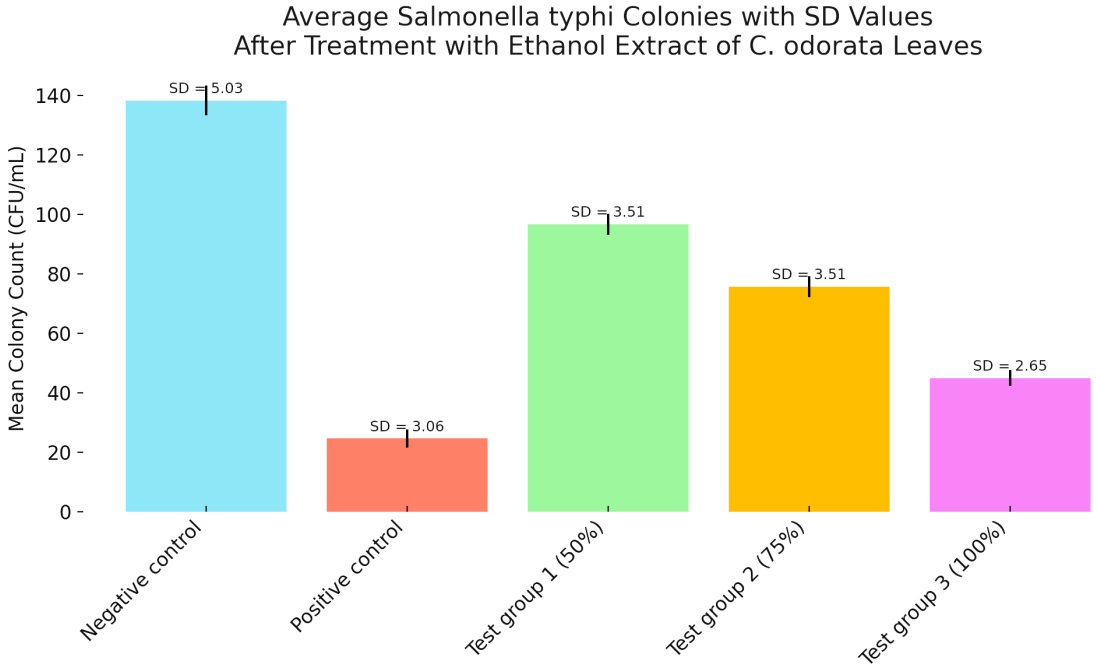

Figure 1. Average colony counts of *Salmonella typhi* after treatment with ethanol extract of *C. odorata* leaves at different concentrations

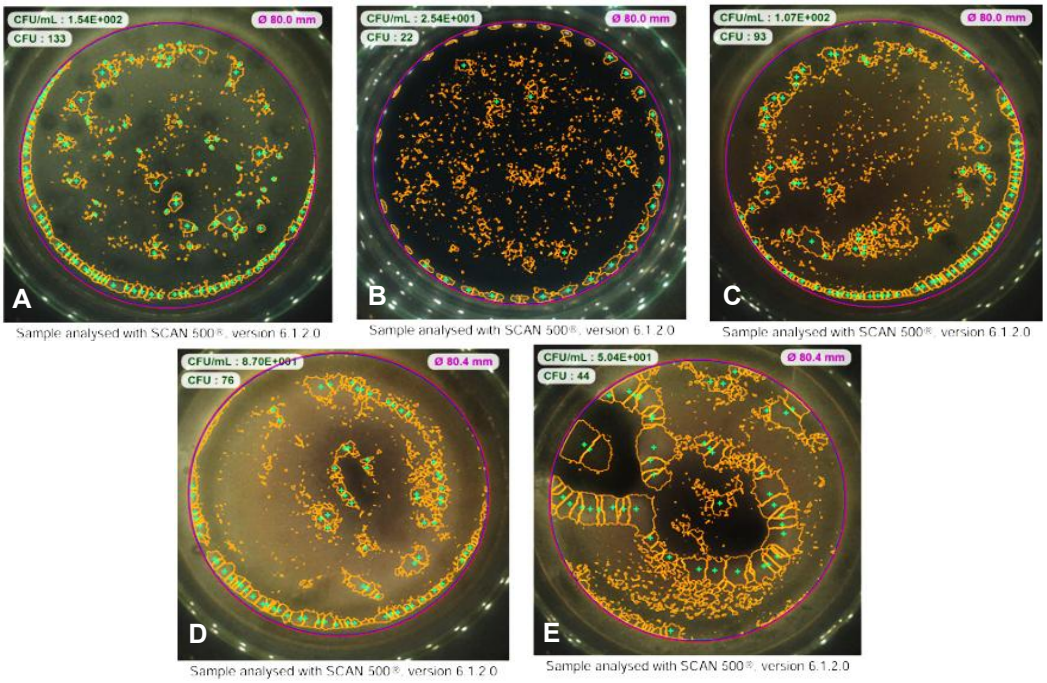

Figure 2. Visual Representation of *Salmonella typhi* Colony Growth and Antibacterial Activity Assay Results for Selected Treatment Groups. (A) Negative control (aquadest), (B) Positive control (ciprofloxacin), (C) Test group 1 (50%), (D) Test group 2 (75%) and (E) Test group 3 (100%)

Table 2. One-Way Analysis of Variance (ANOVA)

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	23726,267	4	5931,567	447,103	,000
Within Groups	132,667	10	13,267		
Total	23858,933	14			

The results demonstrated that the negative control group (aquadest) produced the highest average number of bacterial colonies (138.3 CFU/mL), indicating no inhibitory effect. In contrast, the positive control (ciprofloxacin) showed the lowest colony count (24.7 CFU/mL), confirming its strong antibacterial activity. Among the treatment groups, the ethanol extract of *C. odorata* leaves reduced bacterial growth in a concentration-dependent manner. The inhibitory effect of the extract was concentration-dependent, with the highest efficacy observed at the 100% concentration, resulting in the lowest mean colony count of 45.0 CFU/mL. This effect was followed by the 75% extract (75.7 CFU/mL) and the 50% extract (96.7 CFU/mL). Statistical analysis using One-Way Analysis of Variance (ANOVA) (Table 2) confirmed that there was a highly significant difference in the mean antibacterial effect across the five treatment groups ($F(4,10) = 447.103$, $p<0.001$). This result validates that the differences observed in the bacterial counts among the control groups and the different extract concentrations are statistically significant.

The observed decrease in *S. typhi* colony counts that is dependent on the extract concentration aligns directly with prior findings concerning the antimicrobial spectrum of *C.*

odorata against a variety of gram-positive and gram-negative species (Phetburom *et al.,* 2025; Vijayaraghavan *et al.,* 2017). For example, significant inhibition of *Staphylococcus aureus* and *Escherichia coli* by *C. odorata* extracts has been reported, further supporting the broad-spectrum antibacterial properties of this plant (Theo *et al.,* 2019). Moreover, recent findings demonstrated that *C. odorata*, *Vernonia amygdalina*, and *Cymbopogon citratus* possess strong antibacterial activity, underscoring the therapeutic potential of medicinal plants as promising alternatives to conventional antimicrobials (Ugochi *et al.,* 2025).

The antibacterial activity of *C. odorata* is largely attributed to its phytochemical constituents, particularly flavonoids. These metabolites are known to exert multiple mechanisms of action, including disruption of bacterial cell membranes, inhibition of nucleic acid and protein synthesis, interference with energy metabolism, and suppression of biofilm formation and efflux pump activity (Cushnie & Lamb, 2011; Shamsudin *et al.,* 2022; Zhou *et al.,* 2023). Transcriptomic and molecular evidence further supports these mechanisms, showing that flavonoids can damage bacterial membranes, impair metabolic pathways, and disrupt protein synthesis, thereby contributing to their broad-spectrum antibacterial effects (Zhou *et al.,* 2023).

Beyond conventional extract applications, novel approaches have been developed to enhance the antimicrobial efficacy of *C. odorata*. Topical formulations and nanoparticle-based delivery systems incorporating *C. odorata* extracts have demonstrated improved antibacterial activity (Bishoyi *et al.,* 2024; Olawepo *et al.,* 2024). In addition, in silico studies have revealed that bioactive compounds of *C. odorata* exhibit strong interactions with bacterial protein targets, further reinforcing its therapeutic potential (Lambe *et al.,* 2024).

Taken together, the present study provides additional evidence that *C. odorata* exhibits significant antibacterial activity against *S. typhi*, a clinically relevant pathogen responsible for typhoid fever (Theo *et al.,* 2019). The observed concentration-dependent effect highlights the importance of optimizing extract dosage to maximize antibacterial efficacy. Nevertheless, further investigations, including in vivo experiments and clinical evaluations, are necessary to confirm the therapeutic potential and safety profile of *C. odorata* before its broader application as a natural antibacterial agent.

CONCLUSION

In conclusion, the ethanol extract derived from *Chromolaena odorata* L. leaves was conclusively shown to possess substantial antimicrobial efficacy when tested against *Salmonella typhi*. Extract concentrations of 50%, 75%, and 100% were able to inhibit bacterial growth, with the highest inhibitory effect observed at 100% concentration, which resulted in the lowest mean colony count (45.0 CFU/mL) compared to 75% (75.7 CFU/mL) and 50% (96.7 CFU/mL). These findings confirm that the antibacterial activity of *C. odorata* is concentration-dependent, and its bioactive compounds are capable of effectively reducing the growth of *S. typhi*. This provides scientific support for the potential use of *C. odorata* as a natural antibacterial agent and highlights its relevance in the development of alternative treatments for typhoid fever.

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DECLARATIONS

Author Contributions

Author N.H designed the study, supervised the experimental procedures, and guided the overall research framework. Author E.S.S collected the plant materials, performed the laboratory experiments, and prepared the initial draft of the manuscript. Both authors contributed to data analysis, interpretation of results, and the final revision of the manuscript, and approved the version submitted for publication.

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Ethical Statement

All experimental procedures were conducted in accordance with standard microbiological laboratory protocols. Since this study did not involve human participants or animal testing, ethical approval was not required.

Declaration of Interest

The authors declare that there are no conflicts of interest or financial relationships that could have influenced the outcomes or interpretation of this research.

Data Sharing Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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