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Antibacterial Activity of Moringa (Moringa oleifera L.) Leaf Extract Against Salmonella typhi: A Study for Monograph Development in Biology Education

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Abstract
Salmonella typhi, the causative agent of typhoid fever, has increasingly developed resistance to conventional antibiotics, necessitating research into alternative antimicrobial agents. Moringa oleifera L. is a medicinal plant rich in phytochemical compounds including flavonoids, saponins, tannins, and terpenoids with documented antibacterial properties. This study examined the antibacterial activity of ethanol extract of moringa leaves against S. typhi growth, and evaluated the validity and practicality of a monograph developed from these experimental findings for undergraduate microbiology education. Using a True Experimental Research design with a Completely Randomized Design (CRD), the antibacterial activity was assessed through the Kirby-Bauer disc diffusion method at concentrations of 12.5%, 25%, 50%, and 100%, with Aztreonam as positive control. The monograph development followed the 4-D model (Define, Design, Develop, Disseminate). Results demonstrated that all extract concentrations significantly inhibited S. typhi growth ($p = 0.000$), with the highest inhibition zone diameter of 14.18 mm at 100% concentration, classified as strong inhibition. Higher concentrations consistently produced larger inhibition zones, indicating a dose-dependent antibacterial effect. The developed monograph achieved validity scores of 91.67% (content expert) and 92.85% (media expert), both categorized as valid. Practicality testing with 14 students yielded a score of 87.93%, indicating the monograph is highly practical for use in microbiology courses. These findings confirm moringa leaf extract as a promising natural antibacterial agent and establish its viability as research-based educational material.

Keywords: Antibacterial activity; Disc diffusion; Monograph; Moringa oleifera; Salmonella typhi.

INTRODUCTION

Indonesia, one of Southeast Asia's most biodiverse nations, harbors approximately 20,000 plant species, of which 13,576 have been identified for their medicinal potential (Hasyim et al., 2025; Obeng-boateng et al., 2024; Sun et al., 2024). Herbal plants are generally preferred over synthetic drugs due to their lower risk of side effects and reduced likelihood of inducing bacterial resistance (Parham et al., 2020; Stéphane et al., 2023; Vaou et al., 2021). Among these, Moringa oleifera L. commonly known as the "miracle tree" has attracted significant research interest owing to its rich phytochemical composition and broad spectrum of biological activities.

Moringa oleifera contains a diverse array of bioactive compounds including flavonoids, saponins, tannins, terpenoids, alkaloids, and steroids (Anzano et al., 2021; Obeng-boateng et al., 2024; Pareek et al., 2023). These secondary metabolites have been shown to exert antibacterial activity against multiple gram-negative bacteria by disrupting cell membranes, denaturing proteins, and inhibiting enzymatic functions (El-sherbiny et al., 2024; Kim et al., 2025; Kumar et al., 2025; Sahoo et al., 2024). Previous studies have demonstrated the plant's effectiveness against *Escherichia coli* and *Staphylococcus aureus* at various concentrations (De Fazio et al., 2024; Ngemenya et al., 2024; Saeloh & Visutthi, 2021).

Salmonella typhi is a gram-negative, rod-shaped bacterium and the primary etiological

agent of typhoid fever a systemic infectious disease transmitted through the fecal-oral route, predominantly affecting populations in low- and middle-income countries with inadequate sanitation (A A Maharani & and R M Rukmana, 2023; Buzilă et al., 2025; Shaikh et al., 2023). Globally, *S. typhi* accounts for approximately 76.3% of enteric fever cases. Standard treatment involves fluoroquinolone antibiotics; however, growing multidrug resistance has been documented against pefloxacin, ciprofloxacin, augmentin, gentamicin, co-trimoxazole, and ampicillin (Hrbacek et al., 2020; Mosunmolar, 2025). This alarming resistance pattern necessitates exploration of natural antimicrobial alternatives.

In parallel with the need for novel antibacterials, the Indonesian higher education context faces a shortage of research-based instructional materials. A needs analysis conducted at the Biology Education Department of Universitas Muhammadiyah Maumere revealed that lecturers and students lacked adequate reference books for microbiology courses. Monographs scientific books that comprehensively address a specific topic within a discipline (Geremia et al., 2024; Taher et al., 2025; Williams et al., 2025) present a viable format for translating experimental research findings into educational resources that support context-based learning.

This study therefore pursues a dual purpose: (1) to investigate the antibacterial activity of ethanol extract of moringa leaves against S. typhi growth across varying concentrations using the disc diffusion method, and (2) to develop, validate, and evaluate the practicality of a monograph derived from these experimental findings for use in undergraduate microbiology instruction. The research aims to contribute both to the scientific literature on natural antimicrobial agents and to the development of innovative, research-integrated teaching materials in Indonesian biology education.

METHODS

This study employed a two-phase design: (1) a True Experimental Research phase using a Completely Randomized Design (CRD) to evaluate antibacterial activity, and (2) a Research and Development (R&D) phase following Thiagarajan's 4-D model (Define, Design, Develop, Disseminate) for monograph development (Aghajanyan et al., 2025; Hartanti et al., 2024). Fresh moringa leaves (*Moringa oleifera* L.) were collected, sun-dried, pulverized, and sieved through mesh #40 to obtain fine powder. Extraction was performed by maceration in 96% ethanol for 24-hour intervals with regular stirring. The combined macerates were concentrated using a rotary evaporator at 40°C to yield a thick extract. Phytochemical screening confirmed the presence of tanin, flavonoid, and saponin (alkaloid was absent), as verified by the UMM Central Laboratory (Certificate No. E.7.b/033/Lab.Sentral-UMM/X/2024). *S. typhi* isolates were obtained from the Biomedical Laboratory of Universitas Muhammadiyah Malang and standardized using the 0.5 McFarland standard (1.5×10^8 CFU/mL).

Antibacterial activity was assessed using the Kirby-Bauer disc diffusion method. Six-millimeter paper discs were impregnated with moringa extract at concentrations of 12.5%, 25%, 50%, and 100%. Aztreonam (ATM) served as the positive control, and a treatment-free disc served as the negative control. Plates were incubated at 37°C for 24 hours, after which inhibition zone diameters were measured using a vernier caliper. Each treatment was replicated three times (n = 3). Inhibition zones were classified as: weak (≤5 mm), moderate (6–10 mm), strong (11–20 mm), and very strong (≥21 mm) based on (Abdelhameed et al., 2025). Quantitative data were analyzed using one-way ANOVA after verifying normality ($p > 0.05$) and homogeneity of variance (F-test). All statistical analyses were performed using SPSS 17.0. For the monograph development, content validity was assessed using structured rating sheets completed by two material experts and two media experts. Practicality was evaluated through student questionnaires using a Likert scale, scored using the percentage formula: Score (%) = (Obtained Score / Maximum Score) × 100%. Validity categories followed Arikunto's criteria (valid: ≥75%) and practicality categories followed Permendikbud No. 81A/2013.

RESULT AND DISCUSSION

Antibacterial Activity of Moringa Leaf Extract

Qualitative phytochemical screening confirmed the presence of tannins, flavonoids, and saponins in the ethanol extract of *M. oleifera* leaves. These bioactive compounds are well-documented for their antimicrobial mechanisms: flavonoids disrupt cell membrane integrity and denature bacterial proteins; saponins reduce surface tension of the bacterial cell wall causing cytoplasm leakage; tannins impair cell membrane permeability and inhibit nucleic acid synthesis; while alkaloids destabilize peptidoglycan structures inhibiting cell wall formation (Salman, 2025).

Disc diffusion testing revealed clear inhibition zones around all treated discs, confirming dose-dependent antibacterial activity. Table 1 and Figure 1 summarize the mean inhibition zone diameters across treatments.

Table 1. Inhibition Zone Diameters of Moringa Leaf Extract Against S. typhi

Treatment	Rep. 1 (mm)	Rep. 2 (mm)	Rep. 3 (mm)	Mean (mm)	Category
Positive Control (ATM)	-	-	-	36.85	Very Strong
Negative Control	-	-	-	5.00	Weak
100%	15.55	13.55	13.45	14.18	Strong
50%	10.75	10.25	13.35	11.45	Strong
25%	13.40	10.10	10.50	11.33	Strong
12.5%	10.65	9.85	10.50	10.33	Moderate

** Classification based on Permadani et al. (2014): Weak ≤5 mm; Moderate 6–10 mm; Strong 11–20 mm; Very Strong ≥21 mm*

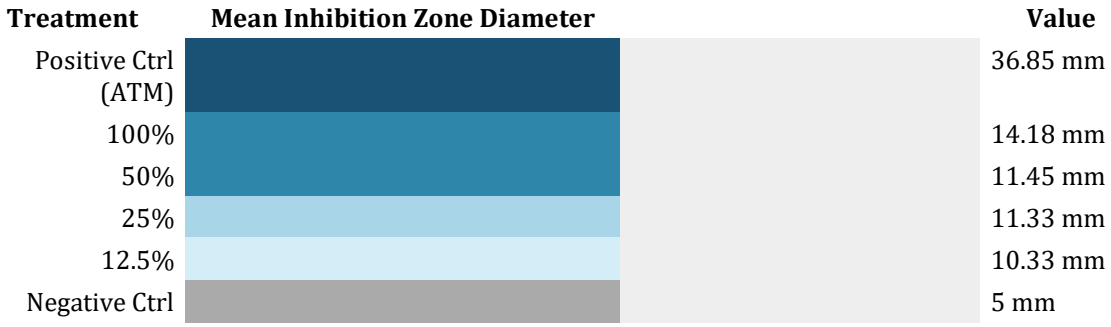

Figure 1. Mean Inhibition Zone Diameter by Treatment Concentration

As illustrated in Figure 1, a clear dose-response relationship was observed. The 100% concentration yielded the highest mean inhibition zone (14.18 mm, strong category), followed by 50% (11.45 mm), 25% (11.33 mm), and 12.5% (10.33 mm, moderate). The positive control Aztreonam produced a substantially larger inhibition zone (36.85 mm, very strong), as expected for a synthetic antibiotic. The negative control showed only 5.00 mm (weak), confirming the absence of inherent growth inhibition from the carrier.

The proportional relationship between concentration and inhibition zone size aligns with the principle that higher extract concentrations deliver increased quantities of bioactive compounds to bacterial cells, enhancing membrane disruption and metabolic interference (Arlin et al., 2025). These results are consistent with findings from , who reported significant inhibition of Staphylococcus aureus at 75% moringa extract concentration, and (P. Saranraj, 2025), who demonstrated concentration-dependent inhibition of S. typhi using Allium cepa L. extract.

Antibacterial Activity of Moringa Leaf Extract

Table 2. One-Way ANOVA of Inhibition Zone Diameter Across Treatment Groups

Source of Variation	Sum of Squares	df	Mean Square	F-value	Sig. (p)
Between Groups	672.627	5	134.525	74.144	0.000*
Within Groups	14.515	8	1.814	-	-
Total	687.142	13	-	-	-

** Significant at p < 0.05*

One-way ANOVA revealed a highly significant difference in mean inhibition zone diameters across treatment groups ($F = 74.144$; $p = 0.000 < 0.05$). This confirms that varying concentrations of moringa leaf extract produced statistically distinct antibacterial effects against S. typhi, validating the experimental hypothesis. The between-groups sum of squares (672.627) far exceeded the within-groups sum (14.515), indicating treatment effects substantially outweighed random variability.

Monograph Validity and Practicality

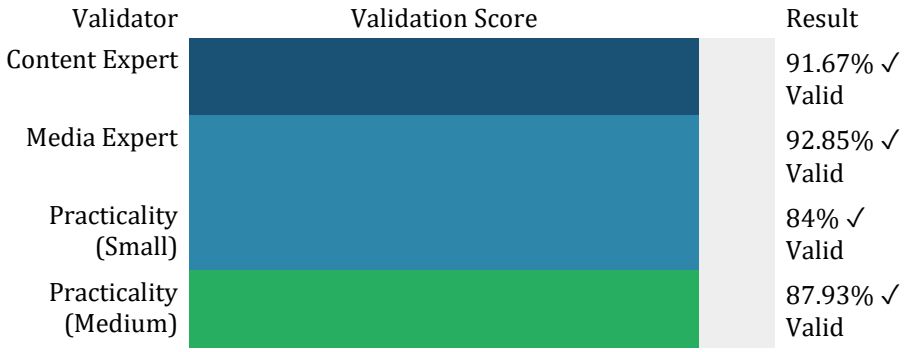

Figure 2. Monograph Validation and Practicality Scores

As shown in Figure 2, the monograph titled 'Properties, Contents, and Antibacterial Activity of Moringa Leaf Extract Against Salmonella typhi Growth' received high validation scores from both expert categories. The content expert awarded 91.67% (44/48 points), while the media expert gave 92.85% (52/56 points), both exceeding the 90% threshold for the 'very good, no revision needed' category (Arikunto & Safruddin, 2009). Validated aspects included content accuracy, systematic presentation, readability, graphic design, layout, and physical book quality.

Student practicality trials proceeded in two stages. The small-group trial (n = 3) yielded 84.00%, while the medium-group trial after revisions (n = 14) achieved 87.93% both categorized as 'very practical.' The improvement between trials reflects the effectiveness of iterative revision based on formative feedback, particularly improvements to conceptual clarity and language accessibility. These results align with Demetriou et al. (2022), who stated that effective monographs must present information in logically organized, cognitively accessible formats appropriate to the reader's developmental level.

The integration of experimental research findings into educational monographs represents a valuable contribution to Biology Education, as research-based instructional materials promote contextual learning and deepen conceptual understanding (Amin, 2010). This approach aligns with the objectives of the Biology Education study program to connect scientific inquiry with real-world applications, preparing students to engage critically with contemporary microbiological challenges.

CONCLUSION

This study demonstrated that ethanol extract of Moringa oleifera L. leaves exhibits significant dose-dependent antibacterial activity against Salmonella typhi, with 100%

concentration yielding the strongest inhibition (14.18 mm, strong category). All concentrations tested (12.5–100%) significantly differed in their inhibitory effects (p = 0.000), confirming moringa extract as a promising natural antimicrobial candidate that warrants further investigation for therapeutic applications. The derived monograph achieved validity scores of 91.67% (content) and 92.85% (media), and a practicality score of 87.93% from student evaluation, all categorized as very valid and very practical. These outcomes establish the monograph as an effective, research-integrated instructional resource suitable for undergraduate microbiology courses. Future studies should investigate Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values, explore synergistic combinations with conventional antibiotics, and extend monograph applicability to additional pathogenic bacteria and broader student populations.

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