

Original Article

A Complementary Health Approach to Tuberculosis Care: Antimycobacterial Activity of Red Betel Leaf and Shallot Ethanol Extracts



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ABSTRACT

Background: Tuberculosis (TB) remains a major global health problem, particularly in low- and middle-income countries. The emergence of drug-resistant *Mycobacterium tuberculosis* has increased interest in alternative antimicrobial agents from medicinal plants. Red betel leaf (*Piper crocatum*) and shallot (*Allium cepa* L.) contain bioactive compounds with potential antibacterial activity. This study aimed to evaluate the inhibitory effect of ethanol extracts of red betel leaf and shallot at different concentrations on the growth of *Mycobacterium tuberculosis* in vitro.

Methods: A pre-experimental one-group study was conducted in the Microbiology Laboratory of Santa Elisabeth School of Health Sciences, Medan. *Mycobacterium tuberculosis* isolates from sputum samples were cultured and tested using the disk diffusion method. Ethanol extracts of red betel leaf and shallot were prepared and tested at concentrations of 40%, 60%, 80%, and 100%. Phytochemical screening was performed, and inhibition zones were measured for three days. Data were analyzed using one-way ANOVA followed by Tukey's HSD post hoc test.

Results: Phytochemical analysis identified alkaloids, flavonoids, tannins, and saponins in the extracts. The mean inhibition zone diameters increased with extract concentration, ranging from 2.67 ± 0.58 mm at 40% to 11.67 ± 0.58 mm at 100%. One-way ANOVA showed a statistically significant difference among concentrations ($F(3, 8) = 134.25, p < .001$), and all pairwise comparisons were significant.

Conclusion: Ethanol extracts of red betel leaf and shallot demonstrated significant, concentration-dependent inhibitory activity against *Mycobacterium tuberculosis* in vitro, supporting their potential as complementary antimicrobial agents and warranting further research in nursing and health sciences.

Keywords: *Mycobacterium tuberculosis*; red betel leaf; shallot; antibacterial activity; inhibition zone.

Implications for Practice:

- Evidence from medicinal plants may support culturally sensitive TB health education led by nurses.
- Findings may inform early-stage development of adjunctive antimicrobial strategies in low-resource settings.
- Results provide a scientific basis for integrating traditional knowledge into nursing-led TB prevention programs

Introduction

Tuberculosis (TB) remains a major global public health problem. The World Health Organization (WHO) estimates that approximately 25% of the world's population is latently infected with *Mycobacterium tuberculosis*, while about 10.8 million people ($\approx 0.13\%$) develop active TB each year. The incidence of TB is higher among men, who account for approximately 55% of cases, a pattern commonly associated with occupational exposure and risk-related behaviors. TB primarily affects the lungs and continues to present significant global health challenges (WHO, 2023). The vision of the End TB Strategy is to achieve a "world free of TB," defined by zero deaths, disease, and suffering caused by tuberculosis, with the global goal of ending the TB epidemic by 2030 (Kemenkes RI, 2023). Tuberculosis is a communicable disease caused by *Mycobacterium tuberculosis*, which is transmitted through airborne droplets released by individuals with active TB. It is estimated that nearly one-quarter of the world's population is infected with *M. tuberculosis*, with approximately 89% of cases occurring in adults and 11% in children (Kemenkes RI, 2024). According to the WHO Global Tuberculosis Report 2023 (based on 2022 data), the global number of TB cases was estimated at 10,556,328, with the highest burden observed in Southeast Asia, followed by Africa and the Western Pacific regions (Directorate General of Disease Prevention, 2023). In Indonesia, TB

remains a critical public health issue. In 2023, an estimated 969,000 TB cases were reported, corresponding to an incidence rate of 354 per 100,000 population, while TB-related mortality was projected at 144,000 deaths, or 52 per 100,000 population (Kemenkes RI, 2023, 2024). At the provincial level, North Sumatra recorded the highest number of TB cases in Indonesia, with 83,949 cases, following West Java, East Java, and Central Java. Within North Sumatra, Deli Serdang Regency reported the highest number of cases (2,967), followed by Medan City (1,697) and Binjai City (1,457). In contrast, Nias Regency reported the lowest number of cases (33), followed by West Nias (58), Gunungsitoli City (76), and South Nias (117) (Dinkes Sumut, 2024).

Recent studies have demonstrated that combinations of plant extracts may exhibit stronger antibacterial inhibitory effects compared to single extracts (Soniman et al., 2022). One plant with known antibacterial properties is red betel leaf (*Piper crocatum*), which contains bioactive compounds such as phenols, flavonoids, saponins, alkaloids, and tannins. These compounds exert antibacterial effects by inhibiting cell wall synthesis, disrupting cell membrane permeability, and interfering with protein synthesis. Extracts of *Piper crocatum* have been shown to exhibit antibacterial activity against *Enterococcus faecalis*, with a minimum inhibitory concentration of 20% and a bactericidal concentration of 25% (Pasril et al., 2014). Shallot (*Allium cepa* L.) is another medicinal plant widely used in traditional medicine for treating bacterial infections, including those caused by *Salmonella typhi* (Jaya Edy et al., 2022). In Indonesia, shallots are commonly used not only as a culinary ingredient but also as a traditional remedy for stabilizing blood glucose levels, treating wounds, diarrhea, headaches, and circulatory disorders (Prima et al., 2023).

Phytochemical studies have shown that *Allium cepa* possesses antibacterial activity, particularly against Gram-negative bacteria such as *Escherichia coli* and *Salmonella typhi*, with the inhibitory effect increasing proportionally with extract concentration ([Widhorini & Rafianti, 2019](#)). Red betel leaf (*Piper crocatum*), characterized by its shiny, silvery-red leaves, has gained increasing attention for its medicinal potential. The plant contains essential oils, phenols, flavonoids, alkaloids, and tannins, which exhibit strong antimicrobial properties capable of inhibiting the growth of various bacterial species ([Sihombing, 2024](#)). Previous studies have confirmed the antimicrobial activity of ethanol extracts of red betel leaf, supporting its potential as a natural antibacterial agent ([Bangun et al., 2023](#)).

Nurses play a pivotal role in TB prevention, treatment adherence, patient education, and community-based health promotion. The increasing complexity of TB management, including multidrug-resistant TB, necessitates evidence-based innovations that can support nursing practice. Research on natural antibacterial agents aligns with nursing's holistic approach to care by integrating biomedical, cultural, and preventive perspectives. Exploring plant-based antibacterial alternatives may expand nurses' knowledge base and contribute to more comprehensive and culturally acceptable TB care strategies. This study is guided by Orem's Self-Care Deficit Nursing Theory, which emphasizes the role of healthcare interventions in supporting individuals' capacity to maintain health and manage illness. Within the public health context, the study also aligns with the Health Promotion Model, which supports preventive and supportive strategies to reduce disease burden. The exploration of plant-based antibacterial agents represents a supportive-educative nursing intervention aimed at enhancing

community self-care resources and strengthening preventive health practices. Most previous studies investigating red betel leaf (*Piper crocatum*) and shallot (*Allium cepa* L.) extracts have focused on their antibacterial activity against non-mycobacterial pathogens, particularly Gram-positive and Gram-negative bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*. These organisms differ substantially from *Mycobacterium tuberculosis* in cell wall composition, growth rate, and intrinsic resistance mechanisms. The lipid-rich mycolic acid layer of *M. tuberculosis* presents a unique barrier to antimicrobial penetration, making extrapolation from non-mycobacterial findings inappropriate. By specifically evaluating activity against *M. tuberculosis*, the present study extends existing phytochemical research into a pathogen-specific tuberculosis context. It provides evidence distinct from prior studies on general bacterial inhibition.

In this study, the independent variables are the concentrations and combinations of *Piper crocatum* and *Allium cepa* L. extracts. In contrast, the dependent variable is the inhibition of *Mycobacterium tuberculosis* growth on MacConkey Agar. Within Orem's framework, effective antibacterial inhibition supports the prevention of disease progression and transmission, thereby reducing self-care deficits related to TB management. The interaction between natural antibacterial agents and bacterial growth reflects the application of scientific evidence to support nursing interventions that promote health maintenance and disease prevention. The findings of this study are expected to contribute to nursing and public health practice by providing evidence on the potential use of locally available medicinal plants as complementary antibacterial agents. The results may enhance nurses' competencies in health education, infection

prevention, and community-based TB control programs. Additionally, the study may inform health managers and policymakers about alternative preventive strategies that are culturally acceptable, cost-effective, and sustainable, particularly in resource-limited settings. Ultimately, this research supports nursing-led initiatives aimed at improving patient outcomes and strengthening TB control efforts.

While this study is contextualized within the national tuberculosis burden, it also aligns with the broader international body of antimycobacterial research that explores plant-derived compounds as potential sources of new antimicrobial agents. Globally, increasing resistance to first-line anti-tuberculosis drugs has intensified interest in ethnobotanically informed plant screening as a complementary research pathway. The novelty of the present study lies in the selection of the plant species investigated, rather than in methodological innovation, as standard *in vitro* antimicrobial assays were employed. By contributing data on underexplored local flora, the study adds incremental value to the global evidence base while remaining methodologically consistent with established antimycobacterial research frameworks.

Based on the rising global and national TB burden reported in the Global Tuberculosis Report 2022, there is a growing need to explore alternative and complementary antibacterial agents derived from natural products. Therefore, this study aims to evaluate the inhibitory effect of a combination of red betel leaf (*Piper crocatum*) and shallot (*Allium cepa* L.) extracts on the growth of *Mycobacterium tuberculosis* using MacConkey Agar (MCA).

Methods

Study Design

This laboratory-based experimental study employed a pre-experimental one-group posttest-only design to evaluate the *in vitro* antibacterial activity of combined ethanol extracts of red betel leaf (*Piper crocatum*) and shallot (*Allium cepa* L.) against *Mycobacterium tuberculosis*. This design was selected because the objective was to assess the inhibitory effect of graded extract concentrations on bacterial growth under controlled laboratory conditions without comparison to a separate intervention group. The posttest-only approach is appropriate for *in vitro* antimicrobial assays where outcome measurement (inhibition zone diameter) occurs after treatment exposure. The study was conducted at the Microbiology Laboratory of the Santa Elisabeth School of Health Sciences, Medan, Indonesia.

Participants

Participants in this study consisted of sputum specimens obtained from patients diagnosed with pulmonary tuberculosis. Samples were selected using a purposive sampling technique to ensure that the specimens met the specific criteria required for microbiological analysis. The inclusion criteria were: (1) patients with a confirmed diagnosis of pulmonary tuberculosis, (2) positive sputum smear for *Mycobacterium tuberculosis*, and (3) sputum specimens suitable for culture and bacterial isolation. The collected sputum samples were processed following standard microbiological procedures to obtain pure isolates of *Mycobacterium tuberculosis*, which were subsequently used for antibacterial susceptibility testing. All specimens were handled in accordance with established biosafety and laboratory protocols.

Instruments

The instruments used in this study consisted of standard microbiological laboratory equipment and materials required for bacterial culture, extract preparation, and antibacterial testing. Laboratory instruments included a biosafety cabinet, incubator set at 37°C, analytical balance, grinder for plant material preparation, glassware for maceration and extraction, filtration apparatus, and sterile Petri dishes. Culture media used in this study included Lowenstein–Jensen medium for the growth and confirmation of *Mycobacterium tuberculosis* isolates and agar media prepared and sterilized according to standard laboratory procedures for antibacterial testing. Sterile paper discs were used to apply the plant extracts in the disc diffusion assay. A vernier caliper was used to measure the diameter of the inhibition zones in millimeters as the primary outcome variable. All laboratory procedures were performed using sterile equipment and standard microbiological techniques to ensure the accuracy and reliability of the results.

Intervention

The intervention in this study consisted of the application of ethanol extracts of red betel leaf (*Piper crocatum*) and shallot (*Allium cepa* L.) at different concentrations to evaluate their antibacterial activity against *Mycobacterium tuberculosis*. The plant materials were first washed, air-dried, and ground into powder. A total of 120 g of powdered red betel leaf was macerated in 300 mL of 96% ethanol for six days with daily agitation. The extract was then filtered and concentrated, after which it was diluted with distilled water to obtain final concentrations of 20%, 40%, 60%, 80%, and 100%.

For antibacterial testing, sterile paper discs were immersed in each concentration of the extract for 10 minutes and then placed onto agar media previously inoculated with bacterial suspensions of *Mycobacterium tuberculosis*. The inoculated plates were incubated at 37°C for 48 hours. The antibacterial effect of the intervention was evaluated by measuring the diameter of the inhibition zones formed around the discs using a vernier caliper, expressed in millimeters (mm). The inhibitory strength was categorized as weak (0–3 mm), moderate (3–6 mm), and strong (>6 mm).

Data Collection

Data were collected through laboratory observation of the antibacterial activity of the plant extracts against *Mycobacterium tuberculosis*. After the bacterial suspensions were inoculated onto agar media, sterile paper discs that had been immersed in different concentrations of the plant extracts were placed on the agar surface and incubated at 37°C for 48 hours. Following the incubation period, the antibacterial activity was assessed by observing the formation of clear inhibition zones surrounding the discs. The diameter of each inhibition zone was measured in millimeters (mm) using a vernier caliper as the primary outcome variable. Each extract concentration was tested under identical laboratory conditions, and the measurements were recorded systematically to ensure consistency and accuracy of the data.

Data Analysis

Data were analyzed using one-way analysis of variance (ANOVA) to determine whether there were significant differences in inhibition zone diameters among the different extract concentrations. Before conducting the analysis, the assumptions of normality and homogeneity of variance

were assessed to ensure the suitability of the data for parametric testing. Descriptive statistics were calculated to present the mean and standard deviation of inhibition zone diameters for each concentration level. When the ANOVA indicated a statistically significant difference, post hoc analysis using Tukey's Honestly Significant Difference (HSD) test was performed to identify specific differences between concentration groups. Statistical analysis was conducted using SPSS version 24, and the level of statistical significance was set at $p < 0.05$.

Ethical Considerations

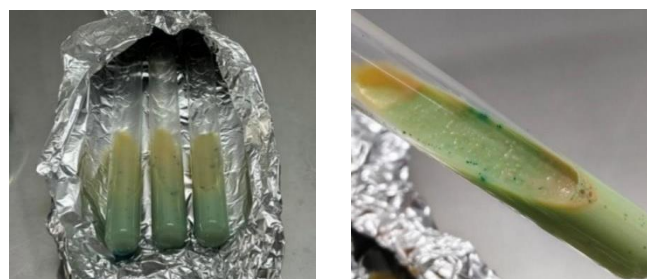
This study was approved by the Ethics Committee of the Santa Elisabeth School of Health Sciences, the research ethics approval letter number No. 006/KEPK-SE/PE-DT/I/2025. The use of sputum specimens complied with institutional and national ethical standards. All patient data were anonymized, and no personal identifiers were recorded or disclosed.

All laboratory procedures involving *Mycobacterium tuberculosis* were performed in accordance with established biosafety regulations. Appropriate personal protective equipment was used throughout the study, and all biological materials and waste were sterilized before disposal.

Results

Growth of Mycobacterium tuberculosis on Lowenstein-Jensen Medium

The study was conducted at the Microbiology Laboratory of the Santa Elisabeth School of Health Sciences, Medan. Preparation of plant extracts using the maceration method was initiated on October 25, followed by phytochemical screening. Sputum samples confirmed positive for tuberculosis were collected and processed in the laboratory.



First week

Second week

Figure 1. Growth of *Mycobacterium tuberculosis* on Lowenstein-Jensen (LJ) medium during the first and second weeks




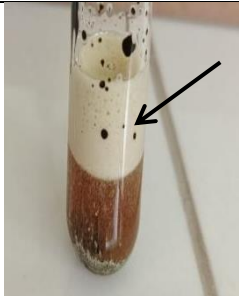
Figure 1 shows the growth of *Mycobacterium tuberculosis* observed on Lowenstein-Jensen (LJ) medium. During the first week of incubation, early bacterial growth was detected, indicated by the initial appearance of isolates. By the second week, bacterial growth was more pronounced, characterized by distinct colonies. The colonies appeared convex, milky white in color, with a rough and dry surface texture, indicating successful adaptation and proliferation of *Mycobacterium tuberculosis* on LJ medium. The use of screw-cap culture tubes was found to be appropriate for culturing *Mycobacterium tuberculosis*, as this method reduced the risk of contamination and supported optimal growth of this slow-growing bacterium.

Phytochemical Screening Results

Phytochemical analysis of the red betel leaf (*Piper crocatum*) extract demonstrated the presence of several bioactive compounds. Alkaloids were identified using Dragendorff reagent, producing a brownish-orange precipitate. Flavonoids yielded a yellow to orange coloration following reaction with NaOH and magnesium. Tannins produced a dark green coloration with ferric chloride (FeCl_3), and the formation of stable foam confirmed saponins. These findings indicate that the

extract contains compounds with known antibacterial potential (**Table 1**).

Table 1. Phytochemical Screening Results

Phytochemical Constituents	Reagent	Result	Description	Figure
Alkaloid	Dragendorff reagent	Positive (+)	Formation of a brownish-orange color with precipitate	
Flavonoid	4% NaOH + Mg	Positive (+)	Development of yellow/orange coloration	
Tanin	1% FeCL3	Positive (+)	Appearance of a dark green to blackish color	
Saponin	Distilled water	Positive (+)	Formation of stable foam	

Inhibitory Activity of Plant Extracts

Antibacterial activity was evaluated by measuring inhibition zones surrounding the paper discs after 24, 48, and 72 hours of incubation. Clear inhibition zones around

the discs indicated sensitivity of *Mycobacterium tuberculosis* to the tested extracts. The diameter of each inhibition zone was measured in millimeters using a vernier caliper (**Figure 2**).



Figure 2. Inhibition zone

Red Betel Leaf Extract

The red betel leaf extract exhibited concentration-dependent inhibitory activity against *Mycobacterium tuberculosis*. At 40% concentration, inhibition zones were consistently categorized as moderate across the three observation days (3.5–4.0 mm). At 60%

concentration, inhibition ranged from moderate to strong (5.0–6.5 mm). The 100% concentration produced strong inhibitory effects on all observation days, with the largest mean inhibition zone observed on day 3 (9.0 mm) (Table 2).

Table 2. Inhibitory Zone of Red Betel Leaf Extract and Shallot (*Allium cepa* L.) Extract against *Mycobacterium tuberculosis*

Time	Red Betel Leaf			Shallot		
	Extract Concentration (%)	Mean Inhibition Zone (mm)	Category	Extract Concentration (%)	Mean Inhibition Zone (mm)	Category
Day 1	40	3.5	Moderate	40	3.0	Weak
	60	5.0	Moderate	60	5.0	Moderate
	100	7.0	Strong	100	6.5	Strong
Day 2	40	4.0	Moderate	40	3.5	Moderate
	60	6.0	Moderate	60	5.0	Moderate
	100	7.5	Strong	100	7.0	Strong
Day 3	40	4.0	Moderate	40	3.5	Moderate
	60	6.5	Strong	60	6.5	Strong
	100	9.0	Strong	100	8.5	Strong

Shallot Extract

The shallot (*Allium cepa* L.) extract also demonstrated antibacterial activity against *Mycobacterium tuberculosis*. At 40% concentration, inhibition was weak to moderate (3.0–3.5 mm). The 60% concentration showed moderate inhibition on days 1 and 2, increasing to strong inhibition on day 3 (6.5 mm). The strongest inhibitory effect was observed at 100%

concentration, with inhibition zones increasing from 6.5 mm on day 1 to 8.5 mm on day 3 (Table 2).

Overall, both red betel leaf and shallot extracts exhibited inhibitory effects against *Mycobacterium tuberculosis*, with inhibition strength increasing proportionally with extract concentration and incubation time. The strongest antibacterial activity for both extracts was

consistently observed at the 100% concentration across all observation days.

The one-way analysis of variance (ANOVA)

A one-way analysis of variance (ANOVA) was conducted to examine differences in inhibition zone diameter (UjiZona) across four extract concentrations (40%, 60%, 80%, and 100%). The analysis demonstrated a statistically significant effect of extract concentration on inhibition zone diameter, $F(3, 8) = 134.25, p < .001$. Descriptive statistics showed a progressive increase in mean inhibition zone diameter with

increasing extract concentration: 40% ($M = 2.67 \pm 0.58$ mm), 60% ($M = 6.67 \pm 0.58$ mm), 80% ($M = 9.33 \pm 0.58$ mm), and 100% ($M = 11.67 \pm 0.58$ mm), indicating a clear dose-response relationship. Post hoc analysis using Tukey's honestly significant difference (HSD) test revealed that all pairwise comparisons between concentration groups were statistically significant ($p < .05$). The homogeneous subsets analysis further confirmed that each concentration level formed a distinct subset, with no overlap between groups, indicating that the antibacterial inhibitory effect increased significantly at each incremental concentration level.

Table 3. One-Way ANOVA of Inhibition Zone Diameter (UjiZona) by Extract Concentration

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	134.250	3	44.750	134.250	.000
Within Groups	2.667	8	.333		
Total	136.917	11			

Table 3 illustrates that a one-way analysis of variance (ANOVA) was conducted to compare inhibition zone diameters across four extract concentrations (40%, 60%, 80%, and

100%). The analysis demonstrated a statistically significant difference among groups, $F(3, 8) = 134.25, p < .001$. Post hoc comparisons were performed using Tukey's HSD test.

Table 4. Post Hoc Multiple Comparisons of Inhibition Zone Diameter (UjiZona) Using Tukey HSD

(I) FUjiZona	(J) FUjiZona	Mean Difference (I-J)	Std. Error	p value	95% CI (Lower-Upper)
40%	60%	-4.00*	0.47	< 0.001	-5.51 to -2.49
	80%	-6.67*	0.47	< 0.001	-8.18 to -5.16
	100%	-9.00*	0.47	< 0.001	-10.51 to -7.49
60%	40%	4.00*	0.47	< 0.001	2.49 to 5.51
	80%	-2.67*	0.47	0.002	-4.18 to -1.16
	100%	-5.00*	0.47	< 0.001	-6.51 to -3.49
80%	40%	6.67*	0.47	< 0.001	5.16 to 8.18
	60%	2.67*	0.47	0.002	1.16 to 4.18
	100%	-2.33*	0.47	0.005	-3.84 to -0.82
100%	40%	9.00*	0.47	< 0.001	7.49 to 10.51
	60%	5.00*	0.47	< 0.001	3.49 to 6.51
	80%	2.33*	0.47	0.005	0.82 to 3.84

Table 4 presents post hoc pairwise comparisons that were conducted using

Tukey's honestly significant difference (HSD) test. All pairwise comparisons



between extract concentrations showed statistically significant differences in inhibition zone diameter ($p < .05$). Negative mean differences indicate smaller inhibition zones in the lower concentration group.

Discussion

The findings of this study demonstrate that both red betel leaf (*Piper crocatum*) and shallot (*Allium cepa* L.) extracts exhibit measurable inhibitory effects against *Mycobacterium tuberculosis*, with inhibition zones increasing proportionally to extract concentration and incubation time. These results are consistent with previous *in vitro* studies reporting that plant-derived phytochemicals, particularly flavonoids, alkaloids, tannins, and saponins, possess antimycobacterial and antibacterial activities through mechanisms such as disruption of bacterial cell walls, inhibition of protein synthesis, and interference with enzymatic pathways (Belete & Akhter, 2024; Cowan, 2024). Several recent studies have documented the antimicrobial potential of *Piper* species, including *Piper crocatum*, attributing their efficacy to high phenolic and flavonoid content, which contributes to oxidative stress within bacterial cells (Bangun et al., 2023). Similarly, extracts from *Allium cepa* have been shown to inhibit both Gram-positive and Gram-negative bacteria, including *Salmonella typhi* and *Escherichia coli*, due to sulfur-containing compounds and polyphenols (Barbu et al., 2023). Recent evidence also indicates that *Allium* species may exhibit antimycobacterial activity by altering mycolic acid synthesis, a critical component of the *M. tuberculosis* cell wall (Nguta, 2025). The present findings align with reports suggesting that higher extract concentrations yield stronger antibacterial effects (Luo et al., 2025). However, unlike some earlier studies that focused on single-plant extracts, this study contributes to the growing body of evidence supporting the

use of combined or comparative herbal approaches to enhance antibacterial efficacy (Rossi et al., 2025; Ulfa et al., 2024). From a theoretical nursing perspective, these results are congruent with holistic and integrative health frameworks that emphasize the use of complementary therapies alongside conventional treatment to support patient outcomes (WHO, 2023).

This study provides new empirical evidence that red betel leaf and shallot extracts demonstrate concentration-dependent inhibitory activity against *Mycobacterium tuberculosis* under controlled laboratory conditions. The identification of multiple bioactive phytochemicals and their association with increasing inhibition zones supports existing antimicrobial theories while extending them to a tuberculosis-specific context. Importantly, this study strengthens the conceptual basis for exploring medicinal plants as complementary agents in TB research, particularly in resource-limited settings where access to advanced pharmacological therapies may be constrained. The findings support current scientific discourse advocating for further exploration of traditional medicinal plants as potential adjuncts to tuberculosis management, especially in the context of rising antimicrobial resistance and prolonged TB treatment regimens (Amingad & Hakkimane, 2025; Praveena et al., 2025; Rossi et al., 2025; Tilburt & Kaptchuk, 2008). While this study does not propose replacing standard anti-TB drugs, it provides foundational evidence that may inform future experimental, clinical, and translational research.

From a scientific and conceptual standpoint, this study contributes to the expanding literature on plant-based antimicrobials by providing focused evidence on *Piper crocatum* and *Allium cepa* against *Mycobacterium tuberculosis*. The results reinforce phytochemical and

antimicrobial theories that emphasize synergistic and concentration-dependent effects of natural compounds (Daglia, 2012; Tilburt & Kaptchuk, 2008). These findings may guide future experimental studies, including fractionation of active compounds, molecular mechanism analysis, and preclinical trials, thereby supporting policy discussions related to integrative and complementary medicine within infectious disease control frameworks (WHO, 2023). Several limitations should be acknowledged. First, the study was conducted *in vitro* using a limited number of samples, which may restrict the generalizability of the findings to clinical settings. Second, the use of a laboratory-based experimental design does not account for host immune responses, pharmacokinetics, or potential toxicity in humans. Third, the study did not include drug-resistant *M. tuberculosis* strains, which limits conclusions regarding effectiveness against multidrug-resistant TB. Future studies with larger sample sizes, standardized extract formulations, and *in vivo* models are therefore recommended.

This study has several methodological constraints that warrant critical reflection. The *in vitro* design limits direct extrapolation to clinical effectiveness, as inhibition zone diameters do not account for host factors, pharmacokinetics, or toxicity profiles. Variability in phytochemical composition due to extraction methods, plant maturity, and environmental conditions may also affect reproducibility. MacConkey Agar was used because of its selective and differential properties, allowing for the targeted isolation and growth of Gram-negative enteric bacteria while inhibiting Gram-positive organisms, thereby improving specificity and reducing background contamination during antimicrobial testing. Biosafety considerations were addressed through standard aseptic techniques;

however, the use of laboratory strains rather than multidrug-resistant clinical isolates constrains real-world applicability and highlights the need for higher-level containment and ethical oversight in future studies. Within low- and middle-income country (LMIC) settings, these findings should be interpreted as preliminary evidence supporting locally available plant resources as candidates for further investigation rather than immediate clinical alternatives. Strengthening laboratory capacity, biosafety infrastructure, and regulatory frameworks in LMICs will be essential before phytochemical research can be responsibly translated into antimicrobial development or public health interventions.

The findings of this study are relevant for nursing and healthcare practice, particularly within community health, infection control, and health promotion domains. Nurses play a critical role in tuberculosis prevention, education, and supportive care, and awareness of evidence-based complementary therapies can enhance culturally sensitive patient counseling. The demonstrated antimicrobial potential of red betel leaf and shallot extracts may inform nursing education on traditional medicine use, support community-based TB awareness programs, and encourage interdisciplinary collaboration between nurses, pharmacists, and public health professionals. While clinical application requires further validation, these results underscore the importance of integrating scientific evidence with traditional health knowledge to strengthen holistic nursing care and public health strategies.

Implications and limitations

Conceptually, this study contributes to the growing body of antimycobacterial research by reinforcing the role of phytochemicals as biologically active

compounds with measurable inhibitory potential against bacterial pathogens. It supports theoretical frameworks that position plant-based antimicrobials as a complementary research pathway within global efforts to address antimicrobial resistance. The findings also strengthen the evidence base for ethnobotanical selection as a valid scientific rationale, particularly in LMIC contexts where indigenous knowledge and local biodiversity intersect with public health challenges.

Several limitations should be acknowledged. The *in vitro* design restricts interpretation to laboratory-based bacterial inhibition. It does not allow conclusions regarding clinical efficacy, safety, or therapeutic applicability—the use of standard laboratory strains rather than clinical or drug-resistant isolates further limits external validity. Additionally, variability in phytochemical composition, potential measurement bias in inhibition zone assessment, and the absence of advanced molecular or mechanistic analyses constrain the depth of interpretation. These limitations highlight the need for cautious interpretation of findings without conflating them with future research directions or clinical recommendations.

Relevance to Practice

The relevance of this study to nursing practice lies primarily in its contribution to education and health policy rather than immediate clinical application. By strengthening nurses' understanding of phytochemical research, antimicrobial mechanisms, and laboratory-based evidence, the findings support the integration of antimicrobial stewardship, infection prevention, and rational use of alternative therapies into nursing curricula. At the policy level, the study underscores the importance of nurse involvement in evidence appraisal, guideline development,

and advocacy for safe, regulated exploration of plant-based antimicrobials, particularly within resource-limited settings. This knowledge equips nurses to contribute meaningfully to interdisciplinary decision-making, patient education, and policy discussions without overstating clinical efficacy.

Conclusion

This *in vitro* study provides preliminary evidence that red betel leaf (*Piper crocatum*) and shallot (*Allium cepa* L.) extracts exhibit measurable inhibitory activity against *Mycobacterium tuberculosis* cultured on Lowenstein–Jensen medium, with statistically significant increases in inhibition zone diameters observed at higher extract concentrations. The identification of bioactive phytochemicals supports their potential contribution to this inhibitory effect. However, these results are limited to controlled laboratory conditions and do not reflect clinical complexity, including host immune responses, pharmacokinetics, toxicity profiles, or interaction with established anti-tuberculosis regimens. Consequently, the findings cannot be interpreted as evidence of clinical efficacy or direct nursing application. From an applied nursing and health perspective, this study enhances foundational knowledge regarding the antibacterial properties of traditional plant extracts and underscores the importance of rigorous evaluation of complementary therapies. Further research is warranted, including standardized extract formulation, *in vivo* studies, and safety assessment, to clarify the relevance of these findings for future therapeutic or health-promotion strategies in tuberculosis care.

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CrediT Authorship Contributions Statement

Seri Rayani Bangun: Conceptualization, Methodology, Writing – Original Draft.

Desriati Sinaga: Software, Validation, Formal Analysis.

R. Oktaviance S.: Investigation, Resources.

Lilis Novitarum: Data Curation, Visualization, Project Administration.

Mestiana Br Karo: Data Curation, Funding Acquisition.

Herlina J. EL-Matury: Supervision, Writing – Review & Editing.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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