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The Effect of Betel Leaf Powder Preparation on the Minimum Inhibitory Concentration (MIC) of Salmonella sp. Growth

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ABSTRACT

Background: The high incidence of gastroenteritis and typhoid fever caused by *Salmonella* in Indonesia is worsened by bacterial resistance to commercial antibiotics. Therefore, herbal plants such as betel leaf preparations need to be investigated as alternative antibacterial agents. **Objective:** This study aimed to determine the effect of betel leaf (*Piper betle* L.) powder preparations on the Minimum Inhibitory Concentration (MIC) of *Salmonella* sp. growth. **Research Methods:** This was a laboratory experimental study with a post-test only control group design. Betel leaf powder was tested at doses of 0.5–10 mg with three replicates. Inhibitory activity was assessed using the well diffusion method on Mueller Hinton Agar by observing clear zones. Ampicillin served as the positive control and sterile distilled water as the negative control. Differences between doses were analyzed using the Kruskal-Wallis test. **Research Results:** The ampicillin positive control produced an inhibition zone of 5 mm, whereas the negative control showed dense bacterial growth without a clear zone. Betel leaf powder at doses of 0.5–9.5 mg left the medium turbid, with an inhibition zone of 0 mm. A thin clear ring was visible only at the highest dose of 10 mg, with a mean diameter of 0.1 mm. The Kruskal-Wallis test showed a significant effect of dose ($p < 0.05$). **Conclusion:** Betel leaf powder suspended directly in sterile distilled water did not inhibit the growth of *Salmonella* sp. at low to moderate doses (0.5–9.5 mg). Inhibition appeared only as a very thin zone at 10 mg, so the exact MIC value could not be determined.

Keywords: *Salmonella* sp., Betel Leaf Powder, Minimum Inhibitory Concentration, Well Diffusion, Sterile Distilled Water.

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Introduction

Salmonella sp. is a facultative anaerobic, Gram-negative, non-spore-forming pathogen of the family Enterobacteriaceae. The bacterium has strong environmental resilience, including under frozen or dry conditions, and can maintain its virulence in various food media with a minimum water activity of 0.94 at a pH below 5.5 (Billah & Rahman, 2024). *Salmonella* sp. infection can cause digestive disorders ranging from gastroenteritis to a severe systemic manifestation known as typhoid fever (Osphronem & Ikan, 2017).

The national prevalence of typhoid fever is around 1.6%, equivalent to 350–810 cases per 100,000 population. Locally, the NTB Provincial Health Office recorded 1,214 typhoid fever cases by early 2023, placing the disease among the top five health complaints of the community (Muflih et al., 2025; Dinkes NTB, 2023). Medical management of *Salmonella* infection still relies on antibiotics. However, massive and irrational antimicrobial use has triggered antibiotic resistance in various foodborne bacterial pathogens, including *Salmonella* (Punchihewage-don, 2024).

This resistance reduces the efficacy of conventional antibiotics and leads to treatment failure. Therefore, the exploration of alternative therapeutic agents is urgently needed. One potential herbal ingredient is betel leaf (*Piper betle* L.), whose antibacterial potential comes from its secondary metabolites. Bioactive compounds such as phenolics (flavonoids, saponins, tannins), alkaloids, and terpenoids play a role in inhibiting pathogenic microbes (Amrillah et al., 2023). Although the antimicrobial activity of betel leaf has been widely documented, studies on the Minimum Inhibitory Concentration (MIC) of raw, water-based simplicia powder preparations against *Salmonella* sp. remain very limited. Therefore, this study aimed to determine the effect of betel leaf powder preparations on the MIC of *Salmonella* sp. growth at doses of 0.5 mg to 10 mg.

Research Methods

The study was conducted at the Microbiology Laboratory of Poltekkes Kemenkes Mataram from March to May 2026. A 24-hour-old *Salmonella* sp. isolate was suspended in 0.9% physiological NaCl to match the 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL).

A post-test only control group design was used, with 60 experimental units: 20 graded dose treatments (0.5–10 mg), each repeated three times and allocated by simple randomization. Commercial betel leaf powder was dissolved in 1 mL of sterile distilled water. Ampicillin was used as the positive control and sterile distilled water as the negative control. Antibacterial testing was performed by the well diffusion method: wells 6 mm in diameter were made perpendicular to the surface of Mueller Hinton Agar (MHA), filled with 75 µL of test solution, and incubated for 24 hours at 37°C. The MIC was assessed

from the appearance of the smallest clear zone around the well. Differences between doses were analyzed using the Kruskal-Wallis test.

Results and Discussion

The results of the *Salmonella* sp. growth test to determine the Minimum Inhibitory Concentration (MIC) of betel leaf powder are presented in Table 4.1.

Table 4.1. Minimum Inhibitory Concentration (MIC) Test Results of Betel Leaf Powder against *Salmonella* sp.

Treatment Group	Dose (mg)	Medium Condition	Mean Inhibition Zone (mm)
Positive Control (C+)	Ampicillin 500 mg	Clear	5.0
Negative Control (C−)	Sterile distilled water	Turbid	0.0
P1–P19	0.5–9.5	Turbid	0.0
P20	10.0	Thin clear ring	0.1

Based on the laboratory measurements in Table 4.1, the medium remained turbid in the treatment groups from 0.5 mg (P1) to 9.5 mg (P19), indicating that no clear zone was formed (inhibition zone of 0 mm) in any replicate. In contrast, a thin clear ring with a mean diameter of 0.1 mm was visually detected only in the highest dose group, 10 mg (P20), in all three replicates.

For comparison, the positive control group (C+) treated with ampicillin showed a clear zone 5 mm in diameter. Meanwhile, the negative control group (C−) treated with sterile distilled water showed a turbid suspension due to dense bacterial growth, without any clear zone (0 mm).

The Kruskal-Wallis test showed a significant effect of betel leaf powder dose on the inhibition of *Salmonella* sp. growth ($p < 0.05$). However, an inhibitory effect appeared only at the highest dose of 10 mg (0.1 mm).

Discussion

The observations showed that betel leaf powder suspended at low to medium doses (0.5–9.5 mg) was unable to form an inhibition zone against *Salmonella* sp. The agar medium that remained turbid indicates that the release and diffusion of active phytochemical compounds from the betel leaf powder matrix into the agar had not occurred effectively.

Theoretically, betel leaf is rich in bioactive secondary metabolites, such as phenolic compounds, eugenol, saponins, tannins, and essential oils, which can damage the integrity of microbial cells. However, the absence of inhibition zones at most doses suggests that these active compounds may still be trapped in the simplicia powder tissue. This is presumably influenced by the sample preparation method, in which the test solution was only a coarse powder suspension in sterile distilled water without maceration or stepwise partitioning, so the active compounds were not optimally extracted (Manarisip et al., 2020).

In addition to extraction, the characteristics of the test bacterium also influenced the results. As a Gram-negative bacterium, *Salmonella* sp. has an outer membrane layered with lipopolysaccharides (LPS) and porin proteins that act as a selective filter, making it difficult for the crude antimicrobial

components of betel leaf powder to diffuse into the cell (Lestari & Ardiningsih, 2016). Therefore, the amount of active substances dissolved by simple mixing with water in this study presumably did not reach the minimum threshold needed to suppress bacterial growth significantly at low doses.

Conclusion

A. Conclusion

1. The inhibitory activity of betel leaf powder against *Salmonella* sp. was visually detected only at the highest dose of 10 mg, as a very thin inhibition zone (mean 0.1 mm), whereas no inhibition zone formed at doses of 0.5–9.5 mg. Thus, the exact MIC value could not be determined.
2. There was a significant effect of betel leaf powder dose on the growth of *Salmonella* sp. based on the nonparametric Kruskal-Wallis test ($p < 0.05$).

B. Suggestions

1. *For future researchers:* it is recommended to process betel leaf into a thick extract using standard extraction methods, such as maceration with a suitable organic solvent (e.g., 96% ethanol), so that the antibacterial active compounds are extracted to the fullest.
2. *For the public:* this information is expected to serve as a basis for knowing that betel leaf has antibacterial potential, although its application still requires appropriate preparation methods and doses.
3. *For educational institutions:* the results of this study are expected to serve as additional references and baseline data in microbiology laboratories on the effectiveness of crude herbal preparations.

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