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Antibacterial Properties of *Piper betle* Ethanolic Extract against Common Pathogenic Microorganism

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Structured Abstract

Background: *Piper betle* is a known medicinal plant in Asia, found specifically in Southest Asian nations. Extract of *P. betle* leaves demonstrated antibacterial activity against Gram-positive and Gram-negative bacteria such as *Escherichia coli (E, coli)* and the fungus *Candida albicans (C. albicans)*. However, there is limited research focusing on its potential against *Salmonella Typhi (S. typhi)* and *Bacillus cereus (B. cereus)*. This study aims to explore *P. betle* ethanolic extract antibacterial activity against *S. typhi* and *B. cereus* using disc diffusion, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) assays.

Methods: A total of 300g of *P. betle* powder was soaked in 96% ethanol for five days, filtered, and concentrated using a rotary evaporator at 52°C. Extract concentrations from 62.5 mg/mL to 1000 mg/mL were prepared in 10% DMSO for antibacterial testing. Antibacterial activity was assessed using the Kirby-Bauer disk diffusion method following CLSI guidelines. The MIC and MBC were determined using a broth microdilution assay in Mueller-Hinton Broth with 2-fold serial dilutions ranging from 62.5 mg/mL to 1000 mg/mL. MBC was confirmed by subculturing onto Mueller-Hinton Agar plates.

Results: The results of this study showed that *P. betle* ethanolic extract exhibited antibacterial activity against *E. coli, B. cereus*, and *S. typhi*. All three bacteria were found to be susceptible at 1000 mg/mL. The MIC for all tested bacteria was < 62.5 mg/mL, indicating that bacterial growth was inhibited at this concentration. Similarly, the MBC values were also < 62.5 mg/mL, confirming the bactericidal effect of the extract. The discrepancies between the disc diffusion and MIC/MBC results for *P. betle* may be attributed to the limited diffusion of bioactive compounds like eugenol in solid media and the qualitative nature of the disc diffusion method.

Conclusion: In conclusion, this study highlights the antibacterial potential of *P. betle* ethanolic extract, which showed strong antibacterial activity, especially against *S. typhi* and *B. cereus*, with the highest inhibition demonstrated at 1000 mg/mL. The MIC and MBC for all tested bacteria were < 62.5 mg/mL, showing that the extract effectively inhibits bacterial growth and kills bacteria at these concentrations.

Keywords: *Piper betle,* Antibiotic Resistance, Antibacterial Activity, *Salmonella Typhi, Bacillus cereus*

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