BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 15, Number 1, April 2026 | Pages: 715-721 | DOI: 10.14421/biomedich.2026.151.715-721 ISSN 2540-9328 (online)

Antibacterial Activity and MIC/MBC Evaluation of an Active Fraction of *Paederia foetida* Leaves Against Pathogenic Bacteria

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Manuscript received:02 November 2025. Revision accepted: 22 May 2026, Published: 26 May 2026.

Abstract

Antibacterial resistance remains a global health concern, increasing the need for natural plant-based alternatives. This study aimed to evaluate the antibacterial activity of the active fraction from *Paederia foetida* Linn. leaves against six pathogenic bacteria: *Staphylococcus aureus, Bacillus subtilis, Shigella dysenteriae, Salmonella typhi, Cutibacterium acnes*, and enteropathogenic *Escherichia coli* (EPEC). The Kirby –Bauer disk-diffusion method was used to measure inhibition zones and the broth dilution method to determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Results showed that the active fraction exhibited variable antibacterial activity, with the highest inhibition zones recorded against EPEC (6.5 mm), *B. subtilis* (6.0 mm), and *S. dysenteriae* (5.0 mm) at 100% concentration. The MIC values ranged from 100 to 200 mg/mL, while MBC values ranged from 200 to 300 mg/mL, confirming bactericidal activity particularly against EPEC and *B. subtilis.* Compared to the crude extract we reported previously, the fraction displayed lower antibacterial activity, suggesting that the antibacterial effect of *P. foetida* may rely on synergistic interactions among multiple secondary metabolites present in the crude extract. These findings indicate that the active fraction of *P. foetida* leaves possesses potential antibacterial properties, suggesting that further studies should focus on exploring synergistic effects among fractions and confirming antibacterial efficacy through in vivo evaluation.

Keywords: antibacterial; Bacillus subtilis; disk-diffusion; EPEC; MBC; MIC.

Abbreviations: EPEC: Enteropathogenic *Escherichia coli*; MBC: Minimum Bactericidal Concentration; MIC: Minimum Inhibitory Concentration

INTRODUCTION

Antimicrobial resistance (AMR) has become one of the most serious public health challenges worldwide, reducing the efficacy of conventional antibiotics and increasing treatment failure in infectious diseases (Ajulo et al., 2024). The World Health Organization (2022) emphasized that antibiotic-resistant pathogens, including *Staphylococcus aureus* and *Escherichia coli*, continue to pose significant threats to global health. Consequently, there is an urgent need to identify novel antimicrobial agents derived from natural and sustainable sources. Medicinal plants are promising candidates because they contain a wide range of secondary metabolites, such as flavonoids, alkaloids, tannins, terpenoids, and saponins, that often act synergistically to inhibit bacterial growth through multiple mechanisms (Khingsai et al., 2025; Angelini et al., 2024; Yunita et al., 2023; AlSheikh et al., 2020).

Paederia foetida Linn. (Rubiaceae), commonly known in Indonesia as *sembukan*, is a climbing herb widely distributed in tropical Asia, including Indonesia (Yunita et al., 2023; Yunita et al. 2025[a]). Traditionally, it has been used to treat diarrhea, dysentery (Dwivedi et al., 2025; Yunita et al., 2025[b]), rheumatism (Barai et al., 2025), and inflammatory diseases (Roy et al., 2024). Phytochemical analyses have revealed that *P. foetida* leaves contain several classes of bioactive compounds associated with antimicrobial and anti-inflammatory effects (Savitri et al., 2024). Several recent studies reported that leaf extracts of *P. foetida* possess antibacterial activity against both Gram-positive and Gram-negative bacteria. For instance, Ugwu et al. (2025) demonstrated that *P. foetida* extract inhibited *S. aureus* biofilm formation by interfering with quorum-sensing signals, while Priyanto et al. (2022) observed moderate antibacterial and antibiofilm activity of ethanolic extracts against *E. coli* and *Mycobacterium smegmatis*. These findings collectively support the potential of *P. foetida* as a source of plant-derived antibacterial compounds.

However, most previous studies have focused primarily on crude extracts, with limited quantitative evaluations such as Minimum Inhibitory Concentration

(MIC) and Minimum Bactericidal Concentration (MBC). The contribution of fractionated extracts and the influence of fractionation on antibacterial potency have rarely been explored. In particular, it remains unclear whether the antibacterial effects of *P. foetida* arise from specific active components or from synergistic interactions among multiple constituents in the crude extract. Our previous investigation (Yunita et al., 2023) reported that the ethanolic extract of *P. foetida* exhibited notable antibacterial activity against several pathogenic bacteria, thereby encouraging further investigation into its active fraction to better characterize its bioactive potential.

In the present study, the active fraction of *P. foetida* leaves was evaluated for its antibacterial activity against six pathogenic bacteria, including *S. aureus, Bacillus subtilis, Shigella dysenteriae, Salmonella typhi, Cutibacterium acnes*, and enteropathogenic *E. coli* (EPEC). Among these, EPEC and *B. subtilis* were selected for MIC and MBC determination because they represent Gram-negative and Gram-positive bacterial models, respectively, and exhibited the most pronounced sensitivity in preliminary screening. This approach provides a focused and quantitative assessment of antibacterial potency while maintaining methodological efficiency. Therefore, the aim of this study was to evaluate the antibacterial activity of the active fraction from *P. foetida* leaves through disk-diffusion and broth microdilution assays, to determine MIC and MBC values against representative bacterial strains, and to compare the antibacterial activity of the fraction with that of the crude extract reported previously.

MATERIALS AND METHODS

Study area

This study was conducted at the Microbiology Laboratory, Faculty of Medicine, Universitas Pattimura, Ambon, Indonesia, from March to August 2025. The plant material of *Paederia foetida* Linn. was collected from the Poka area, Ambon City, Maluku Province, Indonesia (3°39′18″S, 128°11′47″E; Figure 1). The plant was determined at the Herbal Materia Medica Laboratory, Batu, East Java, Indonesia, under reference number 000.9.3/2181/102.20/2024. Ethical approval for this research was obtained from the Ethics Committee of the Faculty of Medicine, Universitas Pattimura, Ambon, Indonesia, with approval number No.152/FK-KOM.ETIK/VII/2025.

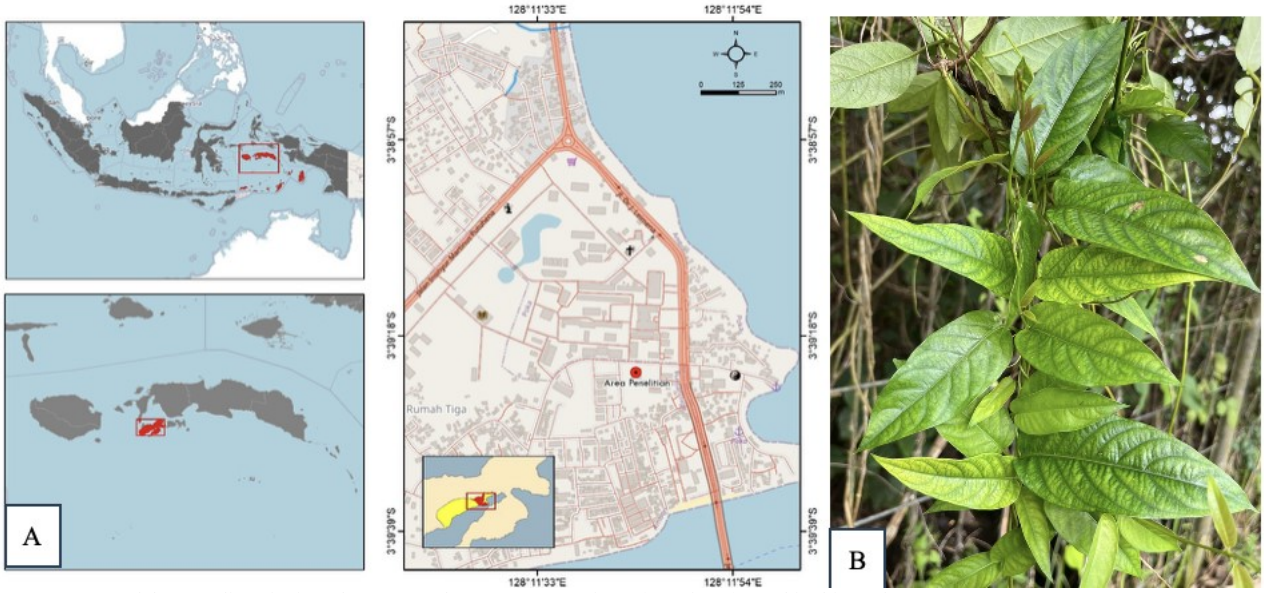

Figure 1. A. Map of the sampling site in Poka area, Ambon; B. Leaves of *Paederia foetida* used in this study.

Procedures

Preparation of plant materials and active fractions

Fresh leaves of P. foetida were collected, air-dried at ambient room temperature (25–28 °C), and finely ground. Approximately 500 g of the powdered leaves was macerated in 96% ethanol for 72 h with occasional stirring, then filtered and concentrated under reduced pressure at 45 °C using a rotary evaporator to obtain the crude ethanolic extract (Yunita et al., 2023). Following extraction, fractionation of the crude extract was carried out by liquid–liquid partitioning using solvents of increasing polarity, namely n-hexane, ethyl acetate, and water, to obtain separate fractions (Priyanto et al., 2022). Each fraction was evaporated to dryness and stored at 4 °C in sealed vials until further use. The fraction showing the highest antibacterial activity in preliminary screening was selected as the active fraction for antibacterial testing.

Bacterial strains and culture conditions

Six bacterial strains were used as test organisms, *namely Staphylococcus aureus, Bacillus subtilis, Shigella dysenteriae, Salmonella typhi, Cutibacterium acnes*, and enteropathogenic *Escherichia coli* (EPEC). All isolates were obtained from the Microbiology Laboratory, Politeknik Kesehatan Jember, Indonesia, and maintained on nutrient agar slants. Prior to testing, the bacterial cultures were sub-cultured at 37 °C for 24 h to obtain fresh active cultures. The bacterial suspensions were then adjusted to a turbidity equivalent to 0.5 McFarland standard (approximately 1×10^8 CFU/mL) using sterile saline solution.

Antibacterial testing

The antibacterial activity of the active fraction was evaluated using the Kirby–Bauer disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2023). Each bacterial strain (*S. aureus, B. subtilis, S. dysenteriae, S. typhi, C. acnes*, and EPEC) was adjusted to 0.5 McFarland standard (≈ 1×10^8 CFU/mL). Mueller–Hinton agar plates were uniformly swabbed with the standardized inoculum and allowed to dry for 10–15 min. Sterile paper disks with 6 mm diameter were impregnated with 20 µL of the active fraction at concentrations of 100%, 80%, 60%, 40%, 20%, and 10% (w/v in sterile distilled water). Disks were placed on the agar surface using sterile forceps, ensuring even spacing. Amoxicillin (25 µg/disc) was used as the positive control, while sterile distilled water served as the negative control. The plates were incubated at 37 °C for 24 h (anaerobic condition for *C. acnes*). Each assay was performed in duplicate, and results were expressed as mean ± standard deviation (SD) for further analysis. After incubation, the inhibition zones were measured in millimeters (mm) using a vernier caliper. The inhibition zone diameter was calculated as the mean of both measurements after subtracting the disk diameter (6 mm), following the formula (Yunita et al., 2023):

$$\frac{(Dv - Dc) + (Dh - Dc)}{2}$$

where:

Dv = vertical diameter (mm)

Dh = horizontal diameter (mm)

Dc = disk diameter (6 mm)

Determination of MIC and MBC

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the active fraction were determined against *B. subtilis* and EPEC, which exhibited the largest inhibition zones in the disk diffusion assay and represent Gram-positive and Gram-negative groups, respectively. MIC determination followed the broth microdilution method as described by Wiegand et al. (2008) with slight modification. Serial dilutions of the active fraction were prepared in Mueller–Hinton broth, and MIC/MBC values were ultimately determined at concentrations of 100–300 mg/mL. Each well of a sterile 96-well microplate was filled with 100 µL of the diluted sample and inoculated with 100 µL of bacterial suspension adjusted to 10^5 CFU/mL. The plates were incubated at 37 °C for 24 h. The MIC value was defined as the lowest concentration showing no visible turbidity (absence of bacterial growth). For MBC determination, 10 µL aliquots from wells showing no growth were spread on nutrient agar plates and incubated for another 24 h at 37 °C. The MBC was defined as the lowest concentration producing no visible colony growth on agar. The ratio of MBC to MIC (MBC/MIC) was used to classify antibacterial activity as bactericidal (MBC/MIC ≤ 4) or bacteriostatic (MBC/MIC > 4), following the interpretive criteria of Kowalska-Krochmal & Dudek-Wicher (2021).

RESULTS AND DISCUSSION

Yield of the Active Fraction

The yield of the active fraction obtained from the ethanolic extract of *Paederia foetida* Linn. leaves was calculated and presented in Table 1. From a total of 45 g of concentrated extract, 18.9 g of active fraction was obtained, corresponding to a yield of 42%

Table 1. Yield of active fraction from *P. foetida* leaves extract.

Weight of crude extract (g)	Weight of active fraction	Yield (%)
45	18.9	42

The yield indicates efficient separation of bioactive components, similar to those reported for polar fractions of other medicinal plants (Priyanto et al., 2022). This fraction was subsequently used for antibacterial activity testing.

Antibacterial activity of the active fraction

The antibacterial activity of the active fraction of *P. foetida* leaves was evaluated against six pathogenic bacteria: *Staphylococcus aureus, Bacillus subtilis, Shigella dysenteriae, Salmonella typhi, Cutibacterium acnes*, and enteropathogenic *Escherichia coli* (EPEC). Amoxicillin (25 µg/disc) was used as the positive control and sterile aquadest as the negative control. The results show a concentration-dependent increase in inhibition zone diameters (Figure 2; Table 2). The largest inhibition zones were observed against EPEC (6.5 ± 0.7 mm) and *B. subtilis* (6.0 ± 1.4 mm), followed by *S. dysenteriae* (5.0 ± 0.0 mm) and *S. aureus* (4.5 ± 0.7 mm). The weakest inhibition was recorded against *S. typhi* (2.5 ± 0.7 mm) and *C. acnes* (2.0 ± 1.4 mm). In comparison, the positive control showed an inhibition zone of 17.5 ± 3.5 mm, whereas the negative control showed no inhibition.

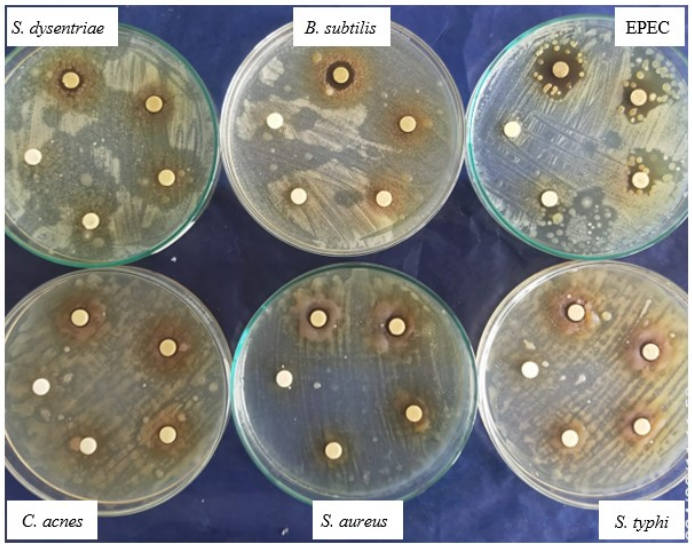

Figure 2. Antibacterial activity of *P. foetida leaf* fraction against six pathogenic bacteria.

Table 2. Inhibition zones of the active fraction of *P. foetida* leaves against pathogenic bacteria.

Bacterial Strain	Average inhibition zone (mm) ± SD				
	10%	25%	50%	75%	100%
Staphylococcus aureus	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	3.5 ± 0.7	4.5 ± 0.7
Bacillus subtilis	1.5 ± 0.7	2.0 ± 0.0	2.5 ± 0.7	3.5 ± 0.7	6.0 ± 1.4
Shigella dysenteriae	1.0 ± 0.0	1.0 ± 0.0	2.0 ± 1.4	3.5 ± 0.7	5.0 ± 0.0
Salmonella typhi	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	2.0 ± 0.0	2.5 ± 0.7
Cutibacterium acnes	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.5 ± 0.7	2.0 ± 1.4
Enteropathogenic *Escherichia coli* (EPEC)	1.5 ± 0.7	2.0 ± 0.0	2.5 ± 0.7	3.5 ± 0.7	6.5 ± 0.7

These findings confirm that the *P. foetida* fraction exhibits a broad-spectrum antibacterial activity. The antibacterial activity varied among bacterial species, with EPEC exhibiting the highest susceptibility. The positive control (amoxicillin) produced a markedly higher inhibition zone (17.5 ± 3.5 mm), as expected from a standard antibiotic, while the negative control (sterile distilled water) showed no inhibition (0.0 mm). This confirms that the observed activity was solely due to the bioactive components of the *P. foetida* fraction. To illustrate the antibacterial patterns more clearly, the comparative activity of the fraction at 100% concentration against all tested bacteria is presented in Figure 2. The visualization highlights differences in susceptibility among the bacterial strains, highlighting the stronger inhibition zones observed for EPEC and *B. subtilis*.

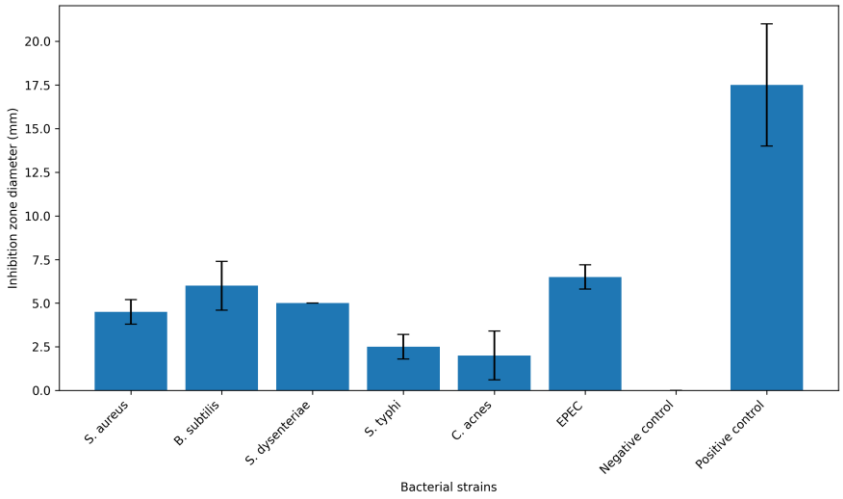

Figure 3. Comparison of inhibition zones of the *P. foetida* active fraction at 100% concentration against six pathogenic bacteria.

Determination of MIC and MBC

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) evaluation were performed against EPEC and *B. subtilis*, which exhibited the largest inhibition zones in the disk diffusion test (Table 3). These two species were selected to represent the structural diversity of bacterial cell walls, where EPEC represents Gram-negative bacteria with an outer membrane containing lipopolysaccharides, while *B. subtilis* represents Gram-positive bacteria with a thick peptidoglycan layer. This selection allows for the assessment of the fraction's antibacterial efficacy across both major bacterial groups while maintaining experimental focus on the most responsive strains.

Table 3. The MIC and MBC evaluation of the active fraction from *P. foetida* leaves against EPEC and *B. subtilis.*

Bacterial Strain	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC Ratio	Interpretation
EPEC	100	200	2.0	Bactericidal
B. subtilis	200	300	1.5	Bactericidal

Table 3 shows that the active fraction exhibited measurable antibacterial potency against both EPEC and *B. subtilis*. The MIC value represents the lowest concentration that inhibits visible bacterial growth, while the MBC corresponds to the minimum concentration that produces complete bacterial killing. The MBC/MIC ratios for EPEC (2.0) and *B. subtilis* (1.5) were both ≤ 4, confirming the bactericidal nature of the fraction according to standard interpretive criteria (Pankey & Sabath, 2004; Kowalska-Krochmal & Dudek-Wicher, 2021).

Discussion

The antibacterial evaluation of the *Paederia foetida* Linn. leaf fraction revealed measurable inhibitory and bactericidal activities against both Gram-positive and Gram-negative bacteria. Among the six tested pathogens, *Bacillus subtilis* and enteropathogenic *Escherichia coli* (EPEC) exhibited the largest inhibition zones in the disk-diffusion assay (6.0 ± 1.4 mm and 6.5 ± 0.7 mm, respectively), prompting further quantitative assessment through MIC and MBC determination. The MIC and MBC values were 100 and 200 mg/mL for EPEC, and 200 and 300 mg/mL for *B. subtilis,* respectively. The corresponding MBC/MIC ratios (2.0 and 1.5) confirmed bactericidal activity, as the ratio was ≤ 4 according to accepted interpretive criteria (Pankey & Sabath, 2004; Kowalska-Krochmal & Dudek-Wicher, 2021).

These findings are consistent with previous reports that *P. foetida* is a source of antimicrobial secondary metabolites. Priyanto et al. (2022) reported MIC values ranging from 23.43 to 125 µg/mL for the leaves extract of *P. foetida* against *E. coli* and *Mycobacterium smegmatis*, with inhibition zones of 26 ± 1.4 mm and 37.6 ± 0.41 mm respectively. Santajit et al. (2024) further demonstrated antibiofilm and anti-quorum sensing activity of the leaf extract against *S. aureus*. Differences observed in inhibition zones between studies may result from extraction solvent, fractionation, and compound diffusion in agar medium, as reported by Balouiri et al. (2016).

The bactericidal effects of the *P. foetida* fraction may arise from synergistic interactions among multiple phytochemicals, including iridoid glycosides, flavonoids, saponins, and phenolic acids, which are known to compromise bacterial cell wall integrity and metabolic processes (Yunita et al., 2023; Priyanto et al., 2022). Flavonoids and phenolic compounds, in particular, disrupt the cytoplasmic membrane and induce leakage of intracellular materials through interactions with lipid bilayers (Cushnie & Lamb, 2011; Balouiri et al., 2016). This dual mechanism, namely membrane disruption and enzyme inhibition, explains the concentration-dependent bactericidal behavior observed in this study.

Compared with peptide-based antimicrobials, such as those derived from *Saccharomyces boulardii* (Venkateswarulu et al., 2019), which demonstrated MIC values of 10–250 mg/mL and MBC/MIC ratios of 1.5–2.0 against *S. aureus* and *B. cereus*, the antibacterial profile of *P. foetida* is remarkably similar in pharmacodynamic behavior. Although the active fraction here is not a peptide, its functional mimicry of antimicrobial peptides (AMPs) – through electrostatic interaction and membrane destabilization – suggests that *P. foetida* metabolites may act as natural AMP analogs. This highlights a promising role for phytochemicals as eco-friendly alternatives to peptide antibiotics.

Furthermore, the correlation between MIC and MBC values observed in this study mirrors patterns described by Rodríguez-Melcón et al. (2021) in *Listeria monocytogenes,* where a strong correlation ($r = 0.85$–0.99) between inhibitory and bactericidal thresholds was noted across diverse biocides. This suggests that agents with strong inhibitory potential are likely to exhibit cidal effects once a two-fold concentration increase is reached, a phenomenon also evident in the present data, where doubling the concentration from 100 to 200 mg/mL converted inhibitory activity into bactericidal action. Thus, the antibacterial response of *P. foetida* follows the classical concentration-dependent pharmacodynamic model, similar to that of conventional antibiotics and biocides.

Interestingly, EPEC exhibited a lower MIC (100 mg/mL) than *B. subtilis* (200 mg/mL), contrary to the general expectation that Gram-negative bacteria are more resistant due to their outer membrane barrier. This observation may be explained by the polarity of the active fraction, which contains hydrophilic phenolics capable of penetrating or disrupting the lipopolysaccharide layer of Gram-negative bacteria (Santajit et al., 2024). In contrast, the thick but porous peptidoglycan of Gram-positive bacteria may slow compound penetration, requiring slightly higher concentrations to achieve equivalent bactericidal effects (Azmy et al., 2025).

Although the inhibition zones observed in the disk-diffusion assay were relatively small, the MIC and MBC results confirmed antibacterial activity. This discrepancy likely arises from the limited diffusion of high-molecular-weight polar compounds through the agar matrix, a well-documented phenomenon in plant-derived extracts (Balouiri et al., 2016). Consequently, the disk diffusion method may underestimate the actual antibacterial potential of *P. foetida* fractions, whereas broth-based MIC and MBC assays reveal their true efficacy under homogeneous conditions. Similar patterns were observed by Priyanto et al. (2022), reporting that *P. foetida* leaf extracts with small inhibition zones still demonstrated significant MIC activity against *E. coli,* underscoring that agar diffusion does not necessarily reflect true bactericidal strength.

When compared to previous studies on the crude ethanolic extract (Yunita et al., 2023), the active fraction displayed a narrower yet more focused antibacterial spectrum, indicating enrichment of potent compounds through solvent partitioning. The 42% yield of the active fraction also highlights the efficiency of the extraction and suggests that large quantities of active metabolites are present in the polar phase. Although crude fractions often require higher concentrations for bacterial killing, their synergistic interactions may contribute to measurable bactericidal activity despite relatively high concentrations (Wiegand et al., 2008).

Taken together, the findings demonstrate that *P. foetida* fractions exert concentration-dependent bactericidal effects (MBC/MIC $\leq$ 2) comparable in pharmacodynamic behavior to natural antimicrobial peptides and synthetic biocides. These results reinforce the ethnomedicinal use of *P. foetida* for treating infections and justify its continued exploration as a phytotherapeutic agent. Future research should focus on bioassay-guided isolation of specific active compounds, synergistic fraction recombination, and *in vivo* validation to assess clinical safety and therapeutic potential. Recent toxicological findings also demonstrated that *P. foetida* leaf extract exhibited acceptable acute oral safety profiles in Wistar rats, supporting its further development as a phytotherapeutic candidate (Yunita et al., 2026)

CONCLUSIONS

The active fraction of *Paederia foetida* leaves exhibited antibacterial activity against six tested pathogenic bacteria, with the strongest inhibition observed against enteropathogenic *Escherichia coli* (EPEC) *(*6.5 ± 0.7 mm) and *Bacillus subtilis* (6.0 ± 1.4 mm) at 100% concentration. Although the inhibition zones were smaller than those shown by the positive control (amoxicillin), the active fraction demonstrated concentration-dependent antibacterial effects, indicating the presence of bioactive compounds with inhibitory potential. The MIC and MBC assays further confirmed antibacterial activity, with MIC values ranging from 100 to 200 mg/mL and MBC values ranging from 200 to 300 mg/mL. The MBC/MIC ratios of 2.0 and 1.5 for EPEC and *B. subtilis*, respectively, indicate a bactericidal mode of action. Overall, these findings support the traditional use of *P. foetida* as a medicinal plant and suggest its potential as a natural source of antibacterial compounds. Further studies are recommended to isolate specific active compounds, evaluate synergistic interactions among fractions, and confirm antibacterial efficacy through *in vivo* investigations.

Acknowledgements: The authors would like to express their sincere gratitude to the Faculty of Medicine, Universitas Pattimura, for providing laboratory facilities and research support. The authors also acknowledge the Herbal Materia Medica Laboratory, East Java, for plant determination.

Authors' Contributions: Melda Yunita designed the study, supervised the research, and revised the manuscript. Muhamad Sadam Safutra & Is Ikhsan Hataul carried out data analysis and interpretation. Siti Nur Azizah & Rosida Rosida performed the extraction, fractionation, and antibacterial assays. All authors contributed to the preparation of the manuscript, read, and approved the final version for submission.

Competing Interests: The authors declare that there are no competing interests.

Funding: This study was financially supported by the Faculty of Medicine, Universitas Pattimura, through the PNBP Grant under the Faculty's Flagship Research Program (Contract No. 1192/UN13/SK/2025).

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