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Original Research

Screening of peat soil bacterial isolates producing antibacterial compounds against *Salmonella* Typhi and Extended-Spectrum Beta-Lactamase (*ESBL*)-producing *Escherichia coli*

Munawarah Fuaddah[1*], Dede Mahdiyah[1], Risyda Komaliya[2], Putri Vidiasari Darsono [1]

[1]*Department of Pharmacy, Faculty of Health, Sari Mulia University, Banjarmasin, Indonesia*
[2]*Department of Pharmacy Profession, Faculty of Health, Sari Mulia University, Banjarmasin, Indonesia*

[*]Corresponding author: fuaddahmunawarah@gmail.com

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ABSTRACT

Background: Peat soil, with its acidic pH and high organic content, supports diverse microorganisms capable of producing secondary metabolites with potential antibacterial activity. These compounds may serve as new sources for antimicrobial development.
Objective: This study aimed to evaluate the antibacterial potential of bacterial isolates from peat soil against *Salmonella typhi* and Extended-Spectrum Beta-Lactamase (ESBL)-producing *Escherichia coli*.
Method: Bacterial isolation was performed using the pour plate method on Trypticase Soy Agar (TSA) medium. Antibacterial activity was assessed using the well diffusion method, while the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined through broth dilution and spread plate techniques. Data were analyzed using descriptive statistics.
Results: Fourteen bacterial isolates were obtained, all of which were Gram-negative bacilli. Isolate 5.1 exhibited the highest inhibitory activity, with inhibition zone diameters of 16.06 mm against ESBL-producing E. coli (intermediate category) and 7.53 mm against S. Typhi (resistant category). The MIC value for active isolates was 5 mg/mL; however, no bactericidal effect was observed within the tested concentration range (5–10 mg/mL).
Conclusion: Peat soil bacteria demonstrated antibacterial potential, with three isolates showing activity against ESBL-producing E. coli and one isolate active against S. Typhi at 5 mg/mL. Further studies are required to identify the active compounds and elucidate their mechanisms of action.

1. Introduction

Bacterial infections remain a major global health issue, increasingly complicated by the rise of antimicrobial resistance. Antibiotic resistance has become one of the greatest health threats of the 21st century. It is projected that antibiotic-resistant infections could cause up to 10 million deaths annually by 2050, a sharp increase from the current estimate of 700,000 deaths per year (Ahmed et al., 2024). This situation necessitates the exploration of novel

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sources of antibacterial compounds, particularly from underexplored environments.

Among drug-resistant pathogens, *Salmonella enterica* serovar Typhimurium (*S.* Typhi) and extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* are of particular concern due to their high morbidity, mortality, and ability to develop multidrug resistance. *S.* Typhi, the causative agent of typhoid fever, accounts for an estimated 11–20 million cases and 128,000–161,000 deaths annually, with its prevalencse strongly linked to poor sanitation (Buzilă et al., 2025). Meanwhile, ESBL-producing *E. coli* is a leading cause of urinary tract infections and diarrhea, with a prevalence of up to 27.3-46.5% in urine samples from patients with urinary tract infections (Vachvanichsanong et al., 2021). The presence of ESBL in *E. coli* inactivates cephalosporin antibiotics and increases therapeutic failure, complicating clinical management (Liu et al., 2022).

Indonesia possesses approximately 21 million hectares of peatland, representing a unique ecosystem characterized by high organic acid content, abundant organic matter, and strong water retention capacity. These extreme conditions make peat soil an ideal habitat for microorganisms adapted to such environments. Microorganisms from extreme environments such as peat soil are known to produce secondary metabolites with unique biological activities, including antibacterial compounds that remain largely unexplored (Mahdiyah et al., 2020; Mahdiyah, Hidayah, et al., 2025). Previous studies have shown that bacterial isolates from peat soil in Kalimantan exhibit antimicrobial activity against various pathogenic bacteria, offering prospects as a source of novel antibacterial agents (Mahdiyah, Darsono, et al., 2025; Mahdiyah et al., 2026). This study aimed to evaluate the antibacterial potential of bacterial isolates derived from peat soil against *S.* Typhimurium and ESBL-producing *E. coli*. This research is expected to contribute to the development of new antibacterial compounds derived from Indonesia's local natural resources.

2. Method

This study employed a true experimental design and was conducted at the Microbiology Laboratory of Universitas Sari Mulia, Banjarmasin, South Kalimantan. The research was carried out in March to November, 2025. All procedures were performed under aseptic conditions to ensure validity and prevent contamination.

Sample and sampling location

Peat soil samples were collected from Jalan Pintas Sambangan No. 16, Benua Raya

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Village, Bati-Bati District, Tanah Laut Regency, South Kalimantan. Sampling was conducted using purposive sampling at a depth of 10–20 cm from the soil surface. Samples were placed into sterile plastic bags, stored in a cool box at 4°C, and immediately transported to the laboratory for processing.

Isolation of peat soil bacteria

Bacterial isolation was performed using the pour plate method. A total of 1 g of peat soil sample was serially diluted up to 10^{-7} using sterile 0.9% NaCl solution. One milliliter from each dilution (10^{-1} to 10^{-7}) was pipetted into sterile Petri dishes, followed by the addition of warm (≈45°C) molten Trypticase Soy Agar (TSA). After the media solidified, plates were incubated at 37°C for 24–48 hours. Colonies exhibiting distinct morphologies were purified using the streak plate method on TSA until pure isolates were obtained.

Morphological identification and Gram staining

Initial identification of bacterial isolates was performed through macroscopic observation (colony shape, color, elevation, and margin) and microscopic observation using Gram staining. Gram staining was conducted using the standard method: smears were heat-fixed, then stained with crystal violet, Lugol's iodine, 96% alcohol, and safranin. Stained smears were observed under a light microscope at 1000× magnification. Gram-positive bacteria appeared purple, while Gram-negative bacteria appeared pink.

Production of bacterial supernatant

Pure bacterial isolates were inoculated into Trypticase Soy Broth (TSB) and incubated at 37°C for 24 hours with shaking at 120 rpm. The culture was then centrifuged at 4000 rpm for 20 minutes.

Antibacterial activity assay

Antibacterial activity was assessed using the well diffusion method. Test bacterial cultures (*S.* Typhi and ESBL-producing *E. coli*) were suspended in sterile 0.9% NaCl to a turbidity equivalent to 0.5 McFarland standard (≈1.5 × 10^8 CFU/mL). The bacterial suspension was evenly swabbed onto the surface of Mueller-Hinton Agar (MHA) using sterile cotton swabs. Wells of 6 mm in diameter were created in the agar and filled with 50 µL of bacterial supernatant. Ceftriaxone (30 µg) was used as a positive control, while sterile distilled water served as a negative control. Plates were incubated at 37°C for 24 hours. The inhibition zone diameters were measured using a vernier caliper. Results were interpreted according to the

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Clinical and Laboratory Standards Institute (CLSI) M100 (Clinical and Laboratory Standards Institute, 2021).

Minimum Inhibitory Concentration (MIC) determination

MIC was determined using the broth dilution method in test tubes. Bacterial supernatants were serially diluted in Mueller-Hinton Broth (MHB) to achieve final concentrations of 10 mg/mL, 7.5 mg/mL, and 5 mg/mL. A 100 µL aliquot of the test bacterial suspension (1.5×10^8 CFU/mL) was added to each tube. Tubes were incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration of supernatant that completely inhibited visible bacterial growth (no turbidity) after incubation.

Minimum Bactericidal Concentration (MBC) determination

MBC was determined by taking 100 µL from clear MIC test tubes (showing no visible growth) and spreading it onto MHA plates using the spread plate method. Plates were incubated at 37°C for 24 hours. The MBC was defined as the lowest concentration of supernatant that resulted in no bacterial colony growth on the solid media.

3. Result

Isolation of peat soil bacteria

Bacterial isolation from peat soil samples using the pour plate method on TSA medium yielded 14 bacterial isolates from seven dilution levels (10^{-1} to 10^{-7}). Figure 1 shows 14 isolates were purified to obtain uncontaminated pure cultures. Gram staining results revealed that all isolates were Gram-negative bacilli, appearing pink under the microscope (Figure 2). This indicates that the bacterial cells possess a thin peptidoglycan layer and an outer membrane that loses the crystal violet stain during decolorization. Macroscopic observation showed that all isolates exhibited cream-colored colonies with convex elevation and smooth margins.

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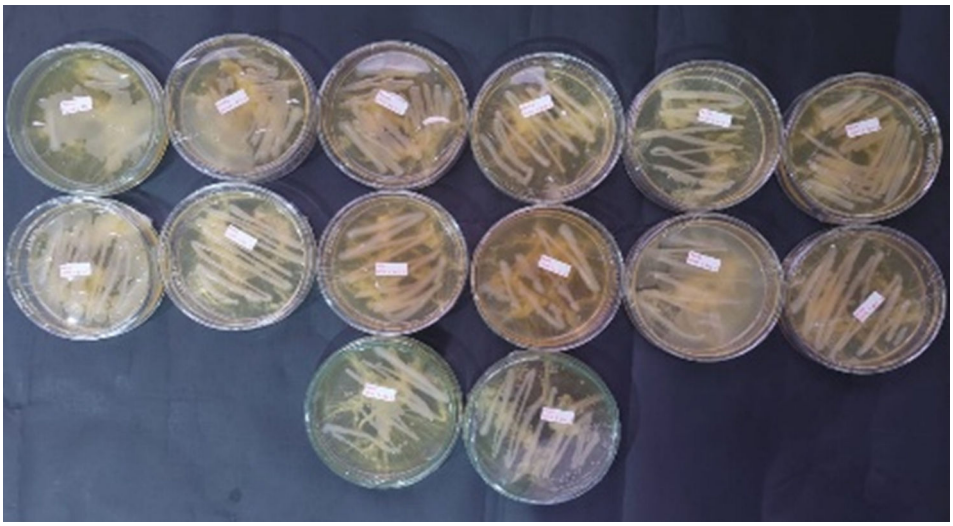

Figure 1. Results of bacterial isolate purification

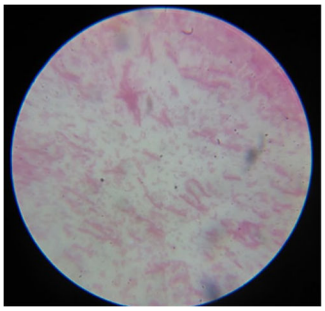

Figure 2. Results of Gram staining

Table 1. Morphological characteristics of peat soil bacterial isolates

Isolate	Characteristics			
	Shape	Color	Elevation	Margin
Isolate 1.1	Rod-shaped (*bacillus*)	Cream	Convex	Smooth
Isolate 1.2	Rod-shaped (*bacillus*)	Cream	Convex	Smooth
Isolate 2.1	Rod-shaped (*bacillus*)	Cream	Convex	Smooth
Isolate 2.2	Rod-shaped (*bacillus*)	Cream	Convex	Smooth
Isolate 3.1	Rod-shaped (*bacillus*)	Cream	Convex	Smooth
Isolate 3.2	Rod-shaped (*bacillus*)	Cream	Convex	Smooth
Isolate 4.1	Rod-shaped (*bacillus*)	Cream	Convex	Smooth
Isolate 4.2	Rod-shaped (*bacillus*)	Cream	Convex	Smooth
Isolate 5.1	Rod-shaped (*bacillus*)	Cream	Convex	Smooth
Isolate 5.2	Rod-shaped (*bacillus*)	Cream	Convex	Smooth
Isolate 6.1	Rod-shaped (*bacillus*)	Cream	Convex	Smooth
Isolate 6.2	Rod-shaped (*bacillus*)	Cream	Convex	Smooth
Isolate 7.1	Rod-shaped (*bacillus*)	Cream	Convex	Smooth
Isolate 7.2	Rod-shaped (*bacillus*)	Cream	Convex	Smooth

Antibacterial activity screening

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Antibacterial activity was assessed using the well diffusion method against *S.* Typhi and ESBL-producing *E. coli*. Among the 14 isolates tested, only isolate 5.1 exhibited activity against *S.* Typhi, with a mean inhibition zone diameter of 7.53 mm (Table 2). Against ESBL-producing *E. coli*, three isolates showed activity: isolate 1.1 (7.49 mm), isolate 5.1 (16.06 mm), and isolate 7.1 (6.66 mm) (Table 3). Isolate 5.1 was categorized as intermediate against ESBL-producing *E. coli* with an inhibition zone of 16.06 mm, while the other isolates were categorized as resistant.

Table 2. Screening of antibacterial activity against *S.* Typhi

No	Isolate	Inhibition Zone (mm)			Mean ± SD (mm)	CLSI
		1	2	3		
1.	Isolate 5.1	6.86	7.89	7.84	7.53 ± 0.579	Resistant
2.	Ceftriaxon	25.00	25.77	26.26	25.67 ± 0.6	Suspectible
3.	Negative control	0	0	0	0	Resistant

Table 3. Screening of antibacterial activity against *E. coli* ESBL

Groups	Inhibition Zone (mm)			Mean ± SD (mm)	CLSI
	1	2	3		
Isolate 1.1	8.32	7.13	7.45	7.49 ± 0.615	Resistant
Isolate 5.1	15.50	16.95	15.85	16.06 ± 0.809	Intermediate
Isolate 7.1	6.34	6.79	7.26	6.66 ± 0.416	Resistant
Ceftriaxon	23.24	23.02	23.59	23.28 ± 0.287	Suspectible
Negative control	0	0	0	0	Resistant

Minimum inhibitory concentration

MIC determination was performed using the broth dilution method at supernatant concentrations of 5 mg/mL, 7.5 mg/mL, and 10 mg/mL. Observations showed that all treatment tubes at the three concentrations exhibited clear media for isolate 5.1 against *S. typhi*, and for isolates 1.1, 5.1, and 7.1 against ESBL-producing *E. coli*. Based on these results (Tables 3 and 4), the MIC value for the active isolates was determined to be 5 mg/mL, as the lowest tested concentration (5 mg/mL) consistently showed clear media across all replicates.

Table 4. MIC results against *S. typhi*

Treatment	Replication		
	1	2	3
Ceftriaxone	Clear	Clear	Clear
Negative control	Turbid	Turbid	Turbid
Isolate 5.1 (5 mg/ml)	Clear	Clear	Clear
Isolate 5.1 (7,5 mg/ml)	Clear	Clear	Clear
Isolate 5.1 (10 mg/ml)	Clear	Clear	Clear

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Table 5. MIC results against *E. coli* ESBL

Treatment	Replication		
	1	2	3
Ceftriaxone	Clear	Clear	Clear
Negative control	Turbid	Turbid	Turbid
Isolate 1.1 (5 mg/ml)	Clear	Clear	Clear
Isolate 1.1 (7,5 mg/ml)	Clear	Clear	Clear
Isolate 1.1 (10 mg/ml)	Clear	Clear	Clear
Isolate 5.1 (5 mg/ml)	Clear	Clear	Clear
Isolate 5.1 (7,5 mg/ml)	Clear	Clear	Clear
Isolate 5.1 (10 mg/ml)	Clear	Clear	Clear
Isolate 7.1 (5 mg/ml)	Clear	Clear	Clear
Isolate 7.1 (7,5 mg/ml)	Clear	Clear	Clear
Isolate 7.1 (10 mg/ml)	Clear	Clear	Clear

Minimum bactericidal concentration

MBC determination was performed by subculturing samples from clear MIC tubes onto MHA media. Observations revealed that at all tested concentrations (5 mg/mL, 7.5 mg/mL, and 10 mg/mL), bacterial colonies grew on the solid media (Table 6). Based on these results, the MBC could not be determined within the tested concentration range (5–10 mg/mL), indicating that the antibacterial compounds produced by the isolates were bacteriostatic at these concentrations.

Table 6. Results of the MBC Test against *E. coli* ESBL & *S. typhi*

Concentration	Replication		
	1	2	3
Isolate 1.1 5mg/ml	Colony growth	Colony growth	Colony growth
Isolate 1.1 7,5mg/ml	Colony growth	Colony growth	Colony growth
Isolate 1.1 10mg/ml	Colony growth	Colony growth	Colony growth
Isolate 5.1 5mg/ml	Colony growth	Colony growth	Colony growth
Isolate 5.1 7,5mg/ml	Colony growth	Colony growth	Colony growth
Isolate 5.1 10mg/ml	Colony growth	Colony growth	Colony growth
Isolate 5.2 5mg/ml	Colony growth	Colony growth	Colony growth
Isolate 5.2 7,5mg/ml	Colony growth	Colony growth	Colony growth
Isolate 5.2 10mg/ml	Colony growth	Colony growth	Colony growth
Isolate 7.1 5mg/ml	Colony growth	Colony growth	Colony growth
Isolate 7.1 7,5mg/ml	Colony growth	Colony growth	Colony growth
Isolate 7.1 10mg/ml	Colony growth	Colony growth	Colony growth

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4. Discussions

Peat soil was selected as the isolation source due to its unique characteristics, including highly acidic pH (3.5–4.5), high organic matter content, and anaerobic conditions, which drive indigenous microorganisms to develop specific adaptive mechanisms, including the production of secondary metabolites with potential biological activities (Mahdiyah et al., 2020). Extreme environmental conditions often correlate with the ability of microorganisms to produce bioactive compounds not found in conventional environments, positioning peat soil as a promising source for the exploration of novel antibacterial agents.

Characterization of bacterial isolates

All 14 successfully isolated bacteria exhibited Gram-negative characteristics with a bacillus shape. The consistent Gram staining results showing pink coloration indicate a cell wall structure with a thin peptidoglycan layer and the presence of an outer membrane containing lipopolysaccharides (LPS). This characteristic is significant as it relates to the bacteria's ability to produce bioactive compounds; many Gram-negative bacteria from extreme environments are known to possess unique secondary metabolite biosynthesis pathways, including genera such as *Burkholderia*, *Pseudomonas*, and *Achromobacter*, which are commonly found in peat soil (Averhoff et al., 2021; Gallo & Aulitto, 2024; Klaus et al., 2020; Mashakhetri et al., 2024; Wilson & Brimble, 2009). The uniform colony morphology (cream color, convex elevation, smooth margins) suggests that the isolates may belong to the same genus or share similar physiological characteristics. However, further identification using molecular approaches such as 16S rRNA gene sequencing is necessary for accurate taxonomic determination.

Antibacterial activity of isolates

The antibacterial activity assay revealed that only three isolates (1.1, 5.1, and 7.1) were capable of inhibiting ESBL-producing E. coli, while only isolate 5.1 showed activity against S. typhi. This variation in activity reflects differences in the antibacterial spectrum produced by each isolate, which is likely due to differences in the type, quantity, and chemical structure of the secondary metabolites produced (Pancu et al., 2021).

Isolate 5.1 exhibited the highest activity, with inhibition zones of 16.06 mm against ESBL-producing *E. coli* (categorized as intermediate according to CLSI) and 7.53 mm against *S. typhi* (categorized as resistant). The difference in inhibition zone diameters between the two

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test bacteria can be explained by differences in cell wall structure and intrinsic resistance mechanisms. ESBL-producing *E. coli* possesses complex resistance mechanisms through the production of β-lactamase enzymes that inactivate β-lactam antibiotics; however, this does not necessarily provide protection against antibacterial compounds with different mechanisms of action (Liu et al., 2022). Meanwhile, *S. typhi*, as an intracellular pathogen, has more complex defense systems, including the ability to survive within macrophages and produce biofilms, which may reduce the effectiveness of antibacterial compounds (Holschbach & Peek, 2018).

The absence of inhibition zones for most isolates does not necessarily indicate that these isolates do not produce antibacterial compounds. Bubonja-Šonje et al. (2020) explained that failure to form inhibition zones in agar diffusion methods can be attributed to several factors, including low compound polarity leading to slow diffusion in aqueous agar media, large molecular size, and compound interactions with agar components. Therefore, the broth dilution method used for MIC determination provides more accurate information regarding antibacterial potential, as it allows direct contact between the test compound and target cells in a homogeneous medium.

Minimum inhibitory concentration and minimum bactericidal concentration

MIC results showed that all three active isolates (1.1, 5.1, and 7.1) had an MIC value of 5 mg/mL against their respective test bacteria. This value is relatively higher compared to the positive control ceftriaxone; however, it should be noted that the supernatant used is a crude extract containing a mixture of various metabolite compounds, not a purified single compound. Thus, the MIC value reflects the total concentration of compounds in the supernatant, not the concentration of specific active compounds. Further purification has the potential to significantly enhance antibacterial activity, as the active fraction can be separated from other components that do not contribute to the antibacterial effect (Hutchings et al., 2019).

MBC results showed that at concentrations up to 10 mg/mL, bacterial colonies still grew on solid media, indicating that the MBC could not be determined. This suggests that the antibacterial compounds produced by the isolates are bacteriostatic within the tested concentration range, rather than bactericidal. An antibacterial compound may exhibit bacteriostatic effects at low concentrations and bactericidal effects at higher concentrations

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(Ishak et al., 2025; Vaisbourd et al., 2025). The failure to achieve bactericidal effects at 10 mg/mL indicates that the concentration required to kill bacterial cells may be above the tested range, or the produced compounds inherently possess a growth-inhibitory (bacteriostatic) mechanism rather than a cell-killing mechanism. Several technical factors that may influence MBC results include pipetting inaccuracies, non-homogeneous inoculum, suboptimal aseptic procedures, and the possibility of cross-contamination. Additionally, biological factors such as test cell viability, bacterial growth phase, and interactions between components within the supernatant may also contribute to the observed results.

Implications and limitations of the study

The findings of this study demonstrate that peat soil from South Kalimantan has potential as a source of antibacterial-producing bacterial isolates, particularly isolate 5.1, which showed activity against antibiotic-resistant pathogenic bacteria. However, this study has several limitations. First, isolate identification was limited to morphological characterization and Gram staining without molecular identification, so the bacterial genus or species remains unknown. Second, the supernatant used was a crude extract, preventing determination of the mechanism of action and identification of active compounds. Third, the concentration range tested in the MBC assay (5–10 mg/mL) may not have encompassed the concentration required to achieve bactericidal effects.

Further research is needed, including molecular identification of isolates using 16S rRNA gene sequencing, characterization and purification of active compounds through chromatography, toxicity testing to evaluate safety, and mechanism of action studies to understand how the compounds inhibit target bacterial growth.

5. Conclusion

This study successfully isolated 14 bacterial isolates from peat soil in South Kalimantan, all of which were Gram-negative bacilli. Among these 14 isolates, three isolates (1.1, 5.1, and 7.1) exhibited antibacterial activity against ESBL-producing *Escherichia coli*, while one isolate (5.1) showed activity against *S.* Typhi. Isolate 5.1 demonstrated the highest potential, with inhibition zone diameters of 16.06 mm against ESBL-producing *E. coli* (categorized as intermediate) and 7.53 mm against *S.* Typhi. The MIC for the active isolates was determined to be 5 mg/mL, while the MBC could not be determined within the tested concentration range (5–10 mg/mL), indicating that the antibacterial compounds produced are bacteriostatic.

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These findings confirm that peat soil bacteria, particularly isolate 5.1, possess potential as a source of novel antibacterial compounds that warrant further exploration.

6. Conflict of interest

All authors declare no conflict of interest.

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