

Antimicrobial Activity of Endophytic Bacteria Isolated from *Chromolaena odorata* (Balakacida)

Helmi Andriyani¹, Asih Triastuti^{2*}, Maya Sari Mutia³, Qori Fadillah⁴

¹ Department of Pharmacy, Universitas Islam Indonesia, Yogyakarta, Indonesia; andriyani2906@gmail.com

² Department of Pharmacy, Universitas Islam Indonesia, Yogyakarta, Indonesia; asih.triastuti@uii.ac.id

³ Department of Medicine, Faculty of Medicine, Dentistry, and Health Sciences, Universitas Prima Indonesia, Medan, Indonesia; mayasarimutia@unprimdn.ac.id

⁴ Department of Medicine, Faculty of Medicine, Dentistry, and Health Sciences, Universitas Prima Indonesia, Medan, Indonesia; qorifadillah@unprimdn.ac.id

* Corresponding author: Asih Triastuti | Email: asih.triastuti@uii.ac.id

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Abstract: Antimicrobial resistance is a pressing global health concern, prompting the search for alternative sources of antimicrobial agents. Endophytic bacteria, which inhabit plant tissues without causing harm, have emerged as promising candidates due to their ability to produce bioactive secondary metabolites. This study aimed to explore the antimicrobial potential of endophytic bacteria isolated from different tissues (roots, stems, and leaves) of *Chromolaena odorata* (Balakacida), a medicinal plant native to Indonesia. Surface-sterilized plant segments were processed to isolate seven endophytic bacterial strains, which were characterized morphologically and biochemically. Antibacterial and antifungal activities were evaluated using the disc diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes*, *Candida albicans*, and *Pityrosporum ovale*. Among the isolates, ABE6 exhibited the highest antibacterial activity, particularly against *S. epidermidis* (17.83 ± 4.79 mm), while ABE3 showed the strongest antifungal activity against *C. albicans* (29.15 ± 2.83 mm). Statistical analyses confirmed significant differences between isolates ($p < 0.05$). These findings suggest that endophytic bacteria from *C. odorata* may serve as a potential source of novel antimicrobial agents to combat drug-resistant pathogens.

Keywords: Endophytic bacteria; *Chromolaena odorata*; antimicrobial resistance; antibacterial; antifungal

1. INTRODUCTION

According to the World Health Organization (WHO), antimicrobial resistance (AMR) is a major global health threat, causing over 1.27 million direct deaths annually due to infections resistant to antimicrobial agents [1]. Bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* have been categorized as high-priority pathogens due to their increasing resistance.

The lack of innovation in antibiotic development, coupled with limited investment from the pharmaceutical industry in drug research and development, is not keeping pace with the rising and often irrational use of antibiotics. Moreover, the increasing incidence of infections caused by resistant pathogenic bacteria poses a serious threat to global health due to the limited availability and effectiveness of new antibacterial agents in clinical practice.

Commonly used antibiotics such as ampicillin, streptomycin, ciprofloxacin, cefotaxime, and azithromycin are beginning to show decreased efficacy against bacterial infections. This situation raises serious concern, as it may hinder infectious disease control and increase the risk of outbreaks that are difficult to treat [2].

Successful antibiotic therapy is only achieved through rational use. Antibiotic resistance is a major public health concern that requires urgent attention. Bacterial resistance refers to the condition in which bacteria no longer respond to treatment with antibiotics, rendering these drugs ineffective

at killing or inhibiting bacterial growth. As a result, the ability of antibiotics to treat infectious diseases in humans is significantly reduced. Furthermore, this contributes to increased morbidity and mortality rates, higher treatment costs, prolonged hospitalization, and a greater risk of adverse effects [3].

In response, numerous studies have focused on finding alternative strategies to reduce the risk of AMR, including the utilization of natural products as alternative therapeutic agents with antimicrobial activity and minimal side effects.

Indonesia, known as one of the world's most biodiverse countries, harbors over 30,000 species of higher plants, many of which possess medicinal properties and potential as raw materials for drug development. One such plant is *Chromolaena odorata* (L.) R.M. King & H. Rob, locally known as Balakacida, a member of the Asteraceae family. Its leaves contain several key compounds, including tannins, phenols, flavonoids, saponins, and steroids. In addition, this plant has been reported to exhibit antibacterial and anti-inflammatory activity. According to previous reports, the leaf extract of *C. odorata* also showed antifungal activity against *Aspergillus flavus* [4].

Endophytic microbes are microorganisms that reside within plant tissues and live in symbiosis with their host plants. These endophytes are capable of producing secondary metabolites that are similar or identical to those synthesized by the host plant. Sari et al. (2023) successfully isolated 19 endophytic bacterial strains from the roots, stems, and leaves

of *C. odorata*, which were characterized based on their morphology, biochemical traits, and Gram staining profiles [5].

Chromolaena odorata has been traditionally used in folk medicine to treat various conditions, such as leech bites, burns, skin infections, and soft tissue injuries. The young leaves are typically crushed to release a sap that is then applied directly to the skin for wound healing [6].

Although *C. odorata* has been shown to contain bioactive compounds with antimicrobial properties, the present study focuses on exploring its endophytic bacteria. This approach is based on the premise that endophytic bacteria can produce similar or even more potent secondary metabolites than those produced by their host plants. Moreover, endophytic bacteria can be isolated and cultivated under laboratory conditions, allowing for efficient and sustainable production of bioactive compounds without the need to directly exploit the plant itself. This strategy is environmentally friendly and aligns with efforts to conserve natural resources. Additionally, endophytes may produce novel compounds that have not yet been identified, offering promising opportunities for the discovery of new antimicrobial agents.

Endophytic bacteria were selected in this study due to their role as non-pathogenic microorganisms that reside within plant tissues and their potential as eco-friendly natural resources. Based on this background, the present study aims to investigate "The Antimicrobial Potential of Endophytic Bacteria Isolated from *Chromolaena odorata* (Balakacida)." The researchers hope that endophytic bacteria may serve as a promising alternative to reduce the negative impacts of excessive exploitation of medicinal plants.

2. MATERIALS AND METHODS

2.1. Place and Time of Research

This research was conducted at the Research Laboratory of Universitas Prima Indonesia, Medan.

2.2. Tools and Materials

The materials used in this study included plant samples of *Chromolaena odorata* (Balakacida), 70% ethanol, NaClO, sterile distilled water, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes*, *Candida albicans*, and *Pityrosporum ovale* fungi, Nutrient Agar (NA) medium, biochemical test media, Gram staining reagents, blank paper discs, and chloramphenicol antibiotic discs. The equipment used included a Class II Biological Safety Cabinet (BSC), autoclave (Gea Autoclave LS-B75), vortex mixer, Petri dishes, analytical balance (Shimadzu), incubator (LabnBCet), and other glassware.

2.3. Sampling and Plant Determination

The *Chromolaena odorata* (Balakacida) plant samples were collected from Medan Marelan District, Medan City. The plant species *Chromolaena odorata* (L.) R.M. King & H. Rob. was identified at the Herbarium Medanense, Universitas Sumatera Utara.



Figure 1. *Chromolaena odorata* (Balakacida) plant

2.4. Surface Sterilization and Isolation of Endophytic Bacteria

2.4.1 Plant Sterilization. All parts of the plant were thoroughly washed under running water to remove adhering soil particles and debris. The plant was then separated into three parts: roots, stems, and leaves.



Figure 2. (a) Balakacida leaves, (b) Balakacida roots, (c) Balakacida stems

Each part of the plant was then rinsed twice with sterile distilled water. Surface sterilization was carried out by immersing the samples in 70% ethanol for 5 minutes, followed by immersion in 2% sodium hypochlorite (NaOCl) solution for another 5 minutes. The samples were subsequently rinsed five times with sterile distilled water to remove residual chemicals, dried using sterile tissue, and aseptically cut into small pieces.

2.4.2 Isolation of Endophytic Bacteria from Plant. Plant tissue fragments were ground in a sterile mortar using 10 mL of sterile physiological saline solution (0.85% NaCl) and homogenized with a vortex mixer for 60 seconds at high speed. The resulting suspension was serially diluted (ten-fold dilutions) using sterile Nutrient Broth, and 100 μ L from each dilution level was inoculated onto Nutrient Agar (NA) plates using the spread plate method.

The Petri dishes were incubated at 37°C and observed periodically until bacterial colonies began to appear. To validate the effectiveness of the surface sterilization process, the final rinse water was inoculated onto NA medium and incubated under the same conditions. The absence of microbial growth on the control media confirmed the success of the surface sterilization.

The growing endophytic bacterial isolates were observed based on their colony morphology. Colonies with distinct

morphological characteristics were separated and re-streaked on fresh NA plates to obtain pure cultures. The resulting pure cultures were subsequently subcultured onto slant NA media and stored at 4°C for further analysis [7].

2.5. Sterilization

The tools were sterilized in an oven at a temperature of 171 °C for 1 hour. The materials used were sterilized by autoclave at a temperature of 121 °C for 15 minutes. Nutrient Agar (NA) and Potato Dextrose Agar (PDA) media were dissolved in an aqueous solution and homogenized with a stirrer at a temperature of 100 °C until boiling. The NA and PDA were sterilized by autoclave at 121 °C for 15 minutes [8].

2.6. Characterization of Bacterial Isolates

All selected bacterial isolates were identified based on morphological characteristics, including colony shape, margin, elevation, and size, as well as microscopic characterization using Gram staining. Biochemical and physiological tests, such as Triple Sugar Iron Agar (TSIA), gelatin hydrolysis, citrate utilization, motility, catalase activity, and blood hemolysis, were performed following standard procedures.

2.7. Inoculation of Test Microbes

Preparation of pathogenic microbial suspensions: *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes*, *Candida albicans*, and *Pityrosporum ovale* were cultured in 10 mL of Nutrient Broth (NB) for 24 hours at room temperature. The pathogenic cultures were then suspended in 10 mL of 0.9% NaCl solution. The turbidity of the suspension was visually compared to the 0.5 McFarland standard. Subsequently, the absorbance was measured using a spectrophotometer at a wavelength of 625 nm, with an absorbance range of 0.08 to 0.13, corresponding to a bacterial concentration of approximately 10^8 CFU/mL [9].

2.8. Antimicrobial Activity Test by Disc Diffusion Method

The test microbes used in this study were *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes*, *Candida albicans*, and *Pityrosporum ovale*. The endophytic bacterial isolates were cultured in Nutrient Broth and incubated for 24 hours. Using sterile cotton swabs, the pathogenic microbes were evenly spread over the surface of Nutrient Agar for antibacterial testing and Potato Dextrose Agar (PDA) for antifungal testing.

Sterile paper discs were immersed in the bacterial supernatant (crude extract) using sterile forceps, then placed onto the surface of the agar media. The plates were incubated in an inverted position at 37°C for 24 hours. The diameter of the inhibition zones was measured using a digital caliper [10].

2.9. Statistical Analysis

Results of antibacterial and antifungal activities were expressed as mean $\pm$ standard deviation. The significance of differences among treatment groups was analyzed using the

Kruskal–Wallis test for non-parametric data and one-way ANOVA for parametric data, depending on the normality distribution. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software version 29.

3. RESULTS AND DISCUSSION

3.1. Isolation of Endophytic Bacteria from *Balakacida*

Endophytic bacteria were isolated from surface-sterilized fragments of roots, stems, and leaves of *C. odorata* (Figure 3). Seven endophytic bacterial isolates were successfully obtained and coded ABE1, ABE3, ABE6, BBE1, BBE2, DBE1, and DBE2.

3.2. Isolate Characterization

Results from the morphological and biochemical characterization of endophyte bacteria isolates ABE1, ABE3, ABE6, BBE1, BBE2, DBE1, and DBE2 can be seen in Tables 1 and 2, and Figure 4.

3.3. Antibacterial Activity

Antibacterial activity testing against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Propionibacterium acnes* showed variations in inhibitory effects among isolates (Table 3). Isolate ABE6 exhibited the highest activity, with inhibition zones of 8.80 ± 2.76 mm against *E. coli*, 17.83 ± 4.79 mm against *S. epidermidis*, and 8.18 ± 2.31 mm against *S. aureus*. Isolates ABE1 and ABE3 also demonstrated moderate activity against *S. epidermidis* and *P. acnes*.

Statistical analysis using the Kruskal-Wallis test revealed significant differences among treatment groups ($p < 0.05$), indicating that certain isolates possess notable antibacterial potential. Although the inhibition zones were smaller than that of the positive control (chloramphenicol), the isolates' ability to inhibit the growth of resistant pathogens highlights their promising therapeutic potential.

These findings are consistent with previous studies reporting that endophytes from *C. odorata* and other tropical medicinal plants are capable of producing antibacterial compounds such as alkaloids, terpenoids, and polyketides [12]. The observed variation in antibacterial activity is likely influenced by species differences and the biosynthetic capacity for secondary metabolite production.

3.4. Antifungal Activity

Antifungal activity assays against *Candida albicans* and *Pityrosporum ovale* revealed that several isolates exhibited varying degrees of antifungal potential (Table 4). ABE3 demonstrated the highest activity against *C. albicans* (29.15 ± 2.83 mm), followed by BBE1 and DBE1. Meanwhile, DBE2 and ABE6 showed moderate inhibitory effects against *P. ovale*.

Statistical analysis (ANOVA and Kruskal-Wallis tests) revealed significant differences ($p < 0.001$ and $p = 0.008$), indicating that certain isolates possess antifungal activity.

This suggests that endophytic bacteria may play a role in protecting their host plants from fungal pathogens and could also serve as potential sources of antifungal agents for human use [13].

These findings support previous reports stating that endophytic bacteria from tropical plants are capable of

producing antifungal compounds such as lipopeptides and volatile organic compounds [14]. The notable antifungal activity of ABE3 and BBE1 suggests that isolates derived from roots and stems may serve as promising candidates for further exploration through genomic or metabolomic approaches.

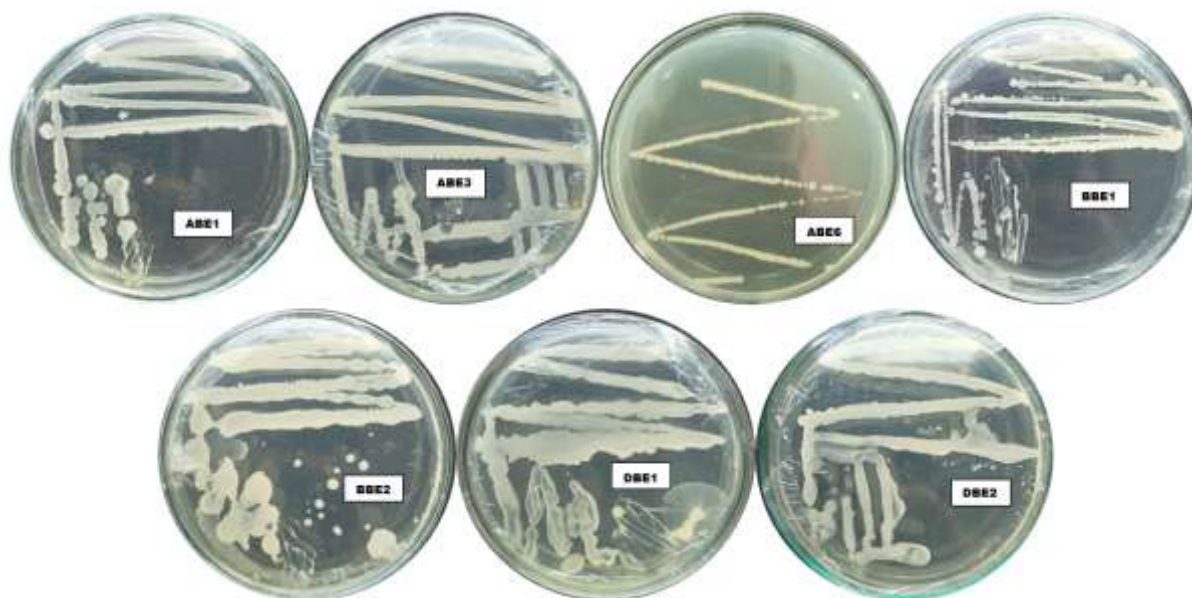


Figure 3. Isolated endophytic bacterial isolates coded ABE1, ABE3, ABE6, BBE1, BBE2, DBE1, DBE2

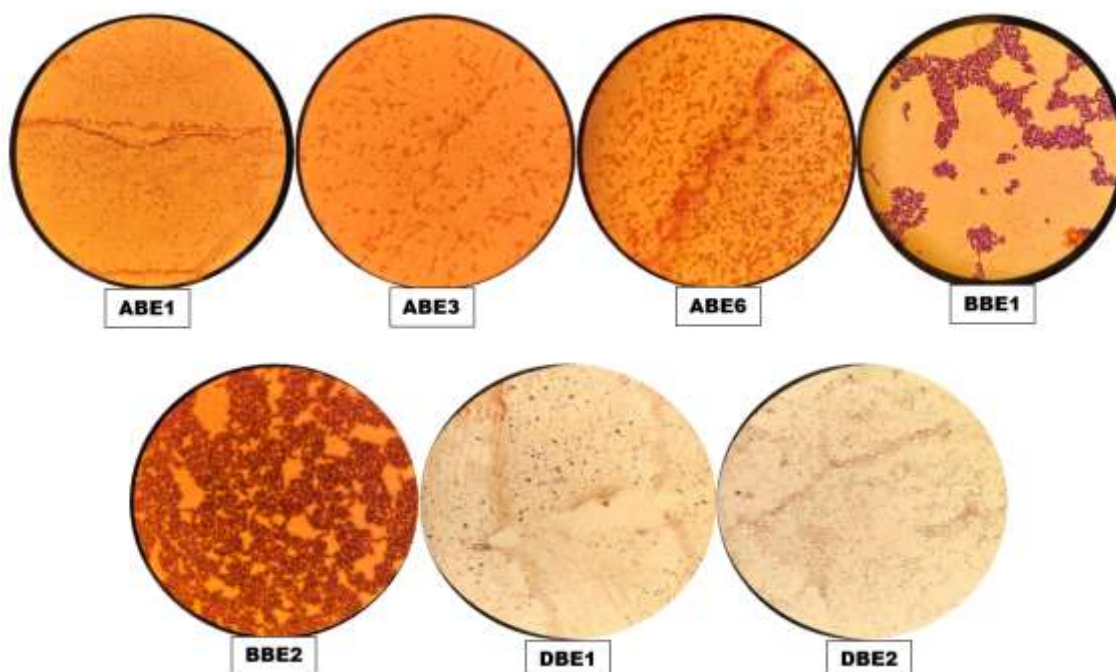


Figure 4. Morphological test results for microscopic observations of isolates coded ABE1, ABE3, ABE6, BBE1, BBE2, DBE1, DBE2

Biochemical tests revealed that all isolates were capable of utilizing citrate and tested negative for gelatin hydrolysis and motility. Most isolates exhibited α -hemolysis, while one isolate showed γ -hemolysis. These findings indicate physiological diversity among the isolates, which may contribute to the production of antimicrobial metabolites. Similar observations have been reported in previous studies on endophytic bacteria from medicinal plants, where diverse biochemical characteristics were correlated with the potential for bioactive compound production [11].

Table 1. Characterization of Endophyte Bacteria Isolates

Isolate Code	Plant Organ	Gelatin	Citrate	Motility	Catalase	Hemolysis	TSIA Butt	TSIA Slant	H ₂ S	Gas
ABE1	Root	-	+	-	-	α	Yellow	Red	-	-
ABE3	Root	-	+	-	-	α	Yellow	Red	-	-
ABE6	Root	-	+	-	+	γ	Yellow	Red	-	-
BBE1	Stem	-	+	-	-	α	Yellow	Red	-	-
BBE2	Stem	-	+	-	+	α	Yellow	Red	-	-
DBE1	Leaves	-	+	-	-	α	Yellow	Red	-	-
DBE2	Leaves	-	+	-	+	α	Yellow	Red	-	-

Morphological observations revealed diversity in colony shape, margin, elevation, and size. All isolates were identified as Gram-negative bacilli (Table 2).

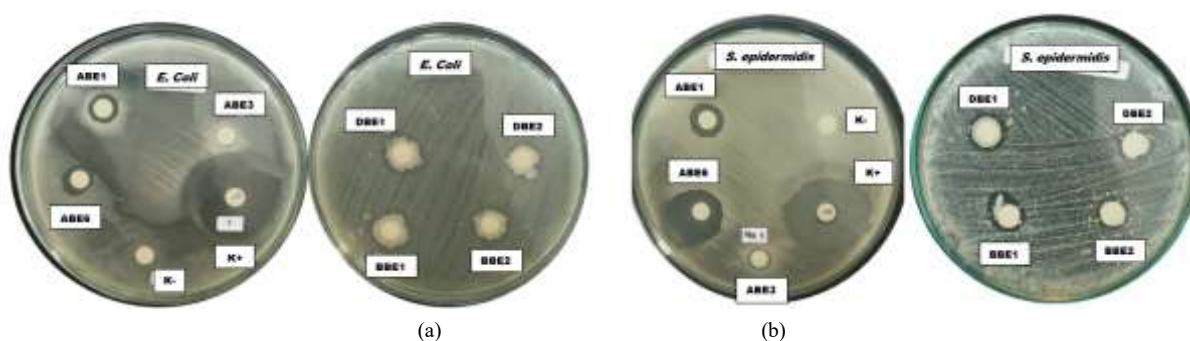
Table 2. Microscopic and Macroscopic Morphological Tests for Endophyte Bacteria Isolates

Isolate Code	Plant Organ	Colony Form	Colony Edge	Colony Elevation	Colony Size	Cell Colour	Cell Shape	Gram Staining
ABE1	Root	Circular	Undulate	Flat	Moderate	Red	Bacilli	Negative
ABE3	Root	Irregular	Curlate	Flat	Moderate	Red	Bacilli	Negative
ABE6	Root	Circular	Entire	Flat	Small	Red	Bacilli	Negative
BBE1	Stem	Irregular	Undulate	Flat	Moderate	Red	Bacilli	Negative
BBE2	Stem	Circular	Undulate	Flat	Moderate	Red	Bacilli	Negative
DBE1	Leaves	Irregular	Undulate	Pulvinate	Moderate	Red	Bacilli	Negative
DBE2	Leaves	Circular	Undulate	Flat	Moderate	Red	Bacilli	Negative

Table 3. Antibacterial Activity of Endophytic Bacterial Isolates — Inhibition Zone Diameter (mm), Mean $\pm$ SD

Treatment Group	<i>E. coli</i>	<i>S. epidermidis</i>	<i>P. acnes</i>	<i>S. aureus</i>
Positive Control	27.85 $\pm$ 1.06	7.30 $\pm$ 5.90	26.30 $\pm$ 1.17	27.54 $\pm$ 0.83
Negative Control	6.00 $\pm$ 0.00	6.00 $\pm$ 0.00	6.00 $\pm$ 0.00	6.00 $\pm$ 0.00
ABE1	7.10 $\pm$ 1.70	8.20 $\pm$ 3.13	7.37 $\pm$ 1.35	6.15 $\pm$ 0.25
ABE3	6.00 $\pm$ 0.00	9.35 $\pm$ 3.72	6.00 $\pm$ 0.00	6.00 $\pm$ 0.00
ABE6	8.80 $\pm$ 2.76	17.83 $\pm$ 4.79	7.22 $\pm$ 0.40	8.18 $\pm$ 2.31
BBE1	6.00 $\pm$ 0.00	8.52 $\pm$ 4.36	6.00 $\pm$ 0.00	6.56 $\pm$ 0.97
BBE2	6.88 $\pm$ 1.52	7.06 $\pm$ 1.84	6.00 $\pm$ 0.00	7.10 $\pm$ 0.96
DBE1	6.00 $\pm$ 0.00	7.89 $\pm$ 3.27	6.00 $\pm$ 0.00	6.55 $\pm$ 0.95
DBE2	6.85 $\pm$ 1.47	7.56 $\pm$ 2.70	6.00 $\pm$ 0.00	6.44 $\pm$ 0.77
P Value	0.029	0.046	0.019	0.011

The P value was obtained from the Kruskal-Wallis test.



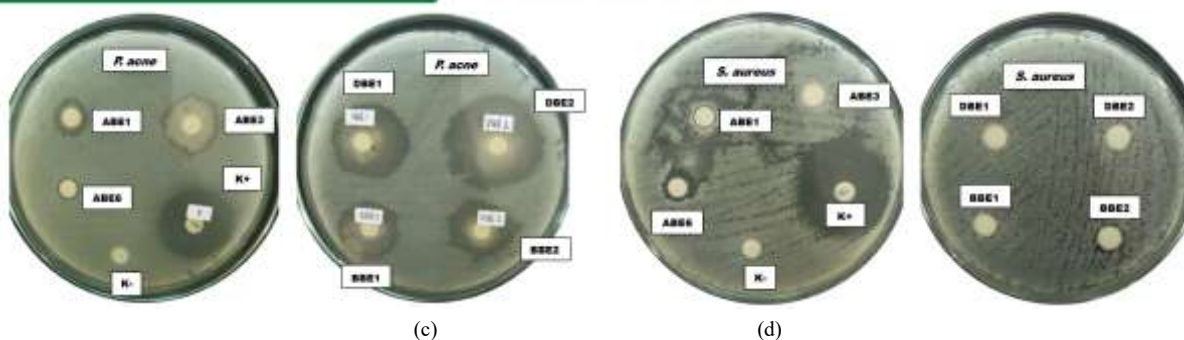


Figure 5. Inhibition zone antibacterial test results for (a) *Escherichia coli*, (b) *Staphylococcus epidermidis*, (c) *Propionibacterium acnes*, (d) *Staphylococcus aureus*, with chloramphenicol antibiotic disc as positive control and sterile aquadest as negative control

Table 4. Antifungal Activity of Endophytic Bacterial Isolates — Inhibition Zone Diameter (mm), Mean $\pm$ SD

Treatment Group	<i>Candida albicans</i>	<i>Pityrosporum ovale</i>
Positive Control	38.70 $\pm$ 0.17	13.27 $\pm$ 1.49
Negative Control	6.00 $\pm$ 0.00	6.00 $\pm$ 0.00
ABE1	18.36 $\pm$ 3.37	6.00 $\pm$ 0.00
ABE3	29.15 $\pm$ 2.83	6.00 $\pm$ 0.00
ABE6	9.93 $\pm$ 0.34	9.45 $\pm$ 0.37
BBE1	27.90 $\pm$ 0.80	6.00 $\pm$ 0.00
BBE2	20.40 $\pm$ 2.02	6.00 $\pm$ 0.00
DBE1	27.24 $\pm$ 1.41	6.00 $\pm$ 0.00
DBE2	25.56 $\pm$ 1.66	7.89 $\pm$ 0.66
P Value	< 0.001*	0.008**

*P value obtained from One-Way ANOVA test; **P value obtained from Kruskal-Wallis test.

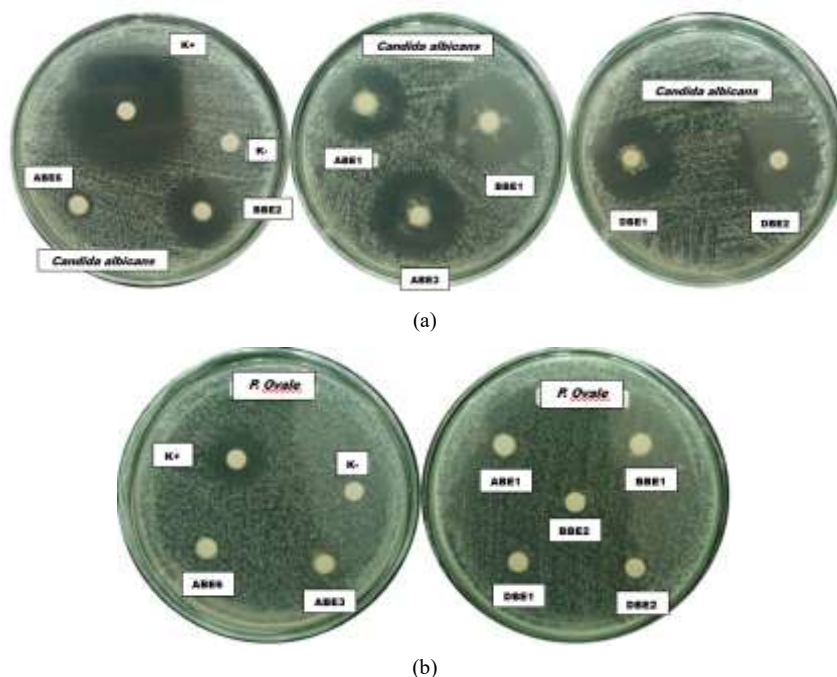


Figure 6. Inhibition zone antifungal test results for (a) *Candida albicans*, (b) *Pityrosporum ovale*, with ketoconazole 2% disc as positive control and sterile aquadest as negative control

4. CONCLUSION

This study demonstrated that endophytic bacteria isolated from different tissues of *Chromolaena odorata* exhibit diverse morphological, biochemical, and antimicrobial characteristics. Among the seven isolates tested, ABE6 and ABE3 showed the most promising antimicrobial activity against both bacterial and fungal pathogens, particularly *Staphylococcus epidermidis* and *Candida albicans*. These findings highlight the potential of endophytic bacteria from *C. odorata* as alternative sources of natural antimicrobial agents. The ability of these isolates to inhibit the growth of clinically relevant pathogens suggests their prospective application in the development of novel therapeutics to address antimicrobial resistance. Further studies involving molecular identification, compound purification, and in vivo efficacy testing are warranted to fully explore the pharmacological potential of these endophytes.

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Conflicts of Interest: The authors declare no conflict of interest.

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