

Potential Antibacterial Activity of Endophytic Bacterial Extracts from the Roots of *Bandotan* (*Ageratum conyzoides* L.) Against Diarrhea-Causing Bacteria

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Abstract

Diarrhea is a disease that can be caused by bacterial, viral, and parasitic infections in the digestive tract. Generally, diarrhea can be caused by two bacteria, namely *Escherichia coli* and *Shigella dysenteriae*. *Bandotan* plants (*Ageratum conyzoides* L.) have been used in several regions as traditional medicinal plants, one of which is for diarrhea medicine. The presence of endophytic bacteria in plants is thought to be able to be used as an alternative to explore their potential in producing bioactive compounds that have activity against bacteria that cause diarrhea. This study aims to determine the characteristics of endophytic bacterial isolates, test their antibacterial activity against *Escherichia coli* and *Shigella dysenteriae* bacteria, and determine the bioactive compounds produced by endophytic bacterial extracts of *bandotan* roots. The research stages began with the isolation of endophytic bacteria, bacterial characterization, antibacterial activity screening, production and extraction of secondary metabolites, preparation of test bacterial suspensions, antibacterial activity testing, MIC and MBC tests, screening of secondary metabolites of the extract, and data analysis. The results showed that there were three isolates that had potential as antibacterials with diverse characteristics and were Gram-negative. The highest antibacterial activity against *Escherichia coli* bacteria was shown by the AC3 extract with a diameter of 10.38 ± 0.27 mm, and against *Shigella dysenteriae* was shown by the AC10 extract with a diameter of 5.02 ± 1.36 mm. The MIC and MBC values of the three extracts were classified as weak at a concentration of 7.5%. Secondary metabolite compounds found in the endophytic bacterial extracts consisted of flavonoids, alkaloids, and triterpenoids.

Keywords: antibacterial; *Escherichia coli*; secondary metabolites; *Shigella dysenteriae*.

INTRODUCTION

Infectious diseases are prevalent in many tropical countries, including Indonesia, and are commonly caused by pathogenic microorganisms (Mariati et al., 2013; Hamtini et al., 2022), with diarrhea being one of the most frequently reported conditions. Diarrhea is a clinical manifestation arising from infections caused by bacteria, viruses, or parasites within the gastrointestinal tract, resulting in watery stools with a frequency of more than three times per day (Mendri & Prayogi, 2017; Halim & Wulandari, 2022). Among the major etiological agents, *Escherichia coli* and *Shigella dysenteriae* are generally recognized as the leading bacterial pathogens responsible for diarrheal cases (Fatayati et al., 2024). Globally, diarrhea ranks as the fifth leading cause of mortality and predominantly affects children under five years of age (Sari et al., 2023). According to the Bangka Belitung Health Office, diarrhea was reported as the most common disease among residents of Bangka Belitung

during December 2023 to January 2024 (Nurhayati, 2024). In 2023, a total of 14,585 diarrhea cases were reported across all age groups (Dinkes Prov. Kep. Babel, 2023).

The treatment of diarrheal diseases is commonly carried out through the administration of oral rehydration salts, adequate nutritional intake, concentrated tea, as well as synthetic antibiotics such as oral rehydration solutions or zinc supplementation (Adeana & Usiono, 2023). However, inappropriate use of synthetic antibiotics may lead to the emergence of resistance among pathogenic microorganisms. Various plant species distributed in nature possess potential therapeutic properties and have long been utilized as traditional remedies by local communities for the management of several diseases. *Ageratum conyzoides* L., locally known as *bandotan*, is traditionally prepared by drying, pounding, or boiling the plant material and has been used to treat abdominal pain, diarrhea, sore throat, fever,

malaria, and other ailments (Djauhariya & Hermani, 2004; Ganus & Sopandi, 2021).

The roots of *A. conyzoides* contain various phytochemical constituents, including flavonoids, saponins, alkaloids, tannins, minerals, vitamins, anthraquinones, cardiac glycosides, and other bioactive compounds (Agbafor et al., 2015). This plant has long been utilized as a traditional medicinal remedy in regions of Africa, South America, and Asia, including Indonesia. In Indonesia, the traditional use of *A. conyzoides* has been documented in South Bangka Regency (Henri et al., 2022), Nias Regency (Harefa et al., 2022), South Hulu Sungai Regency (Elsi et al., 2020), Karawang Regency (Gunarti et al., 2021), the Balinese ethnic community (Ervinia et al., 2020), and several other regions. According to Omole et al. (2019), the ethanol extract of *A. conyzoides* roots exhibited the growth of *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi* at concentration ranging from 6,25 to 200 µg/mL. In addition, Enerijiofi and Isola (2019) reported that the ethanol extract of *A. conyzoides* roots at 25 mg/mL showed inhibition zones of 4 mm against *Salmonella* sp., *Escherichia coli*, and *Pseudomonas aeruginosa*, and 3 mm against *Enterobacter* sp.

The continuous utilization of medicinal plants may exert negative impacts on their growth. Since plants generally exhibit long life cycles, large-scale harvesting requires extended recovery time. Although *A. conyzoides* is not considered an endangered species, its exploitation still needs to be controlled to ensure sustainability. In addition to plants, endophytic bacteria residing within plant tissues are also capable of producing various bioactive metabolites (Leonita et al., 2018). The presence of these endophytes offers an alternative source of antibacterial agents that may be used for treating several infectious diseases. According to Yahya et al. (2017), endophytic bacteria are able to synthesize diverse bioactive compounds with beneficial biological activities, including antibacterial, anticancer, antifungal, anti-inflammatory, antiviral properties, among others.

Previous studies have reported the use of *A. conyzoides* as a traditional medicinal plant, in which both the roots and leaves exhibit antibacterial properties. Research on endophytic bacteria isolated from the roots of *A. conyzoides* has been conducted in India, obtaining endophytic isolates associated with hydrolytic enzyme synthesis (Sagar & Mallesha, 2023), and isolates with Plant Growth-Promoting Rhizobacteria (PGPR) activity (Momin & Ibrahim, 2020). A study in Thailand also reported endophytic bacterial isolates capable of inhibiting the growth of human pathogenic filamentous fungi (Boonman et al., 2023). However, studies focusing on bioactive compounds produced by root-associated endophytic bacteria of *A. conyzoides* and their antibacterial activity, particularly against dysentery-causing bacteria such as *Escherichia coli* and *Shigella*

dysenteriae, have not yet been reported. Therefore, this study was conducted to evaluate the antibacterial activity and secondary metabolites produced by endophytic bacteria isolated from *A. conyzoides* roots against *E. coli* and *S. dysenteriae* as the primary causative agents of diarrhea.

MATERIALS AND METHODS

Materials

The instruments used in this study included a Bunsen burner, Petri dishes, vortex mixer (Thermo Scientific), incubator (Memmert®), microscope, inoculating loop, laminar air flow cabinet, sterile scissors, sterile forceps, spectrophotometer (Shimadzu), vernier caliper, mortar and pestle, test tubes, orbital shaker (Optima), micropipettes with sterile tips, dropper pipettes, spreader, colony counter (Funke Gerber), incubator (Memmert®), and a rotary evaporator (IKA® HB 10 basic).

The materials used during the sampling, plant determination, and sample preparation steps consisted of 70% ethanol, 95% ethanol, 5.25% sodium hypochlorite (NaOCl), distilled water, *A. conyzoides* L. roots, Nutrient Agar (NA), Nutrient Broth (NB), 3% H₂O₂, Sulfide Indole Motility (SIM) medium, Christensen Urea Agar, Triple Sugar Iron Agar (TSIA), Simmons Citrate Agar (SCA), malachite green, safranin, *Escherichia coli* and *Shigella dysenteriae* isolates, ethyl acetate, Mayer, Wagner, and Dragendorff reagents, 2 N H₂SO₄, 70% ethanol, magnesium ribbon, concentrated HCl, 2 N HCl, 1% FeCl₃, chloroform, glacial acetic acid and concentrated H₂SO₄ (Liebermann–Burchard reagent), paper discs, ciprofloxacin, methanol, and 10% DMSO.

Determination of *A. conyzoides* Roots and Confirmation of Test Bacteria

The root samples of *A. conyzoides* were taxonomically identified at the Herbarium Bangka Belitungense (HBB) to verify their taxonomy and relevant morphological characteristics. The confirmation of the test bacteria, namely *Escherichia coli* and *Shigella dysenteriae*, was carried out by Gram staining and biochemical tests, including catalase, urease, citrate utilization, and Triple Sugar Iron Agar (TSIA) assays (Kusuma et al., 2021).

Preparation and Isolation of Endophytic Bacteria

Root samples of *A. conyzoides* L. were washed thoroughly, surface-sterilized, and aseptically processed for the isolation of endophytic bacteria following a modified method of Yandila et al. (2018). Root surfaces (1 × 1 cm) were sterilized using 70% ethanol for 1 min and 5.25% NaOCl for 30 s, followed by rinsing with sterile distilled water three times. The samples were allowed to dry, and the effectiveness of surface sterilization was confirmed by inoculating 1 mL of the final rinse onto Nutrient Agar (NA) and incubating at 37

°C for 24 hours. Endophytic bacterial isolation was performed using a direct plating method by slightly wounding the surface-sterilized roots with sterile scissors. Sterilized root segments from five *A. conyzoides* plants were inoculated into a single Petri dish containing NA medium, with three replications, and incubated at 37–48 hours. Colonies obtained after incubation were subsequently purified to obtain pure endophytic bacterial isolates.

Characterization of Endophytic Bacteria

Colony morphology observations were performed on purified endophytic bacterial isolates through macroscopic and microscopic examinations, as well as biochemical tests. The biochemical tests performed included:

▪ Catalase Test

The endophytic bacterial isolate was streaked onto a glass slide, followed by the addition of 2–3 drops of 3% hydrogen peroxide (H₂O₂). The suspension was gently mixed using an inoculating loop. The catalase reaction was considered positive if oxygen bubbles were observed on the slide (Firdha, 2022).

▪ Urease Test

The endophytic bacterial isolate was streaked on slanted Christensen Urea Agar medium and incubated at 37 °C for 24 hours. A positive urease reaction was indicated by a color change to pink in the medium (Kantari and Ariyanti, 2024).

▪ Citrate Test

The endophytic bacterial isolate was stabbed into the center of the slanted Simmons Citrate Agar medium to a depth of three-quarters of the tube, and subsequently streaked on the slanted surface in a zig-zag manner. The culture was then incubated at 37 °C for 24 hours. A positive result was indicated by a color change from green to blue, while a negative result showed no color change in the medium (Kosasi et al., 2019; Nia, 2024).

▪ Triple Sugar Iron Agar (TSIA) Test

The endophytic bacterial isolate was collected using a loop and was vertically stabbed into the butt of the TSIA medium and streaked on the slanted surface. The medium was incubated at 37 °C for 24 hours. A positive result indicating glucose fermentation was shown by a red color in the slant and a yellow color in the butt, along with gas and H₂S production. When both the slant and butt turned yellow, it indicated the ability of the isolate to ferment sucrose, lactose, and glucose (Fallo and Sine, 2016; Nia, 2024). A red color change in the medium indicated an alkaline (K) reaction, whereas a yellow color indicated an acidic (A) reaction.

Hemolysis Test of Endophytic Bacteria

The purified endophytic bacteria were streaked onto Blood Agar Base to confirm their ability to lyse red

blood cells. The plates were then incubated at 37 °C for 24 hours (Buxton, 2005; Sutandi et al., 2024). Hemolytic characteristics are classified into three types: complete lysis (β -hemolysis), partial lysis (α -hemolysis), and no lysis (γ -hemolysis). The presence of hemolytic activity was indicated by the formation of a clear zone surrounding the bacterial colonies (Sutandi et al., 2024).

Screening of Antibacterial Activity Using the Antagonistic Test

Each test bacterium and endophytic bacterium was cultured in 10 mL of Nutrient Broth (NB) and incubated in a shaking incubator at 120 rpm at 37 °C for 24–48 hours. Each test bacterial culture was inoculated into molten Nutrient Agar (NA), which had been cooled to approximately 40 °C, in sterile Petri dishes. After solidification, the rejuvenated endophytic bacteria were streaked in a circular pattern on the medium and incubated at 37 °C for 24 hours. The presence of antibacterial activity was indicated by the formation of an inhibition zone surrounding the endophytic bacterial colony (Putra et al., 2024). Observations were made on the largest inhibition zones formed by each endophytic isolate, and several isolates with the largest inhibition zones were selected for extraction.

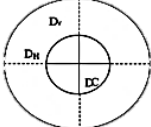
Production and Extraction of Secondary Metabolites from Endophytic Bacteria

Selected endophytic bacterial isolates were cultured as starter cultures in 20 mL of NB medium and incubated for 24 hours. Subsequently, each 20 mL starter culture was inoculated into 1 liter of fresh NB medium. Fermentation was carried out in a shaking incubator at 120 rpm for 72 hours at 28–29 °C. For every 1 liter of bacterial culture, 1 liter of ethyl acetate was added at a ratio of 1:1 (v/v), and the mixture was placed in a shaking incubator at 28–29 °C at 180 rpm for 60 minutes. The solution formed two layers, and the ethyl acetate layer was separated using a separating funnel and evaporated using a rotary evaporator at 150 rpm and 50 °C to obtain the crude extract (Prastya et al., 2019; Putra et al., 2024).

Antibacterial Activity Test Using the Disc Diffusion Method

A total of 1 mL of each test bacterial suspension was transferred into a sterile Petri dish. Nutrient Agar (NA) medium was poured into the Petri dish containing the test bacterial suspension using the pour plate method and allowed to solidify homogeneously. Antibacterial activity was evaluated using the disc diffusion method with sterile paper discs. Sterile paper discs were immersed in the crude extract of endophytic bacterial isolates until fully absorbed, then placed onto the surface of NA medium. Ciprofloxacin 0.01% was used as a positive control, and 10% DMSO was used as a negative control by dropping each solution onto sterile paper discs and placing them onto the NA medium. All plates were

incubated at 37 °C for 24 hours (Sadikin et al., 2021). The inhibition zone diameter was calculated using the following formula.

$$\frac{(D_V - D_C) + (D_H - D_C)}{2}$$


Note :

D_V : Vertical Diameter

D_H : Horizontal Diameter

D_C : Disc Diameter

Figure 1. Measurement of the inhibition zone diameter. (Source: Fiana et al., 2020)

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Assays

The assay was performed using several extract concentrations, namely 7.5%, 5%, 2.5%, 1%, and 0.5%, each supplemented with methanol as the solvent. Each extract concentration and 10 µL of the test bacterial suspension were transferred into a tube and homogenized using a vortex mixer. Ciprofloxacin 0.01% was used as a positive control, while 10% DMSO served as a negative control. The tubes were shaken at 120 rpm for 24 hours, followed by inoculation of 100 µL from each tube onto NA medium using the spread plate method and incubated at 37°C for 24 hours. The MIC value was determined based on the lowest concentration capable of inhibiting bacterial growth, indicated by $\leq 2.5\%$ viable bacterial colonies (Darajat et al., 2022; Insyira, 2024). The MBC value was determined based on the lowest concentration of extract capable of killing the bacterial cells, indicated by $< 0.1\%$ surviving cells relative to the initial inoculum (Salmi and Swandi, 2022).

Qualitative Screening of Secondary Metabolites

▪ Alkaloids Test

The endophytic bacterial extract was added with 2 N H_2SO_4 and shaken thoroughly. Subsequently, three alkaloids reagents—Mayer's, Wagner's, and Dragendorff's—were added dropwise. A positive reaction with Dragendorff's reagent was indicated by the formation of a red precipitate, while Wagner's reagent produced a brownish precipitate, and Mayer's reagent produced a white precipitate (Mailuhu et al., 2017).

▪ Flavonoids Test

The endophytic bacterial extract was placed into a test tube, followed by the addition of a few drops of 70% ethanol and heating. The mixture was then added with a magnesium ribbon and one drop of concentrated HCl. A positive flavonoids reaction was indicated by a color

change to red, pink, or yellow (Parbuntari et al., 2019; Erwan and Parbuntari, 2023).

▪ Saponins Test

The endophytic bacterial extract was mixed with 5 mL of hot water and 2 drops of 2N hydrochloric acid (HCl) and homogenized. A positive saponins reaction was indicated by the formation of stable and persistent foam with strong intensity for up to 10 minutes (Erwan and Parbuntari, 2023).

▪ Tannins Test

The endophytic bacterial extract was added with 2–3 drops of 1% $FeCl_3$. A positive result for tannins was indicated by a color change to dark green (Elviasari and Adam, 2016; Sanatang and Purnama, 2023).

▪ Steroid and Triterpenoid Test

The endophytic bacterial extract was mixed with chloroform and 2 N H_2SO_4 , homogenized, and allowed to stand until two layers were formed, consisting of an upper acidic layer and a lower chloroform layer. The chloroform layer was transferred onto a spot plate and allowed to evaporate, followed by the addition of glacial acetic acid and concentrated H_2SO_4 (Liebermann–Burchard reagent). The formation of a green-blue color indicated the presence of steroids, whereas a red color indicated the presence of triterpenoids (Khafid et al., 2023).

Data Analysis

Qualitative methods were carried out descriptively by presenting the research data using figures and tables for the endophytic bacterial isolation and characterization results. The qualitative secondary metabolite screening data were expressed using the symbols (+) and (–) to indicate the presence or absence of phytochemical compounds. Quantitative methods were applied to analyze the antibacterial activity against *Escherichia coli* and *Shigella dysenteriae* that were inhibited by the endophytic bacterial isolates from the roots of *Ageratum conyzoides* L., expressed as the mean $\pm$ standard deviation (SD). The MIC and MBC data were presented in the form of average values.

RESULTS AND DISCUSSION

Confirmation of *A. conyzoides* L. and Test Bacteria

Based on the morphological determination, the characteristics of the plant used in this study were confirmed to be *A. conyzoides* L., belonging to the family *Asteraceae* and locally known as Bandotan. In addition, the confirmation results of the test bacteria showed that the isolates used were correctly identified as *Escherichia coli* and *Shigella dysenteriae*.

Table 1. Confirmation of the Test Bacteria.

Identification	Test Bacteria	
	<i>Escherichia coli</i>	<i>Shigella dysenteriae</i>
Colony Color	Green Metallic (EMBA)	White (NA)
Gram Staining	-	-
Cell Shape	Bacilli	Bacilli
Catalase	+	-
Motility	Non-motile	Non-motile
Urease	-	-
Citrate	-	-
TSIA		
- Slant/Butt	A/A	K/A
- H ₂ S	-	-
- Gas	+	-

Note: K/A: Glucose fermentation; A/A: Glucose, lactose, sucrose fermentation; + : Positive, - : Negative

Isolation and Characterization of Endophytic Bacteria

Based on the isolation results, a total of 18 endophytic bacterial isolates were obtained from the roots of *A. conyzoides* L., coded as AC1–AC18, using the direct plating method.

Colony Morphology of Endophytic Bacteria

A total of 18 endophytic bacterial isolates were obtained, of which 15 exhibited circular colonies, and one isolate each exhibited rhizoid, filamentous, and irregular colony shapes.

Table 2. Colony Morphology Characterization of Endophytic Bacterial Isolates.

Isolate Code	Colony Morphology					
	Colony Shape	Size	Margin	Elevation	Tekstur	Color
AC1	Circular	Small	Entire	Flat	Smooth	Milky white
AC2	Circular	Small	Entire	Flat	Smooth	Milky white
AC3	Rhizoid	Moderate	Undulate	Raised	Mucoid	Milky white
AC4	Circular	Small	Entire	Flat	Smooth	Milky white
AC5	Circular	Small	Entire	Raised	Smooth	Milky white
AC6	Circular	Small	Entire	Flat	Smooth	Milky white
AC7	Circular	Small	Entire	Raised	Smooth	Milky white
AC8	Circular	Moderate	Entire	Raised	Smooth	Milky white
AC9	Circular	Small	Entire	Raised	Smooth	Milky white
AC10	Circular	Small	Entire	Flat	Smooth	Milky white
AC11	Circular	Small	Entire	Flat	Smooth	Milky white
AC12	Filamentous	Moderate	Filamentous	Raised	Rough	Milky white
AC13	Irregular	Small	Entire	Flat	Smooth	Milky white
AC14	Circular	Small	Entire	Raised	Smooth	Milky white
AC15	Circular	Small	Entire	Flat	Smooth	Crem
AC16	Circular	Small	Entire	Raised	Smooth	Milky white
AC17	Circular	Small	Entire	Raised	Smooth	Milky white
AC18	Circular	Small	Entire	Flat	Smooth	Milky white

Cell Morphology of Endophytic Bacteria

The 18 endophytic bacterial isolates obtained were characterized for their cell morphology and Gram reaction. Among the isolates, 7 were Gram-positive with

a bacilli (rod-shaped) cell morphology, while 11 were Gram-negative, including 16 bacilli-shaped and 2 cocci-shaped isolates. Furthermore, all 18 isolates tested negative for endospore formation.

Table 3. Cell Morphology Characterization of Endophytic Bacterial Isolates.

Isolate Code	Cell Morphology		
	Cell Shape	Gram (+/-)	Endospore
AC1	Bacilli	-	-
AC2	Bacilli	+	-
AC3	Bacilli	-	-
AC4	Bacilli	+	-
AC5	Bacilli	+	-
AC6	Bacilli	-	-
AC7	Bacilli	+	-
AC8	Bacilli	+	-
AC9	Bacilli	+	-
AC10	Bacilli	-	-
AC11	Cocci	-	-
AC12	Bacilli	-	-
AC13	Bacilli	+	-
AC14	Bacilli	-	-
AC15	Bacilli	-	-
AC16	Bacilli	-	-
AC17	Cocci	-	-
AC18	Bacilli	-	-

Note : (+) : indicates a positive result; (-) : indicates a negative result.

■ Biochemical Tests

Based on their biochemical activities, all 18 endophytic bacterial isolates were subjected to biochemical tests, including catalase, urease, citrate, and TSIA tests,

showing varied results. The biochemical test results of endophytic bacterial isolates from *A. conyzoides* L. roots are presented in Table 4.

Table 4. Biochemical Tests of Endophytic Bacterial Isolates from *Ageratum conyzoides* L. Roots.

Isolate Code	Catalase	Motility	Urease	Citrate	TSIA		
					Slant/Butt	H ₂ S	Gas
AC1	+	-	+	-	K/A	-	-
AC2	-	-	+	-	K/A	-	-
AC3	+	+	+	-	A/A	-	-
AC4	+	-	+	-	K/A	-	-
AC5	+	+	+	-	K/A	-	-
AC6	+	+	+	-	K/A	-	-
AC7	+	-	+	-	K/A	-	-
AC8	+	-	+	-	K/A	-	-
AC9	+	+	+	-	K/K	-	-
AC10	+	+	-	+	K/K	-	-
AC11	+	+	+	+	K/A	-	+
AC12	+	+	-	-	K/A	-	-
AC13	+	+	+	-	K/K	-	-
AC14	+	+	+	+	K/K	-	-
AC15	+	+	+	+	K/A	-	-
AC16	+	+	+	+	K/K	-	-
AC17	+	+	+	+	K/A	-	+
AC18	+	-	+	+	K/K	-	-

Note : K/K : no fermentation; K/A : glucose fermentation only; K/A G⁺ : glucose fermentation only with gas production; A/A : glucose, lactose, and sucrose fermentation; + : positive (change observed); - : negative (no change observed).

Hemolysis Test of Endophytic Bacteria

In general, pathogenic bacteria can be identified based on their ability to lyse red blood cells (Alang et al., 2025).

The destruction of red blood cells occurs due to bacterial activity that produces hemolysins (Novriansyah et al., 2025).

Table 5. Hemolysis Test Results of Endophytic Bacterial Isolates.

Code Isolate	Hemolysis Test Results
AC1	β-hemolysis
AC2	α-hemolysis
AC3	γ-hemolysis
AC4	α-hemolysis
AC5	α-hemolysis
AC6	β-hemolysis
AC7	β-hemolysis
AC8	β-hemolysis
AC9	α-hemolysis
AC10	γ-hemolysis
AC11	γ-hemolysis
AC12	β-hemolysis
AC13	γ-hemolysis
AC14	β-hemolysis
AC15	α-hemolysis
AC16	β-hemolysis
AC17	γ-hemolysis
AC18	γ-hemolysis

Note: α-hemolysis = partial hemolysis; β-hemolysis = complete hemolysis; γ-hemolysis = no hemolysis.

The study showed that, among the 18 endophytic bacterial isolates, 5 isolates exhibited α-hemolysis, 7 isolates exhibited β-hemolysis, and 6 isolates showed γ-hemolysis. Bacteria demonstrating α-hemolysis and β-hemolysis have potential pathogenicity to human health; therefore, they were excluded from further tests.

Screening of Antibacterial Activity Using the Antagonistic Test

Preliminary screening of antibacterial activity was conducted to evaluate the inhibitory potential of six endophytic bacterial isolates against *E. coli* and *S. dysenteriae*.

Table 6. Inhibition Zone Diameters of Endophytic Bacteria Against Test.

Test Bacteria	Isolate Code	Mean Inhibition Zone Diameter (mm) ± SD	Category
<i>Escherichia coli</i>	AC3	15,96 ± 0,98	Strong
	AC10	0,00 ± 0,00	None
	AC11	0,00 ± 0,00	None
	AC13	0,00 ± 0,00	None
	AC17	2,35 ± 0,67	Weak
	AC18	0,00 ± 0,00	None
<i>Shigella dysenteriae</i>	AC3	1,63 ± 0,27	Weak
	AC10	3,59 ± 0,28	Weak
	AC11	0,00 ± 0,00	None
	AC13	0,00 ± 0,00	None
	AC17	7,63 ± 1,29	Moderate
	AC18	0,00 ± 0,00	None

Based on the screening results of the endophytic bacteria, only two isolates, AC3 and AC17, exhibited inhibitory activity against *E. coli*, while three isolates, AC3, AC10, and AC17, showed inhibitory activity against *S. dysenteriae*.

Antibacterial Activity of Endophytic Bacterial Extracts Against *Escherichia coli* and *Shigella dysenteriae*

The endophytic bacterial extracts used in the disc diffusion antibacterial test were obtained from isolates

that demonstrated antibacterial activity during the preliminary screening. The extracts showed the highest antibacterial activity for isolate AC3 at a concentration of 7.5% against *E. coli*, with an inhibition zone of 10.38 mm, categorized as strong, and for isolate AC10 against *S. dysenteriae*, with an inhibition zone of 5.02 mm, categorized as moderate.

Table 7. Antibacterial Activity Results of Endophytic Bacterial Extracts from *Ageratum conyzoides* L. Roots.

Test Bacteria	Extract Code	Mean Inhibition Zone Diameter (mm) $\pm$ SD	Category
<i>Escherichia coli</i>	AC3	10,38 $\pm$ 0,27	Strong
	AC10	2,33 $\pm$ 0,25	Weak
	AC17	3,94 $\pm$ 0,68	Weak
	Ciprofloxacin 0,01% (+)	22,94 $\pm$ 0,27	Very strong
	DMSO 10% (-)	0,00 $\pm$ 0,00	None
	AC3	1,95 $\pm$ 0,29	Weak
<i>Shigella dysenteriae</i>	AC10	5,02 $\pm$ 1,36	Moderate
	AC17	3,57 $\pm$ 1,50	Weak
	Ciprofloxacin 0,01% (+)	23,32 $\pm$ 0,08	Very strong
	DMSO 10% (-)	0,00 $\pm$ 0,00	None

The study showed that the endophytic bacterial extracts from *A. conyzoides* roots at a concentration of 7.5% exhibited antibacterial activity, although the antibacterial activity of some extracts produced from the secondary metabolites of endophytic bacteria was not considered strong.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Endophytic Bacterial Extracts from *Ageratum conyzoides* L. Roots

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of endophytic bacterial extracts from *A. conyzoides* L. roots against *E. coli* and *S. dysenteriae* varied among the extracts.

Table 8. MIC and MBC Values of Endophytic Bacterial Extracts from *Ageratum conyzoides* L. Roots.

Extract Code	Treatment Concentration	Average Colony Count	
		<i>E. coli</i>	<i>S. dysenteriae</i>
AC3	Ciprofloxacin 0,01%	0	0
	DMSO 10%	TNTC	TNTC
	0,5%	TNTC	TNTC
	1 %	TNTC	TNTC
	2,5%	TNTC	TNTC
	5%	TNTC	TNTC
	7,5%	100	TNTC
AC10	Ciprofloxacin 0,01%	0	0
	DMSO 10%	TNTC	TNTC
	0,5%	TNTC	TNTC
	1 %	TNTC	TNTC
	2,5%	156	TNTC
	5%	103	TNTC
	7,5%	27	TNTC
AC17	Ciprofloxacin 0,01%	0	0
	DMSO 10%	TNTC	TNTC
	0,5%	TNTC	TNTC
	1 %	TNTC	TNTC
	2,5%	176	TNTC
	5%	23	TNTC
	7,5%	0	247

Note: TNTC (Too Numerous To Count, > 300 colonies)

The determination of the MIC for extract AC3 against *E. coli* was observed at a concentration of 7.5%, with no MBC value detected, while against *S. dysenteriae*, neither MIC nor MBC values were observed. For extract AC10, the MIC against *E. coli* was also at 7.5%, with no MBC detected, and no MIC or MBC values were

observed against *S. dysenteriae*. Extract AC17 showed an MIC against *E. coli* at 5% and an MBC at 7.5%, whereas against *S. dysenteriae*, the MIC was observed at 7.5%.

Screening of Secondary Metabolites in Endophytic Bacterial Extracts from *Ageratum conyzoides* L. Roots

The antibacterial activity results, the that results of inhibition zones as well as MIC and MBC values, were

supported by phytochemical tests these results indicated the presence of secondary metabolites in the endophytic bacterial extracts from *A. conyzoides* L. roots.

Table 9. Identification of Secondary Metabolites in Endophytic Bacterial Extracts from *Ageratum conyzoides* L. Roots.

Extract Code	Secondary Metabolites							
	Alkaloids			Flavonoids	Saponins	Tannins	Steroids	Triterpenoid
	Mayer	Dragendorff	Wagner					
AC3	-	-	-	+	-	-	-	-
AC10	-	-	-	+	-	-	-	-
AC17	+	-	-	+	-	-	-	+

Note: (+) = present in the extract, (-) = absent in the extract.

The study showed that extracts AC3, AC10, and AC17 were positive for flavonoids, as indicated by a color change to yellow. In the alkaloid test using Mayer's reagent, extract AC17 formed a greenish-white precipitate, indicating a positive result for alkaloids. Furthermore, in the triterpenoid test, extract AC17 exhibited a red color change, confirming the presence of triterpenoids. All three endophytic bacterial extracts tested negative for saponins, tannins, and steroids.

Discussion

Confirmation of Plant and Test Bacteria Morphology

Determination was carried out to verify the identity of the plant and to avoid errors in the use of materials in this study. The confirmation results verified that the sample used was *A. conyzoides* L. (*bandotan*), a herbaceous plant with stems covered in fine hairs, green opposite leaves, compound inflorescences forming clusters with light purple flowers, and a fibrous root system.

The confirmation of *E. coli* on EMBA (Eosin Methylene Blue Agar) showed bacterial colonies with a metallic green color, while *S. dysenteriae* on NA (Nutrient Agar) formed white colonies. Biochemical tests were also conducted for further confirmation. The results were consistent with Aufani et al. (2023), which reported that *E. coli* has a bacillus (rod) cell shape, is Gram-negative, forms dark blue colonies resembling metallic green on EMBA, and *S. dysenteriae* has a bacillus cell shape, is Gram-negative, and forms smooth, circular colonies. Similar results were reported by Putra et al. (2024), indicating that *E. coli* is citrate-negative, urease-negative, ferments glucose, lactose, and sucrose, and produces gas in the TSIA test. *S. dysenteriae* was non-motile, citrate-negative, urease-negative, and ferments only glucose (Tjandra, 2018).

Isolation and Characterization of Endophytic Bacteria

The isolation results showed the growth of endophytic bacterial colonies around the roots cultured on Nutrient Agar (NA) medium. This was confirmed by the absence

of microbial growth in the petri dishes containing the final rinse water, which served as a control. Successful surface sterilization was verified by the lack of microbial growth from the final rinse water on the plates (Waheeda and Shyam, 2017; Aqlinia et al., 2020). These findings indicate that the bacteria growing around the roots originated from the internal root tissues, while epiphytic microbes contaminating the surface were eliminated through the surface sterilization process.

Colony and Cell Morphology Characterization of Endophytic Bacteria

Based on the isolation results, the characterization of the endophytic bacterial colonies obtained was consistent with the typical morphology of endophytic bacteria. The characteristics of the endophytic bacteria showed slight differences among isolates. This is because, according to Dalimunthe et al. (2023) and Handayani et al. (2023), endophytic bacteria generally exhibit colony shapes such as circular, rhizoid, irregular, and filamentous, with colony margins that may be entire, undulate, filamentous, or filiform. In addition, these studies reported that endophytic bacteria commonly have flat or raised elevations, with colony colors ranging from white to cream. The diversity of colony morphology is influenced by the ability of bacteria to adapt to environmental conditions, including culture media, temperature, incubation period, and isolate age (Rizqoh et al., 2021).

Differences in Gram staining results can be attributed to variations in the main components of the bacterial cell wall structure. According to Nurhidayat and Silviani (2022), Gram-positive bacteria have a cell wall composed of a thick peptidoglycan layer that serves as the primary protective barrier. The thickness of the peptidoglycan layer allows the crystal violet stain to be retained even after alcohol washing, resulting in the cells appearing purple and classifying them as Gram-positive. In addition, the Gram-positive cell wall contains other components, such as teichoic acids and lipoteichoic acids, which contribute to the stability of the cell wall

structure, its antigenic properties, and adherence to host cells.

According to Nurhidayat and Silviani (2022), Gram-negative bacteria have a two-layered cell wall. First, the inner peptidoglycan layer is much thinner compared to that of Gram-positive bacteria. Second, the outer peptidoglycan layer is accompanied by an additional outer membrane composed of proteins, phospholipids, and lipopolysaccharides (LPS), which are characteristic of Gram-negative cell walls. The LPS consists of three components: the specific O-antigen, the core polysaccharide, and lipid A (endotoxin). The presence of this outer membrane not only protects the cell from harsh environmental conditions but also slows the penetration of antibiotic molecules, which makes Gram-negative bacteria relatively more resistant to antibacterial compounds. The thin peptidoglycan layer allows the crystal violet stain to be easily washed away during alcohol treatment, causing the cells to take up safranin and appear pink, thus classifying them as Gram-negative bacteria.

Endospore staining was performed to determine whether the endophytic bacteria produce spores. According to Apriliani and Zulaika (2021), spore-forming bacteria can undergo sporulation to survive extreme conditions such as high temperature, acidic or basic solutions, desiccation, nutrient deprivation, radiation, and exposure to antibiotics. Generally, only a small portion of bacteria possess endospores, typically Gram-positive rod-shaped (bacillus) bacteria (Fajri et al., 2015). The presence of endospores may indicate that the bacterium belongs to the genus *Bacillus*. Kurniawan et al. (2021) stated that endospore staining is necessary to confirm the possible genus of the isolate. In contrast, Gram-negative bacteria lack the genes necessary for sporulation and therefore do not produce endospores (Beskrovnaya et al., 2021).

▪ Physiological Characterization of Endophytic Bacteria

Based on the results of the study, 17 endophytic bacterial isolates tested positive for catalase, producing oxygen bubbles, while one isolate, coded AC2, did not possess the catalase enzyme as it did not produce oxygen bubbles. Bacteria lacking catalase cannot break down H_2O_2 , thus they are unable to generate oxygen and water. Hydrogen peroxide (H_2O_2) is a toxic compound that can disrupt bacterial metabolic systems, potentially leading to bacterial death (Pulungan and Tumanggar, 2018). According to Kantari and Ariyanti (2024) in Fallo et al. (2025), motility testing is an important factor for determining the ability of endophytic bacteria to form colonies within host plant tissues. In this study, 12 bacterial isolates were motile and possessed flagella, whereas 6 isolates were non-motile. This aligns with the findings of Elokpere (2022), which reported that endophytic bacteria can be either motile or non-motile

because not all bacteria possess locomotory structures such as flagella. Furthermore, Hala et al. (2022) also reported that endophytic bacteria from the roots of *Caesalpinia sappan* exhibited both motile and non-motile characteristics.

Endophytic bacteria that tested positive for the urease test possess the urease enzyme, indicated by a color change of the medium from yellow to pink, whereas a negative urease result is marked by no change in the medium's color (Ulfa et al., 2016). In this study, 16 bacterial isolates were positive for urease, while two isolates, coded AC10 and AC12, were negative for urease. This aligns with the findings of Wati (2018), which reported that endophytic bacterial isolates obtained from the petioles of Taro (*Colocasia esculenta*) were positive for using urea as a carbon source. Hala et al. (2022) also reported that endophytic bacteria from the roots of *Caesalpinia sappan* showed both positive and negative results in the urease test. In this study, seven isolates tested positive for citrate utilization, indicated by a color change of the medium to blue, while the remaining 11 isolates were negative for citrate. This is consistent with the study of Silalahi et al. (2020), which reported that endophytic bacteria obtained from the leaves and stems of *Citrus hystrix* showed three isolates positive and seven isolates negative for citrate utilization.

The TSIA test in this study showed that nine isolates were able to ferment glucose only (K/A), two isolates were able to ferment glucose and produce gas (K/A, G+), one isolate could ferment glucose, sucrose, and lactose (A/A), and six isolates were unable to ferment glucose, sucrose, or lactose (K/K). Gas formation was observed in isolates AC11 and AC17, causing the medium to lift, which was due to gas produced as a result of bacterial fermentation generating formic acid. With the help of the enzyme formate hydrogenase, formic acid is oxidized into hydrogen gas and carbon dioxide. This is consistent with the study by Butar-butur et al. (2023), which reported that some endophytic bacteria isolated from the roots of *Plectranthus amboinicus* (Lour) showed TSIA results indicating the ability to ferment glucose, sucrose, and lactose; inability to ferment glucose, sucrose, and lactose; and fermentation of glucose only.

Hemolysis Test of Endophytic Bacteria

The endophytic bacterial isolates with codes AC3, AC10, AC11, AC13, AC17, and AC18 did not exhibit pathogenic properties and were therefore used for subsequent tests. This is in accordance with the study by Asmoro and Munif (2019), which reported that endophytic bacteria from the roots of *Pteris ensiformis* and *Salvinia molesta* included both pathogenic and non-pathogenic strains; pathogenic bacteria were excluded from further testing due to failing biosafety assessments. According to Novriansyah et al. (2025), partial hemolysis (α -hemolysis) is indicated by a greenish discoloration around the colonies, as red blood cells are not completely

lysed and hemoglobin (Fe^{2+}) is oxidized to methemoglobin (Fe^{3+}). Total hemolysis (β -hemolysis) is shown by the formation of a clear zone around the colonies, indicating complete lysis of red blood cells. Non-pathogenic bacteria (γ -hemolysis) are indicated by the absence of color change in the medium, as the bacteria do not produce hemolysin or exhibit very low hemolytic activity.

Endophytic bacteria, which are known to play important roles in plants, can potentially exhibit pathogenicity in humans. This is because endophytic bacteria possess virulence potential by producing hemolysin toxins, which act as virulence factors under certain environmental changes, such as extreme temperatures, nutrient deficiencies, and drought (Raimi et al., 2025), as well as factors like microbial population and the genotypes of both host and interacting microbes (Hardoim et al., 2015). According to Raimi et al. (2025), virulence studies indicate that some endophytic bacteria in plants can affect human health if the plant is consumed raw. One example of an endophytic bacterium showing high cytotoxicity to human and mammalian cells is *Bacillus cereus*.

Screening of Antibacterial Activity Using the Antagonistic Test Method

The inhibition zones formed by isolates AC3 and AC17 against *E. coli* had average diameters of 15.96 mm (strong category) and 2.35 mm (weak category), respectively. For *S. dysenteriae*, the inhibition zones formed by isolates AC3, AC10, and AC17 had average diameters of 1.63 mm (weak category), 3.59 mm (weak category), and 7.63 mm (moderate category), respectively. The classification of antibacterial activity categories is based on the diameter of the clear zones formed, according to Purnamaningsih et al. (2017), which states that the bacterial growth inhibition response can be categorized as weak (diameter ≤ 5 mm), moderate (5–10 mm), strong (10–20 mm), and very strong (>20 mm).

In the screening test, isolate AC10 did not form an inhibition zone against *E. coli*. This may have occurred because the compounds produced by isolate AC10 were still in low amounts and were not yet sufficient to inhibit the growth of *E. coli*. This is in accordance with the study by A'yun and Nugraheni (2023), which states that bacteria naturally produce secondary metabolites at low concentrations, as the expression of these compounds by each endophytic bacterium is influenced by environmental conditions such as pH, temperature, nutrients, and incubation time. This indicates that the growth of endophytic bacteria needs to be optimized to maximize the production of secondary metabolites. Although isolate AC10 did not show antibacterial activity against *E. coli* in the screening test, it did exhibit antibacterial activity against *S. dysenteriae*. Therefore, isolate AC10 is still considered to have potential antibacterial activity against *E. coli*, which is supported

by the antibacterial activity detected after the extraction process using the disc diffusion method.

This study is in accordance with the research by Naibaho et al. (2023), which reported that three endophytic bacterial isolates from Dayak onion (*Eleutherine bulbosa*) were capable of inhibiting the growth of *E. coli*. In addition, Pratiwi (2015) also stated that endophytic bacteria from the leaves of *Garcinia benthami* Pierre exhibited antibacterial activity against *E. coli* and *S. dysenteriae*. These findings indicate that endophytic bacteria possess antibacterial activity in inhibiting the growth of *E. coli* and *S. dysenteriae*.

Antibacterial Activity of Endophytic Bacterial Extracts Against *Escherichia coli* and *Shigella dysenteriae*

Based on the results, secondary metabolite extracts from endophytic bacteria of *Bandotan* roots at a concentration of 7.5% showed that extract AC3 exhibited an inhibition zone of 10.38 mm (strong) against *E. coli* and 1.95 mm (weak) against *S. dysenteriae*. Extract AC10 produced an inhibition zone of 2.33 mm (weak) against *E. coli* and 5.02 mm (moderate) against *S. dysenteriae*. For extract AC17, the inhibition zones were 3.94 mm (weak) against *E. coli* and 3.57 mm (weak) against *S. dysenteriae*. The positive control, using ciprofloxacin 0.01%, showed a very strong inhibition zone against both test bacteria, indicating that *E. coli* and *S. dysenteriae* are sensitive to ciprofloxacin. The negative control, using 10% DMSO, showed no antibacterial activity as no clear zones were formed, confirming that the observed antibacterial activity originated from the extracts rather than the solvent. The antibacterial mechanisms of these compounds include disruption of cell membrane function, inhibition of protein synthesis, inhibition of cell wall synthesis, inhibition of nucleic acid synthesis, and acting as antimetabolites to block the synthesis of essential metabolites (Oktavia & Pujiyanto, 2018).

The endophytic bacterial extracts from *Bandotan* roots exhibited antibacterial activity. The bioactive compounds from these extracts can penetrate the bacterial cell membrane and disrupt the metabolic processes, leading to cell lysis and eventual death. Differences in antibacterial activity among the extracts can be influenced by variations in concentration, the type of antibacterial compounds present, the diffusion rate of the extract in the agar medium, and the type of solvent used. The concentration of antibacterial compounds affects the resulting activity, as higher extract concentrations generally produce larger inhibition zones (Ayen et al., 2017). The diffusion rate of the extract in the agar can also impact antibacterial activity because different bacteria tend to produce bioactive compounds of varying properties, including molecular size. Bioactive compounds with smaller molecular weights diffuse more rapidly into the agar, allowing wider distribution and resulting in larger inhibition zones (Hening, 2023).

Conversely, bioactive compounds with larger molecular weights tend to produce smaller inhibition zones.

Furthermore, another possible reason is that the compounds produced may be less effective in inhibiting bacterial growth due to the limited ability of the antibacterial compounds to penetrate the bacterial cell wall (Heni et al., 2015). Suboptimal secretion of bioactive compounds by the bacteria into the medium also affects the quantity of compounds produced, thereby reducing their effectiveness in inhibiting the growth of the test bacteria. This study showed that the endophytic bacterial extracts from Bandotan roots (*Ageratum conyzoides* L.) at a concentration of 7.5% exhibited antibacterial activity, although the antibacterial activity of some extracts produced from secondary metabolites of endophytic bacteria cannot yet be considered strong. This finding is consistent with the study by Purwaningsih and Wulandari (2021), which reported that endophytic bacteria from taro tubers exhibited antibacterial activity, although the activity produced from the fermentation of taro endophytic bacteria could not be considered highly potent. In addition, Boonman et al. (2023) reported that endophytic bacterial isolates AconR2 and AconR4 from Bandotan roots showed antibacterial activity against *E. coli* and *S. flexneri*, which was not very effective against human pathogenic bacteria.

Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Endophytic Bacterial Extracts

In the endophytic bacterial extracts from *Bandotan* roots, the MIC of extract AC3 against *E. coli* was observed at a concentration of 7.5%, while the MBC was not detected; against *S. dysenteriae*, neither the MIC nor the MBC was observed. For extract AC10, the MIC against *E. coli* was also observed at 7.5%, with no MBC detected, and neither MIC nor MBC was observed against *S. dysenteriae*. Extract AC17 showed an MIC against *E. coli* at 5% and an MBC at 7.5%, whereas the MIC against *S. dysenteriae* was observed at 7.5%. The decrease in the number of bacterial colonies with increasing concentrations of the Bandotan root endophytic bacterial extracts indicates that the bioactive compounds contained in the extracts exert an antibacterial effect by inhibiting the growth of *Escherichia coli* and *Shigella dysenteriae*.

The differences in antibacterial effects observed between the disc diffusion assay and the MIC and MBC tests are attributed to the use of different methods, as well as the low concentrations of antibacterial compounds in the extracts, which result in limited antibacterial activity. Additionally, the difference may be influenced by the varying diffusion rates of the bioactive compounds in agar media compared to liquid media (Fitriana et al., 2023). The antibacterial compounds in the extracts are presumed to inhibit bacterial growth on solid media but are not yet sufficiently effective at inhibiting

or killing bacteria at a 7.5% concentration in liquid media. This aligns with the findings of Anwar and Rizki (2023), who reported that samples with weak antibacterial activity and bacteria placed together in the same tube may not exhibit a noticeable antibacterial effect.

Based on the MIC and MBC tests, the MIC and MBC values of the extracts at a 7.5% concentration used in this study are still considered weak. According to Saraswati (2015), extracts that exhibit antibacterial activity at concentrations >1000 µg/mL are regarded as having limited potential to be developed as new antimicrobial agents. An extract can be categorized as having antimicrobial potential if it is able to inhibit bacterial growth at concentrations ≤1000 µg/mL.

Research on the use of bandotan root extracts as antibacterial agents is still limited, as most studies have focused on bandotan leaf extracts. The results of this study show that endophytic bacterial extracts have MIC values at concentrations of 5%–7.5% and MBC values at 7.5%. These results are in line with the study by Enerijiofi and Isola (2019), which reported MIC and MBC values of ethanol extracts of bandotan roots at a concentration of 75 mg/mL. Although the MIC and MBC values of ethanol root extracts and endophytic bacterial root extracts coincidentally fall within a relatively similar concentration range, this is likely due to the possibility of targeting similar bacteria, namely Gram-negative bacteria. However, these results may also indicate differences, as the mechanisms of action and the bioactive compounds produced by these two types of extracts can vary. This is consistent with the findings of Enerijiofi and Isola (2019), which reported that ethanol extracts of bandotan roots contain tannins, phenols, glycosides, steroids, and saponins.

Screening of Secondary Metabolites in Endophytic Bacterial Extracts from *Ageratum conyzoides* L. Roots

The results of the antibacterial activity, indicated by the inhibition zones as well as the MIC and MBC values, were supported by phytochemical tests showing the presence of secondary metabolites in the endophytic bacterial extracts from bandotan roots (*A. conyzoides* L.). The compounds present in extracts AC3, AC10, and AC17 tested positive for flavonoids, while extract AC17 also indicated the presence of alkaloids and triterpenoids. However, all three endophytic bacterial extracts tested negative for saponins, tannins, and steroids.

The alkaloid content in endophytic bacterial extracts exhibits antibacterial activity by interfering with the components of peptidoglycan in the bacterial cell wall, thereby preventing the formation of a complex layer and causing bacterial cell death. Additionally, alkaloids can inhibit protein synthesis, which plays a role in bacterial metabolism and affects DNA function (Yan et al., 2021). Alkaloid compounds can inhibit the growth of Gram-negative bacteria, such as *E. coli* and *S. dysenteriae*

(Anggraini et al., 2020). The differences in results using Mayer, Wagner, and Dragendorff reagents are likely due to the alkaloid compounds in the extracts not reacting with the chemical components of Wagner and Dragendorff reagents, as each reagent has a different mechanism and sensitivity toward alkaloid groups. Mayer's reagent contains mercuric chloride and potassium iodide, Wagner's reagent contains iodine and potassium iodide, while Dragendorff's reagent contains potassium iodide and bismuth (III) nitrate in nitric acid. According to Endarini (2016), alkaloid salts have different properties compared to free alkaloids in the base form, as free alkaloids are generally insoluble in water but soluble in organic solvents such as chloroform, ether, benzene, and ethyl acetate.

Flavonoid compounds possess lipophilic properties, allowing them to disrupt bacterial cell membranes. The flavonoids present in extracts AC3, AC10, and AC17 can inhibit bacterial growth by interfering with DNA replication and transcription, damaging the structure and stability of the bacterial cell wall, and binding to extracellular bacterial proteins, thereby disrupting their functions. The presence of the beta ring and hydroxyl (-OH) groups in the flavonoid structure is suspected to contribute to its antibacterial activity (Herlina et al., 2020). In this study, only extract AC17 tested positive for triterpenoids, while all extracts were negative for steroids. Triterpenoids are secondary metabolites derived from terpenoids, composed of carbon units originating from six isoprene (2-methylbuta-1,3-diene) C5 units and derived from the acyclic C30 hydrocarbon, squalene (Hidayah et al., 2023). Triterpenoid compounds in endophytic bacterial extracts can interact with porins in the bacterial cell wall membrane, forming strong polymeric bonds, which leads to porin damage. This porin disruption decreases the permeability of the cell wall, causing nutrient deficiency and inhibiting bacterial growth, ultimately resulting in cell death (Nurulita et al., 2022).

The secondary metabolite screening results of endophytic bacterial extracts from the roots of *A. conyzoides* L. differ from those reported by Agbafor et al. (2015) in Melissa and Muchtaridi (2017), which stated that bandotan roots contain flavonoids, saponins, alkaloids, tannins, minerals, vitamins, anthraquinones, cardiac glycosides, and other compounds known to possess pharmacological activities. According to Omole et al. (2019), ethyl acetate extracts of bandotan roots contain low concentrations of alkaloids, flavonoids, tannins, saponins, phenols, and glycosides, while Ryadi et al. (2023) reported that bandotan roots contain alkaloids, flavonoids, terpenoids, and tannins. The differences in compound content between the endophytic bacterial extracts of bandotan and previous studies may be due to the fact that the secondary metabolite composition of endophytic bacterial extracts does not always match that of their host plant. This variation depends on each endophytic bacterium's biosynthetic

capacity to express secondary metabolites, which is influenced by environmental conditions such as pH, temperature, nutrients, incubation time (A'yun and Nugraheni, 2023), and the type of solvent used.

CONCLUSIONS

Based on the conducted study, it can be concluded that three endophytic bacterial isolates from the roots of *Ageratum conyzoides* L., with isolate code AC3, AC10, and AC17 have potential antibacterial activity. These isolates exhibit similar colony morphology, and all are Gram-negative, rod-shaped (bacilli). Biochemical activities of the isolates varied, including catalase production, motility, positive urease activity, positive and negative citrate utilization, and the ability to ferment glucose, lactose, sucrose, and gas production or not. The highest antibacterial activity against *Escherichia coli* was observed in the AC3 extract with a diameter of 10.38 ± 0.27 mm (strong category), while the highest activity against *Shigella dysenteriae* was shown by the AC10 extract with a diameter of 5.02 ± 1.36 mm (moderate category). The MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values of the three extracts at 7.5% were considered weak. Secondary metabolites present in these extracts contributed to the antibacterial activity against *E. coli* and *S. dysenteriae*. Additionally, AC3 and AC10 extracts contained flavonoids, whereas AC17 extract contained alkaloids, flavonoids, and triterpenoids.

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