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Original Research Article

# Novel Endophytic Bacteria Isolates from Andaliman (*Zancthoxylum acanthopodium* DC.) which Potentially Inhibit *Escherichia coli* and *Staphylococcus aureus*

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**Abstract**

**Background**: Irrational use of antibiotics has various side effects to resistance. Utilization of secondary metabolites of Andaliman plant endophytic bacteria (*Z. acanthopodium* DC) is an alternative known to act as an antibacterial to overcome this problem.

**Objective**: This study aimed to determine the antimicrobial activity of the crude extract of Andaliman endophytic bacteria against *Escherichia coli* and *Staphylococcus aureus*.

**Methods**: This research is an experimental laboratory with qualitative data collection methods. The initial stages will be the isolation of endophytic bacteria from Andaliman, the antagonist test of Andaliman endophyte isolates against *E. coli* and *S. aureus*, and the extraction of endophytic bacterial isolates using ethyl acetate as solvent. An antagonistic test of bacterial isolate extract was performed using the disc diffusion method against pathogenic bacteria *E. coli* and *S. aureus* and observed by an inhibition zone. The final stage is the minimum inhibition test of endophytic isolates by dilution method on three potential endophytic isolates.

**Results**: The results of the extraction of isolates EAA22, EAA28, EAB5, EAB6, and EAB7 Andaliman endophytic bacteria have inhibitory activity against *S.aureus*, and the results of the extraction of isolates EAB5, EAB6, EAA22 have inhibitory activity against *E. coli*.

**Conclusion**: Endophyte bacteria isolates from Andaliman produce antibacterial compounds against *E. coli* and *S. aureus*.

**Keywords**:
*Endophytic bacteria; extraction; antibacterial compound; Andaliman; minimum inhibitory concentration*
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## INTRODUCTION

Infectious diseases occur when interactions with microbes cause damage to the host body; this damage causes various clinical symptoms and signs, one of the causes of which is pathogenic bacteria.[1] Giving antibiotics is still the primary choice for treating bacterial infections. Various studies have found that around 40–62% of antibiotics are misused.[2] The Global Antimicrobial Surveillance System (GLASS) 2018 revealed widespread antibiotic resistance among 500,000 people with suspected bacterial infections in 22 countries.[3] In 2019, antimicrobial resistance indicators were included in the monitoring framework of the Sustainable Development Goals (SDGs). This indicator monitors the frequency of bloodstream infections due to two specific drug-resistant pathogens: *Staphylococcus aureus* and *Escherichia coli*.[2]

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*S.aureus* is a microbiome in the skin, nose and throat. Diseases caused by *S. aureus* bacteria in Indonesia are 28%.[4] *S. aureus* can cause serious infections such as septicemia, pneumonia, endocarditis, osteomyelitis, gastroenteritis and abscesses.[5] Meanwhile, *E. coli* is the most common commensal in the human digestive tract. Diarrhea caused by *E. coli* has the highest prevalence in Indonesia, almost reaching 10%.[6] Other clinical infections, such as urinary tract infections, bacteremia, and septicemia, can also be caused by *E. coli.*[7]

In 2015, as many as 13,300 patients died due to resistant bacterial infections.[8] The discovery of new antibiotics has not offset the increase in cases of bacterial resistance.[9] Efforts that have been made to reduce the incidence of antibiotic resistance include controlling the use of antibiotics, developing research to understand genetic resistance mechanisms better, and discovering new drugs, both synthetic and of natural origin from natural ingredients, it is still being used, one of which is as an antibiotic.[2]

Nature provides many alternative plants with antimicrobial potential against pathogenic microbes, including the Andaliman. Removing bioactive compounds from a plant can be done by extracting parts of the plant. This method is undoubtedly ineffective because if the plant is continuously taken to extract its bioactive compounds, the availability of the plant in the environment will decrease. One way to obtain bioactive plant compounds is to utilize the plant's endophytic bacteria, so there is no need to cut down the original plant to take simplicia, which will most likely take a long time to harvest.[10]

Endophytic bacteria can produce unique natural compounds that are sometimes the same as the compounds produced by their host plants, one of which is the ability to synthesize the same antibacterial compounds as their host plants.[10] The bioactive compounds synthesized by endophytic bacteria are several secondary metabolites such as alkaloids, steroids, terpenoids, peptides, flavonoids and phenols, which are essential in therapeutic applications, one of which is antimicrobial.[11] These bioactive compounds can be further explored for medical and pharmaceutical use, which is very important for further exploration.

Extraction of secondary metabolites of endophytic bacteria from *Anredera cordifolia* leaves shows the ability to produce effective antimicrobials against several pathogenic bacteria such as *S. aureus, E. coli* and *B. cereus.*[12] The results of the antibacterial activity test showed that the BH2 isolate extract from the endophytic bacteria of the Nyawai plant (*Ficus variegate* Blume) could inhibit the bacteria *Bacillus subtilis, S. aureus, E. coli, and Pseudomonas aeruginosa.*[13] Research by Tendani in 2020 reported that crude extracts of endophytic bacteria from *Crinum macowanii* Baker leaves showed inhibitory activity against gram-positive and gram-negative bacterial species.

Antimicrobial activity can be seen in the MIC (minimum inhibitory concentration), the lowest concentration capable of inhibiting the growth and development of bacteria. An increase in the MIC value describes the initial stage of resistance. Antibiotic resistance by bacteria that cause infections requires discovering and developing new antibacterial drugs derived from nature, one of which is by utilizing endophytic bacterial extracts from medicine plants.

Seeing the importance of the function of endophytic bacteria for plants and the antibacterial potential of Andaliman, it is crucial to carry out this research to obtain isolates of endophytic bacteria from Andaliman that have activity as producers of antibacterial compounds that can inhibit pathogenic bacteria activity. Therefore, it is hoped that future results of this research can be used and developed to overcome health problems, mainly due to pathogenic bacterial infections.

## MATERIALS AND METHODS

### *Isolation of Endophyte Bacteria*

The parts of the Andaliman plant used are leaves, stems and roots. Fresh leaves, stems, and roots were cleaned with running water, cut into 2-5 cm long pieces, and separated according to plant parts. The sample pieces were then surface sterilized by immersing them in 70% alcohol for 1 minute, 5.25% sodium hypochlorite (NaOCl) solution for 5 minutes, and 70% alcohol for 2 minutes. The sterilized sample pieces were cut again and then planted in King's B media. The media containing the sample was incubated at room temperature in the dark and observed every day until there was colony growth.

### *Antagonist Test*

Pure isolates of *S. aureus* and *E. coli* (IPBCC) were rejuvenated into Tryptic Soy Broth (TSB) liquid media and incubated at room temperature for 24 hours. Then, the turbidity was measured using spectrophotometry (OD = 0.3, concentration $10^6$ - $10^7$ cells/ml). Bacterial antagonist test for *E. coli* and *S. aureus* used NA media. The bacterial antagonist test of endophytic isolates against the growth of target bacteria was carried out using a two-layer agar media technique consisting of semi-solid and solid media. The target bacteria whose turbidity has been measured using spectrophotometry (OD = 0.3, concentration 10^6 - 10^7 cells/ml) in the broth medium are mixed into the semi-solid medium and then poured over the solid medium that has been previously frozen in a cup. After freezing, the endophytic bacterial isolate was streaked onto it. The culture was incubated for 24 hours at room temperature.

Observations were made after the bacterial sensitivity test culture had been incubated for 24 hours. A bacterial isolate is said to be positive for potential inhibitory power if the isolate produces an inhibition zone in the bacterial antagonist test. The diameter of the inhibition zone measured endophytic bacterial isolates that were positive for producing potential inhibitory activity, then inoculated into NA media and incubated at room temperature for 24 hours.[14]

**Table 1.** Inhibition Category

| Diameter | Category |
|---|---|
| ≥ 20 mm | Very Strong |
| 10-20 mm | Strong |
| 5-10 mm | Moderate |
| ≤ 5 mm | Weak |

**Table 2**. Andaliman Endophyte Bacterial Antagonist Test Results against E. Coli

| No | Isolate Code | Isolate diameter (mm) | Isolate + Clear zone Diameter (mm) | Inhibition zone diameter (mm) | Category |
|---|---|---|---|---|---|
| 1 | EAA28 | 4,2 | 4,9 | 0,7 | Weak |
| 2 | EAB06 | 3,4 | 4,5 | 1,1 | Weak |

**Table 3.** Andaliman Endophyte Bacterial Antagonist Test Results against S. aureus

| No | Isolate Code | Isolate diameter (mm) | Isolate + Clear zone Diameter (mm) | Inhibition zone diameter (mm) | Category |
|---|---|---|---|---|---|
| 1 | EAA03 | 5,6 | 6,7 | 1,1 | Weak |
| 2 | EAA16 | 8,2 | 12,8 | 4,6 | Weak |
| 3 | EAA22 | 5,9 | 8,5 | 2,6 | Weak |
| 4 | EAA26 | 5,7 | 7,6 | 1,9 | Weak |
| 5 | EAB03 | 5,6 | 6,4 | 0,8 | Weak |
| 6 | EAB05 | 6,6 | 15,0 | 8,4 | Moderate |
| 7 | EAB06 | 5,2 | 9,6 | 4,4 | Weak |
| 8 | EAB07 | 5,3 | 10,4 | 5,1 | Moderate |
| 9 | EAB10 | 5,8 | 7,2 | 1,4 | Weak |
| 10 | EAB11 | 5,9 | 7,0 | 1,1 | Weak |
| 11 | EAB16 | 5,6 | 6,8 | 1,2 | Weak |
| 12 | EAB18 | 5,4 | 6,7 | 1,3 | Weak |
| 13 | EAB21 | 5,1 | 6,3 | 1,2 | Weak |
| 14 | EAD07 | 6,3 | 6,8 | 0,5 | Weak |
| 15 | EAD10 | 6,4 | 7,2 | 0,8 | Weak |
| 16 | EAD13 | 5,3 | 5,9 | 0,6 | Weak |
| 17 | EAD14 | 5,6 | 6,7 | 1,1 | Weak |

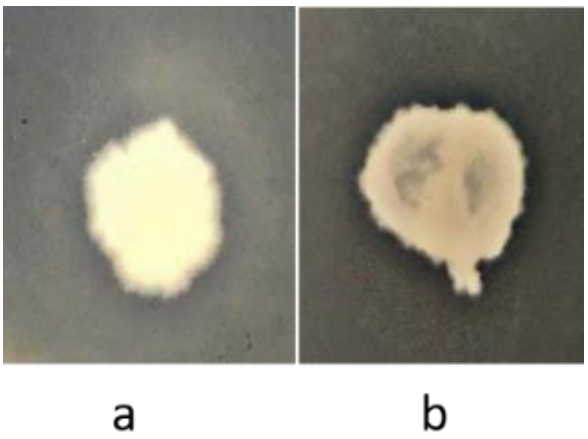


**Figure 1.** Inhibition zone formation in Andaliman Endophyte Bacterial Antagonist Test against *E. Coli*. a. EAA28, b. EAB06

***Minimum Inhibitory Concentration (MIC) Test***

Minimum Inhibitory Concentration (MIC) is the lowest antimicrobial concentration that can still inhibit the growth of certain organisms. The MIC test in this study was carried out using the dilution method. This method is mainly used in determining the minimum inhibitory concentration (MIC). Generally, determining the MIC using the dilution method involves inoculating the target microbe at various concentrations of antimicrobial compounds and incubating for 18 to 24 hours. The MIC test was conducted because a standard concentration of endophytic bacteria isolates from the Andaliman plant had not been found to test its effectiveness as an antimicrobial on *E. coli* and *S. aureus*.

The Andaliman endophytic bacterial isolate was cultured in an NB medium and then incubated for three days at room temperature. Escherichia coli and Staphylococcus aureus were rejuvenated in NB (Nutrient Broth) media and incubated for 24 hours at room temperature. The antimicrobial-producing Andaliman endophytic bacterial isolate that had been incubated was centrifuged at 6000 rpm for 1 hour. The supernatant was taken and then filtered using a 0.22 µm syringe filter and put into 5 test tubes with concentrations of 6.25%, 12.5%, 25%, 50%, and 100%, respectively. Apart from these five concentrations, a positive control was also made by adding an antibiotic in the form of amoxicillin with a concentration of 50 µg/mL, a negative control by not adding antibiotics or endophytic bacterial supernatant, and a media control containing only NB media. After that, each tube was filled with 1 ml of target bacteria, except for the media control.

The MIC test culture was then incubated for 24 hours. Then, the turbidity of each culture was observed in the test tube and measured using spectrophotometry at a wavelength of 620 nm. MIC is determined based on the turbidity of the culture compared to the control.[14] The MIC value is determined by observing the minor concentration at which the test tube appears clear, compared to the control, and confirmed by measuring the OD (Optical Density) of the solution in the test tube using spectrophotometry.

**Table 4.** MIC test results for endophytic bacterial isolates against S.aureus and E.coli

| ***Staphylococcus aureus*** | | | | | |
|---|---|---|---|---|---|
| Concentration | *Optical Density* | | | | |
| | EAB5 | EAB6 | EAB7 | EAA22 | EAA28 |
| 100% | 0,143 | 0,080 | 0,138 | 0,080 | 0,094 |
| 50% | 0,213 | 0,169 | 0,213 | 0,082 | 0,141 |
| 25% | 0,240 | 0,186 | 0,240 | 0,156 | 0,208 |
| 12,5% | 0,241 | 0,227 | 0,241 | 0,290 | 0,232 |
| 6,25% | 0,243 | 0,229 | 0,243 | 0,295 | 0,234 |
| K(-) | 0,271 | | | | |
| K(+) | 0,149 | | | | |

| ***Escherichia coli*** | | | |
|---|---|---|---|
| Concentration | *Optical Density* | | |
| | EAB5 | EAB6 | EAA22 |
| 100% | 0,089 | 0,143 | 0,112 |
| 50% | 0,130 | 0,182 | 0,230 |
| 25% | 0,214 | 0,253 | 0,235 |
| 12,5% | 0,222 | 0,261 | 0,246 |
| 6,25% | 0,281 | 0,288 | 0,258 |
| K(-) | 0,282 | | |
| K(+) | 0,090 | | |

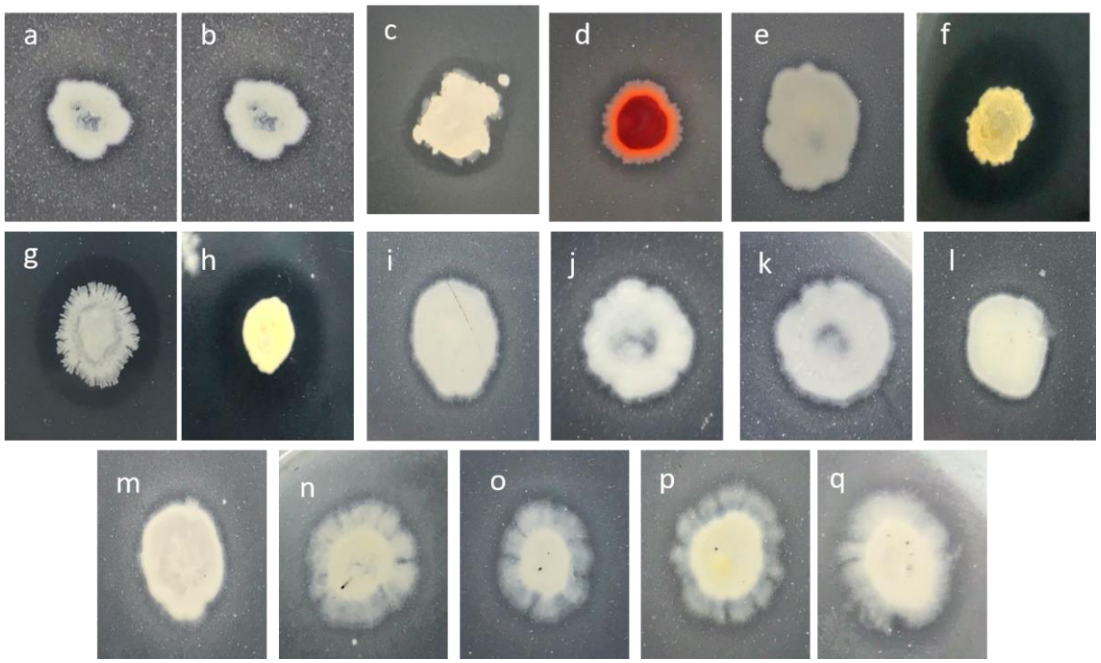


**Figure 2.** Inhibition zone formation in Andaliman Endophyte Bacterial Antagonist Test against *S. aureus*. a. EAA03; b. EAA16; c. EAA22; d. EAA26; e. EAB03; f. EAB05; g. EAB06; h. EAB07; i. EAB10; j. EAB11; k. EAB16, l. EAB18; m. EAB21; n. EAD07; o. EAD10; p. EAD13; q. EAD14.

***Extraction of Andaliman endophytic bacterial isolates***

The six best andaliman endophytic bacterial isolates that had been isolated from the previous step and had been subcultured were each re-cultured into 500 ml of Nutrient Broth (NB) media and incubated on a shaker (170 RPM /Revolutions Per Minute, 30 ºC) for 72 hours so that the bacteria grow uniformly. After that, the culture was centrifuged at 3600 rpm for 10 minutes to separate the supernatant and cells. The supernatant that had been taken was added to 150 mL of ethyl acetate and incubated. Then, it was shaken for 20 minutes and transferred to a separating funnel. After leaving it for 10 minutes, the solvent (transparent top layer) and medium (yellow bottom layer) were separated. The top layer was put into a glass container and evaporated using a vacuum rotary evaporator at a temperature of 40ºC with a speed of 90 rpm.[12] The resulting crude extract of endophytic bacterial isolates in liquid form was stored at four ºC for further use.[15]

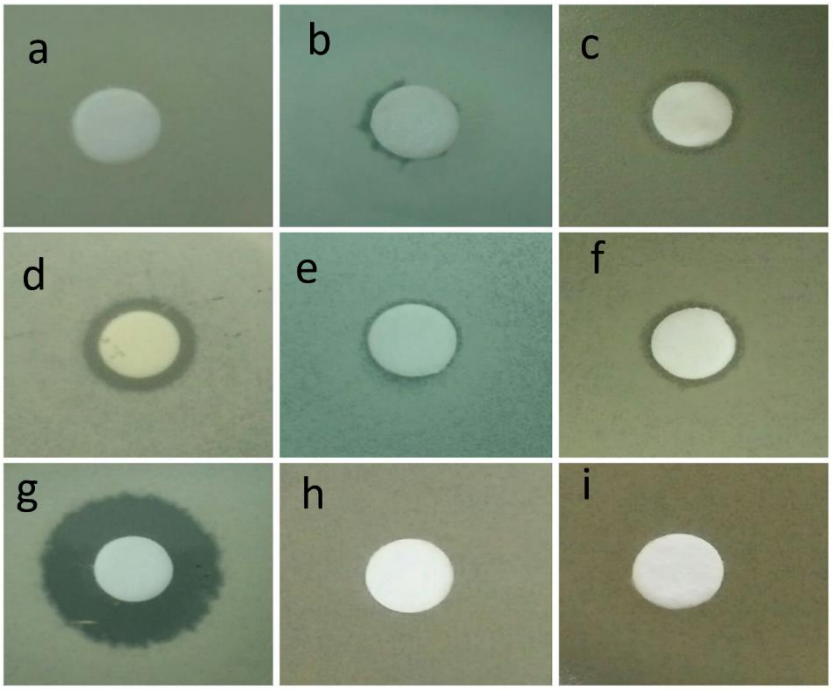


**Figure 3.** Inhibition zone formed from crude extract of isolates of andaliman endophytic bacteria against S. aureus; a = EAA16; b = EAA22; c = EAA28; d= EAB5; e= EAB6; f=EAB7;g=Amoxicilin; h= Ethyl Acetate; i= Aquadest

**Table 5**. Inhibition Test of Crude Extract of Andaliman Endophyte Bacterial Isolate against Staphylococcus aureus and Escherichia coli

| Isolate Code | Isolate diameter (mm) | Isolate + Clear zone Diameter (mm) | Inhibition zone diameter (mm) | Category |
|---|---|---|---|---|
| Inhibition to *Staphylococcus aureus* | | | | |
| EAA16 | 6 | 6 | - | Negative |
| EAA22 | 6 | 6,3 | 0,3 | Weak |
| EAA28 | 6 | 6 | 7 | Weak |
| EAB5 | 6 | 9,2 | 3,4 | Weak |
| EAB6 | 6 | 6,6 | 0,6 | Weak |
| EAB7 | 6 | 7,6 | 1,6 | Weak |
| Amoxicillin | 6 | 12,7 | 6,7 | Moderate |
| Ethyl Acetate | 6 | 6 | - | Negative |
| Aquades | 6 | 6 | - | Negative |
| Inhibition *Escherichia coli* | | | | |
| EAA16 | 6 | 6 | - | Negative |
| EAA22 | 6 | 6,6 | 0,6 | Weak |
| EAA28 | 6 | 6 | - | Negative |
| EAB5 | 6 | 6,35 | 0,35 | Weak |
| EAB6 | 6 | 6,30 | 0,30 | Weak |
| EAB7 | 6 | 6 | - | Negative |
| Amoxicillin | 6 | 14 | 8 | Moderate |
| Ethyl Acetate | 6 | 6 | - | Negative |
| Aquades | 6 | 6 | - | Negative |

***Inhibition Test of Crude Extract of Andaliman Endophytic Bacterial Isolate***

Stocks of pathogenic bacteria were taken and then grown in 10 mL of sterile liquid NB media—next, they were shaken in an incubator at 37°C for 24 hours. Then, the turbidity was measured using spectrophotometry (OD = 0.3 concentration $10^6$-$10^7$ cells/ml). A 2.5 mL pathogenic bacterial culture originating from liquid NB media was put into 50 mL of semi-solid NB media, stirred or stirred, poured into a petri dish of ±10 mL, and cooled. The test was carried out using the diffusion method; crude extracts of endophytic bacterial isolate compounds dissolved in ethyl acetate were dropped onto paper discs 100 µl each. Next, the paper disc on which the extract has been dripped is waited to dry to eliminate water access, then placed on the surface of NB semi-solid media containing indicator microbes and incubated for 24 hours. The formation of a clear zone indicates positive

test results.[15] The diameter of the inhibition zone around the test disc is measured using a ruler or a calliper.

## RESULTS

### *Isolation of Endophyte Bacteria*

The first stage of this research was isolating the Andaliman plant using two parts of each of the roots, stems, and leaves from three different plants. From the isolation using the cutting-planting method, 252 colonies were produced from all the parts taken. The details of the number of colonies that grew on King's B media were that on the roots, the results were 150 colonies; on the stems, the results were 81; on the leaves, the results were 21.

### *Antagonist Test*

After observing the microscopic characteristics of the Gram stain, a bacterial sensitivity test using the spot technique on Double Layer Agar media was followed. Of the eighty-five isolates tested against pathogenic bacteria, 18 isolates were obtained which showed inhibitory activity against the pathogenic bacteria *E. coli* and *S. aureus*, with details on the roots of 5 isolates from 28 isolates (17.8%), on the stems nine isolates from 40 isolates (22.5%) and four isolates from 17 isolates (23.5%) on leaves. Table 2 and Table 3 display the results of the Antagonist Test for Andaliman endophytic bacteria against the pathogenic bacteria *E. coli* and *S. aureus.* Two isolates have inhibition activity against *E. coli* (Figure 1) and 17 isolates have inhibition activity against *S. aureus* (Figure 2).

### *Minimum Inhibitory Concentration (MIC) Test*

Based on measuring the absorbance value using spectrophotometry, the MIC (minimum inhibitory concentration) value was obtained for *S. aureus* bacteria at a concentration of 6.25% for isolates EAB7, EAB5, EAB6, EAA28 and a concentration of 25% for isolate EAA22 (Table 4). Meanwhile, the MIC value for *E. coli* bacteria was at a concentration of 6.25% for isolate EAA22 and 12.5% for isolate EAB5 and EAB6. The MIC value is based on the isolate concentration's optical density value, which is lower than the negative control.

### *Extraction and Inhibition Test of Andaliman Endophytic Bacterial Isolates*

The extraction results for each isolate EAB5, EAA16, EAA28, EAB5, EAB6, and EAB7 resulted in less than 5 mL of extraction stored in a tube bottle. Antagonism test of the six extract isolates tested, it was found that five isolates, EAA22, EAA28, EAB5, EAB6, and EAB7, had weak inhibitory activity against the pathogenic bacteria *S.aureus*, and three isolates, namely EAB5, EAB6 and EAA22, had weak activity against *E. coli.*

## DISCUSSION

Endophytic bacteria live in host plant tissues without causing disease symptoms.[16] The mechanism of endophytic bacterial invasion into tissue can be carried out in several ways, namely through roots, stomata, lenticels, natural wounds, and damaged leaves. Bacteria in plant tissue then colonize at the point where they enter or spread to all parts of the plant through the xylem.[17] This study found endophytic bacterial isolates in roots, stems and leaves. Based on the isolation results, it was found that there were more endophyte isolates from the roots than stems and leaves, following research conducted by Balosi et al.[18] that the population of endophytic bacteria is more abundant in roots and decreases in stems and leaves. The number of endophytic bacteria in plants cannot be determined with certainty, but these bacteria can be isolated using agar media.[19] In this study, the agar medium used to isolate endophytic bacteria was King's B medium. This medium is rich in

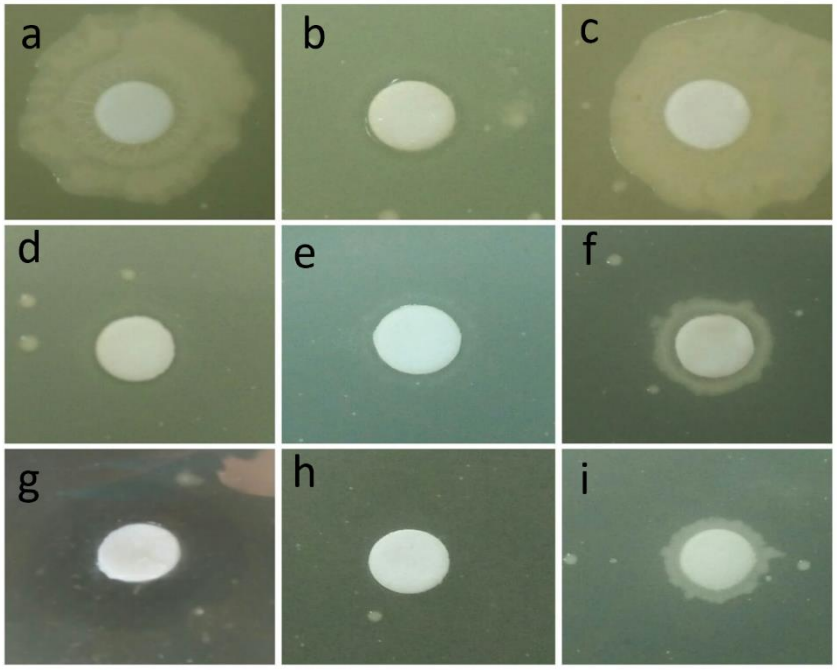


**Figure 4.** Inhibition zone formed from crude extract of isolates of andaliman endophytic bacteria against E. coli; a = EAA16; b = EAA22; c = EAA28; d= EAB5; e= EAB6; f=EAB7;g=Amoxicilin; h= Ethyl Acetate; i= Aquadest

peptone, glycerol, magnesium sulfate, dipotassium phosphate, and agar, which helps bacterial growth. Endophytic bacteria can live on this media due to its complex nature, which allows it to have a composition similar to conditions in plants and is a suitable medium for the non-selective isolation of bacteria.[20]

Bacterial sensitivity testing was carried out to determine the activity of Andaliman endophytic bacterial isolates in inhibiting the growth of *E. coli* and *S. aureus*. Before a sensitivity test, the target bacteria will be rejuvenated into agar media. The media used is *Tryptic Soy Broth* (TSB) agar media because this media helps support the growth and development of various microorganisms, predominantly anaerobic and facultative aerobic bacteria. This sensitivity test was carried out by growing endophytic and pathogenic bacteria in petri dishes using a two-layer agar technique.[14] This technique is helpful so that the results of the inhibition zone obtained are more clearly visible and the nutrition obtained by the target and endophytic bacteria is sufficient. Following research by Oktavia et al.[21] in a sensitivity test, the concentration of target bacteria can influence the inhibition zone formed, the concentration of endophytic bacteria being tested, temperature and incubation time, and other factors such as the type of medium used.

The pathogenic bacteria used in this study were *E. coli* and *S. aureus*. *Escherichia coli* was chosen to represent Gram-negative pathogenic bacteria, and *S. aureus* was chosen to represent Gram-positive pathogenic bacteria.[22] The inhibitory activity of endophytic bacteria against pathogenic bacteria is characterized by the appearance of a clear zone around the area of endophytic bacteria that is spotted on media that already contains pathogenic bacteria. The inhibition zone that appears indicates that there is secondary metabolite activity at work. The inhibitory mechanism of antibacterial compounds will generally work by damaging cell wall synthesis, disrupting membrane function, protein synthesis, nucleic acid synthesis, and antimetabolites.[23]

Based on the sensitivity test carried out in this study, it was found that of the 18 isolates that gave rise to the inhibition zone, 17 isolates could inhibit the growth of *S. aureus* bacteria, and two isolates could inhibit *E. coli*. Isolate EAB6 could inhibit both pathogenic bacteria. It is supported by several studies that their research show the potential inhibitory power of the Andaliman plant against *S. aureus*. Several studies report that isolates isolated from endophytic bacteria of a plant will have higher inhibitory zone activity against Gram-positive pathogenic bacteria such as *S. aureus*.[19,24] It can happen because Gram-negative pathogenic bacteria have more robust defence capabilities when attacked by inhibitory compounds than Gram-positive pathogenic bacteria. Gram-negative bacteria have good defence because they have different cell wall components. The cell walls of Gram-positive bacteria are relatively thinner because most of them are only composed of peptidoglycan. At the same time, Gram-negative bacteria are not only composed of peptidoglycan. However, they are also composed of other components such as lipoproteins, outer membranes, and lipopolysaccharides, making inhibitory compounds from Andaliman endophytic bacterial isolates not to enter Gram-negative bacterial cells.[22]

The dilution method determines the MIC because it is easier and more efficient, does not require agar media, and only uses liquid media in a tube.[25] The MIC test results showed that various isolated supernatants had different minimum inhibitory concentrations. For Staphylococcus aureus bacteria, the optical density value of isolates EAB7, EAB5, EAB6, and EAA28 at a concentration of 6.25%, and isolate EAA22 at a concentration of 25% had a value that was smaller than the optical density value of 0.271 which was a negative control. In Escherichia coli bacteria, the optical density of isolates EAB5 EAA22 at a concentration of 6.25% and isolate EAB6 at 12.5% was 0.282 smaller than that of a negative control. The MIC value is determined based on the isolate concentration's optical density value, which is lower than the negative control. Spectrophotometry is used because of the tool's detailed capabilities regarding the absorption of chemical energy by chemical species, thus allowing greater accuracy in qualitative details and measurements.[23]

The incubation process produces a suspension in NB media in the form of bacterial secondary metabolites in the stationary phase. During the stationary phase, namely when the number of bacterial cells is constant, meaning the number of bacteria that die is the same as the number of bacteria that grow, when the nutrients for the bacteria in the medium begin to run out, competition between bacteria occurs and produces secondary metabolites to defend themselves which are also beneficial for the host plant.[26] The supernatant taken from each liquid culture of endophytic fungi from the Javanese Ginseng Plant (*Talinum paniculatum* Jag.) contains several types of compounds that are antimicrobial, namely flavonoids, alkaloids, terpenoids, and tannins. These antimicrobial compounds have the potential to cause damage to the test bacterial cells, thereby inhibiting their growth.[27]

Endophytic bacterial isolates grown in Nutrient Broth (NB) medium were shaken for three days (72 hours) with the aim of aeration, and it is hoped that they will obtain optimal cell biomass. Based on research conducted by Kuntari et al.[26] on root endophytic bacteria of the *Moringa oleifera* L, the three-day incubation process produced a suspension in NB media in the form of bacterial secondary metabolites in the stationary phase. The shaker with a speed of 170 rpm is also intended so that the bacteria can secrete the antibacterial compounds they produce into the culture medium optimally.[25]

The supernatant was extracted using ethyl acetate, the process of extracting metabolite compounds with the aim of the metabolite compounds diffusing into the solvent. Ethyl acetate is an excellent solvent for extraction because it can be quickly evaporated, is not hygroscopic, has low toxicity, and is semi-polar, so it is expected to attract metabolites of both polar and non-polar compounds.[28] Ethyl acetate macerate was separated from the culture medium using a separating funnel and evaporated using a vacuum rotary evaporator to obtain an isolate extract of endophytic bacteria.

The inhibition test results of Andaliman endophytic bacterial isolate extract against *S. aureus* showed that five endophytic bacterial isolates could inhibit the growth of *S. aureus* bacteria. Meanwhile, the results of the

antagonist test of endophytic bacterial isolates against *E. coli* showed that three endophytic bacterial isolates could inhibit the growth of *E. coli* bacteria. Andaliman plant extract contains alkaloids, flavonoids, glycosides, saponins, tannins, triterpenes/steroids, and glycosides, which have antimicrobial activity in inhibiting the growth of *S. aureus.*[29]

The flavonoid content in andaliman extract effectively inhibits the growth of *S. aureus*. Flavonoids are polar, so they more easily penetrate the peptidoglycan layer of microbes, which are also polar than the non-polar lipid layer, thereby disrupting the permeability of cell membranes.[30] Saponin compounds reduce the surface tension of bacterial cell walls and damage membrane permeability. Lysis of cell membranes can disrupt the survival of bacteria so that saponins can be used as antibacterials.[31]

Secondary metabolites from the endophytic bacterial isolate of *Anredera cordifolia* CIX1 leaves were extracted, and the antibacterial potential of the extract was evaluated against selected bacteria. The extract showed activity against Gram-positive bacterial strains. Gram-negative bacteria and a thin peptidoglycan layer (2 to 7 nm) have about 7 to 8 nm of outer membrane. This outer membrane consists of an additional protective lipopolysaccharide layer that exhibits toxicity and antigenicity to antibacterial agents. *E. coli*, which are Gram-negative bacteria, have a more complex cell wall structure than *S. aureus.*[12]

The difference between the initial screening results of Andaliman endophytic bacterial isolates and crude extracts was caused by several things. The diffusion speed of different substances and differences in the response of microbes to the substance being tested cause the resulting inhibitory diameter.[32] The differences in bacterial growth that occur at each fermentation time are caused by the different abilities of the bacteria to reproduce. The specific growth rate for each bacteria is different because the enzyme content in each bacteria is different, which influences the bacterial metabolic process in producing secondary metabolites.[33]

## CONCLUSION

Based on the isolation of Andaliman endophytic bacteria, 252 colonies and 85 isolates with different characteristics were obtained. The bacterial antagonist test showed that 18 isolates of Andaliman endophytic bacteria had the potential to inhibit the growth of *E. coli* and *S. aureus* bacteria. The test results of Andaliman endophytic bacterial isolate extracts observed five bacterial isolate extracts that could inhibit *S. aureus* bacteria in the weak inhibitory category with the highest inhibitory diameter value in the EAB5 isolate. Three isolate extracts could inhibit *E. coli* in the weak inhibitory category with the highest inhibitory diameter value for isolate EAA22. In the MIC test, the MIC value of isolates EAB7, EAB5, EAB6, and EAA28 at a concentration of 6.25% and isolate EAA22 at a concentration of 25% inhibited *S. aureus.* For *E. coli* bacteria, the MIC value of isolates EAB5 and EAA22 was at a concentration of 6.25% and isolate EAB6 was 12.5%.

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