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Antibacterial Activity of *Dendrophthoe pentandra* (L.) Leaves Extract Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract. *Sapuran, Wonosobo is a popular area with several local tea plantations, infested by mistletoe, such as Dendrophthoe pentandra (L.) Miq. Mistletoe has been widely reported to have antibacterial activity due to its secondary metabolite content. Therefore, this study aimed to identify and explore the antibacterial activity of D. pentandra leaves extract against Staphylococcus aureus and Escherichia coli. The test samples were extracted using the maceration method, followed by phytochemical screening and a total assay of flavonoids, phenols, and tannins. Antibacterial activity tests were carried out using disk diffusion, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC). The yield from the maceration method using 96% ethanol solvent was 8.78%, containing secondary metabolite compounds, including alkaloids, saponins, steroids, terpenoids, and flavonoids. The total flavonoid, phenol, and tanin content obtained were 4.09 ± 0.70 mg QE/g, 1.76 ± 0.16 g GAE/g, and 1.37 ± 0.14 mg TAE/g, respectively. In addition, the extract showed medium inhibition of S. aureus at a concentration of 400 and 525 mg/m, with no inhibitory effect against E. coli. The MIC and MBC from the microdilution method for S. aureus were at 4400 and 8800 mg/mL extract, while values of 28000 and 56000 mg/mL were obtained for E. coli. Based on these results, the inhibition activity of ethanol extract of D. pentandra against S. aureus was more significant compared to E. coli.*

Keywords: *Antibacterial, Escherichia coli, Mistletoe, Dendrophthoe pentandra, Staphylococcus aureus*

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INTRODUCTION

In Sapuran, Wonosobo, many resident-owned tea plantations often grow without maintenance, leading to their infestation by parasitic plants, such as mistletoe. Several studies have shown that mistletoe is a hemiparasitic plant that is detrimental to the agricultural sector due to its ability to absorb nutrients and water from the host. Despite the negative impacts, it is still believed by the community to be an alternative medicine, including a cure for diarrhea, cancer, cough, pain relief, and skin infections (Alviani et al., 2022). The pharmacological activity found in the plant is due to its constituent compounds, such as flavonoids, alkaloids, tannins, terpenoids, polyphenols, and saponins, which have antibacterial activity (Christalina et al., 2013). Nasution et al. (2013) revealed that Scurrulla sp. mistletoe leaves at a concentration of 25% had inhibitory effect against *Salmonella typhi*.

One of the mistletoe species found in Sapuran tea plantation, Wonosobo, is *Dendrophthoe pentandra*. This mistletoe has been reported to have antibacteria activity, leading to various pharmacological studies on its potential, specifically focusing on clinical trials in humans. The quercetin in *D. pentandra* has inhibitory activity against several bacteria, such as *Escherichia coli*, *S. typhi*, *Staphylococcus aureus*, and *Pseudomonas* (Hardiyanti et al., 2019).

In general, *S. aureus* can be found on the surface of human skin, leading to its rapid transfer through various means, such as touch or exchange of objects. Some strains of S. aureus can also be found in food and dirty environments, and their excessive presence often causes bacteremia, endocarditis, skin,osteoarticular, and pleuropulmonary infections (Gnanamani et al., 2017). One of the long-term effects that occur due to this infection is blood complications. Another bacteria associated with the digestive system and commonly found in food and human clinical samples, such as blood, hair, and skin, is Escherichia coli. Dirty environments can over-invade E. coli, causing various diseases, including diarrheal respiratory, and urinary tract infections (Basavaraju & Gunashree, 2022). The broad metabolic capacity of the bacterium allows for rapid growth in a wide range of environments, and can survive for a long time in many conditions. These traits favor the pathogenic strains of *E. coli*, which can infect humans more easily.

The use of antibiotics has been widely developed in the medical world to address the infection caused by bacteria. However, these antibiotics cause various side effects, such as increased blood pressure, shortness of breath, and bacterial resistance (Ratman et al., 2019). In some countries, an alternative medicine has been developed using plants to cure diarrheal and itching. One of the plants proven to have the potential to inhibit bacteria is the tea mistletoe plant. Despite its potential, its use has yet to be developed scientifically. One host can also be overgrown with various mistletoe species, causing differences in antibacterial activity.

D. pentandra has been shown to have anti-inflammatory and anticancer activities (Mustarichie et al., 2017), leading to its widespread use in traditional medicine. The tea mistletoe found in Sapuran, Wonosobo, has not been further studied regarding its antibacterial activity. Therefore, this study aimed to explore and identify the antibacterial activity of tea mistletoe in Sapuran, Wonosobo against *S. aureus* and *E. coli*. The results can help local community develop traditional medicine using the plant as well as contribute to the development of *D. pentandra* herbal medicine studies in the future.

MATERIALS AND METHODS

The study was conducted from March to May 2023 at the Biotechnology Laboratory Faculty of Biotechnology, Universitas Kristen Duta Wacana. The sample of tea mistletoe was obtained from the local community tea garden, Sapuran, Wonosobo, Central Java. The bacterial isolates used were *Staphylococcus aureus* FNCC 0047 and *Escherichia coli* ATCC 25922.

Sample Preparation

The tea mistletoe samples were sorted based on the quality of the plant samples, sorting the stem, leaves, and flower parts. The sorted leaves were then washed and their wet weight was recorded. Additionally, the samples were dried using the wind-dry technique for 1×24 hours at room temperature, then continued with dried in an oven at 40°C for 12 hours.

Sample Extraction

The extraction was performed by following Kristiningrum et al.(2020), and the shade-dried materials were then weighed. A total of 250 g of samples was extracted in 96% ethanol using the maceration method, and the solvent used was 2.5 L with the ratio between sample and solvent 1:10 (w/v). The solution mixture was macerated at room temperature for 3 days, and every 24 hours, the solvent was replaced, and the pulp was re-dissolved with a new solvent. Furthermore, the macerate was concentrated by evaporating the solvent using a rotary evaporator, and the result was the crude extract of mistletoe leaves. The yield was calculated by comparing the weight of the extract and the weight of samples using this formula:

$$Yield = \frac{weight\ of\ crude\ extract\ (g)}{weight\ of\ sample\ (g)} \times 100\%$$

Phytochemical Screening

The extracts of *D. pentandra* leaves were tested with specific reagents to determine the content of phytochemical compounds. These included the assay of flavonoids, phenols, tannins, alkaloids, saponins, steroids, and terpenoids (Wirasti, 2019, Wulandari et al., 2020).

Total Phenol Content

Several concentrations of standard solutions were needed to determine the concentration of compounds in the test sample. In the phenol test procedure, gallic acid was used as a standard because it was more stable and belonged to the type of simple phenol. The concentration variations used for making phenol standard curves were 100, 200, 300, 400, 500, 600, 700, 800, and 900 ppm. The linear equation was obtained from the absorbance value of the solution observed with a UV-Vis spectrophotometer at a wavelength (□) 662.85 nm by plotting absorbance value (y) against sample concentration (x).

A total of 10 mg of extract was dissolved with ethanol 90%, and the Folin-Ciocalteu reagent 0.4 mL was added and incubated for 8 minutes. Furthermore, 4 mL Na_2CO_3 10% solution and distilled water were added until reaching the final volume of 10 mL, and the solution was incubated for 2 hours. The absorption value was obtained using a UV-Vis spectrophotometer at □ 662.85 nm (Tahir et al., 2017)

Total Flavonoids Content

Quercetin was the standard chemical for the total flavonoid assay procedure due to its potential antibacterial mechanism, commonly found in leaves parts. The concentration variations used for flavonoid standard curve preparation were 100, 200, 300, 400, 500, 600, 700, 800, and 900 ppm.

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The linear equation was obtained by plotting the absorbance value (y) against sample concentration (x). These were observed using a UV-Vis spectrophotometer at 415 nm. In addition, a linear regression equation was used to calculate the total flavonoid content of the sample.

A total of 5 mg of extract was dissolved with 10 mL of 80% ethanol, and a total of 0.05 mL of sample was added to 96% ethanol, 1.5 mL of 10% $AlCl_3$, 0.1 mL of 0.1 mL potassium acetate 1 M, and 2.8 mL of distilled water. The sample was homogenized and allowed to stand at room temperature for 30 minutes. Additionally, the absorption value was obtained using a UV-Vis spectrophotometer at 415 nm (Hartati, 2016).

Total Tannins Content

Tannic acid was the standard solution to determine total tannin compounds. The hydrolysis properties of tannic acid produced chemical reactions that could help to measure total tannin levels (Hartati, 2016). The concentration variations of tannin acid were 1, 2, 4, 8, 20, 40, 80, and 160 ppm. The linear regression equation was obtained by plotting absorbance value (y) against sample concentration (x), which was observed using a UV-Vis spectrophotometer at 760 nm. A total of 100 mg of extract was dissolved with 10 mL of diethyl ether, and the sample was soaked for 20 hours in a closed state. The solution was filtered, and the solvent was removed by evaporation, which was added with 10 mL of distilled water, then 1 mL of sample was added to 0.1 mL Folin-Ciocalteu. Additionally, the solution was vortexed and allowed to stand for 5 minutes, then added with 2 mL Na_2CO_3 15% and allowed to stand for 2 minutes, and then distilled water until the final volume of 10 mL. The absorbance was obtained using a UV- Vis spectrophotometer at 760 nm (Fajarizki et al., 2022)

Antibacterial Activity Disk Diffusion Method

Pure isolates of bacteria were inoculated on NA media and incubated at 37°C for 24 hours. Mueller Hinton Agar (MHA) media was sterilized using an autoclave at 121°C, and sterile media was poured into 6 sterile Petri dishes. Before the inoculation stage, the bacterial suspension corresponding to McFarland 0,5 turbidity was ensured, and the bacteria could be inoculated evenly on MHA media using a cotton swab. Each paper disk was placed on a petri dish with a different concentration, and the antibiotic ampicillin 10% was also placed as a positive control, and the negative control was DMSO. After it was incubated for 48-72 hours at 37°C, the inhibition zone was observed and measured using a caliper. Inhibition zone grouping was determined based on the standard zone diameter to the nearest millimeter. Additionally, antibiotic ampicillin had a weak inhibition zone diameter group (< 5 mm), moderate or medium inhibition (5 - 10 mm), strong inhibition (10 - 20 mm), and very strong inhibition (> 20 mm) (Inusa et al., 2018).

In this study, a variety of concentrations were used, and according to Inusa et al. (2018), the ability of mistletoe extract to inhibit the growth of S. aureus was at a concentration of 10 mg/mL, which was categorized in medium activity. According to Diningsih and Aswan, (2019), a concentration of 300 mg/mL of D. pentandra extract already inhibited the growth of *E. coli* bacteria in the strong activity. The sample concentrations for these 2 bacteria included 25, 150, 275, 400, and 525 mg/mL.

Minimum Inhibitory Concentration (MIC)

Broth microdilution was used to find the Minimum Inhibitory Concentration (MIC) value. This method utilized serial dilutions (log dilutions) procedure of extracts of antibacterial agents in Brain-heart Infusion

Broth (BHIB) media (Balouiri et al., 2016). The minor concentration used in the MIC test was a level below the concentration variation with less inhibition, and the largest concentration did not reach 100% concentration. The disk diffusion test resulted in the use of various concentrations for both bacteria. In the MIC test, the extract against *E. coli* was determined based on the highest concentration because, in the inhibition zone test, there was no inhibitory reaction from the extract. Microplates were incubated in an incubator for 20 hours at 35°C. The optical density (OD) value was obtained, and the MIC value was determined using an ELISA microplate reader. The wavelength required in the MIC test of S. aureus and E. coli isolates was 570 nm. The MIC value was the minimum concentration of extract that could inhibit the growth of organisms in the well (the smallest OD value was seen from the lowest extract concentration), while the results of various concentrations were compared with the OD value of the blank and positive control.

Minimum Bactericidal Concentration (MBC)

The concentrations selected for the Minimum Bactericidal Concentration (MBC) test were below and above the MIC. Sterile MHA media was inoculated with bacteria from microplates from the previous MIC test using the streak plate method. However, the petri dish was incubated for 20 hours at 35°C, and the MBC concentration was determined qualitatively by comparing the growth between the 3 concentrations and equated with the growth in the positive control treatment. MBC concentration was the lowest antibacterial agent concentration required to kill a bacterial isolate (Parvekar et al., 2020; Soelama et al., 2015).

Data Analysis

The analysis used in the disk diffusion test was descriptive and quantitative, based on the category of inhibition. The data obtained were analyzed using the One-way ANOVA test. The MIC value obtained from the difference in concentration was carried out with a Post-Hoc (Pairwise Comparisons) test in a non-parametric test to determine the statistical data group. The probability (p-value) used was 95% (Anita et al., 2014). MBC results could be seen qualitatively, namely by the formation of bacterial colonies on petri disks.

RESULTS AND DISCUSSION

Phytochemical Screening of Extracts

The solvent used in the extraction process was 96% ethanol, and in the study of Diantika et al. (2016), ethanol 96% was also used to dissolve D. pentandra leaves extract because of its polarity. The toxicity of ethanol solvents was more tolerable than some other solvents, such as hexane and ethyl acetate, and the method used was maceration.

The crude extract weighed 21.96 g after the evaporation of solvent from the macerate. According to this result, the yield of ethanolic crude extract from 250 g of tea mistletoe leaves was 8.78%. This yield had met the requirement of the Indonesian Herbal Pharmacopoeia yield standard, which was more than 7.2%. However, the results were relatively low because of the closeness of the standard. A high yield indicated many obtained bioactive components during the extraction (Dewatisari et al., 2018). Moreover, other factors determined the yield, such as the formulation of the amount between the solvent and sample and also maceration time. Additionally, it was also possible that different yielded results from the same solvent but differed in the species of plant, the amount of solvent used, and the extraction time.

Table 1. Secondary metabolite content of ethanol extract of D. pentandra leaves

Secondary Metabolite Group	Phytochemical Results	Total Level ± SD
Flavonoids	+	4.09 ± 0.70 mg QE/g
Tannins	+	1.37 ± 0.14 mg TAE/g
Phenol	+	1.76 ± 0.16 g GAE/g
Saponins	+	-
Alkaloids:		
Meyer's Reagent	+	-
Dragendorf reagent	+	-
Wagner's Reagent	+	-
Terpenoids and Steroids	+	-

Notes: (+) present, (-) not tested

The phytochemical screening of ethanolic of *D. pentandra* leaves was shown in Table 1, and flavonoids, alkaloids, terpenoids, steroids, saponins, and tannins were detected in this extract. Secondary metabolite compounds in *D. pentandra* extract in this study were consentient with Nirwana et al. (2015), who also found flavonoids, alkaloids, terpenoids, tannins, steroids, and saponins in the leaves extract of *D. pentandra.* According to Kong et al. (2023), mistletoe *D. petandra* was identified that it had a group of tannin compounds, alkaloids, and flavonoids that were dominant with quercetin, steroids, and tannins such as tannic acid.

The diversity of secondary metabolite compounds in the extract affected the pharmacological activity of the extract. The antibacterial potential in secondary metabolite compounds in *D. pentandra* extract was found (Christalina et al., 2013). The results of phytochemical screening could be further identified quantitatively using the total content test (Othman et al., 2019). According to Kong et al. (2023) and Hardiyanti et al. (2019), flavonoids, tannins, and polyphenols were abundant compounds in the leaves of *D. pentandra*. In addition, these 3 compounds had a strong inhibition mechanism because tannins and flavonoids could form protein complexes and polysaccharide compounds (Othman et al., 2019), while phenols had an inhibitory mechanism that attacked the protoplasm and blocked cell replication (Rohdiana et al., 2013). In this study, total tannin content of 1.37 ± 0.14 mg TAE/g extract, total flavonoid content of 4.09 ± 0.70 mg QE/g extract, and total phenol content of 1.76 ± 0.16 g GAE/g extract were identified (Table 1). Based on these results, it was known that in D. pentandra extract, the phenol equivalent of gallic acid was the most commonly found compound, followed by the tannin equivalent of tannic acid and flavonoid equivalent of quercetin.

The total phenol content in this sample was higher compared to Rantesalu et al. (2022) which was 291.36 mg GAE/g from the same mistletoe species. The production and availability of various secondary metabolite contents in plants could be affected by environmental factors, such as light intensity and soil nutrients. Additionally, phenol was a compound that functioned as antioxidants. This defense mechanism could be formed whenever the plant encountered stress, such as UV-B radiation (Sembiring et al., 2018). According to Ramakrishna et al. (2011), the light intensity of 301-600 Lux was a medium

928.8 hPa which was categorized into the high light intensity category and was presumed that the production of abundant secondary metabolites was phenol.

Yee et al. (2017) identified the total polyphenol content using the tannic acid standard in *D. pentandra* which was 14.9 ± 0.2 µg TAE/g. The total content of the study was lower compared to the total content identified in the *D. pentandra* tea mistletoe in this study, which was 1.37 ± 0.14 mg TAE/g. Tannin production in these 2 studies was different due to differences in the species of host of each sample. Mistletoe could absorb nutrients from its host by using its haustorium, and the compounds nutrients absorbed by this type of mistletoe were different. This statement was also supported by the study of Yismairai et al. (2019) which detected differences in the polyphenol and flavonoid content of *D. pentandra* in 3 different hosts using the same solvent, namely methanol. This was related to the ability of mistletoe to absorb nutrients from its host plant.

The total flavonoid content in D. pentandra leaves extract was 4.09 ± 0.70 mg QE/g (Table 1). This value was lower than the previous study by Lekal and Watuguly (2017), which was 144 mg QE/g. Based on SNI 19-7030-2004, the minimum standard of the growth media for nitrogen was 0.4%, 0.1% for phosphate, and 0.2% for potassium. In this study, the nitrogen, phosphate, and potassium levels in the soil were 0.165, 0.073 and 0.145% respectively. The nutrient that played a role in forming phospho-anhydride bonds in adenosine triphosphate (ATP) was phosphorus, and phosphorus deficiency caused an increase in free radical compounds (ROS) due to the excessive production of electrons and the mitochondrial oxidative phosphorylation system. In addition, phosphorus deficiency inhibited the photosynthetic electron transport chain which affected the availability of flavonoid compounds needed by plants. The nutrient associated with the formation of flavonoid content was nitrogen through the activity of the enzyme phenylalanine ammonia-lyase (PAL), and this enzyme activity was related to the presence of ammonia in the soil. Ammonia remained stable in the environment because it had positive ions and negatively charged soil colloids bind more easily. Increasing nitrogen fertilizer concentration spurred plant stem growth but decreased flavonoid production. Inhibition of flavonoid accumulation flavonoid accumulation was caused by suppression (downregulated) of PAL enzyme activity, which resulted in decreased flavonoid synthesis (Amir et al., 2013).

Inhibition of *D. pentandra* Extract against Bacteria

The potential antibacterial activity of plant extracts could show based on the diameter of the inhibition zone formed. This test was used as an initial test to determine the concentration range that could be used for further tests, namely the MIC and MBC tests. Table 2 shows that the inhibitory potential of S. aureus was found in concentrations of 150 mg/mL and 275 mg/mL classified into the weak inhibition category. 400 mg/mL and 525 mg/mL concentrations were categorized into moderate inhibition, while the positive control was included in the robust category. A different result was presented by Hardiyanti et al. (2018) that showed moderate activity for 1000 µg/mL of *D. pentandra* extract against *S. aureus*. The inhibitory potential of E. coli (Figure 1. B) was not found in the same 5 concentrations as for *S. aureus*, and there were 5 additional concentrations of 750, 1000, 1250, 1500, and 1750 mg/mL. However, out of 10 concentrations, no inhibition was formed

around the disk (Table 2). Based on Diningsih and Aswan (2019), D. pentandra extract had potent inhibitory ability against *E. coli* as seen from the diameter of the inhibition zone at a concentration of 150 mg/mL reaching 8 mm. However, the extract showed different results in this study.

Table 2. Inhibition test results of S. aureus FNCC 0047 and E. coli ATCC 25922 ethanol extract of *Dendrophthoe pentandra* leaves by Disk Diffusion method.

Treatment	Inhibition Zone Diameter (mm) ± SD *S. aureus*	Category *S. aureus* Inhibition Zone	Inhibition Zone Diameter (mm) ± SD *E. coli*	Category *E. coli* Inhibition Zone
Positive Control	36.35 ± 0.02	very strong	26.19 ± 0.08	very strong
Negative Control	0 ± 0.00	ND	0 ± 0.00	ND
25 mg/mL	0 ± 0.00	ND	0 ± 0.00	ND
150 mg/mL	4.23 ± 0.02	weak	0 ± 0.00	ND
275 mg/mL	4.78 ± 0.01	weak	0 ± 0.00	ND
400 mg/mL	5.82 ± 0.006	medium	0 ± 0.00	ND
525 mg/mL	6.35 ± 0.02	medium	0 ± 0.00	ND
750 mg/mL	-	-	0 ± 0.00	ND
1000 mg/mL	-	-	0 ± 0.00	ND
1250 mg/mL	-	-	0 ± 0.00	ND
1500 mg/mL	-	-	0 ± 0.00	ND
1750 mg/mL	-	-	0 ± 0.00	ND

According to the antibacterial activity, S. aureus was relatively more sensitive to bioactive ingredients of ethanol extract of *D. pentandra* tea bark. This was caused by differences in cell wall structure between Gram-positive and Gram-negative bacteria. Gram-negative bacteria had a more complex wall structure compared to Gram-positive bacteria. Gram-negative bacteria had 3 layers of cell walls, namely the outer membrane, peptidoglycan cell wall, and the inner membrane in the third layer (Breijyeh et al., 2020).

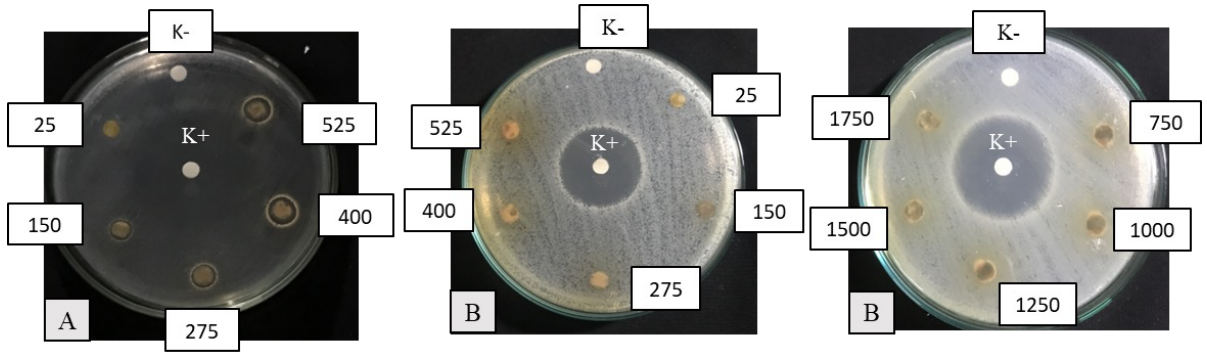

Figure 1. Zone of bacterial inhibition formed from *D. pentandra* extract treatment. (A) *S. aureus* FNCC 0047 (B) *E. coli* ATCC 25922

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According to Othman et al. (2019), a common mechanism of compound in inhibiting *E. coli* pathogenic bacteria was by blocking the ATP activity of *E. coli* by binding to GyrB-type proteins therefore inhibiting E. coli biosynthesis. This mechanism was found in the flavonoid compound, especially the quercetin group. The total flavonoid that was equivalent to the total of quercetin in this study was found to be the lowest among tannins and phenol compounds. The diversity of total compounds was influenced by the mechanism when mistletoe's antibacterial ability inhibited bacteria. This difference in mechanism was supported because the structure of the *E. coli* cell wall was complex to break when viewed from its structure (Breijyeh et al., 2020), and it was less appropriate to use the mechanism of destroying the cell wall that happened in very high concentration conditions (Othman et al., 2019). The phenol equivalent of gallic acid was the highest compound found in the ethanol extract of *D. pentandra* leaves. Additionally, it was known that the phenol mechanism in inhibiting bacteria was by poisoning the protoplasm part of the bacterial cell wall (Rohdiana et al., 2013).

Antibacterial Activity of *D. pentandra* Tea Mistletoe Leaves Extract

The results of the disk diffusion test in the form of concentrations were further tested for antibacterial ability using the broth micro-dilution method to identify the minimum concentration that could potentially inhibit pathogenic bacteria. In the growth diagram of *S. aureus* (Figure 2. A), it could be seen that the concentrations of 4400 mg/mL and 17600 mg/mL had a range that did not exceed the negative control and was not too far from the positive control. However, at 8800 mg/mL concentration, there was an extreme decrease in value and it doubted the value of 17600 mg/mL as the MIC value, and this concentration was also included in the high concentration. This showed that the MIC value of *D. pentandra* extract was 4400 mg/mL. In addition, based on the data in Figure 2. A, it could be seen that the smaller the concentration of ethanol extract of *D. pentandra* leaves, the higher the OD value of the bacteria increased, even at a concentration of 2200 mg/mL and above the mean OD value of bacteria exceeded the OD value of the negative control which also influenced the negative control which was too small.

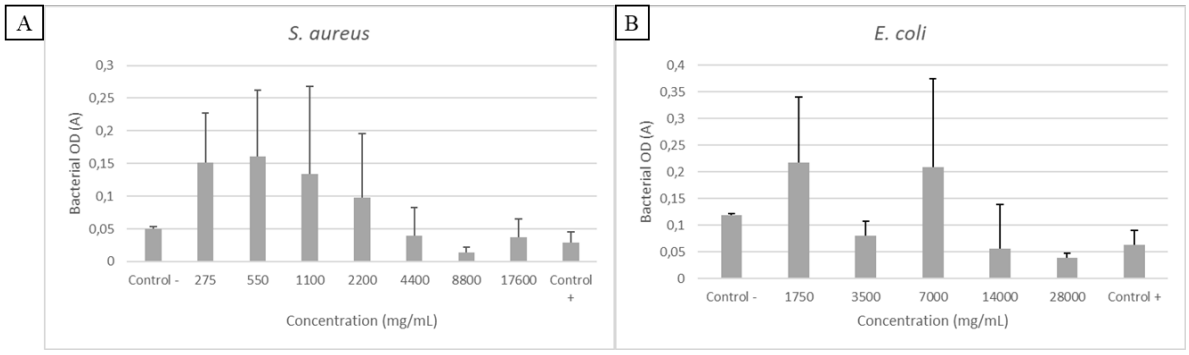

Figure 2. Bacterial growth in varying concentrations of *D. pentandra* extractant by broth micro-dilution method. (A) *S. aureus* FNCC 0047 (B) *E. coli* ATCC 25922

In the diagram of *E. coli* growth, it could be found that the concentration of 28000 mg/mL was the MIC concentration (Figure 2. B) because when it was compared to the negative control, the OD value at a concentration of 28000 mg/mL was lower and near to the OD value of the positive control bacteria. In addition, the MIC value was determined based on the smallest standard deviation value of 28000 mg/mL concentration. From the data, fluctuations could be seen, and this could be influenced by the diversity between data, namely the standard deviation value. For example, at a concentration of 7000 mg/mL, the standard deviation was very high as shown by the red line.

The study previously conducted by Diba et al. (2021) identified the MIC in *D. pentandra* species in lime host plants against S. aureus was smaller, namely 1250 µg/mL when compared to this study on *D. pentandra* tea mistletoe extract, while against *E. coli* there was a smaller concentration of 625 µg/mL. The difference in inhibitory potential between previous studies and this study could be based on differences in hosts. Mistletoe could absorb nutrients from its host by using its haustorium and the conditions of the environment of each host plant and the compounds and nutrients absorbed by this type of mistletoe were different. This statement was also supported by the study of Yismairai et al. (2019) which detected differences in the phenolic and flavonoid content of *D. pentandra* in 3 different hosts using the same solvent, namely methanol.

The MIC results of the microdilution test method were in line with the inhibition zone study of the disk diffusion method. However, in the inhibition zone test, no inhibitory potential was found from the ethanol extract of *D. pentandra* against *E. coli* starting from a concentration of 25 mg/mL to 1750 mg/mL. Furthermore, it could be concluded that the ability of D. pentandra ethanol extract as an antibacterial agent for E. coli ATCC 25922 bacteria was feeble. Factors that could influence secondary metabolite compounds could activate antibacterial activity. The compound most contained in the extract had a higher potential to inhibit bacterial growth, and the antibacterial mechanism could be more dominantly used.

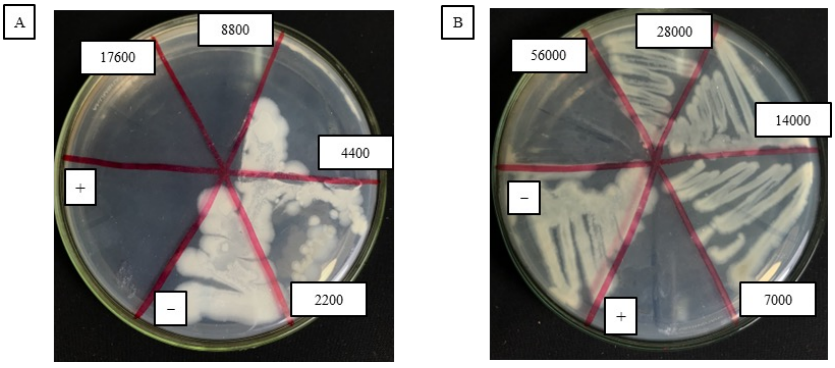

Figure 3. MBC results of *D. pentandra* extract against bacteria by broth micro-dilution method. (A) *S. aureus* FNCC 0047 (B) E. coli ATCC 25922

The minimum inhibitory level was determined in the previous MIC test using the micro-dilution method, and then the maximum level of an antibacterial agent was needed in inhibiting pathogenic bacterial infections to see the effectiveness of a sample in killing bacteria. The way to find out was by determining MBC, which was the lowest concentration of antibacterial agent needed to kill 98% - 99.9% (Balouiri et al., 2016). The concentration used in the MBC test was determined based on the MIC results that were done before, with the MIC concentration maintaining the standard.

Based on the results of inoculation of samples in agar media (Figure 3. A), it was shown that there was no growth at concentrations of 17600 mg/mL, 8800 mg/mL, and positive control (ampicillin). A little bit of S. aureus growth was observed at a concentration of 4400 mg/mL, while dense growth was seen at a concentration of 2200 mg/mL. This was in line with the OD value of bacteria from a concentration of 8800 mg/mL which had less decrease than the positive control. In addition, it showed that a higher concentration of the extract was more lethal for *S. aureus* FNCC 0047. In Figure 3. B, it could be seen that no growth of E.coli was observed at 56000 mg/mL, while other concentrations showed the growth of E. coli colonies.

The growth of *S. aureus* and *E. coli* colonies on MHA media appeared transparent, slimy, and white following Utomo et al. (2018). According to data, ethanolic extract of mistletoe (*D. pentandra*) tea leaves had an MBC value against S. aureus and E. coli respectively, at 8800 mg/mL and 56000 mg/mL. In addition, the antibacterial activity of D. pentandra ethanolic extract was greater for *S. aureus* than *E. coli*, even though it was still classified in the low category. Taking into account its pharmacological potential, more studies were still needed to optimize the extraction of mistletoe active compounds and found out the effect of ecological factors in the production of its secondary metabolites.

CONCLUSION

In conclusion, *D. pentandra* leaves extract contained flavonoids, tannins, alkaloids, saponins, terpenoids, steroids, and phenols as the most compounds found in the extract. The antibacterial activity of *D. pentandra* extract was higher in inhibiting the growth of *S. aureus* compared to *E. coli*. The MIC and MBC values of the extract against *S. aureus* were 4400 and 8800 mg/mL, while against *E. coli* were 28000 and 56000 mg/mL, respectively.

AUTHOR CONTRIBUTION

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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