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Antibacterial Activity of Simpur Leaves Methanol Extract (*Dillenia* sp.) Againts *Staphylococcus aureus*

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ABSTRACT

Simpur (Dillenia sp.) is a plant that can be used as traditional antibacterial medicine. This study aims to determine the inhibitory activity of Staphylococcus aureus bacteria when exposed to a concentration of methanol extract from Simpur leaves (Dillenia sp.). This study utilized a completely randomized design with five concentration levels of Dillenia sp. specifically, the study included a control group and methanol extracts of Simpur leaves at concentrations of 5%, 10%, 15%, and 20%. The methods used included total plate count, hemocytometer, and UV-Vis spectrophotometry, which were then analyzed using ANOVA (Analysis of Variance). The results showed that the greatest inhibition occurred in the methanol extract of Simpur leaves (Dillenia sp.) at a concentration of 20%, there was a decrease in the number of colonies ranging from 90% to 94%, while the decrease in the number of cells ranged from 17% to 18%. The lowest inhibition was found in the methanol extract of Simpur leaves (Dillenia sp.) with a concentration of 10%, the proportion of decreasing the number of colonies ranged from 63% to 70%, while the proportion of decreasing the number of cells ranged from 6% to 7%. The highest optical density (OD) value at a 20% concentration ranged from 0.482 to 0.547, while the lowest value at a 5% concentration ranged from 0.127 to 0.131. The conclusion of this study is that the methanol extract of Simpur leaves (Dillenia sp.) Bacteriostatic antibiotics only inhibit bacterial growth and cannot kill bacteria.

Keywords: Antimicrobe; bacteriostatic; *Dillenia*; Staphylococci

INTRODUCTION

Simpur (*Dillenia* sp.) is commonly found in various regions, including Sumatra, Java, and Kalimantan. Traditionally, these leaves are used as a wrapper for rice. The fruit is used as a laxative for stomach aches, a cooling drink for fever, and an ingredient in cough medicine. Based on literature studies and previous research, Simpur contains secondary metabolite compounds with pharmacological effects. In research on the phytochemical tests of Simpur leaves extract, various active compounds were found, including alkaloids, phenols, tannins, flavonoids, steroids, terpenoids, and saponins (Prananda et al., 2015). These compounds have activity in inhibiting bacteria that cause infections.

Infectious diseases remain a health issue in both developing and developed countries. Microorganisms that cause infectious diseases include parasites, viruses, and bacteria. *S. aureus* is the most common cause of nosocomial infections, which patients acquire after being admitted to the hospital (Jawetz, 2004). Several types of diseases that can be caused by *S. aureus* infection include mastitis, dermatitis (skin inflammation), respiratory tract infections, impetigo, abscesses, toxic shock syndrome, and food poisoning. Symptoms may include nausea, vomiting, and diarrhea (Affifurahman, 2014).

The research results of Yakop et al. (2020) showed that *Dillenia suffruticosa* leaf extract can inhibit the growth of *S. aureus* at a concentration of 300 mg/ml, resulting in an inhibition zone of 7.44 mm. Research by Apu et al. (2010) demonstrated antimicrobial and cytotoxic activities in various fractions of *Dillenia* indica. The research results of Wiart et al. (2004) showed that the methanol extract from D.

suffruticosa has the potential to be antimicrobial and antifungal against *Bacillus cereus*, *Bacillus subtilis*, *Candida albicans*, and *Pseudomonas aeruginosa*. One milligram of *D. suffruticosa* extract exhibits antibacterial activity against *B. subtilis* and *P. aeruginosa*, resulting in inhibition zones of 7 mm and 9 mm, respectively. The research results of Syafrana et al. (2021) showed that *Dillenia* sp. leaves extract has the potential as an antimicrobial for *S. aureus*, showing a high inhibition zone of 10.52 mm at a concentration of 40%. Meanwhile, leaf extracts cannot demonstrate an inhibition zone or inhibit the growth of *Escherichia coli* and *Candida albicans*.

Based on several previous research findings regarding the antibacterial activity of *Dillenia* sp., it exhibited activity that inhibits bacterial growth, resulting in the formation of a clear zone. Even though the antibacterial properties have been proven, few studies have investigated the inhibitory effect on the growth of *S. aureus* colonies and cells using methods such as total plate count, hemocytometer, and UV-Vis spectrophotometry. Based on this, further testing is needed to assess the effect of administering methanol extract of *Dillenia* sp. against the growth of *S. aureus* bacteria with several different concentrations. This study aims to determine the inhibitory activity of *S. aureus* bacteria when exposed to a concentration of methanol extract from Simpur leaves (*Dillenia* sp.). It is hoped that this research can provide basic information for the development of simpur plants as a source of natural metabolites that can be used as alternative medicine or for the development of medicinal preparations.

METHODS

Research Design

The experimental design used was a completely randomized design (CRD). This research involved two levels of treatment. The first level was the control treatment using distilled water, while the second level involved the methanol extract of Simpur leaves with concentrations of 5%, 10%, 15%, and 20%. Each treatment was repeated three times, resulting in a total of 15 trials.

Research Tools and Materials

The tools used in this research include aluminum foil, autoclave, stirring rod, glass beaker, Biological Safety Cabinet (BSC), Bunsen burner, Petri dish, funnel, Erlenmeyer flask, measuring cup, hot-stir plate, incubator, test tube, filter paper, refrigerator, micropipette, spectrophotometer, analytical scale, and vacuum rotary evaporator. The materials used in this research were sterile distilled water, 70% alcohol, hot water, and Simpur leaves (*Dillenia* sp.), $FeCl_3$, concentrated HCl, concentrated H_2SO_4, pure culture of *S. aureus*, Nutrient Agar (NA), Nutrient Broth (NB) Methanol, NaOH, Liebermann-Burchard reaction, Wagner's reagent, and sterile saline.

Sample Preparation and Extraction of Simpur Leaves (*Dillenia* sp.)

Three kilograms of fresh green Simpur leaves are washed, sliced, and air-dried indoors, then blended into powder. Two hundred grams of Simpur leaves powder were soaked in 2 liters of methanol as a solvent. Maceration was carried out for three days and repeated every 24 hours using the same procedure. The maceration results are then filtered using filter paper. The filtrate obtained from the extract is

evaporated using a rotary evaporator to yield a concentrated extract (Syafriana, 2021).

Media Preparation

Nutrient Agar (NA) is prepared by dissolving 20 grams in 1000 ml of distilled water, while Nutrient Broth (NB) media is made by weighing 8 grams of nutrient broth (NB) dissolve the powder in 1000 ml of distilled water. Each medium is heated using a hot plate. The media was then sterilized in an autoclave at 121°C with a pressure of 1 atm for 15 minutes.

Bacterial Rejuvenation

Rejuvenating bacterial isolates was performed by transferring the *S. aureus* isolate onto Nutrient Agar media in a test tube using the streak method. The isolate was then incubated for 24 hours at 37°C (Hermawan, 2007).

Bacterial Suspension Preparation

The *S. aureus* was taken with a loop and inoculated into an Erlenmeyer flask containing 25 ml of sterilized Nutrient Broth (NB) Media. The sample was then incubated at 37°C and homogenized with a shaker at room temperature for 6-18 hours. The suspension was then measured every 2 hours until the optical density (OD) value of 0.8-1, obtained using a UV-Vis spectrophotometer at a wavelength of 600 nm. The OD value is equivalent to the standard of McFarland 0.5 with an estimated cell number of 1.5x10^8 CFU/ml (Claudia et al., 2021).

Preparation of Extract Concentration

Simpur leaves methanol extract solution was prepared by weighing 10 grams of the extract. 10 mL of distilled water was added to obtain a stock solution with a concentration of 10 g/10 mL. This study compared the extract concentrations of 5%, 10%, 15%, and 20% with a control group (0%). The test was carried out using the tube dilution method (Waluyo, 2008). The test procedure is as follows:

1. 5% concentration, 0.5 ml of extract and 9.5 ml of distilled water.
2. 10% concentration, 1 ml of extract and 9 ml of distilled water.
3. 15% concentration, 1.5 ml of extract and 8.5 ml of distilled water.
4. 20% concentration, 2 ml of extract and 8 ml of distilled water.
5. Concentration 0% (control), 10 ml of distilled water.

The next step involved filling each tube with 1 ml of bacterial suspension, which had been previously diluted with a syringe. Subsequently, the tube was homogenized using a vortex until it reached a homogeneous state.

Viability Test of *S. aureus* using the Total Plate Count Method

This research was carried out at concentrations of 5%, 10%, 15%, 20%, and a control. Observations were carried out twice, namely after 24 and 48 hours. Each 1 ml extract concentration of suspension was poured into a petri dish using the pour plate method, followed by adding 20 ml of nutrient agar (NA) was added and incubated at 37°C for 24 and 48 hours. After the incubation period, the bacterial colonies were counted using the total plate count (TPC) method. The number of colonies in the sample is calculated using the following formula (Waluyo, 2008):

$$\text{Colonies per ml} = \frac{\text{Number of colonies per plate}}{\text{Dilution factor}}$$

Measurement of *S. aureus* Growth

The bacterial cell growth was measured using the UV-Vis spectrophotometry method at concentrations of 5%, 10%, 15%, 20%, and control (without extract addition). Observations were conducted twice, specifically after 24 and 48 hours. Observations were made by examining the turbidity at each concentration and measuring the Optical Density (OD) Value determined using UV-Vis spectrophotometry at a wavelength of 600 nm. The cell population density of *S. aureus* was calculated at 24 and 48 hours. Cell numbers were counted using a hemocytometer placed under the microscope objective lens. In this method, microscopic counts are carried out in grid boxes. Each scale box has an area of 1 mm^2, comprising 25 large boxes with an area of 0.04 mm^2, and each large box consists of 16 small boxes. The thickness of the sample located between the two slides and the cover glass is 0.02 mm. The number of cells in several large boxes can be counted, and then the number of cells per milliliter of sample can be calculated as follows (Waluyo, 2008).

Number of cells per ml of sample = Number of cells per large box × 1.15 × 10^6

Phytochemical Screening of Simpur Leaves Extract

a. Alkaloid Test

The methanol extract of Simpur leaves was dropped onto a drip plate and then treated with Wagner's reagent for 10 minutes. A brown precipitate will form if the result is positive (Hanani et al., 2005; Farnsworth & Cordell, 1976).

b. Flavonoid Test

The methanol extract of Simpur leaves was dropped onto a drip plate and then treated with NaOH solution. If the result is positive, a yellow, orange, or red color will appear (Farnsworth & Cordell, 1976).

c. Saponin Test

The methanol extract of Simpur leaves was dropped onto a drip plate, dripped with hot water, and then left for 10 seconds before being left for 10 minutes. The foam will form if the result is positive (Farnsworth & Cordell, 1976).

d. Phenol Test

The methanol extract of Simpur leaves was dropped onto a drip plate and then dripped with FeCl3 solution. Positive results will produce a green or blue-green color, indicating the presence of phenol content.

e. Terpenoid Test

The methanol extract of Simpur leaves was applied to a drop plate and then treated with an H_2SO_4 reagent. Positive results will lead to the formation of a blue color (Marliana et al., 2005).

Data analysis

Data from measuring incubation time and bacterial numbers are represented in growth graphs. In the graph, the X-axis represents the incubation time (in hours),

while the Y-axis represents the percentage reduction in the number of colonies and the number of *S. aureus* cells. Data from observations of the number of colonies and number of cells are presented in tables and graphs showing percentage reduction. Data on the number of colonies and bacterial cells after treatment were analyzed using ANOVA (Analysis of Variance) with a significance level of 0.5%. If the results shown are significantly different, proceed with the Duncan test with a significance level of 5%.

RESULTS AND DISCUSSION

The results of treatment with methanol extract of Simpur leaves (*Dillenia* sp.) on the growth of *S. aureus* colonies showed that the concentration of Simpur leaves extract influenced the growth of the number of *S. aureus* colonies. Observations at the incubation time of 24 hours and 48 hours showed that the number of *S. aureus* colonies experienced growth in the number of colonies in the control and extract treatments. However, in the extract concentration treatment, there was a decrease in the percentage of colony numbers compared to the control treatment (**Table 1**, **Figure 1**).

Table 1. Percentage reduction in the number of *S. aureus* colonies after treatment with methanol extract of Simpur leaves (*Dillenia* sp.) using the total plate count method

Treatment	Mean and percentage decrease in number of colonies during incubation time ($\times 10^{-5}$ CFU/mL)			
	24h	(%)	48h	(%)
Control	$506{,}33 \pm 53{,}78^{c}$	0	$561{,}67 \pm 39{,}50^{c}$	0
5%	$145{,}00 \pm 13{,}75^{b}$	71,36	$193{,}67 \pm 5{,}51^{b}$	65,52
10%	$148{,}33 \pm 20{,}74^{b}$	70,70	$206{,}00 \pm 50{,}27^{b}$	63,32
15%	$74{,}00 \pm 32{,}91^{a}$	85,39	$107{,}33 \pm 35{,}10^{a}$	80,89
20%	$31{,}67 \pm 19{,}50^{a}$	93,75	$57{,}67 \pm 10{,}69^{a}$	89,73

Note: Values followed by different letters on the same line indicate significantly different results at the α level: 0.05.

Based on the results of statistical analysis, it shows that the addition of the concentration of methanol extract of Simpur leaves (*Dillenia* sp.) has a natural effect on the growth of *S. aureus* as seen from the number of colonies at 24 hours incubation time ($F4.10 = 107.001$, $p = 0.000$; Anova), and 48 hours ($F4.10 = 107.424$ $p = 0.000$; Anova). Duncan's further test results showed that the control treatment was significantly different from all treatments. The 20% and 15% treatments showed results that were not significantly different but were significantly different from the other treatments. The 5% and 10% treatments also showed results that were not significantly different but were significantly different from the other treatments.

The graph of the growth of *S. aureus* bacteria using the Total Plate Count (TPC) method shows that the bacteria can grow in all treatments. The results of the research show that the graph of the percentage decrease in the number of colonies shows that as the concentration value of the extract used increases, the growth of the *S. aureus* bacteria decreases. The best percentage reduction occurred during 24-

hour observations in each extract treatment compared to the control treatment (**Figure 1**).

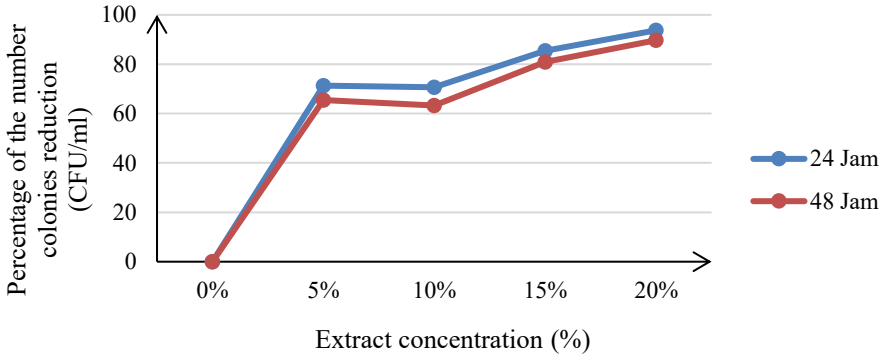

Figure 1. Percentage reduction in the number of colony growth of *S. aureus* bacterial isolates by giving methanol extract of Simpur leaves (*Dillenia* sp.) using the total plate count method

The results of treatment with methanol extract of Simpur leaves (*Dillenia* sp.) on the growth of *S. aureus* cells showed that increasing the concentration of Simpur leaves extract affected the growth of the number of *S. aureus* cells. Observations at the incubation time of 24 hours and 48 hours showed that the number of *S. aureus* cells experienced growth in cell number. However, in the extract treatment, it was seen that there was a decrease in the percentage of cell number compared to the control treatment (**Table 2**).

Table 2. Percentage reduction in the number of *S. aureus* cells after treatment with methanol extract of Simpur leaves (*Dillenia* sp.) using the hemocytometer method

Treatment	Mean and percentage decrease in cell number at incubation time (× 10^6 cell/mL)			
	24h	(%)	48h	(%)
Control	90,67±4,04^{d}	0	94,67±3,05^{b}	0
5%	79,67±0,58^{ab}	12,13	84,33±4,16^{ab}	10,92
10%	85,00±5,29^{bc}	6,25	87,67±8,14^{ab}	7,39
15%	78,00±3,61^{ab}	13,97	81,33±4,16^{a}	14,09
20%	75,67±3,51^{a}	16,54	77,67±6,81^{a}	17,96

Note: Values followed by different letters on the same line indicate significantly different results at the α level: 0.05.

Based on the results of statistical analysis, it shows that the addition of the concentration of methanol extract of Simpur leaves (*Dillenia* sp.) has a natural effect on the growth of *S. aureus* as seen from the number of cells at 24 hours incubation time (F4.10 = 7.006, p = 0.000; Anova), and 48 hours (F4.10 = 4.024 p = 0.000; Anova). Duncan's further test results showed that at the 24-hour incubation time, the control treatment was significantly different from all treatments. However, the 5% and 15% treatments were similar to the 19% and 20% treatments. At an

incubation time of 48 hours for all treatments, the extract concentration did not make a significant difference between treatments.

The graph of the growth of *S. aureus* bacteria using the hemocytometer method shows that the bacteria can grow in all treatments. The research results showed that as the concentration value of the extract used increases, the growth of *S. aureus* bacterial cells decreases. The growth graph in Figure 2 shows that bacteria given the extract have different growth patterns. The best percentage reduction occurred during 24-hour observations in each extract treatment compared to the control treatment.

The results of the optical density value (OD) of the treatment of methanol extract of Simpur leaves (*Dillenia* sp.) on the turbidity of *S. aureus* show that the concentration treatment of Simpur leaves extract affects the Optical density value of *S. aureus* using the UV-Vis Spectrophotometry method. 24-hour and 48-hour observations of *S. aureus* OD values have increased. High growth was shown in the 20% treatment with OD values of 0.482 (24 hours) and 0.547 (48 hours), while the lowest OD values were shown in the 5% treatment with amounts of 0.127 (24 hours) and 0.131 (48 hours) (**Table 3**).

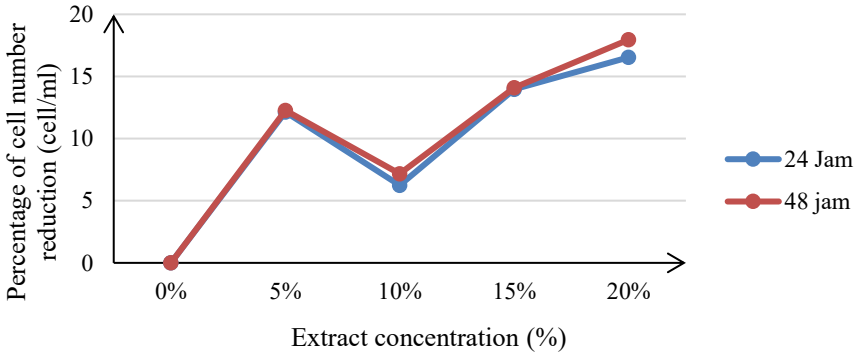

Figure 2. Percentage reduction in the number of growth cells of *S. aureus* bacterial isolates by giving methanol extract of Simpur leaves (*Dillenia* sp.) using the hemocytometer method

Table 3. Turbidity value of *S. aureus* after treatment with methanol extract of Simpur leaves (*Dillenia* sp.) using the UV-Vis Spectrophotometric Method

Treatment	Optical Density value	
	24h	48h
Control	0,458	0,448
5%	0,127	0,131
10%	0,285	0,274
15%	0,330	0,388

The graph of the growth of *S. aureus* using the UV-Vis Spectrophotometry method shows that the bacteria can grow in all treatments. The research results showed that as the concentration value of the extract used increased, the OD value of the *S. aureus* bacteria increased. The growth graph in Figure 3 shows that bacteria given the extract have different growth patterns. A concentration of 5% indicates

that growth has decreased compared to the control treatment. Meanwhile, compared with the control treatment, other concentration treatments showed that growth increased as the treatment concentration increased.

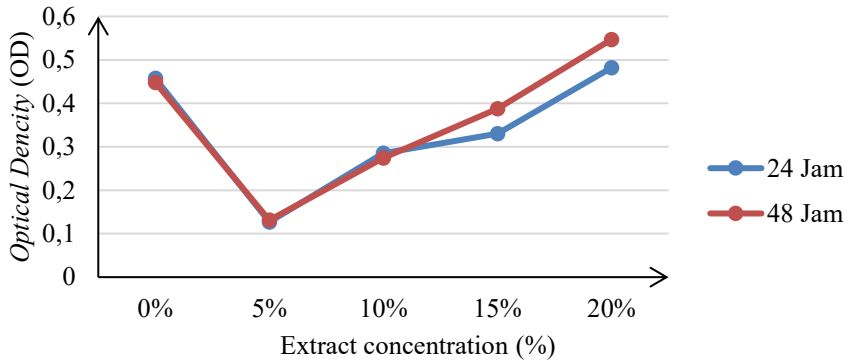

Figure 3.Decreased Optical Density Value of *S. aureus* by adding Methanol Extract of Simpur Leaves (*Dillenia* sp.) Using the UV-Vis Spectrophotometry Method.

A phytochemical screening test for the methanol extract of Simpur leaves (*Dillenia* sp.) was carried out to determine the content of secondary metabolite compounds contained in the methanol extract of Simpur leaves by observing the color change after being given the test reagent. The high or low levels of secondary metabolites can be seen from the intensity of the color change and the large amount of precipitate formed. The results of the phytochemical test of the methanol extract of Simpur leaves (*Dillenia* sp.) showed several secondary metabolite compounds such as alkaloids, flavonoids, and phenols (**Table 4**).

Table 4. Phytochemical screening results of Simpur leaves extract (*Dillenia* sp.)

No	Secondary metabolite	Type of test	Results
1.	Alkaloid	Wagner	+
2.	Flavonoid	NaOH	+
3.	Saponin	Vortex in hot water	-
4.	Phenol	$FeCl_3$	+
5.	Terpenoid	H_2SO_4	-

Information:
(-) does not contain secondary metabolite compounds
(+) contains secondary metabolite compounds

Based on the results of the phytochemical test in **Table 4** showed that the methanol extract of Simpur leaves with the administration of Wagner's reagent showed positive results (+), there were alkaloid compounds with a color change to dark brown, and there was a precipitate. Flavonoid compounds with NaOH administration showed positive results (+) with a brown color change and a precipitate. Testing for phenolic compounds by administering $FeCl_3$ showed positive results (+) with a bluish-green color change.

Viability is maintaining life in competition between individuals against nature (Nurkartika, 2001). In this study, bacterial viability was influenced by adding

methanol extract from Simpur leaves (*Dillenia* sp.). This extract was added to see how a bacteria's viability affects the number of colonies and the number of *S. aureus* cells.

Based on **Tables 1** and **2**, it was observed that *S. aureus* growth decreased in the number of colonies and cells after treatment with a concentration of Simpur leaves methanol extract, causing the growth of the number of colonies to decrease. These results indicate that Simpur leaves extract at all treatment concentrations can inhibit the growth of *S. aureus* compared to the control treatment. It is likely because the control concentration of 0% did not contain Simpur leaves extract, so the number of colonies that grew was greater than at other concentrations treated with Simpur leaves methanol extract. According to Khunaifi (2010), microbial death is related to antibacterial concentration, meaning that the higher the extract concentration, the faster the bacteria die. This result is in line with the opinion of Jawetz (2008), namely that antibacterial activity depends on concentration, temperature, and time. Deficient concentrations can stimulate bacterial growth, higher concentrations can inhibit it, and even higher concentrations can kill certain organisms.

Inhibition of *S. aureus* bacteria growth by administering methanol extract of Simpur leaves (*Dillenia* sp.) was measured using the total plate count, hemocytometer, and UV-Vis spectrophotometry methods. In the total plate count and hemocytometer methods, the best concentration for inhibiting bacterial growth occurred at 20% (**Table 1** and **Table 2**). In Table 1, the total plate count method shows that the higher the concentration of Simpur leaves methanol extract, the smaller the number of colonies, so the percentage reduction in the number of colonies increases. In contrast, in **Table 2**, the Haemocytometer method also shows that the higher the concentration of Simpur leaves methanol extract, the higher the number of cells. It gets smaller so that the percentage decrease in cell number increases. It is thought that the higher the extract concentration is caused by secondary metabolite compounds contained in the methanol extract of Simpur leaves, the greater the inhibition of the growth of *S. aureus* bacteria. According to Rakhmanda (2008), the lower the concentration of the extract, the less the number of active compounds in the extract, so the ability of the extract to inhibit bacterial growth is reduced. On the other hand, the higher the extract concentration used, the more inhibited bacterial growth activity. It is due to more antibacterial compounds in the extract with a high concentration.

The growth of *S. aureus* observed and measured using the UV-Vis spectrophotometric method showed that the higher the concentration of Simpur leaves methanol extract, the higher the optical density (OD) value. It is thought that the high O.D. value is not only due to the growth of bacterial cells, but also there is a possibility that there is an influence of giving a high extract concentration on the color of the media, which can be absorbed by light waves and results in the absorbance value being high as the concentration of Simpur leaves methanol extract increases. It follows the opinion of Dewi (2010) that the increase in absorbance values in the research results is not entirely caused by bacterial growth. However, there are other factors, such as concentrated extract residues and the absorption of light by bacterial cells, both living and dead. Thus, the results of increasing the absorbance value cannot be interpreted as an indicator of bacterial growth but can be influenced by several other factors.

The inhibition graph of the percentage reduction in the number of *S. aureus* bacterial colonies and cells was compared between the control treatment and the treatment given methanol extract of Simpur leaves at incubation times of 24 hours and 48 hours. The research results using the total plate count and hemocytometer methods showed that the percentage decrease in the number of colonies and the number of cells increased along with the addition of the concentration of Simpur leaves methanol extract (**Figure 1** and **Figure 2**). It is thought to be due to secondary metabolite compounds in the methanol extract of Simpur leaves. According to (Gan et al., 1995; Preta et al., 2014), the activity of secondary metabolite compounds contained in plant extracts can attack various bacterial cells, namely cell walls, cell membranes, proteins, and bacterial cell nucleic acids. It can also inhibit the growth of bacterial cell metabolites.

Based on the results of phytochemical screening in this study, there were alkaloid, flavonoid, and phenol compounds. These results follow Prananda's research (2015), which stated that the ethanol extract of Simpur leaves contains alkaloids, phenols, tannins, flavonoids, steroids, terpenoids, and saponins. These secondary metabolites are expected to inhibit the growth of *S. aureus* in this study, namely alkaloids, flavonoids, and phenols. The results of the phytochemical screening showed that alkaloids, flavonoids, and phenols are the compounds that play a role in bacterial growth. Flavonoids are one of the secondary metabolite compounds often found in plant tissues, which have antioxidant properties and antibacterial and antifungal effects, containing phenol groups believed to play a role in their activity as antimicrobial agents (Abdi, 2010). Alkaloid compounds play a role in inhibiting cell wall synthesis in bacteria. The mechanism of action of this alkaloid compound causes instability in the bacterial cell wall, which causes various essential functions such as selective permeability, active transport function, and control of the protein structure of the bacterial cell to be disturbed, resulting in loss of shape and lysis (Lamothe et al., 2009).

Based on research, methanol extract of Simpur leaves (*Dillenia* sp.) can inhibit the growth of *S. aureus* bacteria or is bacteriostatic up to a concentration of 20%. Based on several previous studies, Binahong (*Anredera cordifolia*) leaves extract can inhibit the growth of *S. aureus* at a concentration of 25%. According to research by Syafrana et al. (2021), Daum Simpur ethanol extract can inhibit the growth of *S. aureus* at a concentration of 40%. This research shows that bacterial viability is disturbed by the addition of methanol extract from Simpur leaves (*Dillenia* sp.), which can be seen from the decrease in the number of bacterial colonies and cells.

CONCLUSION

The most significant inhibition occurred in the methanol extract of Simpur leaves (*Dillenia* sp.) at a concentration of 20% with a percentage reduction in colony number ranging from 90-94%, while the percentage reduction in cell number ranged from 17-18%. The lowest inhibition was found in the methanol extract of Simpur leaves (*Dillenia* sp.) with a concentration of 10%. The percentage reduction in colony number ranged from 63-70%, while the percentage reduction in cell number ranged from 6-7%. The highest optical density (OD) value at a concentration of 20% ranges from 0.482-0.547, and the lowest value at a concentration of 5% ranges from 0.127-0.131

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