

Effectiveness Ofantibacterial extract Bawang Suna (*Allium schoenoprasum* L.) Against *Methicillin-Resistant* *Staphylococcus aureus* (MRSA) Using Total Plate Count

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ABSTRACT

Methicillin-Resistant Staphylococcus aureus (MRSA) infection can cause death which has caused the World Health Organization (WHO) in 2017 to issue a list of priority pathogens (one of which is MRSA) for the search for new antibiotic research. Bawang suna (*Allium schoenoprasum* L.) is believed to be able to inhibit the growth of the number of MRSA bacterial colonies because it contains saponins, alkaloids, flavonoids, tannins, triterpenoids, and steroids. The research was aimed to prove extract of bawang suna (*Allium schoenoprasum* L.) has effectiveness as an antibacterial to inhibit the growth of MRSA. True experimental design research design with cup count method using posttest only control design to compare the results of observing the number of MRSA bacterial colonies with and without treatment (control group) with serial dilution sampling technique serial dilution using a ratio of 1:5. The treatment used 1 ml an extract of bawang suna (*Allium schoenoprasum* L.) in the control group. 1 ml an extract of bawang suna (*Allium schoenoprasum* L.) gave a significant and closely related effect to inhibiting the growth of MRSA bacteria at a dilution level of 10^{-3} of $3288,50 \pm 1117,98$, a dilution level of 10^{-4} of $379,25 \pm 33,75$, and a 10^{-5} dilution rate of $42,00 \pm 8,68$. The decrease in the number of MRSA bacterial colonies was due to the extract of bawang suna (*Allium schoenoprasum* L.) containing compounds: saponins, alkaloids, flavonoids, tannins, triterpenoids, and steroids that could prevent the growth of MRSA bacterial colonies. The extract of bawang suna (*Allium schoenoprasum* L.) as much as 1 ml using Total Plate Count (TPC) with serial dilution testing technique has a significant effect on dilutions of 10^{-3} , 10^{-4} , and 10^{-5} as an antibacterial to inhibit the growth of MRSA bacteria

Keywords : *Methicillin-Resistant Staphylococcus aureus* (MRSA); Extract of Bawang Suna (*Allium schoenoprasum* L.); Colony Number; Total Plate Count (TPC); Serial Dilution.

INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) can cause a variety of illnesses and different symptoms depending on where the infection occurs in the body. MRSA infection causes several types of skin and soft tissue infections such as impetigo, abscess, necrotizing fasciitis, erysipelas, cellulitis, spinal osteomyelitis, long bones of the upper and lower extremities through the local extension of infection from wounds or as part of hematogenous infections (Clebak, 2018 and Prabhoo, 2019), and is also a major cause of hospital-acquired pneumonia and ventilator-associated pneumonia (Haddadin, 2021). According to Larry (2021), MRSA infection is an important cause of bacterial endocarditis that can cause death in about one third of infected patients (30-37%).

MRSA is a type of *Staphylococcus aureus* that is resistant to methicillin antibiotics. Meanwhile, bacteria that are still sensitive to methicillin are called *Methicillin Sensitive Staphylococcus aureus* (MSSA). Besides being resistant to methicillin antibiotics, MRSA is also resistant to beta-lactam antibiotics, macrolides, tetracyclines, chloramphenicol, and quinolones (Yuwono, 2012). The glycopeptide vancomycin, which is the drug of choice for MRSA infection, has a slow bactericidal effect and often leads to therapeutic failure. The problem is further complicated by the discovery of vancomycin-resistant MRSA strains and vancomycin-resistant MRSA strains. The evolution of bacterial species that are increasingly resistant to antimicrobials is due to the sometimes inappropriate use of antibiotics by the wider community and the ability of bacteria to adapt to different environmental conditions (Putra, 2014).

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In 2017 the World Health Organization (WHO) issued a list of priority pathogens to increase research efforts to find new antibiotics, in the list of priority pathogens there is MRSA which has a high priority for research in the search for new antibiotics against it.

According to Maureen (2019), there are 51 medicinal plants from 35 types of families that have medicinal plant extract activities for the treatment of MRSA infections. From Swandari's research (2018) it is stated that the use of ethanol extract of bawang tiwai (*Eleutherine bulbosa* (Mill.) Urb.) which contains sugar, alkaloids, terpenes, steroids and flavonoids compounds has the potential as good antibacterial activity against MRSA with a minimal inhibitory level (MIC) of 1 mg/mL. Another study conducted by Tiasarah (2017) showed that extract of bawang batak (*Allium Chinense* G. Don) which contains allicin, flavonoids, and saponins compounds can inhibit the growth of MRSA bacteria with an inhibition zone diameter of 8,695 mm at a concentration of 50%, and a concentration of 100 % can inhibit the growth of MRSA bacteria with an inhibition zone diameter of 10,545 mm. A similar study conducted by Sinta (2019) stated that the 96% ethanol of extract bawang suna (*Allium schoenoprasum* L.) which contains saponins, alkaloids, flavonoids, steroids and tannins can inhibit the growth of *Staphylococcus aureus* bacteria at concentrations of 10 grams, 8 grams, 6 grams, 4 grams and 2 grams of extract concentrated added 10 ml of DMSO (100%, 80%, 60%, 40% and 20%) and *Staphylococcus saprophyticus* bacteria at a concentration of 10 grams, 8 grams and 6 grams of extract concentrated added 10 ml DMSO (100%, 80%, and 60%)

According to Lakhundi (2018), the mechanism of action of flavonoids as antimicrobials can inhibit nucleic acid synthesis, inhibit cell membrane function and inhibit energy metabolism. According to Darsana (2012), the mechanism of action of tannins as antibacterial can inhibit the enzyme reverse transcriptase and DNA topoisomerase resulting in bacterial cells cannot be formed. The mechanism of action of saponins according to Phyliria (2017) can inhibit bacterial growth by forming complex compounds with cell membranes through hydrogen bonds so that they can destroy the permeability properties of bacterial cell walls and cause bacterial cell death. According to Pelczar (2008) the mechanism action of alkaloids that have antibacterial abilities is because they have a quaternary aromatic group capable of intercalating with DNA and alkaloids can disrupt the integrity of the peptidoglycan constituent components in bacterial

cells. The mechanism of action of steroids as antibacterial according to Rahmawati (2018) is related to membranes, lipids and sensitivity to steroid components leading to leakage of liposomes.

The literature above, the research conducted is to prove that extract of bawang suna (*Allium schoenoprasum* L.) which contains saponins, alkaloids, flavonoids, steroids and tannins can be used as an alternative antibacterial material in the future to inhibit the growth of *Methicillin-Resistant Staphylococcus aureus* bacteria. (MRSA) using the total plate count (TPC) test.

METHODOLOGY

Materials and Equipment

Bawang suna (*Allium schoenoprasum* L.) which has a planting age of \pm 3 months from Basarang Village Km.6 Basarang District, Kapuas Regency, Central Kalimantan Province. *Methicillin-Resistant Staphylococcus aureus* (MRSA) bacteria were obtained from the Biomolecular Laboratory at the Department of Clinical Microbiology, dr. Soetomo Surabaya. 96% ethanol, 0,9% NaCl, equates, Blood Agar (BAP) medium, Mueller Hinton Agar (MHA) medium, Brian Heart Infusion Broth (BHI-B) medium, staph aureus latex agglutination, and Plate Count Agar (PCA) were obtained from the Laboratory of the Faculty of Health Sciences, University of Muhammadiyah Palangka Raya. The equipment used is all analytical chemistry of equipment.

Method

Preparation of Extract Bawang Suna Suspension (*Allium schoenoprasum* L.)

Bawang suna (*Allium schoenoprasum* L.) used is a white tuber, still fresh, with no traces of animal or pest bites and has been cleaned from the roots, then cleaned under running water to remove dirt and dried under the hot sun for 5 days until it does not contain water, is easily brittle and crumbles when gripped by covering it with a black cloth. After drying, the bulbs of bawang suna (*Allium schoenoprasum* L.) were ground in a blender and filtered through a filter to get the surface of the bulbs to come into contact with the solvent. When it is smooth, bawang suna (*Allium schoenoprasum* L.) are weighed as much as 1,4 kg and put into a jar then mixed with 5 liters of 96% ethanol and stirred until homogeneous. After being homogeneous, the jars were tightly closed with aluminum foil and then macerated for 1x24 hours at room temperature. After the maceration process, the filtrate and pulp are separated by filtering using filter paper to produce filtrate. If it is

still colorless, the filtered dregs are macerated again until they get a colored filtrate. The resulting filtrate is then concentrated and separated from the solvent using a rotatory evaporator at a speed of 100-110 rpm. After that, the filtrate was poured into a 100 ml glass beaker and continued with a water bath for 3,5 hours at 70°C to produce an extract of bawang suna (*Allium schoenoprasum* L.). The finished viscous extract was then weighed as much as 10 grams and put into a test tube together with 100 ml of distilled water and homogenized to produce a suspension of extract bawang suna (*Allium schoenoprasum* L.) (Sinta, 2019 and Pebrinata, 2020).

Preparation of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Pure cultures of MRSA bacteria were first rejuvenated using BHI-B media by transferring 2 ose of MRSA bacterial cultures into 100 ml of BHI-B media by suspension and incubating at 37°C for 24 hours (Rahmawati, 2014). After the rejuvenation process, 1 ose of MRSA bacteria was transferred by scraping evenly over the entire surface of the BAP media, then incubated at 37°C for 24 hours so that the MRSA bacterial media grew. If the media has not grown, the process can be continued for up to 48-72 hours. MRSA bacteria on BAP media that has grown, then performed a catalase test, coagulase test and cefoxitin test.

Catalase Test

The catalase test was carried out by taking 1 ose of MRSA bacteria that had grown aseptically and scratched on a petri dish until they are flat and do not accumulate, then 1-2 drops of 3% H₂O₂ are dripped and tightly closed so that there is no contamination or maximizes microbes to remodel H₂O₂. Make observations to determine whether there are small bubbles, if there are bubbles then the media is a positive catalase bacteria (*Staphylococcus aureus*), or conversely there are no bubbles then the media is catalase-negative bacteria (Ibrahim, 2016).

Coagulase Test

The coagulase test was carried out by taking 1 ose of MRSA bacteria that had grown aseptically into a test tube that already contains staph aureus latex agglutination reagent and then stirring until homogeneous, then incubated for 4-24 hours. Make observations to determine whether there is a clot formation, if it shows the results of the formation of a clot then it is called positive *Staphylococcus aureus*, whereas if there is no clot it is called negative *Staphylococcus aureus* (Ibrahim, 2016).

Cefoxitin Test

The cefoxitin test was carried out by taking MRSA bacteria that had grown using a sterile cotton swab, then streaked evenly on the MHA media and allowed to dry for 5 minutes at a closed room temperature. Then place the cefoxitin 30 g disc on the surface of the medium by pressing gently (do not press the disc until it sinks) using a sterile cotton swab to ensure the disc adheres to the agar surface and incubated at 37°C for 24 hours. After incubation, observe and record the inhibition zone around the disc (in mm), declared resistant if the diameter of the inhibition zone is ≤14 mm, intermediate if the inhibition zone is 15-17 mm and sensitive if the inhibition zone is ≥18 mm (Ibrahim, 2016).

Colony Test

Research has proven that extract of bawang suna (*Allium schoenoprasum* L.) which contains saponins, alkaloids, flavonoids, steroids and tannins can inhibit the growth of MRSA bacteria using the cup count method. External variables that affect the course of the experiment (treatment) can be controlled, because there are significant differences between the experimental group and the control group so the results of the treatment given have a significant influence (Sugiyono, 2013). The samples for the control group and the experimental group (treatment) in this research came from the same medium, namely a suspension of MRSA bacteria that had grown. The control group is the result of observing the number of MRSA bacterial colonies before treatment, and the experimental group is the result of observing the number of MRSA bacterial colonies after the addition of 1 ml an extract of bawang suna (*Allium schoenoprasum* L.) suspension. The sampling method used the serial dilution technique, which is to lessen or reduce the number of microbes suspended in the liquid. Determination of the magnitude or number of dilution levels used in this research is to use a dilution level with a ratio of 1:5 for the first and subsequent dilution samples, so that the next dilution contains 1/10 of the microorganism cells.

Preparation of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Bacterial Suspension Media

MRSA bacteria on BAP media that had grown were taken as much as 1 ose and dissolved in 10 ml of sterile 0.9% NaCl solution. Then homogenized using a vortex mixer for 15 seconds until it reaches the same turbidity as the standard turbidity Mc. Farland 0,5. After that, the media was then covered with sterile aluminum foil and

incubated with an incubator at 37°C for 24 hours to produce a suspension of MRSA bacteria (Ibrahim, 2016).

Control Group Test

The MRSA bacterial suspension was transferred as much as 1 ml aseptically into a test tube, then 9 ml of distilled water was added and then homogenized using a vortex (samples were marked with a dilution of 10^{-1}). From the 10^{-1} dilution, 1 ml was taken using a sterile measuring pipette and inserted into the second tube containing 9 ml of distilled water and then homogenized using a vortex (the sample was marked with a dilution of 10^{-2}). This step is repeated for a dilution level of 10^{-3} (third tube) to obtain a dilution level of 10^{-5} (fifth tube). From each dilution of 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5} , then transfer 1 ml aseptically into a petri dish containing PCA media evenly and cover it. Homogenize the mixture by shaking or rotating it slowly to form a figure of eight (8) on a flat table under aseptic conditions. After the media condenses, invert the petri dish and incubate at 37°C for 24-48 hours. Make observations and count the number of colonies that grow using the colony counter and record the results separately. Repeat this process 5 times to get data on the number of colonies in the control group (Practicum Guide, 2020).

Experimental Group Testing (Treatment)

In the experimental (treatment) group, the experimental group's colony testing method was the same as the control group's colony testing method. The test media used as the test medium was taken from the remaining test media for the MRSA bacterial suspension of the control group and given treatment with the addition of 1 ml an extract of bawang suna (*Allium schoenoprasum* L.) (the extract of bawang suna used was 10 grams of thick extract and 100 ml of distilled water).

Activity Data Analysis Extract of Bawang Suna (*Allium Schoenoprasum* L.) Against Methicillin-Resistant *Staphylococcus aureus* (MRSA) Bacteria

The research data were tested using the SPSS 21.0 program with the paired sample t-test analysis method. According to Sugiyono (2013), before the analysis test, the data is tested whether the data is normally distributed or not using the tests of normality (Shapiro Wilk), the histogram plot test and the Normal P-P Plot test, as well as the Outliers test (boxplot). The basis for the decision to prove "extract of bawang suna (*Allium schoenoprasum* L.) has effectiveness as an

antibacterial to inhibit the growth of MRSA bacteria" which is characterized by if the significance value of $t_{count} < 0,05 t_{table}$, the decision : extract of bawang suna (*Allium schoenoprasum* L.) has a significant effect on the growth of MRSA bacteria, and if the significance value of $t_{count} > 0,05 t_{table}$, the decision : extract of bawang suna (*Allium schoenoprasum* L.) has no significant effect on the growth of MRSA bacteria.

RESULTS AND DISCUSSION

Test Results Of Bawang Suna (*Allium Schoenoprasum* L.)

The results of the identification of the bawang suna (*Allium schoenoprasum* L.) used in this research are Kingdom : *Plantae* (plants); Subkingdom : *Tracheobionta* (vascular plants); Subdivision : *Spermatophyta* (producing seeds); Division : *Magnoliophyta* (flowering plants); Class : *Liliopsida* (single/monocot); Subclass : *Commelinidae* Order : *Asparagales*; Tribe : *Amaryllidaceae*; Clan : *Allium* Species : *Allium schoenoprasum* L.; Local Name : Bawang Suna.

The yield extract of bawang suna (*Allium schoenoprasum* L.) was taken from tubers with a wet weight before drying weighing 1400 grams and macerated by soaking simplicia cells of bawang suna (*Allium schoenoprasum* L.) as much as 420 grams in 5 liters of 96% ethanol solvent for 3x24 hours, yielded 92,26 grams extract of bawang suna (*Allium schoenoprasum* L.) which is dark brown, has a characteristic odor of bawang suna (*Allium schoenoprasum* L.), and has no taste. The interpretation of the extraction results can be seen in figure 1.

The results of quantitative phytochemical screening examinations were carried out by carrying out phytochemical screening examinations at the Biochemistry and Biomolecular Laboratory of Faculty Medicine, Lambung Mangkurat of University Bajar Baru. Based on the results of quantitative phytochemical screening, the extract of bawang suna (*Allium schoenoprasum* L.) contains chemical compounds as shown in table I.

Validation Test Results *Methicillin-Resistant Staphylococcus aureus* (MRSA)

Before being used as a test medium, MRSA bacteria were tested using the catalase test, coagulase test, and cefoxitin test with the following results:

Results of Catalase Test and Coagulase Test

The results of the catalase test using 3% H_2O_2 reagent produced bubbles in the test media, and the results of the catalase test using the staph



Image A

Image B

Description : Image A = Weigh extract of bawang suna (*Allium Schoenoprasum L.*) after the maceration process is implemented; Image B = Extract of bawang suna (*Allium Schoenoprasum L.*) which is ready to be used.

Figure 1. Extract of bawang suna (*Allium Schoenoprasum L.*)

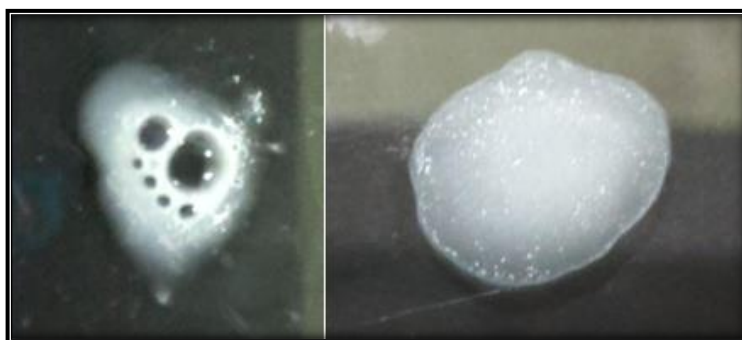


Image A

Image B

Description : Image A = Catalase test results; Image B = Coagulase test results.

Figure 2. Results of Catalase Test and Coagulase Test

Table I. Compound Content Extract of Bawang Suna (*Allium schoenoprasum L.*)

| No. | Compounds tested | Average Level |
|-----|----------------------|---------------|
| 1. | Saponin (%) | 46,787 |
| 2. | Alkaloid (%) | 53,820 |
| 3. | Flavonoid (mg/ml QE) | 17,417 |
| 4. | Tanin (mg/ml GAE) | 0,322 |
| 5. | Triterpenoid (mg/ml) | 54,467 |
| 6. | Steroid (mg/ml) | 40,781 |

Source : Phytochemical screening examination data from the Laboratory of Biochemistry and Biomolecular, Faculty Medicine, University of Lambung Mangkurat Bajar Baru.

aureus latex agglutination reagent resulted in the formation of lumps in the test medium, so it can be concluded that the test medium contained MRSA

bacteria. The interpretation of the results of the catalase test and the coagulase test can be seen in figure 2.



Image A

Image B

Image C

Description : Image A = *Methicillin-Resistant Staphylococcus aureus* (MRSA) bacteria before incubation; Image B and Image C = Test results using cefoxitin antibiotic disc (disk diameter = 0,6 cm and inhibition zone = 1,13 cm) after incubation.

Figure 3. Cefoxitin Test Results

Table II. Test Results Total Plate Count (TPC) of *Methicillin-Resistant Staphylococcus aureus* (MRSA) Before and After Treatment Addition of Extract Bawang Suna (*Allium Schoenoprasum* L.)

| Dilution | Control Group (Before Treatment) | | | | | Experiment Group (After Treatment) | | | | |
|----------|----------------------------------|--------------------|------------------|------------------|------------------|------------------------------------|--------------------|------------------|------------------|------------------|
| | 10 ⁻¹ * | 10 ⁻² * | 10 ⁻³ | 10 ⁻⁴ | 10 ⁻⁵ | 10 ⁻¹ * | 10 ⁻² * | 10 ⁻³ | 10 ⁻⁴ | 10 ⁻⁵ |
| Repeat | | | | | | | | | | |
| B | ∞ | ∞ | 3916,00 | 428,00 | 56,00 | - | - | 2,00 | 5,00 | 7,00 |
| C | ∞ | ∞ | 4536,00 | 384,00 | 38,00 | - | - | 4,00 | 4,00 | 3,00 |
| D | ∞ | ∞ | 2540,00 | 348,00 | 42,00 | - | - | 5,00 | 7,00 | 8,00 |
| E | ∞ | ∞ | 2176,00 | 376,00 | 52,00 | - | - | 3,00 | 3,00 | 2,00 |
| Average | - | - | 3292,00 | 384,00 | 47,00 | - | - | 3,50 | 4,75 | 5,00 |
| SD | - | - | 1117,72 | 33,15 | 8,41 | - | - | 1,29 | 1,71 | 2,94 |

Description : * = Data not used for analysis.

Cefoxitin Test Results

The results of the cefoxitin test using the antibiotic cefoxitin disk (disk diameter = 0,6 cm and inhibition zone = 1,13 cm) resulted in the test medium having an inhibition zone = 0,533 cm or 5,33 mm. From these results, it was concluded that the test medium was resistant to antibiotics (inhibition zone 14 mm). The interpretation of the cefoxitin test results can be seen in figure 3.

Colony Test Results

The results of calculating the number of MRSA bacterial colonies before and after treatment with the addition extract of bawang suna (*Allium Schoenoprasum* L.) using the total plate count (TPC) test can be seen in table II.

The table II, it can be seen that the number of MRSA bacterial colonies before treatment had unlimited colony growth (infinity) at dilution levels of 10⁻¹ and 10⁻², at a dilution level of 10⁻³ had colony growth of 3292,00 ± 1117,72, at the dilution

level of 10⁻⁴ had colony growth of 384,00 + 33,15, and at the dilution level of 10⁻⁵ had colony growth of 47,00 + 8,41. Interpretation of colony growth before treatment can be seen in figure 4.

From table II, it can also be seen that the number of MRSA bacterial colonies after treatment (addition of 1 ml an extract of bawang suna (*Allium schoenoprasum* L.) into the control group MRSA bacterial suspension test media) showed a significant decrease in the number of MRSA bacterial colonies, namely at a dilution level of 10⁻³ had colony growth of 3,50 ± 1,29, at the dilution level 10⁻⁴ had colony growth of 4,75 ± 1,71, and at the dilution level 10⁻⁵ had colony growth of 5,00 ± 2,94. Interpretation of colony growth after treatment can be seen in figure 5.

Based on the data above, the comparison of the average number of MRSA bacterial colonies before and after treatment (addition of 1 ml an extract of bawang suna (*Allium schoenoprasum* L.) into the control group MRSA bacterial suspension test media) can be seen in table III.

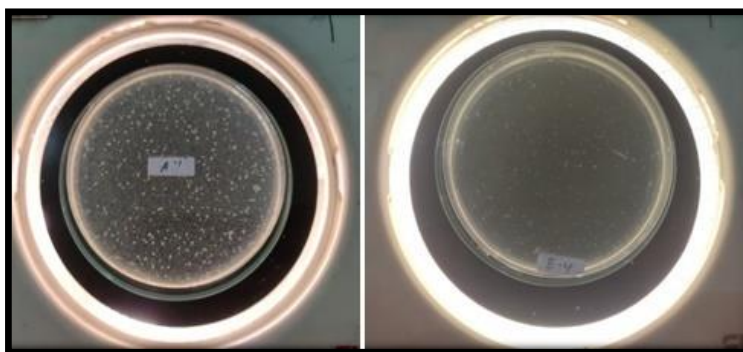


Figure 4. Overview of *Methicillin-Resistant Staphylococcus aureus* (MRSA) Colony Growth Before Treatment Has Infinity and Countable Colony Numbers



Figure 5. Overview of *Methicillin-Resistant Staphylococcus aureus* (MRSA) Bacterial Colony Growth After Treatment Has Count The Number Of Colonies Can Be Count

Table III. Comparison of *Methicillin-Resistant Staphylococcus aureus* (MRSA) Colony Numbers Before and After Treatment

| Dilution | Number of Samples | Colony Average \pm SD | | |
|-----------|-------------------|-------------------------|------------------|---|
| | | Control Group | Experiment Group | Comparison of Bacterial Colony Numbers Experiment Group and Control Group |
| 10^{-3} | 4 | 3292,00 \pm 1117,72 | 3,50 \pm 1,29 | Lower |
| 10^{-4} | 4 | 384,00 \pm 33,15 | 4,75 \pm 1,71 | Lower |
| 10^{-5} | 4 | 47,00 \pm 8,41 | 5,00 \pm 2,94 | Lower |

Activity Extractofbawang Suna (*Allium Schoenoprasum L.*) Against *Methicillin-Resistant Staphylococcus aureus* (MRSA)

The results of the analysis of the paired sample t-test with a 95% confidence level, there is a close correlation between the results of calculating the number of MRSA bacterial colonies before and after treatment (addition extract of bawang suna (*Allium schoenoprasum L.*) as much as 1 ml into the *Methicillin-Resistant Staphylococcus aureus* (MRSA) bacterial suspension test medium (control group) which shows the results of the correlation value for the 10^{-3} dilution level of -0,204 (sig. correlation = 0,796), the 10^{-4} dilution level of -0,330 (sig. correlation = 0,670), and the

dilution level of 10^{-5} is +0,081 (sig. correlation = 0,919). The correlation value marked minus (-) indicates the direction is inversely proportional and the correlation value is marked positive (+) indicates the direction is proportionally unidirectional.

The administration extract of bawang suna (*Allium schoenoprasum L.*) as much as 1 ml using a technique the serial dilution of method (graded/serial dilution) can have a significant and closely related effect on inhibiting the growth of MRSA bacteria as indicated by the results of the Total Plate Count (TPC) test of the occurrence of the decrease in the number of MRSA bacterial colonies at the 10^{-3} dilution level was 3288,50 \pm

Table IV. The Effect of Giving Extract Bawang Suna (*Allium schoenoprasum* L.) to prevent the Growth of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Bacteria Growing Based on the Paired Sample T-Test

| | Dilution | Experimental Group (Treatment) | | |
|---------------|------------------|--------------------------------|--------------|---------------------------------------|
| | | Sig.t-test | Relationship | Effect |
| Control Group | 10 ⁻³ | 0,010 | Significant | Closely- Oppositely |
| | 10 ⁻⁴ | 0,000 | Significant | Closely- Oppositely |
| | 10 ⁻⁵ | 0,002 | Significant | Closely- Comparable Unidirectional |

Number of Samples = 4

1117,98, the 10⁻⁴ dilution level was 379,25 ± 33,75, and the 10⁻⁵ dilution level was 42,00 ± 8,68. The effect of decreasing the number of MRSA bacterial colonies after administration extract of bawang suna (*Allium schoenoprasum* L.) was also shown by the results of the paired sample t-test, namely the sig value. t-test < 0,05 t_{table} for dilution levels 10⁻³, 10⁻⁴, and 10⁻⁵. The interpretation of the paired sample t-test results can be seen in table IV.

The decrease in the number of MRSA bacterial colonies before and after treatment (addition of 1 ml an extract of bawang suna (*Allium schoenoprasum* L.)) was due to the extract of bawang suna (*Allium schoenoprasum* L.) containing chemical compounds, namely 46,787% of saponins, 53,820% of alkaloids, 17,417 mg/ml QE of flavonoids, 0,322 mg/ml GAE of tannins, 40,781 mg/ml of steroids, and 54.467 mg/ml of triterpenoids. According to Amalia's research (2017) the content of chemical compounds in the ethyl acetate extract of daun sembung (*Blumea Balsamifera* (L.) DC.) is the content of steroid compounds with a working mechanism that can damage the cell membrane of MRSA bacteria; the content of alkaloid compounds can interfere with the peptidoglycan constituent components in MRSA bacterial cells so that the cell wall layer is not fully formed and causes cell death; the content of terpenoid compounds is suspected to be able to damage the outer membrane of the bacterial cell wall of MRSA because terpenoid compounds can react with porin (transmembrane protein) to form strong polymer bonds and damage the porin and reduce the permeability of the bacterial cell wall, causing bacterial cells to lack nutrients and their growth will be stunted or die; and the content of flavonoid compounds can form complex compounds with extracellular and dissolved proteins so that they can break the cell membrane of MRSA bacteria followed by the release of intracellular compounds. According to research by Meitasari (2017), the content of chemical compounds in guava leaf extract (*Psidium guajava*), namely the content of saponin

compounds with a working mechanism can reduce surface tension, resulting in increased permeability or leakage of MRSA bacterial cells and resulted in the release of intracellular compounds; and tannin compounds with the mechanism of action can inhibit the reverse transcriptase and DNA topoisomerase enzymes so that MRSA bacteria cannot be formed.

CONCLUSION

Based on the research results, administration of 1 ml an extract of bawang suna (*Allium schoenoprasum* L.) using TPC test with the serial dilution testing technique with a ratio of 1:5 has a significant effect on dilution of 10⁻³, 10⁻⁴ and 10⁻⁵ as an antibacterial for inhibiting the growth of *Methicillin-Resistant Staphylococcus aureus* (MRSA) bacteria.

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