

Antibacterial Effectiveness Test of Shallot Extract (*Allium Cepa*) Against *Staphylococcus Aureus* Growth

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ABSTRACT

Background: Infectious diseases are a difficult health problem in many countries especially infectious diseases with ever-increasing antibiotic resistance such as *Staphylococcus aureus* infection. Treatment using antibiotics gives rise to various side effects such as hypersensitivity reactions. Based on this, it is necessary to replace antibiotic therapy with alternative therapies that have minimal side effects and are easy to find, such as the use of shallots as an alternative therapy.

Objectives: The purpose of this study was to test the antibacterial effect of shallots ethanol extract against the growth of *Staphylococcus aureus*.

Methods: The type of research used is true experimental design with a posttest only control group test research design. Testing of antibacterial activity of shallots extract using the liquid dilution method. The study sample consisted of a positive control of Clindamycin, a negative control of aquades, and a concentration group of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56% with three repetitions for each group.

Results: Based on the results of bacterial testing with the shallots extract dilution method, the results of the test were obtained for minimum inhibitory concentration(MIC) at a concentration of 25% and a minimum bakterisid concentration(MBC) at a concentration of 50%.

Conclusion: Shallots extract 95% alcohol has an antibacterial effect against the growth of *Staphylococcus aureus*.

KEYWORDS: Shallot extract, antibacterial, *Staphylococcus aureus*

INTRODUCTION

Infectious diseases are a serious health problem in Indonesia. According to the 2019 Indonesian Health Profile Data, infectious diseases are the leading cause of death in children aged 29 days to 11 months. A total of 1,725 deaths were reported, including respiratory infections, gastrointestinal infections, nervous system infections, malaria, tetanus, and other causes.⁽¹⁾ One of the causes of this infection is the bacteria *Staphylococcus aureus*.

One of the dangerous *Staphylococcus aureus* infections comes from the MRSA strain. (*Methicillin Resistant Staphylococcus aureus*) has high morbidity and mortality rates. This is due to its ability to hydrolyze beta-lactam antibiotics such as penicillin.⁽²⁾ According to data from the United States Centers for Disease Control and Prevention, more than 98 thousand people out of 1.7 million patients died due to *Staphylococcus aureus* infections.⁽³⁾

According to Paterson K. Iona et al., (2016), the treatment of *Staphylococcus aureus* bacterial infection cases by administering antibiotics must be replaced or adapted with a combination of other treatment regimens and reduce the use of antibiotics as well as eradicate antibiotics in the body.⁽⁴⁾ Long-term use of antibiotics can cause side effects such as disruption of the digestive system microbiota.⁽⁵⁾

The East Nusa Tenggara (NTT) region has reported a significant increase in shallot production. Shallots, a seasonal crop, are growing, from 1.5 million tons in 2018 to 27,753 tons in 2020. 2.7 million tons of shallots were harvested.⁽⁶⁾⁽⁷⁾ One of the areas in NTT where onions are a commodity The red area is very big, namely Rote Ndao, where the distribution of shallots covers Kupang Regency, South Central Timor (TTS), North Central Timor (TTU) and Belu.⁽⁸⁾

This antibacterial effect is due to the various active compounds contained in shallots, such as saponins and flavonoids. These compounds can aid in the circulatory system, digestion, and antioxidant activity. As a natural antioxidant, shallots can suppress carcinogenic effects and free radicals.^(9,10,11) Switching from antibiotic-resistant agents to plant extracts with antibacterial effects is considered to be a better substitute for conventional therapy.⁽¹²⁾ Based on the things that have been described previously, researchers are interested in conducting research on the antibacterial effectiveness of shallots on the growth of *Staphylococcus aureus* bacteria.

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RESEARCH METHODS

The type of research used is descriptive research. *true experimental design* with research design posttest only control group design. Antibacterial testing is carried out by looking at the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration using the dilution method, then a post hoc test is carried out using the test *Mann-Whitney*. This research was conducted at three different locations: the Laboratory of the Faculty of Medicine and Veterinary Medicine, Nusa Cendana University, the Integrated Laboratory Unit (UPT) of Nusa Cendana University, and the Laboratory of Widya Mandira Catholic University, Kupang. The research period was March–June 2022.

The shallots used in the research came from Rote Ndao village Oelaba. Bacterial sample *Staphylococcus aureus* obtained from the Food and Drug Monitoring Agency (BPOM) of Kupang City. The sample size used in this study consisted of 9 different groups, namely: 7 types of shallot concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, a positive control of clindamycin, and a negative control of distilled water with a total of 3 repetitions.

Shallot furthermore cleaned, aired Then The maceration results were then evaporated and then subjected to ethanol- free and phytochemical tests.

The instruments used in this antibacterial activity study were first sterilized. Glass instruments and MSA media were wrapped in aluminum foil and then autoclaved at 121°C for 15-20 minutes. Loop needles were sterilized by burning them over a flame using rubbing alcohol. Plastic instruments were sterilized with 70% alcohol.

Bacteria *Staphylococcus aureus* done test confirmation bacteria using gram staining. Next, prepare the media *Nutrient broth* in a test tube after 24 hours followed by a confirmation test with MSA media.

RESULTS AND DISCUSSION

Shallot Extraction

Shallot (*Allium fast*) as much as 2 kg of red onion extract was obtained (*Allium cepa*) as much as 175.87 gr.

Etanol Free Test

Orange reaction or color of the extract mixture, K₂Cr₂O₇, and H₂SO₄ so that the extract does not contain ethanol.

Phytochemical Test

The results of phytochemical tests found that the extract contained active compounds such as: Alkaloids, Flavonoids, Saponins, Tannins, and Terpenoids.

Bacterial Confirmation Test

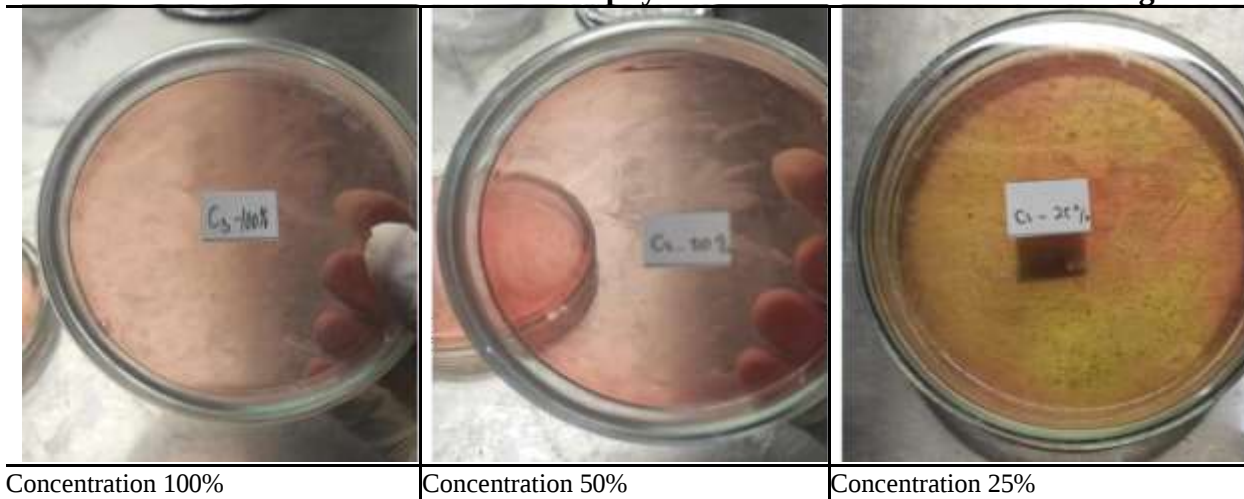
The results of gram staining show that the morphology of the bacteria is in the form of *coccus* purplish in color which is the morphology of gram-positive bacteria *Staphylococcus aureus*.

Antibacterial Test

Minimum Growth Inhibitory Concentration Test *Staphylococcus aureus* on the media Nutrient Bro



Growth Confirmation Test Results *Staphylococcus aureus* on Mannitol Salt Agar



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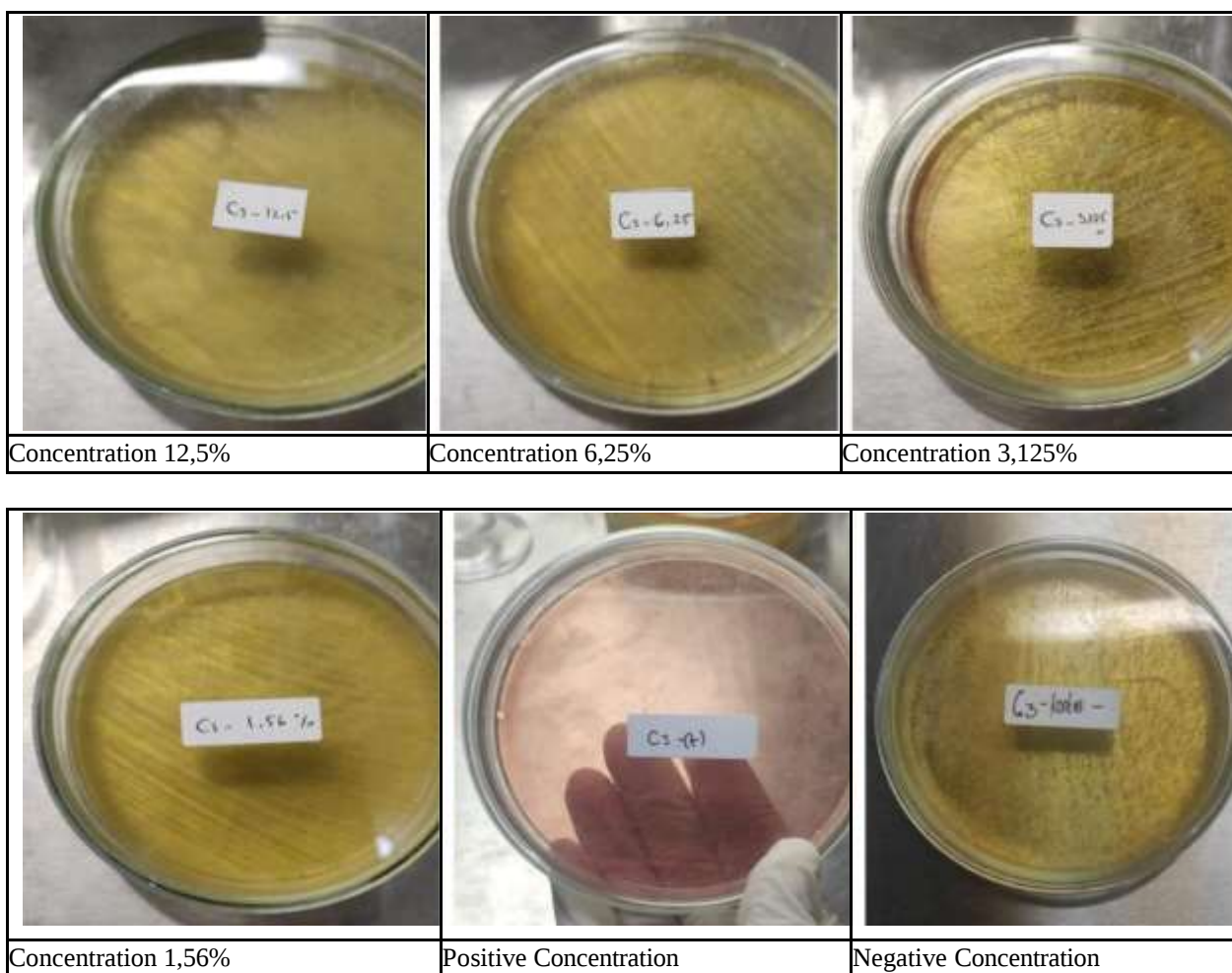


Table 1 Results of the confirmation test of red onion extract on growth *Staphylococcus aureus*

Concentration	Repetition		
	1	2	3
100%	-	-	-
50%	-	-	-
25%	+	+	+
12,5%	+	+	+
6,25%	+	+	+
3,125%	+	+	+
1,56%	+	+	+
Kontrol positif (Klindamisin)	-	-	-
Kontrol negatif (aquadest)	+	+	+

Keterangan : + : There is growth *Staphylococcus aureus*
 - : There is no bacterial growth

Data analysis

Table 2 Test results *Mann Whitney* between treatment groups based on the results of the confirmation test of red onion extract on growth *Staphylococcus aureus*.

Concentration	Concentration								
	100%	50%	25%	12,5%	6,25%	3,12%	1,56%	K+	K-
100%		1,000	0,025*	0,025*	0,025*	0,025*	0,025*	1,000	0,025*
50%		1,000	0,025*	0,025*	0,025*	0,025*	0,025*	1,000	0,025*

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25%	0,025*	0,025*	1,000	1,000	1,000	1,000	0,025*	1,000
12,5%	0,025*	0,025*	1,000	1,000	1,000	1,000	0,025*	1,000
6,25%	0,025*	0,025*	1,000	1,000	1,000	1,000	0,025*	1,000
3,12%	0,025*	0,025*	1,000	1,000	1,000	1,000	0,025*	1,000
1,56%	0,025*	0,025*	1,000	1,000	1,000	1,000	0,025*	1,000
K+	1,000	1,000	0,025*	0,025*	0,025*	0,025*	0,025*	0,025*
K-	0,025*	0,025*	1,000	1,000	1,000	1,000	1,000	0,025*

Keterangan:

* : There is a significant difference between groups

DISCUSSION

The shallots used in this study were collected from Oelaba, Rote, East Nusa Tenggara. They were sorted to separate damaged and intact shallots, then cleaned. The cleaned shallots were thinly sliced and air-dried for 10 minutes without exposure to direct sunlight. Next, the sliced shallots were placed in a glass container and 95% ethanol was added. 95% ethanol was used because this solvent is more selective in dissolving the desired substances or compounds, has good absorption by the herbal medicine, prevents the growth of mold or yeast in the extract, and can produce a thicker extract more quickly compared to 70% ethanol.⁽¹³⁾ After 3 days soaking, the ethanol extract of shallots will be extracted using the evaporation method using *Rotary evaporator* for 24 hours and the result is a thick red extract.

The extract was then subjected to ethanol-free and phytochemical tests to determine the content of secondary metabolites and to ensure the absence of ethanol in the extract, which could cause bias in the study. The ethanol-free test revealed no ethanol content in the extract, while the phytochemical test revealed secondary metabolites such as saponins, tannins, alkaloids, flavonoids, and terpenoids in the ethanol extract of shallots.

These test results are consistent with research by Hasibuan et al. (2020) tested the content of secondary metabolite compounds contained in ethanolic red onion extract.⁽¹⁴⁾ The results of this test are also in accordance with the research of Setiani et al. (2017) who tested the Flavonoid test using the maceration and MAE methods (*Microwave Assisted Extraction*).⁽¹⁵⁾ However, this test differs from the results of the phytochemical test conducted by Misna (2016) which did not find any saponin content in shallot skin extract.⁽¹³⁾

These active compounds function to inhibit and even kill bacteria that damage body cells through the infection process.⁽⁹⁾ Simaremare(2017),in His research stated that the existence organosulfur components that play an important role in the antibacterial activity of shallots are: *Dipropyl disulfide* *Anddipropyl trisulfide*.⁽¹⁶⁾ Organosulfur compounds together with alkaloids will penetrate the bacterial cell membrane and then damage the bacterial DNA, then other secondary metabolic compounds will damage the cell surface and protein synthesis by the bacterial cells which will ultimately affect the death of the bacterial cells.⁽¹⁶⁾

Bacteria *Staphylococcus aureus* is a gram positive bacteria which in gram staining will show results in the form of a coccus (round) with a purple color. This is because the bacterial cell wall is composed mostly of peptidoglycan, unlike gram-positive bacteria whose cell wall is composed of a lipid layer which causes the gentian violet staining to not be maintained, especially when alcohol is added. The lipid layer will fade so that when stained it will show a red color.⁽¹⁷⁾

On the wall cell bacteria *Staphylococcus aureus* consists of a peptidoglycan layer which, due to the alkaloid and tannin compounds contained in the extract, will cause the components of the peptidoglycan layer to not form completely, resulting in bacterial cell death due to damage to the barrier, The saponin, flavonoid, and tannin compounds found in shallot extract cause bacterial cell lysis and inactivate aureus bacterial enzymes like catalase, killing the bacteria.⁽¹⁸⁾

In this study, antibacterial testing used 7 treatment groups consisting of concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.56%; positive control with clindamycin and negative control using sterile distilled water with all groups repeated three times. clindamycin as a positive control based on IDSA guidelines (Infectious Diseases Society of America)⁽¹⁹⁾ and the antibacterial effect of clindamycin has similarities with secondary metabolite compounds in extract.⁽²⁰⁾ Based on the results of research that has been carried out, 95% ethanol shallot extract has an antibacterial effect against bacteria. *Staphylococcus aureus* This can be seen from the presence of turbidity in the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests at several treatment concentrations. In the minimum inhibitory concentration (MIC) test, the color of the extract causes turbidity. treatment group with high extract concentration, therefore a minimum bactericidal concentration (MBC) test is required to confirm the presence of bacterial growth in nutrient broth.

In the research data related to antibacterial tests, it can be seen that the ethanol extract of red onions has antibacterial activity against the growth of *Staphylococcus aureus* where in the minimum inhibitory concentration test it was found at a concentration of 25% based on the turbidity test while for the minimum killing concentration test it was found at a concentration of 50% based on the number of colonies growing on the petri dish which can be seen by the absence of a change in color from red to yellow on the MSA medium as well as in the microscopic test which did not show any growth of bacterial colonies. The presence of organosulfur content and other secondary metabolite compounds causes the ethanol extract of shallots to have the ability to inhibit and kill *Staphylococcus aureus*. This is in accordance with research Previously, Wijaya (2019) and Simaremare (2017) conducted a test on the antibacterial ability of shallots against the growth of *Staphylococcus aureus*.

Based on Table 1, it can be seen that there was an increase in antibacterial effectiveness in the treatment group with high concentrations, where the higher the concentration, the greater the antibacterial activity. Using the liquid dilution method,

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turbidity was observed at 100% concentration due to the absence of a solvent. reduce the viscosity level of the extract as well as at positive concentrations where the antibiotic Clindamycin in soluble in Nutrient broth different from the concentration of the solvent Aquades High turbidity levels are lower and clearer, therefore, a confirmatory test using the plate diffusion method is required to detect bacterial colony growth. This is consistent with research conducted by Yolla et al. (2019) and Lu'lu et al. (2020) who used the dilution method to test the MIC of the extracts studied.^(20,21)

Table 2 shows a significant difference between the treatment groups with 100% and 50% concentrations and the other treatment groups. Based on this, H0 rejected and H1 accepted means it can be concluded that there is a significant difference in the administration of red onion extract on growth *Staphylococcus aureus*.

CONCLUSION

- 1) The ethanol extract of shallots obtained was 175.87 grams.
- 2) Phytochemical test results of red onion ethanol extract were found in the extract compound saponins, alkaloids, terpenoids and flavonoids.
- 3) Based on the test concentration minimum resistance(KHM) with the referenced liquid dilution method on turbidity, concentration 25% set as concentration resistor minimum in research.
- 4) Based on the Minimum Killing Percentage (MBC) test which refers to the growth of bacterial colonies on Mannitol Salt So that, concentration 50% set kill as concentration minimum in research.

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