THE COMPARISON OF ANTIBACTERIAL EFFECTS ON *CINNAMOMUM BURMANNII* WATER EXTRACT WITH PENICILLIN AGAINST *STAPHYLOCOCCUS AUREUS* IN VITRO

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ABSTRACT

Introduction: Along with the development of science, many varieties of microorganisms are resistant to antibiotics. To avoid cases of resistance grew severe, researchers developed alternative medicines as a substitute for antibiotics. One of the ingredients of herbs researched that has antibacterial activity is Cinnamon (*Cinnamomum burmannii*).
Aim: This study aimed to compare antibacterial effects water extract of *Cinnamomum burmannii* with *penicillin* against *Staphylococcus aureus*
Methods: This an experimental studies witd non-equivalent control group design. Research using the bacteria *S. aureus* are divided into two groups, the control group and experimental group. The concentration of *Cinnamomum burmannii* and *penicillin* are used that is 80- 150 mg/L. Antibacterial test used dilution method and a microplate reader for reading absorbance. This research conducted in the laboratory of Microbiology, Faculty of Pharmacy Widya Mandala Catholic University Surabaya for 2 weeks.
Result: Based on visual observations, *Cinnamomum burmannii*'s MIC is located at concentration 320- 600 mg/L while *penicillin*'s MIC is located at concentration 160- 300 mg/L. Based on the results of microdilution test, *Cinnamomum burmannii*'s MIC is located at concentration 160- 300 mg/L while *penicillin*'s MIC is located at concentration 640- 1200 mg/L.
Conclusion: The conclusion of this research is giving *Cinnamomum burmannii* water extract in *Staphylococcus aureus* has efficacy or inhibitory that is not significantly different with *penicillin*.

Keywords: Antibacterial, *Cinnamomum burmannii,* Penicillin, *Staphylococcus aureus*

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INTRODUCTION

Staphylococcus are Gram-positive spherical cells which are usually arranged in irregular groups shaped like grapes. The Staphylococcus genus has at least 40 species. The three species most frequently encountered and of clinical importance are Staphylococcus aureus, Staphylococcus epidermidis and Staphylococcus saprophyticus. [1] Some Staphylococcus bacteria are normal flora in places exposed to the outside world, namely the skin, respiratory tract, and human digestive tract. Normal flora are microorganisms that occupy an area without causing disease in the host that is occupied. This bacterium is also found in the air and the environment.[2,3] *Staphylococcus aureus* (*S. aureus*) is one of the main pathogens for humans. Almost everyone experiences some type of *S. aureus* infection. Throughout life ranging from food poisoning or minor skin infections to severe life-threatening infections.[1] Pathogenic *S. aureus* is invasive, causes hemolysis, forms positive coagulase, and is able to disperse mannitol.[2,3]

Microorganisms can have both good and bad effects on humans. The substance that can destroy pathogenic microorganisms is antibiotics. Along with the development of science, it was found that many varieties of microorganisms that are resistant to antibiotics.[4,5] The main cause of antibiotic resistance is its widespread and irrational use.[6,7]

In 1944, most *S. aureus* was sensitive to penicillin, although several resistant strains were found. After the widespread use of penicillin, in 1948, it was found that *Staphylococcus* was isolated in the hospital, apparently 65-85% produced β-lactamase, so it was resistant to *penicillin G*. In 1986, penicillin-resistant *Staphylococcus* was found not only in hospitals, not only 80% of it produced β-lactamase, so that it was resistant to *penicillin*, but also 90% are isolated from the community.[7,8]

Methicillin-resistant Staphylococcus aureus (MRSA) is a type of multidrug resistant organism (MDRO) which has a high number and has become a problem in the medical world. MRSA progressivity in Indonesia shows a significant number from year to year. In 1986, the incidence of MRSA in Indonesia was 2.5%. In 1993, it was 9.4% and in 2006 it was 23.5%.[9,10]

As resistance cases develop, researchers find that if bacteria don't consistently cooperate with antibiotics, they will start to forget how to become resistant to antibiotics. Bacteria that are naturally resistant and mutated, not only can survive antibiotics, but also become stronger so that the diseases caused are

more serious and result in a higher death rate than previously produced diseases. To avoid the case of resistance getting worse, the use of alternative medicines was developed as a substitute for antibiotics to treat most diseases to ensure that antibiotics are needed in serious conditions can still be used effectively.[5,11]

One of the herbal ingredients that have been investigated to have antibacterial activity is cinnamon. Based on the results of research conducted by researchers, it is mentioned that herbal cinnamon oil and ethanol extract (50%) *Cinnamomum zeylanicum* have antibacterial activity against 10 types of bacteria. [5,12] Other studies state that *(E)-cinnamaldehyde* (volatile oil) and proanthocyanidins (polyphenols), which contain cinnamaldehyde cinnamon bark oil (antibodies) have an antibacterial effect.[5,13] *Cinnamomum osmophloeum* also contains cinnamaldehyde which has antibacterial activity.[5,14] Many researches have found that *Cinnamomum burmannii* has an antibacterial effect, it makes researchers interested in conducting further research to determine the effectiveness and optimal levels of *Cinnamomum burmannii* water extract as a potential bactericidal against *S. aureus* in vitro.

METHOD

This study uses an experimental study with non equivalent control group design. The study used S. aureus bacteria which were divided into two groups: the control group and the treatment group. The control group was divided into 4 groups namely K1 = Mueller Hinton Broth, K2 = Mueller Hinton Broth + *S. aureus*, K3 = Mueller Hinton Broth + *penicillin*, and K4 = Mueller Hinton Broth + *Cinnamomum burmannii* extract. The treatment group was divided into 4 groups namely P1 = Mueller Hinton Broth + *S. aureus* + *Cinnamomum burmannii* extract, P2 = Mueller Hinton Broth + *S. aureus* + *penicillin*, P3 = *S. aureus* + *Cinnamomum burmannii* extract, and P4 = *S. aureus* + *penicillin*. In all control groups and treatment groups, the Minimum Inhibitory Level (MIC) and the Minimum Kill Rate (MBC) were calculated.

The making of *Cinnamomum burmannii* extract is by adding 10 g dried *cinnamomum burmannii* bark into boiling water 100 ml (94°-96° C) for 15-20 minutes then filtering using filter paper. After filtering, the mixture is dehydrated to form hygroscopic powder.

Then, treparation of bacteria test is carried out by regenerating bacteria, identifying bacteria macroscopically, identifying bacteria microscopically and identifying bacteria biochemically.

The test of antibacterial activity of *Cinnamomum burmannii*

1. Microdilution method

Antibacterial activity testing was carried out by the microdilution method. The MHB media, *Cinnamomum burmannii* extract test solution, *penicillin* solution, and *S. aureus* bacterial suspension were inserted into the microplate hole and a series of dilutions were carried out.

2. Deteminantion of MIC and MBC

After settling for 24 hours, the microplate was observed with turbidity using a microplate reader. Then, the TTC concentration of 0.1% was added as much as 30 µl into each well in the microplate and put in an incubator and then observed after 30 minutes. The red-well is a positive area covered with bacteria. The clear colorless area at the smallest extract concentration is the MIC of the extract.

The determination of MIC is quantitatively determined based on the smallest concentration of the drug or test solution of plant aquades extract which does not show macroscopic growth in a minimum amount of ≤10% or a minimum percentage inhibition of ≥90%. MBC determination is determined on the solid media with the smallest concentration that is not overgrown with bacterial colonies or there is a minimal amount of bacterial colonies that is ≤0.1% or a minimum percentage of growth inhibition of ≥99.9% the number of initial inoculum. MIC and MBC were measured by calculating the percentage of resistance from the absorbance or optical density (OD) value obtained from the microplate reader or microplate spectrophotometer

RESULTS

Table 1 The Results of *S. aureus* bacteria identification

No	Treatment	Literature Review	Observation Result	Conclusion
1.	Bacterial regeneration on MSA media	The solution fermentation test on *S. aureus* is a change in the color of the medium from red to yellow. It shows that *S. aureus* changes mannitol which produces lactic acid so that it can change the pH of the medium to acidic. [15,16,17] positive mannitol	MSA media change its color from red to yellowish orange which indicates the growth of *S. aureus*	+
2.	Gram staining	*S. aureus is a* Gram-positive bacterium that will turn purple with Gram staining with rounded cells, usually arranged in groups like grapes	Bacteria change color to purple, round, and clustered cells	+

		that are irregular. [1]		
3.	Catalase test	The catalase test is important to distinguish between *Streptococcus* and *Staphylococcus*, where the *Staphylococcus* group is a positive catalase that produces gas bubbles. [15,18,19]	Gas bubbles are formed	+

Note: Positive results (+) indicate compliance with the literature review

Table 2 The pattern of filling the solution on the microplate

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Cinnamomum burmannii extract + *S. aureus* bacteria

Cinnamomum burmannii extract + *S. aureus* bacteria + MHB

Cinnamomum burmannii extract + MHB (sample control C. burmannii)

Penicillin + *S. aureus* bacteria

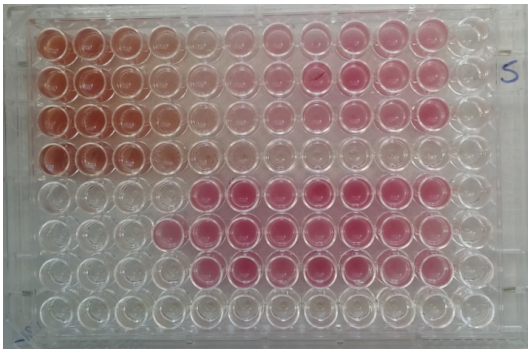
Penicillin + *S. aureus* bacteria + MHB

Penicillin + MHB (sample control *penicillin)*

S. aureus bacteria + MHB (control positive)

MHB (control negative)

Figure 1 The observation of microplate holes after the addition of 0.1% TTC and after incubation of 37° C for 30 minutes



Note : The pink microplate holes indicate growth of bacteria

Table 3 The observation of microplate holes after the addition of 0.1% TTC and after incubation of 37° C for 30 minutes

	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	-	-	-	+	+	+	+	+	+	-
B	-	-	-	-	-	+	+	+	+	+	+	-
C	-	-	-	-	-	+	+	+	+	+	+	-
D	-	-	-	-	-	-	-	-	-	-	-	-
E	-	-	-	-	+	+	+	+	+	+	+	-
F	-	-	-	+	+	+	+	+	+	+	+	-
G	-	-	-	-	+	+	+	+	+	+	+	-
H	-	-	-	-	-	-	-	-	-	-	-	-

Note: (+) means it turns into pink (there is bacterial growth); (-) means it does not turn into pink (no bacterial growth).

The pattern of filling the solution on the microplate is as same as Table 2.

Determination MIC dan MBC

From the absorbance results, it will be obtained a percent resistance as in the formula:

$$\%Resistance = \frac{(control\ (+) - control\ (-)) - (sample - control\ sample)}{(control\ (+) - control\ (-))} \times 100\%$$

DISCUSSION

As the case for resistance develops, the researchers are developing the use of alternative medicines instead of antibiotics. One of the herbal ingredients that can be used as alternative medicine and has been investigated to have antibacterial activity is in cinnamon.

Cinnamomum burmannii bark is extracted using water solvents. They are chosen because they are easily available, inexpensive, stable, non-volatile, non-toxic, natural and non-flammable. 10g of *Cinnamomum burmannii* bark that has been dried is put into 100 ml boiling water (94°-96° C) for 15-20 minutes then filtered using filter paper. Once filtered, the mixture will undergo a dehydration process so that it is in the form of hygroscopic powder. To make the *C.burmanii* extract solution, the hygroscopic powder of 0.05 g was added to a 5 ml sterile distilled water and homogenized (with vortex if necessary) into a 10,000 ppm solution.

S. aureus bacteria need to be identified to ensure that the bacteria used are actually *S. aureus* bacteria. The regeneration of *S. aureus* in MSA for three consecutive days resulted in the color of MSA changing from red to yellow-orange indicating the growth of *S. aureus*. The results of identification of *S. aureus* which are gram-positive bacteria in gram staining give a purple color with cells that are round, usually arranged to form a group like irregular grapes.[1] The catalase test results give positive results with arising of gas bubbles. The coagulase test was not carried out because the regeneration results on MSA and gram staining along with the catalase test have proven the identity of the bacteria used.

The bacteria used must be made equivalent to 0.5 McFarland first by mixing *S. aureus* from the stock of bacteria regenerated on day 3 into 10 ml of sterile distilled water, if necessary, they need to homogeneous with vortex.

Penicillin as a comparison was weighed as much as 0.05 g and added to 5 ml sterile distilled water and homogenized (with vortex if necessary) to 10,000 ppm. In this study, the *penicillin* solution used was 2,000 ppm, to convert 10,000 ppm to 2,000 ppm, then 200 µl of penicillin solution was added to 800 µl sterile distilled water and homogenized to 2,000 ppm.

Researchers worked on aseptic filling of microplates in LAF that had previously been cleaned with alcohol, then sterilized with UV light for about 1 hour before use. Microplate filling is started in column 2 up to column 12 filled with MHB as much as 100 µl. *Cinnamomum burmannii* extract was filled in rows A to D column 1 totaling

10,000 ppm 100 µl and continued with dilution as much as half times in rows A to D column 2 and so on up to rows A to D column 10. *Penicillin* was filled in rows E to H column 1 as much as 2,000 ppm 100 µl and followed by dilution as much as half times in rows E to H column 2 and so on up to rows E to H column 10. 80 a. *S. aureus* bacteria were filled in row rows A to C columns 1 to column 11 and filled in rows E to G column 1 to column 11. In row D, it is used as a sample control from *Cinnamomum burmannii* and row H is used as a sample control from p*enicillin*. At line D and line H, another 80 µl MHB is added to replace the bacterial suspension so that it can be used as a control sample of *Cinnamomum burmannii* and *penicillin*. Control samples are used to ensure the color produced by the treatment group is the result of bacterial growth and distinguishes between the color of the *Cinnamomum burmannii* solution and the penicillin without bacteria. In column 11, which contains a mixture of MHB and *S. aureus*, except row D and row H are used as positive controls. In column 12, the containing MHB is used as a negative control. From the results of dilution in a row then a concentration range is taken, hereinafter referred to as concentration x to facilitate the comparison of *Cinnamomum burmannii* and *penicillin* solutions.

After all the microplate holes have been filled, the microplate is inserted into the microplate reader with a wavelength of 595 nm to do a turbidity reading (absorbance) that begins with shaking for 15 seconds and then the results of turbidity will appear on a computer monitor and print the results. The disadvantage of using a microplate reader is that the sample used must be really clear because it affects the microplate reader that absorbs turbidity.

From the visual observation of the microplate before being incubated for 24 hours, the results showed that all the microplate holes were clear, indicating there was no bacterial growth. The microplate visually observed after being incubated for 24 hours obtained the result that column 12 which is a negative control remains clear which indicates no bacterial growth. Column 11 except row D and H which are positive controls turn into turbid which indicates the growth of bacteria. Rows A to C column 7 through column 11 turn into turbid indicating there is bacterial growth. Row F column 4 and row E to G column 5 until column 10 turns into turbid which indicates that there is a bacterial growth.

In visual observations after the addition of 0.1% TTC (as an indicator of

living microorganisms) and after incubation of 37° C for 30 minutes, the results obtained are column 12 which is a non-pink negative control indicating no bacterial growth. Column 11 except row D and H which are positive controls turn pink which indicates bacterial growth. Row B column 5 and row A to C column 6 to column 10 turn pink which indicates bacterial growth. Row F column 4 and row E to G column 5 to column 10 turn pink which indicates bacterial growth. The MIC is visually determined by a clear microplate that does not show macroscopic growth. Based on visual results, it can be concluded that the MIC of *Cinnamomum burmannii* is located in a four times concentration while the MIC of *penicillin* is located in a two times concentration.

The results of the microdilution test with optical density reading using a microplate reader with a wavelength of 595 nm obtained the MIC of *Cinnamomum burmannii* was located in a two times concentration while the MIC of *penicillin* was located in a more than eight times concentration. In the percentage of inhibition of *Cinnamomum burmannii* against *S. aureus*, it is found that the trend of inhibition continues to fall which indicates that the smaller the concentration of *Cinnamomum burmannii*, the inhibitory power given to *S. aureus* bacteria is getting smaller. These results are consistent with research conducted by researchers which states that the content of (E)-cinnamaldehyde (essential oils) and proanthocyanidins (polyphenols) which is an ingredient in cinnamon bark. *Cinnamomum burmannii* herbal oil has an antibacterial effect.[5,13] On the percentage of inhibition *penicillin* against *S. aureus* showed that the percentage of obstacles experienced incline and decline at a concentration of 0.5 to 8 times and experienced a trend of the percentage of obstacles that continued to fall starting from a concentration of 0.25 times.

Penicillin was chosen as a comparison because it was based on J.C. UWAEZUOKE and L. E. ARIRIATU's research (2004). From 48 isolates of *Staphylococcus aureus*, were found to be sensitive to gentamicin (91.7%), cloxacillin (85.4%) and most resistant to penicillin (95.8%) and ampicillin (89.6%). In that study, the percentage value of sensitivity was 4.2%, 10.4%, 12.5% and 25% of penicillin, ampicillin, tetracycline and chloramphenicol.[20]

Penicillin has the effect of killing germs in a time dependent manner, which only has a minimal relationship with drug concentrations greater than the minimum inhibitory concentration. These drugs have relatively slow bactericidal action,

and a slight increase in bactericidal activity is seen when the concentration increases to more than one point of maximum killing action, which is about four times the minimum inhibitory concentration.[21] *C.burmanii* kills germs by concentration dependent, the percentage of inhibition of *Cinnamomum burmannii* against *S. aureus* has increased in line with the increase in concentration.

The results of data analysis using the mann whitney on SPSS were not significant, it was found that the administration of *Cinnamomum burmannii* water extract to *Staphylococcus aureus* had efficacy or inhibition which were not significantly different from penicillin. Thus, the research hypothesis was not accepted. Researchers have a variety of research scope namely the wide concentration range of *Cinnamomum burmannii* and *penicillin* so that there is no specific number is obtained in determining the inhibitory concentration, and researchers have not increased the concentration of Cinnamomum burmannii and penicillin yet, so that they do not get enough data of the MBC of *Cinnamomum burmannii* and *penicillin*.

CONCLUSION

Based on the results of research that has been done it can be concluded that:

1. Based on visual observations, the MIC of *Cinnamomum burmannii* is located in a four times concentration while the MIC of *penicillin* is located in a two times concentration.
2. Based on the results of the microdilution test, the MIC of *Cinnamomum burmannii* is located in a two times concentration while the MIC of *penicillin* is located in a more than eight times concentration.
3. The administration of *Cinnamomum burmannii* water extract to *Staphylococcus aureus* has efficacy or inhibition that is not significantly different from *penicillin.*
4. *Cinnamomum burmannii* as a bacteriostatic potential against *Staphylococcus aureus*.

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