ANALYSIS OF ANTIBACTERIAL EFFECT OF *CURCUMINOID* WITH MESOPOROUS SILICA NANOPARTICLES ON *STAPHYLOCOCCUS EPIDERMIDIS*

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ABSTRACT

Introduction: The rate of antibiotic resistance in *Staphylococcus epidermidis* is high. The previous study shows that 79% of *Staphylococcus epidermidis* bacteria isolated were resistant to Methicillin, while 98% were resistant to Penicillin. The active substance *Curcuminoid* in Turmeric is known to have an antibacterial effect. Nowadays, nanoparticle-based medicine has been developed which can increase bioavailability and solubility in the water, as well as the cellular uptake.
Aim: The study aimed to analyze the inhibitory and killing effect of *Curcuminoid* from Turmeric *(Curcuma longa L.)* with mesoporous silica nanoparticles against *Staphylococcus epidermidis*
Methods: This research performs a microdilution test of *Curcuminoid* with mesoporous silica nanoparticles at concentration 2000 – 32000 µg/mL against *Staphylococcus epidermidis* on the microplate. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were obtained from its optical density value after evaluating its turbidity on the spectrophotometer.
Results: In this research found the MIC of *Curcuminoid* with mesoporous silica nanoparticles against *Staphylococcus epidermidis* was at the concentration of 32000 µg/mL.
Conclusion: *Curcuminoid* from turmeric *(Curcuma longa L.)* with mesoporous silica nanoparticles has an inhibitory effect against Staphylococcus epidermidis.

Keywords: Antibacterial effect, *Curcuminoid,* mesoporous silica nanoparticles, *Staphylococcus epidermidis*

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INTRODUCTION

Staphylococcus epidermidis is a gram-positive bacteria from the genus *Staphylococcus*. *Staphylococcus epidermidis* is a coagulase-negative *Staphylococcus,* and it is the most normal flora in the human skin and mucous membrane[1,2]. Infection due to coagulase-negative staphylococci (CoNS) is a nosocomial infection that can cause infection on implanted medical devices, such as prostheses in the knee, cerebrospinal fluid shunts, central venous catheter, intravascular catheter, cardiac pacemaker, and urinary tract catheter especially in patients with very young or old age, or patients with immunocompromised conditions. About 75% of infections caused by coagulase-negative staphylococci and cases of bacteremia in medical device implants are caused by *Staphylococcus epidermidis*[1–6].

Infection caused by *Staphylococcus epidermidis* is difficult to treat because *Staphylococcus epidermidis* can form biofilms on the surface of prosthetic devices that can protect bacteria from antibiotics and the immune system. There is a correlation between biofilms and the resistance of *Staphylococcus epidermidis* bacteria to several types of antibiotics [1,5,7,8]. The research results by Gordon in 2012 showed that out of 100 *Staphylococcus epidermidis* bacteria isolated, 79% were resistant to Methicillin, while 98% were resistant to Penicillin[9].

Indonesia is a tropical country that has the potential of plants that are hereditary used as herbal medicines [10]. One of the traditional plants that have been studied and has an antibacterial effect is turmeric or Curcuma longa L [11]. Turmeric is commonly used as a coloring agent, flavoring, and food deodorizer [12–14]. In the turmeric rhizome, there is curcumin, which is a polyphenolic compound which is included in Curcuminoid which is hydrophobic. Curcumin content in turmeric has several benefits, besides being beneficial as an antibacterial, it can also be used as an antioxidant, anti-inflammatory, antiviral, antifungal, antiparasitic, anti-allergic, antiarthritis, Alzheimer's treatment, anticancer, hepatoprotective, neuroprotective, nephroprotective, and anti-HIV[15,16].

Curcumin inhibits bacterial cell proliferation and breaks bacterial cell walls, causing cell rupture which leads to bacterial cell death [11,17]. Curcumin has a problem in the low level of solubility in water which then causes a decrease in absorption, increased metabolism, and increased excretion which causes low bioavailability of curcumin[11,18].

To overcome the low level of solubility and bioavailability of curcumin, nanoparticle-based drugs are developed. These drugs can increase bioavailability and solubility in water, and increase cellular uptake[11,14]. Mesoporous silica nanoparticles have been studied and are known to have interesting properties, such as hydrophilic surface, longer blood circulation time, very low toxicity, and better absorption[19–21]. Therefore, this study was conducted to determine inhibitory activity and kill Curcuminoid originating from Curcuma longa L. with mesoporous silica nanoparticles against *Staphylococcus epidermidis* bacteria, in the hope of being developed into an antibacterial candidate.

METHODS

This study used an experimental study with a nonequivalent control group design. This research was conducted in the Microbiology Laboratory of Faculty of Medicine, Research Laboratory Faculty of Pharmacy Widya Mandala Catholic University of Surabaya, and Laboratorium Mikrobiologi Klinik Balai Besar Laboratorium Kesehatan (BBLK) Surabaya. The population of this study was *S. epidermidis* bacteria. The samples taken were *S. epidermidis* ATCC 14990 obtained from the BBLK Clinical Microbiology Laboratory. The concentration of Curcuminoid with mesoporous silica nanoparticles used was 2000 - 32000 µg / mL.

Preparation of Curcuminoid with Mesoporous Silica Nanoparticles

Add 480 mg of Curcuminoid with mesoporous silica nanoparticles obtained from the Chemical Engineering Department into a test tube. Add 5 mL tween 20 to the test tube filled with Curcuminoid with mesoporous silica nanoparticles then mix until homogeneous using vortex. Curcuminoid with mesoporous silica nanoparticles solution was obtained with a concentration of 96000 µg / mL.

Preparation of Bacterial Suspension

S. epidermidis ATCC 14990 was taken with a sterile ose and then suspended into a tube containing 5 mL 0.9% NaCl solution to obtain bacterial turbidity similar to 0.5 McFarland standard turbidity on Mc Farland densitometer to obtain a bacterial suspension containing 1.5 x 10^8 CFU / mL.

Antibacterial Test

Antibacterial activity testing was performed by the microdilution method. This study was divided into a control group and a treatment group with a volume of each well of 150 µL.

K_{1-4} = The control group consists of:

K1 = Mueller Hinton Broth

K2a-e = Mueller Hinton Broth + Curcuminoid with mesoporous silica

nanoparticles on concentration 2000 – 32000 µg/mL

K3 = Mueller Hinton Broth + *S. epidermidis* + Penicillin

K4 = Mueller Hinton Broth + *S. epidermidis* + tween 20

P_{0-5} = The treatment group consists of:

P0 = Mueller Hinton Broth + *S. epidermidis*

P_{1-5} = Mueller Hinton Broth + *S. epidermidis* + Curcuminoid with mesoporous silica nanoparticles on concentration 2000 – 32000 µg/mL.

Then the microplate was incubated for 24 hours at 37 ^{o}C. After incubation, turbidity was observed by using a spectrophotometer to evaluate the optical density value. After that, the results of microdilution were planted in Mueller Hinton Agar and then incubated for 24 hours at 37 ^{o}C to evaluate bacterial growth.

RESULTS

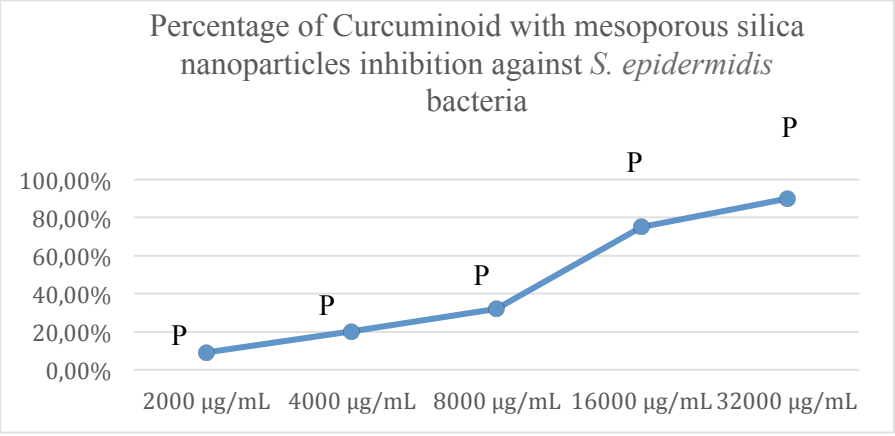
The optical density value (OD) on the spectrophotometer reading (Table 1) shows that the higher the concentration of Curcuminoid with mesoporous silica nanoparticles, the lower the value of OD. Concentration in the range 2000 - 32000 µg / mL with the lowest concentration is at P1, and the highest concentration is at P5.

Table 1. Results of optical density Curcuminoid with mesoporous silica nanoparticles values on a spectrophotometer

Concentration	$\bar{x} \pm SD$
P0	0,16 ± 0,026
P1	0,23 ± 0,03
P2	0,25 ± 0,038
P3	0,22 ± 0,042
P4	0,07 ± 0,028
P5	0,01 ± 0,01

In Figure 1 shows that the higher the concentration of Curcuminoid with mesoporous silica nanoparticles, the higher the percentage of inhibition to the *Staphylococcus epidermidis* bacteria. The highest percentage inhibition was at a concentration of 32000 µg / mL which was equal to 90%.

Figure 1. Percentage of Curcuminoid with mesoporous silica nanoparticles inhibition *S. epidermidis*



In Figure 2 shows that after planting on MHA and incubating for 24 hours at 37 ^{o}C, all bacteria in the treatment group grew on MHA. There was no bacterial growth in K1 and K2a - K2e. In the K3 there was no bacterial growth. In the K4 appears bacterial growth.

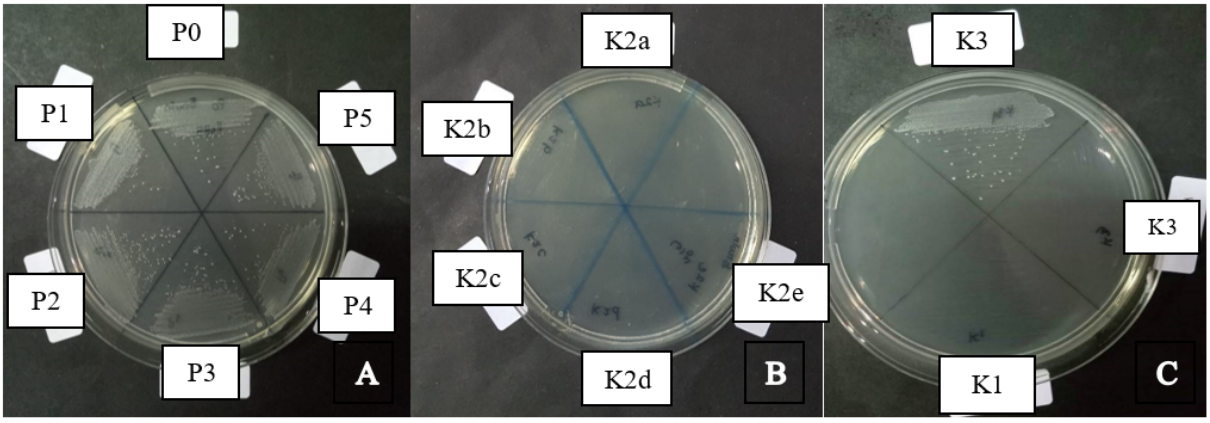


Figure 2. Observation of treatment group (A), control of K2a - K2e (B), and control K1 - K4 (C) after growing on MHA and incubating for 24 hours at 37 ^{o}C.

DISCUSSION

The results of this study show that Curcuminoid with mesoporous silica nanoparticles has an antibacterial effect on *Staphylococcus epidermidis* bacteria. Based on the results of observations on the spectrophotometer, it was shown that the higher the concentration of Curcuminoid with mesoporous silica nanoparticles would also be followed by a decrease in the value of OD. This means that more bacteria die if the concentration of Curcuminoid with mesoporous silica nanoparticles is increased[(22)].

The calculation of the percentage of resistance to *Staphylococcus epidermidis* also shows a trend that increases along with the increase in the concentration of Curcuminoid with mesoporous silica nanoparticles. In this study found MIC Curcuminoid with mesoporous silica nanoparticles against *Staphylococcus epidermidis* located at a concentration of 32000 µg/mL. Several factors are thought to play a role in influencing this study including the effectiveness of Curcuminoid as a single antibacterial preparation, thermolabile Curcuminoid properties, the pore size of mesoporous silica nanoparticles, and Curcuminoid release process from mesoporous silica nanoparticles.

The effectiveness of Curcuminoid as a single antibacterial preparation is thought to be less effective when compared with water extract from turmeric rhizome as in the study conducted by Niamsa, N. (2009). Allegedly this is because, in addition to

containing Curcuminoid, water extract from turmeric rhizome also contains sequesters and isoflavones which have antibacterial effects[23–26]. The thermolabile Curcuminoid properties are also thought to play a role in the occurrence of changes in the structure of Curcuminoid during the loading process into the pores of mesoporous silica nanoparticles that require heating at a temperature of 55 ° C. In a study conducted by Kharat (2017), the percentage of curcumin retention in fat emulsions in water will decrease with increasing temperature, the greatest decrease in retention of curcumin is found in heating at 55 ° C [27].

Another factor that is thought to influence the results of this study is that the pore size of mesoporous silica nanoparticles that contain Curcuminoid is not uniform, causing a difference in the number of particles of Curcuminoid in the same measurement of concentration. This can cause differences in the effectiveness of Curcuminoid with mesoporous silica nanoparticles as antibacterial preparations. In this study, it was assumed that the release process of Curcuminoid from mesoporous silica nanoparticles was hampered so that it could affect the antibacterial effect of Curcuminoid. The blockage of curcuminoid release from mesoporous silica nanoparticles causes a longer time needed so that the Curcuminoid can be completely released and interact with bacterial cells to cause an antibacterial effect.

The antibacterial mechanism of Curcuminoid with mesoporous silica nanoparticles is thought to originate from the ability of Curcuminoid to inhibit bacterial cell proliferation and has the ability to breaks bacterial cell walls causing bacterial cell rupture which leads to bacterial cell death[12,15]. Curcuminoid also has an antibiofilm effect, especially in bacteria that can form biofilms such as the *Staphylococcus epidermidis* bacteria. Curcuminoid inhibits the expression of biofilm-forming and quorum sensing genes from bacteria. The ability of bacteria to form a biofilm depends on quorum sensing, such as the ability to produce alginate, motility, and moving in a colony[12,15]. Turmeric (Curcuma longa L). also has the ability to reduce the attachment of bacteria to the surface of the substrate and reduce the adhesion to the biofilm surface so that microorganisms are more difficult to attach to previously formed biofilms.

CONCLUSION

Based on the results of the study it can be concluded that Curcuminoid with mesoporous silica nanoparticles has an inhibitory effect on the growth of *Staphylococcus epidermidis* bacteria at a concentration of 32000 µg / mL.

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