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Potential of Turmeric Rhizome (*Curcuma longa*) as an antibacterial agent against *Staphylococcus aureus*: A Lab Experimental Study

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ABSTRACT

Introduction: Staphylococcus aureus can cause various clinical manifestations in humans. With the rising number of antibiotic-resistant pathogens, the widespread of Staphylococcus aureus is troublesome so new alternative antimicrobial substance is needed. One of the potential substances is turmeric (Curcuma longa) which contains tannins and flavonoids that have been proven to have antibacterial effects. This study evaluated the antibacterial effects of Curcuma longa rhizome extract against Staphylococcus aureus. ***Methods:*** This study is a true experimental with a posttest-only control group design using tube dilution and agar dilution methods. Treatment groups are groups of Staphylococcus aureus that were given Curcuma longa extract with concentrations 300, 150, 75, 37.5, 18.75, and 9.375 mg/ml. The replication used in this research was four replications. MIC (Minimum Inhibitory Concentration) was observed visually by comparing the turbidity of the solution before and after incubation at 370C for 24 hours. The MBC (Minimum Bactericidal Concentration) was observed visually by observing the presence of Staphylococcus aureus colonies growth. ***Results:*** MIC cannot be observed due to no change in turbidity before and after treatment. Cultures on nutrient agar plates had no colony growth in concentrations of 300, 150, and 75 mg/ml. Thus, the MBC is 75 mg/ml. ***Conclusion:*** There is an antibacterial effect of Curcuma longa extracts against Staphylococcus aureus with a Minimum Bactericidal Concentration of 75 mg/ml.

Keywords: antibacterial; *Curcuma longa* extracts; *staphylococcus aureus*; antimicrobial activity.

INTRODUCTION

Staphylococcus aureus is one of the main human pathogens that can cause various diseases such as lung infections like pneumonia or empyema, skin infections like impetigo, furuncles, carbuncles, folliculitis, or cellulitis, gastroenteritis, meningitis, urinary tract infections, and toxic shock syndrome.[1] *Staphylococcus aureus* is one of the bacteria that cause gastroenteritis, which is generally characterized by diarrhea symptoms stemming from food poisoning[2]. Centers for Disease Control and Prevention (CDC) estimates that 1.5 million Americans are infected with *Staphylococcus aureus* each year[3]. In Indonesia, *Staphylococcus aureus* bacterial infections account for approximately 4.4% of the total number of acute bacterial gastroenteritis infections, indicating an increase in infections compared to other bacteria[4].

One of the most common problems encountered in the management of bacterial infections such as those causing diarrhea is the excessive use of antibiotics by the public. The effectiveness of antibiotics used to treat bacterial infections is generally threatened by the global growth of antibiotic-resistant bacteria. The concerning levels of resistance among bacterial infections were reported by the Global Antimicrobial Resistance and Use Surveillance System (GLASS) in 2022. About 35% of *Staphylococcus aureus* are resistant to methicillin and approximately 42% of *E. coli* are resistant to third-generation cephalosporins in 76 countries[5]. In 2020, 1 in 5 cases of *E. coli* urinary tract infections showed decreased sensitivity to common antibiotics such as ampicillin, cotrimoxazole, and fluoroquinolones[5].

As a result, the recovery rates for bacterial infections have decreased. Antimicrobial resistance (AMR) will certainly cause significant problems for the national health system and economy as a whole. For example, this will create more expensive and intensive treatments, as well as higher mortality rates.

Herbal plants from the Zingiberaceae family or ginger family have been proven to have antibacterial properties against various bacteria. Findings show that turmeric (Zingiber officinale) contains phenolic compounds, sesquiterpenoids, monoterpenoids, and their derivatives, including alcohols, ketones, esters, and aldehydes. These beneficial substances offer various antibacterial activities against different microbes, making turmeric a viable alternative to synthetic antimicrobial use[6].

Turmeric (*Curcuma longa*) has similar antibacterial effects. Curcumin, tannins, and flavonoids, compounds in turmeric, provide strong antibacterial activity against Bacillus cereus, Clostridium perfringens, Listeria monocytogenes, *Staphylococcus aureus*, Salmonella Typhimurium, and Vibrio parahaemolyticus with thermal and pH stability, and can be effectively used as a natural preservative to prevent the growth of food pathogens.[7]

Based on the explanation above, and to enhance the utilization of herbal plants in Indonesia, as well as to reduce the increasing rate of antibiotic resistance, the researcher wants to study the antibacterial activity of yellow turmeric rhizome extract (*Curcuma longa*) against *Staphylococcus aureus* bacteria in vitro using the dilution method.

METHODS

This was an experimental study with a randomized controlled method and post-test control group design using tube dilution and agar dilution methods. Data collection was conducted only at the end of the study period after administering treatments. This study received ethical clearance from the Ethics Committee for Health Research, Airlangga University, Surabaya.

EXTRACTION OF TURMERIC RHIZOME

Yellow turmeric rhizomes weighing 1500 grams were obtained from Tuban, East Java, and then extracted at the Balai Materia Medika, Batu, using the maceration method with 96% ethanol for three days at room temperature. The extraction product was then filtrated to separate the sediment from the filtrate and was condensed using a rotary evaporator at 40°C, followed by drying. After being processed through extraction, 42 grams of yellow turmeric rhizome extract were obtained and dissolved in 96% ethanol with a smooth, solid texture, and reddish-yellow color. The extract was then stored in a plastic bottle and kept in a refrigerator at 4°C.

PREPARATION OF *STAPHYLOCOCCUS AUREUS* BACTERIA

The *Staphylococcus aureus* bacterial culture is taken with a sterilized loop and then inoculated into the Mueller Hinton broth medium. Next, the mixture is vortexed and standardized to McFarland 0.5. Once standardized, the concentration of *Staphylococcus aureus* colonies at 1.5 x 108 CFU/ml is ready for use. The isolate was then incubated at 37°C for 24 hours.

CONTAMINATION TEST OF YELLOW TURMERIC RHIZOME EXTRACT

A contamination test of yellow turmeric extract was conducted. The contamination test was performed by planting or streaking the yellow turmeric extract to form wells on nutrient agar media. Then, the nutrient agar was incubated in an incubator for 24 hours at a temperature of 37°C. The results of the contamination test showed that the yellow turmeric extract used did not experience contamination.

DATA ANALYSIS

This study used eight dilution tubes consisting of two control tubes (positive and negative) and six treatment tubes filled with yellow turmeric extract (*Curcuma longa*) dissolved in 50% DMSO, with decreasing concentrations in each tube. This study conducted 4 times replications based on the frederer equation. This study uses the dilution method to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of yellow turmeric extract (*Curcuma longa*) against *Staphylococcus aureus* bacteria. To assess the MIC, the turbidity level of the yellow turmeric extract was observed with the naked eye. To assess the MBC of yellow turmeric extract (*Curcuma longa*) against *Staphylococcus aureus* bacteria, the yellow turmeric extract from each tube was streaked onto a Nutrient Agar Plate, then incubated for 24 hours in an incubator at 37°C. Subsequently, the growth of *Staphylococcus aureus* bacterial colonies on the Nutrient Agar Plate was observed.

RESULTS

This study was conducted at the Microbiology Laboratory, Faculty of Medicine, Airlangga University, Surabaya for 30 days.

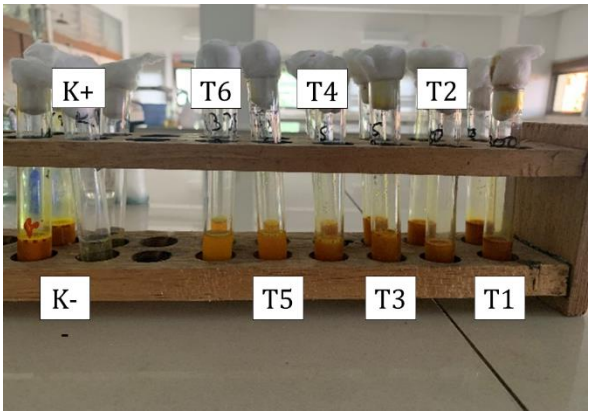


FIGURE 1: Replication Tube 1 After being incubated for 24 hours in an incubator at 37°C to measure minimum inhibitory concentration.

The determination of the MIC value used bacterial control as a comparison for the turbidity level. The clearest tube with the lowest extract shows the Minimum Inhibitory Concentration (MIC) of yellow

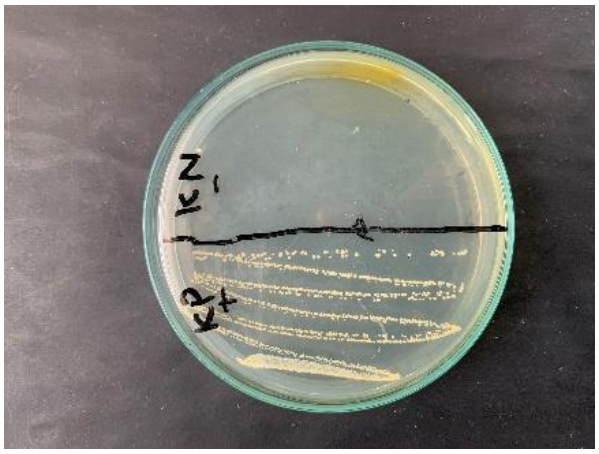
turmeric extract against the Staphylococcus aureus bacteria.

TABLE 1: Result of Minimum Inhibitory Concentration.

Group	Color of the tube liquid
K+	Clear Whitish
K-	Brown Murky Orange
T1	Brown Murky Orange
T2	Brown Murky Orange
T3	Brown Murky Orange
T4	Murky Yellow Orange
T5	Murky Yellow Orange
T6	Murky Yellow Orange

Source: Research data, processed
T1 = 300 mg/ml; T2 = 150 mg/ml; T3 = 75 mg/ml; T4 = 37.5 mg/ml; T5 = 18.75 mg/ml; T6 = 9.375 mg/ml.

There were four replications that had been conducted with similar results on each replication. Based on Figure 1 and Table 1, the observation results regarding the turbidity of the tubes did not show any difference in turbidity between one tube and another, so in this study, the minimum inhibitory concentration (MIC) of yellow turmeric extract against *Staphylococcus aureus* bacteria could not be concluded.



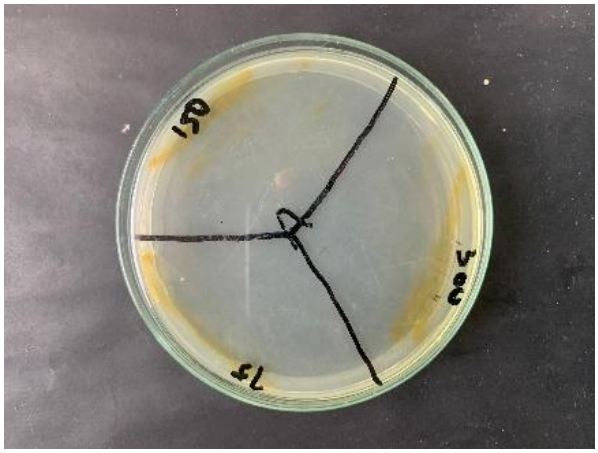


FIGURE 2: Replication Nutrient agar plate 1 after being incubated for 24 hours in an incubator at 37°C to measure minimum bactericidal concentration.

To assess the antibacterial activity of yellow turmeric extract (*Curcuma longa*) against *Staphylococcus aureus*, the yellow turmeric extract was streaked in each tube on a Nutrient Agar Plate, then incubated for 24 hours in an incubator at 37°C. Subsequently, the growth of *Staphylococcus aureus* colonies on the Nutrient Agar Plate was observed.

TABLE 2: Result of Minimum Bactericidal Concentration.

Group	Bacterial growth
K+	+
K-	-
T1	-
T2	-
T3	-
T4	+
T5	+
T6	+

Source: Research data, processed
T1 = 300 mg/ml; T2 = 150 mg/ml; T3 = 75 mg/ml; T4 = 37.5 mg/ml; T5 = 18.75 mg/ml; T6 = 9.375 mg/ml. + = bacterial growth was present; - = bacterial growth was absent.

There were four replications that had been conducted with similar results on each replication. Based on Figure 2 and Table 2. With the increasing concentration, a decrease in the growth of *Staphylococcus aureus* was observed. There was absent bacterial growth at concentrations of 300 mg/ml, 150 mg/ml, and 75 mg/ml of *Curcuma longa* extract. The minimum inhibitory concentration (MIC) of *Curcuma longa* extract that effectively kills *Staphylococcus aureus* is at a concentration of 75 mg/ml.

DISCUSSION

This study used eight dilution tubes consisting of two control tubes (positive and negative) and six treatment tubes filled with yellow turmeric extract (*Curcuma longa*) dissolved in 50% DMSO with decreasing concentrations in each tube.

This study employed the dilution method to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of yellow turmeric extract (*Curcuma longa*) against *Staphylococcus aureus*, the results of which can be seen in Tables 1 and 2.

Table 1 shows the results of the dilution test to determine the Minimum Inhibitory Concentration (MIC), which is the lowest concentration needed to inhibit bacterial growth as visually assessed by the researcher, and there is a significant difference compared to the positive control in either liquid or solid medium8. In this study, the yellow turmeric extract (*Curcuma longa*) in the six dilution tubes did not show significant differences in turbidity and clarity before and after treatment. The yellow turmeric extract (*Curcuma longa*) in the dilution tubes was murky orange-yellow, making it impossible to determine the Minimum Inhibitory Concentration (MIC).

The Minimum Bactericidal Concentration (MBC) of this study was assessed by inoculating the six extracts of yellow turmeric (*Curcuma longa*) and *Staphylococcus aureus* bacteria into the treatment tubes on Nutrient Agar Plate (NAP) media using the streaking method, followed by incubation for 24 hours in an incubator at 37°C. The Minimum Bactericidal Concentration (MBC) is defined as the lowest concentration of a compound that can kill 99.9% of bacterial colonies compared to the bacterial colonies that grow in the positive control9. In the four replications conducted in this study, the same results were obtained, namely the growth of *Staphylococcus aureus* bacterial colonies in tubes T4, T5, and T6, as well as the control tube. Meanwhile, in tubes T1, T2, T3, and the negative control tube, there was no growth of *Staphylococcus aureus* bacterial colonies. Thus, in this study, it can be concluded that the Minimum Bactericidal Concentration (MBC) of yellow turmeric extract (*Curcuma longa*) against *Staphylococcus aureus* bacteria is at a concentration of 75 mg/ml.

As indicated in Table 1, the Minimum Inhibitory Concentration (MIC) could not be determined because there was no significant change in the turbidity levels of the dilution tubes that had been incubated for 24 hours in an incubator at 37°C before and after treatment. The turbidity of the tubes T1 to T6 could not be distinguished in their growth before and after incubation, because the turmeric extract itself was already orange-turbid, making it difficult to determine the minimum inhibitory concentration. (MBC).

The Minimum Bactericidal Concentration (MBC) of the yellow turmeric extract *Curcuma longa* was obtained from the culture of bacterial colonies on nutrient agar plates (NAP) for 24 hours in an incubator at 37°C. Ideally, the streaking process is carried out on tubes that have been determined as the Minimum Inhibitory Concentration (MIC) up to the tube with the highest concentration. However, since the MIC could not be determined in this study, the streaking process was carried out on all tubes. The culture results, as presented in Table 2, show growth at concentrations of 37.5, 18.75, and 9.375 mg/ml in all replicates. At concentrations of 300, 150, and 75 mg/ml, no bacterial growth was observed in all replicates, resulting in a Minimum Bactericidal Concentration (MBC) of 75 mg/ml.

To further confirm the presence or absence of the effect of *Curcuma longa* turmeric extract on *Staphylococcus aureus*, a colony count was performed as presented in Table 2. The results showed that *Curcuma longa* turmeric extract has bacteriolytic (killing) and bacteriostatic (inhibiting) effects on *Staphylococcus aureus*. The inhibitory effect can be seen from the smaller number of colonies at concentrations of 37.5 mg/ml, 18.75 mg/ml, and 9.375 mg/ml compared to the number of colonies in the Positive Control (PC). Meanwhile, at concentrations of 300, 150, and 75 mg/ml, no growth of *Staphylococcus aureus* colonies was observed.

These results align with the researchers' expectations, as generally, the number of colonies is inversely proportional to the concentration of the extract. The higher the concentration of the extract, the fewer the number of colonies.

Alkaloids, flavonoids, curcumin, essential oils, saponins, tannins, and terpenoids were found in turmeric (*Curcuma longa*) as active ingredients. Previous research has shown that curcumin functions as an antioxidant, anti-cholesterol, anti-tumor, and anti-bacterial agent. This is done by the substance damaging the cell wall. Antimicrobial compounds, such as phenol and its derivatives, cause lysis or inhibit the formation of cell wall components in growing cells, alter the permeability of the cytoplasmic membrane, resulting in the leakage of nutrients from the cell material, denaturation of cell proteins, and cessation of enzyme activity within the cell. (Sari dan Maulidya., 2016).

Based on this research, which shows a decrease in the number of *Staphylococcus aureus* bacterial colonies with increasing concentrations of yellow turmeric extract (*Curcuma longa*), it can be concluded that yellow turmeric extract (*Curcuma longa*) has an antibacterial effect on *Staphylococcus aureus* bacteria. This supports the hypothesis that the author has formulated as correct.

STRENGTH AND LIMITATIONS

There are several limitations in this research that should be acknowledged. The limitations were this study was not able to use spectrophotometry to assess the growth density of *Staphylococcus aureus* colonies. This research is also not able to investigate the antibacterial potential of turmeric rhizome (*Curcuma longa*) extracts in vivo. Future research is expected to address this limitation, especially to determine the pharmacokinetic and pharmacodynamic effects, as well as the toxicity of the active ingredients in the turmeric extract (*Curcuma longa*).

CONCLUSION

Based on the results, it can be concluded that turmeric rhizome (*Curcuma longa*) ethanol extract has antibacterial activity against *Staphylococcus aureus* with MBC 75 mg/ml.

ACKNOWLEDGMENTS

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ETHICAL CLEARANCE

This study received ethical clearance from the Ethics Committee for Health Research, Airlangga University, Surabaya, Indonesia.

AUTHORS' CONTRIBUTIONS

Designed the study and drafted the manuscript: MMAA, RJS, and YS. Collected data and performed statistical analysis: MMAA. Supervised results and discussion: MMAA, RJS, YS, and KEP. All authors reviewed and approved the final version of the manuscript.

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