Antibacterial Activity Test of Turmeric Extract (Curcuma longa) From Madura Island Against Staphylococcus aureus

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Received: 30 January 2021/Accepted:19 February 2022/Published Online: 28 February 2022
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Abstract
Staphylococcus aureus is a gram-positive bacteria, a normal flora of the body that can cause infection in various organs and has the potential to become resistant to antibiotics. Turmeric (Curcuma longa) is one of the herbs that has antibacterial effects and is often used by Madurese people as a traditional medicine. It is suspected that geographical differences and the natural conditions in which turmeric grows can affect its compounds and properties. This study aims to analyze the ethanolic extract of turmeric from Madura as an antibacterial against S. aureus. An in vitro laboratory research using agar well diffusion method was conducted with the concentration of turmeric extract used 20%, 40%, 80%, and 100%. The positive controls used was penicillin. The negative control in this study was 100% DMSO. The results showed inhibition zone at concentrations of 20% (11.86 mm), 40% (12.04 mm), 80% (12.53 mm), and 100% (10.97 mm). The diameter obtained was lower than the positive control penicillin which was 27.59 mm. Turmeric extract (Curcuma longa) from Madura has antibacterial activity against Staphylococcus aureus.

Keywords: Turmeric; Curcuma longa; Madura; Antibacterial; Staphylococcus aureus

INTRODUCTION
Staphylococcus aureus is a gram-positive bacteria, a normal flora of the body found in the nose, throat, skin and other body tissues. S. aureus as the main cause of bacterial infections can affect the integrity of human body tissues, can cause a wide spectrum of clinical disease such as bacteremia, infective endocarditis, skin and soft tissue infections, osteoarticular infections, lung and pleural infections (Tong et al., 2015). People with methicillin resistant S. aureus (MRSA) infections are 64% more likely to die than people with drug sensitive infections (WHO, 2021). Southeast Asia is one of the countries with the highest number of antibiotic resistance cases in the world, especially infection by MRSA, so that the function of these antibiotics has decreased (World Health Organization, 2015). MRSA is a strain of S. aureus that is susceptible to narrow-spectrum beta-lactam antibiotics which are known as the main pathogens causing nosocomial infections. The mechanism of resistance occurs because there is a gene encoding mecA in SCCmec that encodes penicillin binding protein (PBP2a), signal controlled expression proteolytic transduction, namely sensor protein (MecR1), and repressor protein (MecI). It should be noted that the SCCmec gene is only found in MRSA. This is due to the exotoxin released by S.
Aureus is a pyogenic toxic superantigen (PTSAg). PTSAg is a multifunctional protein that has pyrogenic activity, superantigenicity, and the ability to induce hypersensitivity causing endotoxin death. *S. aureus* also expresses hemolysins (Rai A., 2021). Antibiotics are now being used in ways that are not in compliance with their recommendations, resulting in an increase in antibiotic resistance. One way to prevent antibiotic resistance in this country is to use natural extracts as antibacterial medications instead of antibiotics.

Turmeric (*Curcuma longa*) is one of the traditional plant species in Indonesia that has anti-inflammatory, antioxidant, antihyperlipidemic, antiseptic and antibacterial effects (Soleimani v., 2018). These effects make turmeric widely used as an herb for the treatment of several cases such as respiratory disease, gastrointestinal and liver disorders, rheumatism, anorexia, wounds, and burns (Prasad and Aggarwal, 2011; Adamczak et al., 2020). As an antibacterial, it is known that turmeric is able to inhibit the growth of *S. aureus* (Pulido-Moran et al., 2016). This antibacterial activity is thought to be a working effect of the compounds contained in it. Turmeric contains bioactive curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin). Curcumin is a lipophilic polyphenol compound with anticancer, antibacterial, anti-inflammatory, and anti-aging properties. Turmeric’s volatile oil and phytochemical molecule also acts as an antibacterial, limiting bacterial metabolism by disrupting the cytoplasmic membrane and denaturing proteins, causing bacterial cells to die or have their growth slowed in the same way that curcumin does (Soleimani v., 2018). According to Lawhavinit et al. (2010), ethanol is one of the solvents that can produce a strong antibacterial effect on turmeric extract because of its excellent ability to bind active substances.

Madurese people have the perception that turmeric has a fairly high antibacterial effect. As many as 46% of the Madurese community, especially of Sapoloh village, Bangkalan district, use turmeric as traditional medicine (Rohmah, 2017). The chemical nature of the soil has an impact on the content of secondary metabolites of turmeric. Curcumin has a positive correlation with N nutrients found in Madura soil, where in the pathway of curcumin formation several enzymes such as PAL (Phenylalanine Ammonia Lyase) are needed which catalyze the conversion of phenylalanine to cinnamic acid. PAL enzyme activity will increase in extreme environmental conditions such as low soil nutrient levels. The levels of curcumin obtained from Madura turmeric from lowest to highest were in Bangkalan (1.073%), Pamekasan (2.75%) and Sumenep (2.90%) districts. Meanwhile, the highest essential oil content is in Geger sub-district, Bangkalan district, which is 2.78% (Sholelah, N D., 2016).

The compound and effectiveness of turmeric as an antibacterial is affected by various factors such as topography, environment, and climate factors that have an impact on various conditions of soil, water, pH, and nutrients (Li et al., 2020). There have been many studies that have investigated and proven the antibacterial activity of turmeric extracts from various regions against *S. aureus* (Dosoky and Setzer et al., 2018). However, it is still not clear how strong the antibacterial activity of turmeric that grows in Madura is, because no research has investigated it directly. Therefore, this study aims to analyze the ethanolic extract of turmeric from Madura Island as an antibacterial against *S. aureus*.

**METHOD**

1. **Research Design**

This study is an in vitro experimental laboratory research to determine the activity of...
turmeric extract (Curcuma longa) against S. aureus bacteria using the agar well diffusion method which was carried out at the Laboratory of Microbiology, Faculty of Medicine, Universitas Airlangga from November 2020 to February 2021. Turmeric extract was tested in five different concentration groups. Based on Federer's formula (1963), the diffusion method antibacterial test was carried out three times in each group. The data was collected using a post-only group design. The research had been approved and passed ethics by the Health Research Ethics Committee, Faculty of Medicine, Universitas Airlangga No.69/EC/KEPK/FKUA/2021.

2. Materials and Tools
The research materials were Mueller Hinton Agar (MHA) medium, turmeric extract (Curcuma longa), S. aureus bacteria whose culture was obtained from the Department of Microbiology, Faculty of Medicine, Airlangga University, ethanol as a solvent, and penicillin discs. The research instruments were a petri dish, micropipette tip, tweezers, measuring cup, sterile cotton swab, scalpel knife, centrifuge, mill, caliper, incubator, ose, spiritus, autoclave, maceration vessel, vacuum evaporator, and phone camera.

3. Extraction of Turmeric
Turmeric collected from local farmers in Bangkalan, East Java as much as 5 kg then peeled and cleaned under running water. The clean turmeric is sliced using a knife and then placed in a container to dry in the sun. After drying, the turmeric will be processed into powdered simplicia and then macerated. Maceration was carried out by immersing turmeric rhizome simplicia into an ethanol solution for 3—24 hours and allowed to stand in an impermeable container, stirring with a stirrer between soaking times, then the maceration results were filtered using filter paper. The results of the filtering obtained the first liquid extract, then the residue from the first extra liquid was macerated again by immersing it in an ethanol solution for 24 hours and stirred and then filtered. The results of the filtering obtained a second extra liquid which was then combined with the first extra liquid. Then, evaporation was carried out using a rotary evaporator to obtain a thick extract (Kumara et al., 2019).

4. Steps of Diffusion Method (Agar Well)
First, prepare standardized bacterial cultures with 0.5 Mc. Farland (1.5 -108 CFU/ml) then drip a sterilized cotton stick into the bacterial liquid culture and wipe a cotton swab over the entire surface of the Mueller Hinton agar medium, repeated 2 times while rotating the plate 60º. Then the plate is left for 3-5 minutes at room temperature. It should not be more than 5 minutes so that the bacteria are completely dry before placing the antibiotic disc. Thereafter make 5 wells with a perforator to test turmeric extract (Curcuma longa) and drop 1 ml of turmeric extract (Curcuma longa) in 5 parts of wells with different concentrations (0%, 20%, 40%, 80%, 100%) (Ramadhani et al., 2018). Take the penicillin or vancomycin disc with tweezers, then place the antibiotic disc on the surface of the medium so that it is slightly pressed, so that it can adhere completely. Incubate at 37ºC for 24 hours and the last look and calculate the diameter of the resulting inhibition zone.

5. Statistical Analysis
Statistical Analysis was performed using IBM SPSS Statistics 25 software. All data were presented as mean ± standard deviation. One-way ANOVA test and post-hoc Fisher’s LSD were used for comparative analysis with significance level of p value < 0.05.

RESULT AND DISCUSSION
Turmeric extract showed activity against Staphylococcus aureus which was indicated by the formation of inhibition zones on agar well diffusion method. At a concentration of 20%, the inhibition zone diameters were ranging from 11 mm, 12 mm,
and 12.60 mm. At a concentration of 40%, the inhibition zone diameters were 11.54 mm, 12.20 mm, and 12.40 mm. At a concentration of 80%, the inhibition zone diameters were 12.08 mm, 12.53 mm, and 13 mm. While at a concentration of 100%, the inhibition zones were 10.5 mm, 11.13 mm, and 11.28 mm. The inhibition zone had different diameters due to variance in the length of activity in inhibiting bacterial growth and the time required to reach the peak of antibiotic production.

The antibacterial activity test using the diffusion method was carried out three times (n=3). K1: replication 1, K2: replication 2, K3: replication 3.

![Picture 1: The antibacterial activity test using the diffusion method was carried out three times (n=3). K1: replication 1, K2: replication 2, K3: replication 3.]

The inhibition zone diameters produced by turmeric extract against *S. aureus* increased in each treatment, with the average obtained from the highest to the lowest respectively, at a concentration of 80% (12.53 ± 0.46 mm), 40% (12.04 ± 0.45 mm), 20% (11.86 ± 0.80 mm), and 100% (10.97 ± 0.41 mm). The positive control used was 10µg penicillin with a mean diameter of 27.59 ± 0.33 mm. Showed in the table 1.

**Table 1. The results of inhibition zone diameter of turmeric extract and 10µg penicillin against *Staphylococcus aureus***

<table>
<thead>
<tr>
<th>Extract Concentration</th>
<th>Mean of Inhibition Zone Diameter (mm)</th>
<th>One-Way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0 (0%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>M1 (20%)</td>
<td>11.86 ± 0.80</td>
<td>p = 0.000</td>
</tr>
<tr>
<td>M2 (40%)</td>
<td>12.04 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>M3 (80%)</td>
<td>12.53 ± 0.46</td>
<td></td>
</tr>
<tr>
<td>M4 (100%)</td>
<td>10.97 ± 0.41</td>
<td></td>
</tr>
<tr>
<td>Penicillin 10µg</td>
<td>27.59 ± 0.33</td>
<td></td>
</tr>
</tbody>
</table>

The normality test was carried out using Shapiro-Wilk, obtained significant results for each data (p > 0.05) which means that all data are normally distributed. Then, the homogeneity test was carried out using the laven test. The result was significant (p > 0.05), meaning that the data variance was homogeneous. In the one-way ANOVA test, it was known that the dependent variable has a significance value (p <0.05) which means that there is a significant difference between group. The results of post-hoc Fisher’s LSD test are presented in table 2. It can be seen that there is a significant difference between each treatment group and the penicillin group as positive control.

**Table 2. The results of post-hoc Fisher’s LSD test (p value) between groups. *p<0.05***

<table>
<thead>
<tr>
<th>Group</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>Penicillin 10 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>-</td>
<td>0.680</td>
<td>0.145</td>
<td>0.060</td>
<td>0.000*</td>
</tr>
<tr>
<td>M2</td>
<td>-</td>
<td>0.274</td>
<td>0.029*</td>
<td>0.000*</td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>-</td>
<td>0.004*</td>
<td>0.000*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>-</td>
<td>0.000*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillin 10 µg</td>
<td>-</td>
<td></td>
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</tbody>
</table>

Turmeric has been widely used as herbal medicine because the active compounds contained in it are proven to have various pharmacological effects.
A review by Dosoky and Setzer et al. (2018) showed that turmeric extracts from various regions in countries such as India, Pakistan, Malaysia, China, Brazil, Nigeria, and France had different levels of compounds. These findings indicate that the content and efficacy of turmeric is affected by various factors such as geographical, topographic, environmental, and climatic factors. The environmental factors can be in the form of water and nutrients availability, soil conditions, and pH levels (Li et al., 2020).

In this study, turmeric from Madura Island was chosen for research because the Madurese community has been using turmeric for generations as a traditional medicine which is usually mixed with other herbs to make herbal medicine (known as ‘jamu’) (Utami et al., 2020). The herbal concoction containing turmeric is commonly used to treat various health problems, one of which can occur due to bacterial infection (Widyawaruyanti et al., 2007). Previous studies have proven that the compounds contained in turmeric have a potent antibacterial effect against several types of bacteria, including *S. aureus* (Adamczak et al., 2020). According to Tonin et al. (2021), turmeric extract is strong bactericidal against *S. aureus* because of its characteristics as a gram-positive bacteria that only has a single layer of peptidoglycan in cells so it is more sensitive to antimicrobial substances. The purpose of this study was to analyze how strong the antibacterial activity of turmeric grown in Madura Island against *S. aureus* compared to other turmeric from the existing studies.

The results obtained showed that the ethanolic extract of turmeric from Madura produced the maximum inhibition zone against *S. aureus* at a concentration of 80%, which was 12.53 ± 0.46 mm. However, this inhibitory power was not greater than the penicillin antibiotic 10µg as a positive control (27.59 ± 0.33 mm). The inhibition zone produced by antibiotics against *S. aureus* is said to be sensitive if 29 mm and resistant if 28 mm (Clinical and Laboratory Standard Institute, 2013). Therefore, turmeric extract in this study was still relatively weak in inhibiting *S. aureus* because the average diameter obtained was less than 28 mm. Similarly, Ramadhani et al. (2018) found that the ethanolic extract of turmeric from West Sumatra, Indonesia, cause a maximum inhibition zone at a concentration of 80% against *S. aureus*, which was 14.25 mm. However, amoxicillin had a stronger effect by showing the zone of inhibition of 26.50 mm. The antibacterial activity of penicillin as a control in this study was also reported to be stronger than turmeric methanol extract from Cyprus, Turkey (Ogbonna and zgör, 2021). Another research by Khatun et al. (2021) reported that the ethanolic extract of turmeric from Jashore, Bangladesh produced antibacterial activity against *S. aureus* with the largest inhibition zone of 17.03 ± 0.30 mm at a concentration of 500 mg/ml. The zone of inhibition was still lower than the erythromycin 15 g. At lower concentrations, 2 mg/ml of turmeric ethanol extract from Sindhuli, Nepal could inhibit *S. aureus* biofilms up to 77.72 ± 3.32% (Suwal et al., 2021). Aqueous as a solvent in turmeric extraction is also widely used in several studies. It is known that the antibiotics ciprofloxacin and tetracycline also produce stronger antibacterial activity than the aqueous extract of turmeric (Oghenejobo and Bethel, 2017; Hosea et al., 2018). While Gupta et al. (2015) found that turmeric extract from India with various fractions (methanol, chloroform, water, benzene, and petroleum ether) produced an inhibition zone against three *S. aureus* strains of 9mm-21mm. As a comparison, gentamicin was used which showed an inhibition zone of 20mm – 25mm. On electron microscopy examination, it was seen that *S. aureus* had membrane damage and cytoplasm release resulting in bacterial cell damage. The difference in the value of antibacterial activity between this study and other studies could be due to
variance in several points such as the bacterial strain, the content of turmeric in different varieties, the type of extraction solvent, and the antibacterial test method. (Teow et al., 2016).

The antibacterial activity of turmeric is thought to be due to the presence of various compounds in it such as essential oil, curcumin, turmerone, curlone, ar-curcumene, -zingiberene, valeric acid, curdione, curzerene, and -elemenone (Ahaveethunisa and Hopper, 2012; Gul and Bakht, 2015; de Oliveira Filho et al., 2021). Essential oils contain hydrophobic compounds that can infiltrate bacterial cell membranes that have lipophilic ends of lipoteichoic acid. This causes cell death due to increased membrane permeability which triggers leakage of ions and cytoplasmic material (Avanço et al., 2017). Damage to membrane proteins, cell wall degradation, cytoplasmic coagulation, and proton motive force depletion are other working points of essential oils as antibacterial (Burt, 2004). Curcumin is a major component that gives turmeric its yellow color which has antimicrobial, antidiabetic, anti-inflammatory, anticancer, and antioxidant properties (Priyadarsini, 2014). As an antibacterial, curcumin works by binding to the FtsZ protein, resulting in inhibition of bacterial proliferation and cytokinesis (Rai et al., 2008). The bond between curcumin and cell wall peptidoglycan also causes damage to the cell wall and membrane of S. aureus (Mun et al., 2014). In MRSA bacteria, curcumin can inhibit the mecA gene and reduce PBP2α protein so that the sensitivity of bacteria to -lactam antibiotics increases (Tyagi et al., 2015). Another known mechanism is that curcumin can inhibit DNA replication and gene expression, reduce motility, and interfere with the cytoskeleton GTPase activity of bacteria so that bacteria fail to proliferate and divide cells (Kaur et al., 2010). In addition, curcumin is known to have a synergistic effect with the antibiotics cefixime, cefotaxime, vancomycin, and tetracycline against S. aureus so that the consumption of turmeric together with these antibiotics can be helpful in cases of infection (Moghaddam et al., 2009).

Phytochemical test of ethanolic extract of turmeric showed that turmeric also contains alkaloids, flavonoids, phenols, tannins, glycosides, and carbohydrates. These various active components contribute to the pharmacological effect of an herb (Irshad et al., 2018; Khatun et al., 2021). Flavonoids and phenols are compounds that have potent antimicrobial, antioxidant, and anti-inflammatory activities (Amine et al., 2021). While alkaloids and tannins are also efficacious as antibacterial (Gupta et al., 2015). Tannin is able to inactivate the reverse transcriptase enzyme and destroying proteins in bacterial cell. Tannins also have a target on cell wall polypeptides, so that the formation of cell walls is less than perfect (Sapara and Waworuntu, 2016). Tannins can bind iron more than gallic acid, microorganisms that grow under aerobic conditions require iron to perform their functions such as reduction of DNA ribonucleotide precursors, formation of haem, and other important functions. Therefore, bacterial cells cannot be formed by the strong iron-binding ability of tannins (Akiyama et al., 2001).

The mechanism of the various components in turmeric causes turmeric to have broad-spectrum antibacterial activity. Besides S. aureus, turmeric extract also has antibacterial properties against Klebsiella pneumoniae, Escherichia coli, V. parahaemolyticus, V. cholerae, Bacillus subtilis, B. cereus, Aeromonas hydrophila, Streptococcus agalactiae, Staphylococcus intermedius, and Staph. epidermidis (Ungphaiboon et al., 2005; Lawhavinit et al., 2010). This antibacterial effect is known to be more optimal in turmeric which has extracted active compounds and biosynthesis of nanoparticles compared to whole crude extract as in
this study which used ethanol as a solvent in conventional extraction methods. (Khan et al., 2021)

Based on this discussion, ethanolic extract of turmeric from Madura has antibacterial activity which is relatively weak compared to penicillin. Supporting data from previous studies also showed that the majority of antibiotics have a stronger antibacterial effect than turmeric. The limitation of this study is that there is no bioactive compound test for turmeric extract, so the levels of compounds that act as antibacterial are not clear. In addition, this research only proves the antibacterial effect in vitro using a simple extraction method that produces components that are still pure and has not produced more specific active compounds. However, this study is the first to find out the inhibition zone diameter of the turmeric ethanol extract, specifically from Madura, against *S. aureus*. The author suggests that the use of turmeric as an alternative is worth considering because of the potential synergistic effect with antibiotic and has fewer side effects.

CONCLUSION

Turmeric extract (*Curcuma longa*) has antibacterial activity against *Staphylococcus aureus* which is gram-positive bacteria. It was demonstrated by the presence of an inhibition zone on Mueller Hinton Agar (MHA) media. The optimum concentration to inhibit *S. aureus* growth was obtained at 80% turmeric extract. However, the antibacterial activity of this plant is still lower than penicillin antibiotics. Further research should be carried out by examining the content of turmeric extract from Madura to ensure the levels of antibacterial compounds in it as have been found in previous studies. In addition, in vivo study of turmeric as antibacterial is needed by using more advanced extraction method in order to obtain the bioactive compounds optimally.

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