

## ANTIBACTERIAL EFFECT OF CURCUMA DOMESTICA ETHANOLIC EXTRACT ON GROUP A STREPTOCOCCUS $\beta$ HEMOYTICUS

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### ABSTRACT

**Introduction :** Pharyngitis is one of the most common infection that general practitioners handled. It is estimated in one year there are 15 million people with pharyngitis will come to the doctor. American Society of Microbiology said that 94,3 % from 402 patient who were suspected pharyngitis received antibiotic therapy even without indication of antibiotic therapy. Antibiotic resistance is becoming a great threat for the world today. World Health Organization state that one of the plan to fight antibiotic resistance is to research and develop new drugs. Curcuma domestica is a plant that is widely used in Indonesia and some research said that it has anti-inflammatory, antioxidant, and antimicrobial effect.

**Aim:** The aim of this study is to find the antibacterial effect of Curcuma domestica ethanolic extract on Group A Streptococcus  $\beta$  hemolyticus and find the Minimum Bactericidal Concentration (MBC) value.

**Methods :** This was in vitro experimental study with broth microdilution method and inoculation on agar blood media to find the MBC. Treatment group consisting of, media (Mueller Hinton Blood Broth), Group A Streptococcus  $\beta$  hemolyticus bacteria, and 5 concentrations (312.5  $\mu$ g/ml, 625  $\mu$ g/ml, 1250  $\mu$ g/ml, 2500  $\mu$ g/ml, 5000  $\mu$ g/ml) of Curcuma domestica extract.

**Result :** There is no bacterial growth at 5000  $\mu$ g/ml concentration on solid media, but there are less bacterial growth at 2500  $\mu$ g/ml than at concentration 312.5  $\mu$ g/ml, 625  $\mu$ g/ml, 1250  $\mu$ g/ml.

**Conclusion:** There is an antibacterial effect of Curcuma domestica ethanolic extract on Group A Streptococcus  $\beta$  hemolyticus with MBC value in range 2500 – 5000  $\mu$ g/ml.

**Keywords :** Curcuma domestica ethanolic extract, Group A Streptococcus  $\beta$  hemolyticus, MBC

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## INTRODUCTION

*Streptococcus* is a Gram-positive spherical bacteria with a paired or chain-shaped arrangement. By their ability of hemolysis in blood these bacteria are divided into 3 types: *Streptococcus a hemolyticus*, *Streptococcus β hemolyticus*, and *Streptococcus non hemolyticus*<sup>(1)</sup>. Group A *Streptococcus β hemolyticus* can cause various diseases, ranging from mild diseases such as pharyngitis and impetigo to dangerous diseases such as *necrotizing fasciitis* and *streptococcal toxic shock syndrome*<sup>(2)</sup>.

Pharyngitis is one of the most commonly treated diseases by general practitioners, it is estimated that there are 15 million patients in the United States who come to the doctor every year due to this disease<sup>(3)</sup>. *The American Society of Microbiology* states that 94.3% of 244 patients received antibiotics even without an indication of antibiotics<sup>(4)</sup>. One of the reasons of giving inappropriate antibiotics is to avoid complications<sup>(5)</sup>.

Antibiotic resistance has become a big problem for the world today. This is a consequence of inappropriate use of antibiotics. *The World Health Organization* (WHO) states that one strategy in overcoming resistance is the discovery and development of new types of drugs<sup>(6)</sup>. Indonesia is a tropical country with potential plants that are traditionally

used for traditional medicine. Jamu is a traditional Indonesian beverage that has become a culture of Indonesian society since centuries ago which functions to maintain health<sup>(7)</sup>. Based on 2013 Basic Health Data Research, 30.4% of Indonesians use traditional health services, 49% consume jamu, and 52.7 % reason to consume herbs to maintain health and fitness<sup>(8)</sup>.

Turmeric (*Curcuma domestica*) is one of the traditional plants from the Southeast Asian region. Indonesian people generally use *Curcuma domestica* as a food flavoring, natural coloring, and as a traditional medicine ingredient<sup>(9)</sup>. Deepika et al. found that several modern studies have shown that *Curcuma domestica* has anti-inflammatory, anti-microbial, and antioxidant effects.<sup>(10)</sup> The research conducted by Ankur found that the leaves of *Curcuma domestica* contain several compounds, including curcumin, alkaloids, tannins, flavonoids, glycosides, carbohydrates and the results showed that alkaloids and flavonoids in curcuma have antibacterial and inhibitory effect on *Staphylococcus aureus*.<sup>(11)</sup>

## METHODS

This research is an experimental study in vitro with a post-test only control group design. This research was carried out in the Microbiology Laboratory of the

Faculty of Medicine, Research Laboratory of the Faculty of Pharmacy, Widya Mandala Catholic University of Surabaya, and the Clinical Microbiology Laboratory of *Balai Besar Laboratorium Kesehatan* (BBLK) Surabaya. *Group A Streptococcus  $\beta$  hemolyticus* bacteria was used, with type of ATCC 19615 *Streptococcus pyogenes*, provided by BBLK.

Extraction was carried out using the maceration method which involved putting 1 kg of dried simplicia of *Curcuma domestica* into a glass, then filled it with ethanol 90% to 2 cm above the simplicia powder and macerated for 7 days and *waterbath* to evaporate the solvent until 124.5 grams semisolid extract were obtained.

Preparation of bacteria starting with taking 1 culture of bacterial *group A Streptococcus  $\beta$  hemolyticus* colonies using sterile kit and then put it in 5 ml 0.9% NaCl then *vortexed* and densitometer test until it reached the standard 0.5 McFarland ( $1.5 \times 10^8$  CFU / mL).

The antibacterial effect test was carried out *in vitro* with the *broth microdilution* method and subsequently inoculated on solid media to obtain the KBM value. The anti-bacterial effect test with *broth microdilution* was carried out at concentrations of 5000 (P1), 2500 (P2), 1250 (P3), 625 (P4), 312.5 (P5)  $\mu\text{g} / \text{ml}$ , replicated 4 times with negative control of

the media without bacteria and positive control of the media *Group A bacteria Streptococcus  $\beta$  hemolyticus*. The next step was to incubate *microplates* for 24 hours at 37°C and CO<sub>2</sub> levels of 5%. The last step was after incubating, *streaking* or inoculating on solid media (*Mueller Hinton Blood Agar*) to determine whether there is a bacterium or not so that it can show the value of KBM.

## RESULT

The Minimum Killer Level Test was carried out by *streaking* samples that had been incubated for 24 hours with 4 replications. In the KBM test the extract concentrations used were 5000, 2500, 1250, 625, and 312.5  $\mu\text{g} / \text{ml}$ .

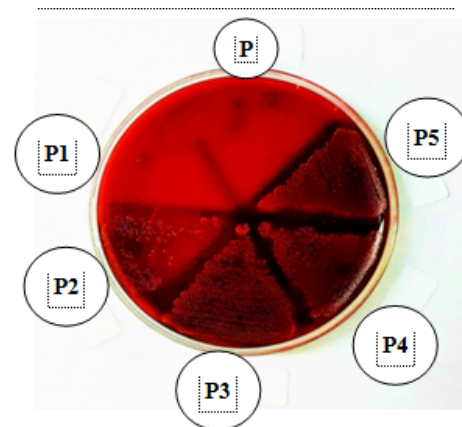


Figure 1 Observation of treatment group after *streaking* on *Blood Agar* media and incubated 24 hours (P = without *streaking*, P1 = 5000  $\mu\text{g} / \text{ml}$ , P2 = 2500  $\mu\text{g} / \text{ml}$ , P3 = 1250  $\mu\text{g} / \text{ml}$ , P4 = 625  $\mu\text{g} / \text{ml}$ , P5 = 312.5  $\mu\text{g} / \text{ml}$ )

The results after 24 hours observation of incubation to all treatment groups, it appears that in Column P there

was no growth because no sample was streaked on the column. The P1 column with the highest concentration of 5000 µg / ml does not appear to have colonies (white spots) or zones of hemolysis (blackish color). In P2 column with a concentration of 2500 µg / ml, there were some colonies, but the numbers were not as many as columns P3, P4, and P5. The P3 column (1250 µg / ml), P4 (625 µg / ml) and P5 (312.5 µg / ml) appear to have colonies that grow closely and the zone of clear hemolysis. This shows that KBM for *Curcuma domestica* extract against Group A *Streptococcus β hemolyticus* bacteria is in the concentration between 2500 - 5000 µg / ml.

## DISCUSSION

The results of this study indicate that *Curcuma domestica* ethanol extract has an antibacterial effect on the bacteria Group A *Streptococcus β hemolyticus*, this can be seen in the results of inoculation at the highest concentration (5000 µg / ml) where there was no bacteria colonies. This discovery is consistent with the theory that *Curcuma domestica* has several antibacterial active compounds such as alkaloids, flavonoids, curcumin, essential oils and tannins.<sup>(11)</sup> Alkaloids have several mechanisms to inhibit bacteria such as inhibiting nucleic acid synthesis by inhibiting the enzyme *dihydrofolate*

*reductase*, disrupting the process of cell division through the Z-ring, damaging the cell wall and disrupting the integrity of the cytoplasmic membrane.<sup>(12)</sup> Flavonoids also have several antibacterial mechanisms such as inhibiting nucleic acid synthesis, inhibiting cell membrane function, and inhibiting cell energy metabolism.<sup>(13,14)</sup>

Curcumin is one of the important compounds in turmeric plants. This compound has an antibacterial mechanism by inhibiting bacterial proliferation, damaging the cell walls and membranes and increasing the sensitivity of beta lactam antibiotics.<sup>(15)</sup> Essential oils can damage cell structure, causing increased permeability and decreased cell membrane integrity.<sup>(16)</sup> Tanin has the ability to damage bacterial cell walls, bind iron, which disrupts bacterial cell function.<sup>(17)</sup>

## CONCLUSION

The result of this study showed that *Curcuma domestica ethanol extract* has antibacterial effect against Group A *Streptococcus β hemolyticus* in concentration between 2500 – 5000 µg/ml.

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