Folia Medica Indonesiana Vol. 48 No. 4 October-December 2012 : 163-166

ANTIBACTERIAL ACTIVITY OF *KUNYIT* (*Curcuma longa*) LEAVES EXTRACT ON *Staphylococcus aureus* EXAMINED USING DILUTION METHOD

Usamah[1], Juniastuti[2], Ramadhani RB[3]
[1]Medical Doctor Program, [2]Department of Microbiology,
[3]Department of Pharmacology,Airlangga University, Surabaya

ABSTRAK

Infeksi yang disebabkan oleh Staphylococcus aureus adalah infeksi serius yang berhubungan dengan morbiditas dan mortalitas yang tinggi dan sering mengakibatkan infeksi metastatik seperti endokarditis infektif, yang memiliki dampak negatif pada hasil pasien. Kami penelitian pentingnya penggunaan antibiotik yang tepat untuk membunuh atau menghentikan perkembangan Staphylococcus aureus dengan ekstrak daun longa Curcuma. Secara tradisional, pengobatan pilihan untuk infeksi Staphylococcus aureus bergantung untuk sebagian besar pada kerentanan penisilin patogen. Namun, sebagian besar resisten dengan Penisilin aureus Staphylococcus. Curcuma longa ekstrak daun ekstrak sudah menguji sensitivitas terhadap kelompok Streptococcus A dan bekerja. Jadi kita penelitian Curcuma longa ekstrak daun untuk sensitivitas terhadap Staphylococcus aureus. Tujuan dari penelitian ini dilakukan untuk menguji aktivitas antibakteri Kunyit (Curcuma longa) ekstrak daun pada Pertumbuhan terhadap Staphylococcus aureus. Penelitian ini merupakan penelitian eksperimental. Spesimen adalah isolat Staphylococcus aureus yang diambil dari Departemen Mikrobiologi Fakultas Kedokteran Universitas Airlangga. Anti uji aktivitas bakteri dilakukan dengan menggunakan pengenceran Kunyit ekstrak daun dengan konsentrasi tertentu untuk mengetahui konsentrasi hambat minimum. Kemudian tabung keruh melesat di piring agar nutrisi untuk mengetahui konsentrasi bakterisida minimum. Hasil penelitian ini menunjukkan bahwa ekstrak daun Kunyit memiliki aktivitas antibakteri untuk Staphylococcus aureus. MIC Kunyit ekstrak daun tidak dapat dilihat karena warna ekstrak gelap dan MBC adalah 100%. Kesimpulannya, Kunyit ekstrak daun tidak cukup efektif lagi Staphylococcus aureus. ***(FMI 2012;48:163-166)***

Kata kunci: *endokarditis, Staphylococcus aureus, daun Kunyit, aktivitas antibakteri*

ABSTRACT

Infection caused by Staphylococcus aureus is a serious infection associated with high morbidity and mortality and often results in metastatic infections such as infective endocarditis, which have a negative impact on patient outcomes. We research the importance of the use of precise antibiotic to kill or stop the development of Staphylococcus aureus with the extract of Curcuma longa leaves. Traditionally, the treatment of choice for Staphylococcus aureus infection has depended to a large extent on the penicillin susceptibility of the pathogen. However, most of the Staphylococcus aureus are resistant with Penicillin. Curcuma longa leaves extract extract are already tested the sensitivity against Streptococcus group A and it works. So we research Curcuma longa leaves extract for sensitivity against Staphylococcus aureus. The purpose of this study was performed to test the antibacterial activity of Turmeric (Curcuma longa) leaves extract on The Growth against Staphylococcus aureus. This study was an experimental study. The specimen was the isolates of Staphylococcus aureus taken from Microbiology Department of Medical Faculty of Airlangga University. Anti bacterial activity test was performed using the dilution of Turmeric leaves extract in specified concentrations to know the minimum inhibitory concentration. Then the turbid tube streaked on Nutrient agar plate to know the minimum bactericidal concentration. The result of this research shows that Turmeric leaves extract has antibacterial activity to Staphylococcus aureus. The MIC of Turmeric leaves extract can't be seen because the extract color is dark and MBC is 100%. In conclusion, turmeric leaves extract is not effective enough again Staphylococcus aureus. ***(FMI 2012;48:163-166)***

Keywords: *Endocarditis, Staphylococcus aureus, Turmeric leaves, antibacterial activity*

Correspondence: Usamah, Medical Doctor Program, Faculty of Medicine, Airlangga University, Jalan Prof dr Moestopo 47, Surabaya 60131, Indonesia. Phone: 62-31-5020251

INTRODUCTION

Nosocomial infections are acquired infections in hospitalized patients in the hospital. *Staphylococcus aureus* is a major cause of nosocomial infections such. Every person infected with *Staphylococcus aureus* infection, although certain groups of people are at greater risk, including those with chronic conditions such as diabetes, cancer, vascular disease, eczema, foodborne and lung disease. In health, the risk of *Staphylococcus aureus* infection is higher because patients often have a decreased immune system (Centers for Disease Control and Prevention, 2011). In the United States, two million patients/year have

nosocomial infection by spending at least $ 4.1 billion - $ 11 billion (Klein et al 2007). In Indonesia, a study carried out in eleven hospitals in Jakarta in 2004 showed that 9.8% of inpatients received a new infection during treatment. Most of these patients were infected by *Staphylococcus aureus*. It was also reported that nosocomial infections caused by *Staphylococcus aureus* resulted in 88,000 patients die each year in the world (Wahid 2007). *Staphylococcus aureus* is also causing osteomyelitis, septic arthritis, skin infections, endocarditis and meningitis. More than 95% of patients with *Staphylococcus aureus* infections worldwide do not respond well to first-line antibiotics such as penicillin or ampicillin (Neu 1992). Therefore, other antibiotics are needed to reduce the threat of bacterial microbes.

Turmeric (*Curcuma longa*) as a tropical plant that grows in Indonesia is still not widely used. Traditionally use to treat *Staphylococcus aureus* infections has not been scientifically proven. The content of turmeric leaf extract contains flavonoids, essential oils, turmeron, atlanton and gingibaron. Polyphenols flavonoids are compounds having 15 carbon atoms consist of two benzene rings are linked together by a linear chain consisting of three carbon atoms. Polyphenols provide color in plants containing anti-oxidants also contains anti-allergic, thereby reducing the likelihood of allergies in humans. In addition, antibiotics are also contained in essential oils and flavonoids (Bhardwaj et al, 2011). Activity of leaf extract of turmeric have been done by the method of diffusion (Kirby-Bauer method) against four types include *Bacillus cereus* bacteria, *Streptococcus pneumoneae*, *Streptococcus pyogenes* and *Micrococcus glutamicus*. The results of this study revealed that all five species of leaf turmeric (Curcuma amada, *Curcuma longa*, Curcuma aromatic, caesia Curcuma, Curcuma zedoary) showed antioxidant activity and antibiotic activity (Bhardwaj et al, 2011). In this study, turmeric leaf extract to test the potential of antibiotics against *Staphylococcus aureus*. Testing the effects of turmeric leaf antibiotics against *Staphylococcus aureus* infection is an effort to use and innovation in the field of medicine. This study aims to determine the potential of turmeric leaf antibiotics on the growth of *Staphylococcus aureus* with dilution method.

MATERIALS AND METHODS

This study design was observational research laboratory to determine whether the leaf extract Turmeric (*Curcuma longa*) can inhibit the growth of *Staphylococcus aureus*, determine the minimal inhibitory concentration. *Staphylococcus aureus* isolates obtained from the Department of Microbiology, Faculty of Medicine, University of Airlangga. Extracts from the leaves of turmeric (*Curcuma longa*) is obtained by maceration. Turmeric leaf extract were tested potential antibiotics against *Staphylococcus aureus* with MIC dilution method and calculated her. The incubation temperature of turmeric leaf extract dilution test against *Staphylococcus aureus* is 37ºC with incubation time is 18-24 hours. Colonies were subcultured (aged 18-24 hours) on agar plates Nutrin (Oxoid), taken by ose (*sengkelit*) sterile 3-5 colonies made 5 ml suspension in a liquid medium with Muller Hinton 0.5 MacFarland turbidity. The suspension was divided into 10 sterile tubes, each tube filled with 0.5 ml. Preparation of the bacterial suspension was made 2 groups, 2 rows of tubes for the control (without treatment) and 8 rows of tubes for treatment. Each isolate was made 2 series and 8 series control treatment. After the suspension of 0.5 MacFarland equivalent dilution tested and ready for immediate treatment and direct observation without treatment. Turmeric leaves obtained from the garden house in Surabaya. Turmeric leaves (500 gr) is dried, finely powdered and soaked with a solution of 50% methanol. The extraction process is done by cold extraction is by maceration. The process is done by soaking the leaves of turmeric powder with methanol. Maceration process is done for 3x24 hours with solvent fluid replacement every day and while stirring once. The precipitate obtained was separated and then the filtrate was concentrated. Then the filtrate was separated from the solvent using a rotary evaporator (Bhardwaj et al 2011)

This study takes 8 treatment groups in the form of different levels of leaf extract so it can be calculated that the number of repetitions required. This study was conducted with three replications. Concentrations used in the study is a 100% tube, tube B 90% C 80% tube, tube D 70%, 60% E tube, tube F 50%, 25% G tube, tube H 12.5%, control (only contains extracts), tube A control (containing only the bacterial suspension). All tubes were incubated at 37 degrees C for 18-24 hours. After incubation we observed a clear tube to determine the Minimal Inhibitory Concentration (minimum concentration to inhibit the growth of bacteria). Data from the control group and the treatment group were processed and tested by descriptive statistics. Research conducted at the Laboratory of Microbiology, Faculty of Medicine, University of Airlangga. The study was conducted from August 2012 to December 2012.

RESULTS

Dilution test (minimum inhibitory concentration (MIC)) with the initial concentration of leaf extract of Turmeric

and cultivation on agar plates (Test kill minimum concentration (MBC)) showed in Table 1.

Table 1. Results of MIC and MBC dilution test with turmeric leaf extract in different concentrations on the first replication.

Replication	KHM	KBM
I	Not determined	100%
II	Not determined	100%
III	Not determined	100%
Mean	Not determined	100%

In the first experiment, the minimum inhibitory concentration of leaf extract Turmeric (*Curcuma longa*) can not be determined, because when extracts from the leaves of Turmeric (*Curcuma longa*) is mixed with a liquid medium Mueller Hinton, the mixture became turbid and therefore can not distinguish between an overgrown tube *Staphylococcus aureus* those who did not. Therefore, the determination of the minimum inhibitory concentration (MIC) can not be determined. While the control variables obtained turbidity change in control (+) which is the control of the growth of germs in the media and can not be determined changes in turbidity in the control (-) control material containing turmeric leaf extract as murky since mixing.

The same dilution test results are also obtained in the replication of the 2nd and 3rd where the outcome remains, tube 1 to tube 8 and controls (-) can not be determined because the mixture is cloudy from the first mixing. In the control tube (+) changes in turbidity of the original clear becomes cloudy. Because the research conducted there is difficulty in distinguishing turbidity obtained from extracts of materials and of the growth of bacteria. Therefore, in order to plate planting, cultivation on agar plates of this, we can also find bakteriosid effect of leaf extract Turmeric (*Curcuma longa*). In replication to-1, the planting of the tube 1-8 was on agar plates. Results on the tube 1 not found any bacterial growth on agar plates, while the tube 2-8 obtained the growth of bacteria on agar plates. The planting of control (+) obtained growth of bacteria colonies in agar plate. While the cultivation of the control plate (-) are not obtained the growth of bacteria colonies. In a replication of the 2nd and 3rd planting as in the first experiment, the planting of the tube 1 to tube 8, the second and third replication also obtained the same results as in the first trial, which began tube 2 is overgrown colonies *Staphylococcus aureus* . Thus the minimum kill concentration (MBC) of the leaf extract of Turmeric (*Curcuma longa*) is found on the tube to-1 (100%).

DISCUSSION

The survey results revealed that the Minimum Kill Concentration (MBC) of the leaf extract Turmeric (*Curcuma longa*) against Staphylococcus is 100% ie 1 ml of aqueous extracts of leaves Turmeric in 1 ml of solution (1 ml extract/ml). Although the Minimal Inhibitory Concentration (MIC) was observed with the research difficult, it can be ascertained that the antimicrobial effects of turmeric leaf extract against *S. aureus*.

According to CLSI, media dilution remains clear after incubation showed that bacterial growth is inhibited by the addition of turmeric leaf extract. However, in this study the MIC can not be known because the leaf extract of Turmeric has a murky color. This disturbed observation dilution test. Therefore, other methods should be considered in further research to determine the MIC value such that the dilution method, but the study was not used because this method requires a long time.

With the experiment three times, got the same result in the first experiment, second, and third is the MIC of turmeric leaf extract against *S. aureus* bacteria can not be determined in the dilution test. Therefore, the test results are grown in agar plate dilution showed turmeric leaf extract has a bactericidal effect at 100% concentration. This means that, at a concentration of 100% extract key has killed *S. aureus* bacteria that can not grow despite grown on agar plates.

In this study has proven that the leaf extract of Turmeric has a bactericidal effect at a concentration of 100%. This is probably due turmeric leaf extract has activities such as penicillin antibiotics that inhibit mucopeptide cell wall. While Penicillin is rarely used to treat bacterial infections due to *Staphylococcus aureus* has been a lot going on resistance. While antibiotics Penicillin is used to treat infections of Streptococcus group A and Group B Group (Zafar, Medscape). Therefore, turmeric leaf extract is less effective at inhibiting and killing of *Staphylococcus aureus* but still effective against the bacteria Streptococcus Group A and Group B.

CONCLUSION

Leaf extract of turmeric (*Curcuma longa*) has an antimicrobial effect against *Staphylococcus aureus* in minimum concentration of 100%.

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