Antibacterial Activity of Several Indonesian Endemic Plants against *Staphylococcus epidermidis, Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus*

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Abstract: In this current era, infectious diseases worldwide is increasing and due to misuse of antibiotics for treating infections eventually leads to the emergence of antibiotic resistant microbes. As a country with abundance of natural resources, Indonesia must be the forefront on research in finding new antibacterial candidates resourced from endemic medicinal plants. The objective of this research is to assesed the activities of several Indonesian endemic plants extract to inhibit several bacteria in-vitro by microdilutin method to obtain Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) and agar diffusion method to obtain inhibition zone. In this study, extract of *Syzigium aromaticum, Piper betle* and *Aleurites moluccana* were show anti bacterial activity against MRSA (Methicillin-resistant *Staphylococcus aureus*), *Staphylococcus aureus* and *Staphylococcus epidermidis.* On the other hand, extract of *Curucuma longa* and *Samanea saman* didnt show any anti bacterial properties. This study show the potency of several endemic plants extract to inhibit Staphylococcal bacteria.

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1 INTRODUCTION

Antimicrobial agents in today medicine play a very important role in treating infectious disease that was once fatal and incurable (Katzung B *et al*, 2012) In this modern age, there is a rising concern of antimicrobial resistance due to extensive and unregulated antimicrobial used in clinical setting by medical professionals (Fraise A P, 2002; Nikaido H, 2014). Consequently, more and more antimicrobial resistance is being reported and in addition, it is discovered that the highest rate of antimicrobial resistance is located in developing world (Nikaido H. 2014). Nevertheless, the demand of new novel effective anti-microbial to combat pathogenic microorganisms in clinical setting has increased (WHO, 2002).

Staphylococcus epidermidis is a gram-positive bacteria belonging to the coagulase-negative staphylococci group. This bacteria is a normal flora of human skin but also the most common cause of infection in the use of medical devices (WHO, 2002). Increased resistance of *S. epidermidis* to various antibiotics led to the treatment of nosocomial infections more difficult (Yuwono H, 2010). Based on a study conducted by Najar-Peerayeh *et al*, 2014, 92.2% of the 64 isolates of *S.epidermidis* have mecA genes that play a role in penicillin-binding expression protein (PBP2a) that decreases the affinity of the beta-lactam antibiotic. In addition, the ability of S. *epidermidis* to form biofilms makes this bacteria able to avoid the immune system and antibacterial drugs (Solati SM *et al*, 2015; Abidi *et al*, 2015).

Staphylococcus aureus is a bacteria that can cause various diseases because of the toxin it produces or direct invasion that damages the tissue (Murray PR *et al*, 2013). In early 1940, the infection caused by *S. aureus* was successfully treated with penicillin, a beta-lactam antimicrobial group, which rapidly replaced by the new resistant strain encodes a betalactamase enzyme. This new strain is actually can be resolved through the administration of new antimicrobial methicillin (Yuwono H, 2010) but in the early of 1980s, methicillin resistant *S. aureus*

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(MRSA) strains spread rapidly and alter the therapies available for *S. aureus* infection. In 2003, a new strain of MRSA caused outbreak of cutaneous infection and pneumonia.

Compared to other resistant bacteria, MRSA infection is epidemiologically significant. Studies conducted by CDC more than half Staphylococci bacteria ehich caused Hospital Acquired Infections are resistant to oxacillin (De Angelis G *et al*, 2010) and its infection nowadays is an endemic in US hospitals and communities (Klein E *et al*, 2007; Crum NF*et al*, 2006). Furthermore, MRSA in Cipto Mangukusumo Hospital, Indonesia also show an increase from 28.5% in 2009 to 32% in 2010 (Liana P., 2014).

Several research to find a novel anti Staphyococcal bacteria from plants is also progressing. Pradhan D *et al*, 2013 and Dwivedi V*et al*, 2014 reported *Piper betle* leaves and leaves extract have antimicrobial, anti-inflammatory, antioxidant and antiseptic properties. Specifically, *P. betle* shown to have antibacterial activity against *S. aureus*, *Streptococcus pyogenes*, *E. coli*, *Pseudomonas aeruginosa*, *Enterococcus fecalis*, *Klebsiella pneumoniae*, and others. The content of sterols in betel leaf extract interact with bacterial cell wall, disturbing its permeability.

Other plant that also potential to be an antibacterial are bark of candlenut (*Aleurites moluccana*) which is used traditionally for the treatment of diarrhea and thypoid fever (Alimboyoguen AB, *et al* 2014). Research shows 3acetyl aleuritolic acid from bark extract has an antimicrobial activity. Moluccanin from *A. moluccana* also has antibacterial including *S. aureus* and antiviral activity (Othman AS and Rasyidah MR, 2010).

Albizia saman (Jacq.) Merr. formerly known as *Samanea saman* is having several phytochemical components which are flavonoids, alkaloids, tannins, carbohydrates, glycosides, saponins, steroids, and reducing sugar are widely used as the remedy for colds, diarrhea, headache, and stomach ache. According to Perry in 1980, the alcoholic extract of *S. saman* is also proven to inhibit the growth of *Mycobacterium tuberculosis* (Kirithika T.2013).

The clove plant (*Syzygium aromaticum*) contain chemical compounds that provide its aromatic and antibacterial nature. The active compound being studied is eugenol, one of many phenolic compounds. Eugenol has been widely used in dental care settings, and has been proven as an effective anesthetic and antiseptic (Cortés-Rojas D*et al*, 2016; Neveu V*et al*, 2010).

With its promising properties as antibacterial and its abundancy worldwide, we assessed several concentration of *P. betle* leaves extract, *A. moluccana* stem bark extract, *S. saman* extract, *C. longa* extract and *S. aromaticum* flower bud extract against *S. epidermidis*, *S. aureus* and MRSA. As our result show that several extracts have a good potency as anti-staphylococcal infection.

2 MATERIAL AND METHODS

2.1 Bacteria, Medium and Extract

S. epidermidis, *S. aureus* and MRSA bacteria were grown in nutrient agar. All bacteria were from Microbiology Department culture collection, Faculty of Medicine Universitas Indonesia, which identified using commercial Vitex identification kit and tested for its resistancy according to CLSI. Broth Brain Heart Infusion (BHI) medium and Muller Hinton Agar (MHA) for antibacterial testing and Plate Count Agar (PCA) were provided by Department of Microbiology, Faculty of Medicine, Universitas Indonesia. Extracts of *P. betle* leaves, *A. moluccana* stem bark, *S. saman*, *C.longa* and *S. aromaticum* flower bud in ethanol were prepared by Medical Pharmacy Department, Faculty of Medicine, Universitas Indonesia. Antibiotic ciprofloxacin or clindamycin was used for positive control.

2.2 Antibacterial Assay

2.2.1 Agar Diffusion Method

An overnight bacteria culture was diluted into 0.9% NaCl to reach McFarland value of 0.5. Bacterial suspension was then applied into MHA followed by creating 7 diffusion wells in the media using blue tips. Each extract at several concentrations was applied in to the well which are : *P.betle* extract at 62,5; 125; 250; 500 and 1000 mg/ml was tested against *S. epidermidis* and *A.moluccana* extract at 50; 100; 200; 400 and 800 µg/ml against MRSA. With addition for aquadest and antibiotics at 20 µg/ml as negative and positive control respectively. Plate was then incubated for 16 – 18 hours at 37 ^{o}C. Observed inhibitory zone was measured using calipers.

2.2.2 Dilution Method

An overnight bacteria culture were diluted into BHI media followed by addition of final concentration of *S. saman* and *C. longa* extract at 12.5%, 6,25%, 3,125%, 1.563%, 0.782% and 0.391% meanwhile for *S. aromaticum* extract at 0.0488%, 0.0977%, 0.19530%, 0.3906%, 0.7813%, 1.563%, and 3.125% all against MRSA and non-MRSA. Aquadest and antibiotic at 20 µg/ml were used as negative and positive control respectively. Culture were then incubated for 18 – 24 hours at 37 ^{o}C.
MIC value defiened as the smallest extract concentration that inhibits bacteria growth in BHI media. Two cultures at higher and lower concentration of MIC value were smeared at PCA followed by incubation for 18 – 24 hours at 37 ^{o}C to obtain number of bacterial colony from the tested concentration. Lowest extract concentration giving smaller amount of 30 CFU/ml (colony forming unit) bacteria defined as MBC value.

3 RESULT AND DISCUSSION

Several plants extract tested in this study are briefly conclude in table 1. Extract of *C. longa, A. moluccana* and *S. saman* didn't show any antibacterial activity against *S. aureus* and MRSA by dilution method. Our result is contrary with several studies which show anti bacterial inhibition of *S.* saman and *C. longa* against several bacteria including *S. aureus* and MRSA due to lower extract concentration that we use in this study (Rita *et al*, 2013, Thippeswamy *et al*, 2011, Prasad *et al*, 2008, Bengmark *et al*, 2009 and Moghadamtousi *et al*, 2014, Othman *et al*, 2009). Obasi *et al*, 2011, found that tannin is one of phytochemical compund found in *S. saman* that can inhibit the growth of microorganism by precipitating the microbial protein needed for their growth, resulting in protein deprivation of the microorganism. Experiments performed by Ibrahim A, 2011 showed *A. moluccana* bark extract produced an antimicrobial effect against *Salmonella thyphii* and *Vibrio cholerae* but not tested against MRSA or other positive gram cocci bacteria.
Our research show the potency of *P. betle* leaf extract formed the inhibitory zone of *S. epidermidis* on MHA for all tested concentrations as listed in table 2. The results were greater than the positive control of ciprofloxacin which resulted in an average inhibitory zone diameter of 31.70 ± 0.94. Based on the Pearson correlation test, it was found that the concentration of *P. betle* leaf extract correlated with moderate (r = 0.642) to the large increase in inhibition zone diameter of *S. epidermidis* bacteria (Tumbelaka AR *et al*, 2011). Chakraborty, et al, 2011 tested metalloic leaf extract of *P. betle* against *S. aureus* and proved an increase in antibacterial activity assessed by measurement of inhibitory zone diameters along with increased concentration of extract (5 mg/ml, 10 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml).
Our result for *S. aromaticum* flower bud extract show anti *S. aureus* and MRSA with similar MIC and MBC value at 0.7813% and 0.3906% respectively. Interestingly, our result show inhibition and bactericidal value of *S. aromaticum* extract against MRSA is lower than *S. aureus* which may need further research. We hypothesize the effect of mecA and change in the cell wall structure of MRSA increased sensitivity towards eugenol, confirmed phenolic compund of *S. aromaticum* (Cortés-Rojas D*et al*, 2016; Neveu V*et al*, 2010).
A research on the effect of Indian spices on food borne pathogens showed that an aqueous extract of *S. aromaticum* has inhibitory activity against *S aureus* at 1% concentration, and complete bactericidal activity at 3% concentration (Sofia P et al, 2007). Another research compared aqueous clove extracts from Sri Lanka and Zanzibar, and its results suggested that the end-point of antimicrobial activity against *S aureus* is at 6.25% (Nzeako BC*et al*, 2006). These results suggest that multiple external factors influence the content and potency of the herb. These factors also influence the inhibitory and bactericidal effects. In this research, *S aureus* is inhibited at 0.7813%, which is much lower to other results.

4 CONCLUSION

Our study shows that extract from *Piper betle* and *Syzygium aromaticum* were found to have a good anti Staphylococcal activity which can be further analyed for purification and bacterial inhibition mechanism.

Table 1. Plant extract, antibacterial method and result

Plant extract	Antibacterial method	Bacterial tested	Inhibition
Curcuma longa	Dilution	*S. aureus*, MRSA	-
Aleurites moluccana	Diffusion	MRSA	-
Samanea saman	Dilution	*S. aureus*, MRSA	-
Piper betle	Diffusion	*S. epidermidis*	+
Syzigium aromaticum	Dilution	*S. aureus*, MRSA	+

Table 2. Inhibition zone of ciprofloxacin and *P.betle* leaf extract against *S. epidermidis*

Treatment	Concentration (mg/ml)	Average inhibition zone (nm) ± SD
Aquadest	-	0
Ciprofloxacin	5	31,70 ± 0,94
P. betle extract	1000	32,38 ± 2,63
P. betle extract	500	30,05 ± 1,38
P. betle extract	250	27,85 ± 1,91
P. betle extract	125	23,80 ± 2,1
P. betle extract	62,5	23,78 ± 0,47

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