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Short Communication: Inhibitory effect of *Sapindus rarak* ethyl acetate extract on *Staphylococcus aureus*

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Abstract. *Silviani S. 2017. Short Communication: Inhibitory effect of Sapindus rarak ethyl acetate extract on Staphylococcus aureus. Bioteknologi 14:1.* The research aimed to know the Minimum Inhibitory Concentration (MIC), the Minimum Killing Concentration (MKC) and the inhibitory fungal zone of *Sapindus rarak* - ethyl acetate extract on *Staphylococcus aureus*. The research used an experimental descriptive design by using controlled post-test approach by measuring the value of MIC, MKC and the inhibitory fungal zone. This research was performed in 2016 at the Bacteriology Laboratory of Akademi Analis Kesehatan Nasional (Academy of National Health Analyst) by using dilution method and diffusion disc. The result of this research showed that the value of MIC was not able to be concluded, the value of MKC was 80% and the greatest inhibitory effect zone achieved at 100% concentration was 10.3 mm. The ethyl acetate extract claimed medium to strong inhibitory effect on *Staphylococcus aureus*.

Keywords: Inhibitory effect, *Staphylococcus aureus*, ethyl acetate extract, MIC, MKC

INTRODUCTION

Indonesia is rich in natural resources which provide benefit for human health. *Sapindus rarak* (Sapindaceae), because of its chemical content like alkaloid, tannin, polyphenol, flavonoid, and saponin is believed to be a useful bio-resource with antibacterial potentialities (Silviani and Puspitaningrum 2015). The plant can used as batik washing material and is easily available in Indonesia.

Acne is a blockage of skin and hair oil gland channel marked by skin inflammation. (Rahmi et al. 2015). Acne is familiar to society, especially to teenagers, and might cause psychological problems (Uhlenhake et al. 2010). Eighty-five percent of acne cases belong to females of age 14-17 years and males of age 16-19 years (Hasan et al. 2015).

Staphylococcus aureus inflicts acne by invading hair follicle tissue, multipling and then causes necrosis (Razak et al. 2013). In some countries, the bacteria has been recognized to be resistant to penicillin group, including methicillin (Hilda et al. 2015). Khusnan et al. (2016) states that *S. aureus* is resistant to penicillin 78%, doxycycline 56%, gentamicin 26%, tetracycline 22%, erythromycin 13% and methicillin 9%. Morell and Balkin (2011) state that there is a higher resistance of *S. aureus* to antibiotic from 2% to 64% in 2004. Recently, natural bioresources are chosen by people as one of the alternatives to prevent resistance and minimize the side effect (Fatisa 2013).

Based on the above, this research was performed to explore the antibacterial properties of *S. rarak* against *S. aureus* base on the value of Minimum Inhibitory Concentration (MIC), Minimum Killing Concentration (MKC) and the diffusion disc.

MATERIALS AND METHODS

Materials

Materials used in this research were the fresh fruit of *Sapindus rarak*, obtained from Sky Argo Distributor in Solo. While the *S. aureus* isolate was obtained from agents with acne. This research was performed by using experimental analytical design and controlled post-test approach. *Quota sampling* was the technique chosen.

Extraction preparation

Brown *S. rarak* fruit with diameters of 2 cm were chosen. The fruit were then cleaned, seeds were taken out, and then dried. Dried *S. rarak* were meshed using a 20-mesh sieve and kept in a closed container (Samsuharto 2010).

Making ethyl acetate extract

Ethyl Acetate Extraction was done by using maceration method. Maceration is a process of extracting simplicia by shaking or stirring with ethyl acetate solvent in room-temperature for 5 days. Then, the extract was obtained after filtration and evaporation of ethyl acetate solution until a constant weight. The ethyl acetate extract was then tested by using qualitative phytochemical test.

Checking the Minimum Inhibitory Concentration

1 ml nutrient broth was added into 8 test tubes. Tubes 1-6 were set for concentration sample of 75% to 100% , tube 7 was set for positive control and tube 8 was for negative control. A suspension of 1 ml bacteria, adjusted to Mc Farland standard no 0.5 which had been diluted 100X,

was then added to each of the tubes, number 1-8. Tubes number 1-7 were filled with *S. rarak* ethyl acetate extract with varied concentration and tube number 8 was filled with *S. rarak* ethyl acetate extract of 100% concentration. All tubes were then incubated at 37°C for 24 hours. The turbidity of each tube was noted.

Checking the Minimum Killing Concentration

36 ose (1 mm diameter) of each tube was inoculated to a Nutrient Agar Plate media by streaking. All tubes were incubated at 37°C for 24 hours. The colony growth in every plate was noted.

Checking Diffusion Disc

The bacterial suspension was inoculated with density 1.0 x 10^8 CFU/ml into a Nutrient Agar plate evenly. Then the blank disc that had been filled with *S. rarak* ethyl acetate extract was set. All tubes were then incubated at 37°C for 24 hours (CLSI 2006) .

RESULTS AND DISCUSSION

The phytochemical test of ethyl acetate extract was shown in Table 1. Based on Table 1, it was confirmed that *S. rarak* - ethyl acetate extract has antibacterial properties as indicated by its secondary metabolites substances or active materials.

Table 2 revealed that the value of MIC could not be concluded because there was turbidity in *S. rarak* extract control, thus the turbidity in the concentration treatment might result from *S. rarak* - ethyl acetate extract.

Based on Figure 1, it is shown that increasing concentration of extracts formed a wider inhibitory effect zone. *S. aureus* is a gram-positive bacteria, coccus and grouped like grapes (Radji 2011). *S. aureus* has optimum growing temperature at 37^0C, best pigment formation takes place at 20-25^0C. The bacteria has a grayish to golden yellow, round-shaped, smooth, distinctive, and shiny colony (Jawetz 2008). *S. aureus* can be differed from other Staphylococcus based on mannitol fermentation test, coagulation and the pigment produced (Toele and Lenda 2008). Coagulation enzyme is pathogenic factor *S. aureus* (Andreasen 2008). *S. aureus* cell wall is composed of peptidoglycan which is a polysaccharide polymer. Peptidoglycan can be damaged by strong acid and lysozyme.

Sapnidus rarak consists of active compound, tannin, saponin, polyphenol and flavonoid. The result of qualitative Test (Table 1) shows that ethyl acetate extract contains all those active compounds, it is similar to the research performed by Silviani and Puspitaningrum (2015) which states that *S. rarak* contains polyphenol, tannin, saponin and flavonoid.

The tannin present in the extract gives antibacterial to *S. aureus* by disturbing the permeability of bacteria cell walls (Retnowati et al. 2011). Payne et all (2012) reported that tannic acid in tannin inhibited *S. aureus* biofilm formation. It can also inhibit the works of DNA enzyme topoisomerase and reverse transcriptase so that induces the death of bacteria cell (Amelia, 2015). Flavonoid is a phenol compound with a hydroxyl group, this compound has polar characteristics and works by forming an extracellular complex with bacteria protein so that it damaged bacteria cell wall (Bansode and Chavan 2012).

Alkaloid works as antibacterial substance by disturbing the forming layers of bacteria cell wall, especially on peptidoglycan site. (Paju 2013). The inhibition of the cell wall formation causes the death of bacteria. Tannin and flavonoid compounds are mostly drawn by using ethyl acetate solvent. Because ethyl acetate is a semi-polar solvent, tannin and flavonoid concentration are higher in ethyl acetate than in any other extracts (Tanaya et al. 2015).

Table 1. Phytochemical test on ethyl acetate extract of *S. rarak*

Active material	Result	Conclusion
Flavonoid	Red-yellow color	Positive
Alkaloid	Orange sediment after the addition of HCL & dragendroff	Positive
Saponin	Forming stable height of foam 1-3 cm for 10' after shaking	Positive
Tannin	Bluish green after the addition of water and $FeCl_3$	Positive
Polyphenol	Black-blue color after the addition of $FeCl_3$	Positive

Table 2. MIC and MKC ethyl acetate extract to *Staphylococcus aureus*

Concentration	MIC	MKC
100%	+	0
95%	+	0
90%	+	0
85%	+	0
80%	+	0
75%	+	1
Sapindus rarak control	+	0
Bacteria control	+	UC
Media control	-	0

Note: Uc = Uncountable, + = Turbidity,-= Non-Turbidity. MIC = Minimum Inhibitory Concentration, MKC = Minimum Killing Concentration

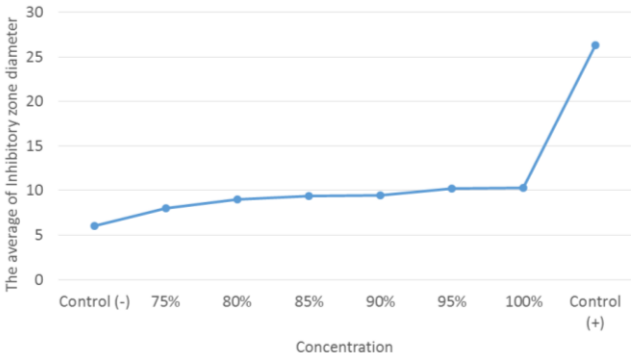

Figure 1. Average of inhibitory zone diameter of ethyl acetate extract to *Staphylococcus aureus*

The result of *S. rarak* - ethyl acetate extract diffusion disc to *S. aureus* (Figure 1) could not be compared to control (+), that is ciprofloxacin. As previously mentioned by Davis and Stouts (1971), antibacterial inhibitory level of natural materials can be classified into 4 categories. They are: weak if the inhibitory level is ≤ 5 mm, medium if the inhibitory level is 5-10 mm, strong if the inhibitory level is 10-19 mm, and very strong if the inhibitory level established is more than 20 mm. In this research, the inhibitory zone of ethyl acetate extract was medium to strong. 80% extract concentration resulted medium inhibitory level, 85%-100% concentration resulted strong inhibitory level.

The results support the earlier report by Marsa's (2010) which stated that *S. rarak* extract was able to inhibit the growing of *Enterococcus faecalis*, while Silviani and Puspitaningrum (2015) stated that both ethanol and ethyl acetate extracts were able to inhibit enteropathogenic *Escherichia coli* and enterotoxigenic *E. coli*.

Conclusions drawn from this research are: (i) MIC of ethyl acetate extract to *S. aureus* could not be concluded. (ii) MKC of ethyl acetate extract to *S. aureus* was 80%. (iii) The greatest Inhibitory Zone formed was 10.3 mm for 100% concentration. Some suggestion for further research including fractionation test and toxicity tests should be done on active compounds of any extracts.

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