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The Antibacterial Potential of Pineapple Core Extract (*Ananas comosus (L.) Merr*) Against Methicillin-resistant *Staphylococcus aureus* (MRSA)

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Staphylococcus aureus is one of major pathogens causes serious infection. Penicillin antibiotic is one of therapies against *Staphylococcus* infection. However, inadequate and irrational use of antibiotic causes resistance and emerges incidence of methicillin-resistant *Staphylococcus aureus* (MRSA). Herbal medicine from pineapple, especially from its core extract, is hopefully can reduce the incidence of antibiotics resistance because it contains bromelain, flavonoid, saponin, and tanin, which have antibacterial effect. This research was conducted to investigate the antibacterial potentiality of pineapple core extract against MRSA. This research is true experimental with post-test controlled group design. Pineapple core was extracted by maceration method. Pineapple core extract's concentrations used were 750, 500, 250, 187.5, 125, and 62.5 mg mL^{-1}. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by broth dilution test with five replications. Vancomycin was used as control group. MIC was determined visually by comparing turbidity of solutions after incubation at 37 ^{o}C for 24 h. Then these solutions were cultured on nutrient agar plates at 37 ^{o}C for 24 h. MBC was determined visually by inspecting the presence of bacterial colonies growth. The Minimum Inhibitory Concentration (MIC) could not be determined due to no turbidity changes. Vancomycin control cannot be used for determining MIC. Cultures on nutrient agar plates had no colonies growth in concentrations of 750 and 500 mg mL^{-1}. In summary, pineapple core extract has antibacterial potentiality against methicillin-resistant *Staphylococcus aureus* (MRSA) with MBC of 500 mg mL^{-1}.

Key words: antibacterial, dilution susceptibility test, methicillin-resistant *Staphylococcus aureus* (MRSA), pineapple core, vancomycin

Staphylococcus aureus merupakan salah satu patogen yang menyebabkan infeksi serius. Penisilin merupakan salah satu terapi infeksi *Staphylococcus*. Penggunaan antibiotika yang tidak tepat dan irrasional mengakibatkan resistensi dan munculnya *methicillin-resistant Staphylococcus aureus* (MRSA). Obat herbal dari nanas, terutama ekstrak dari bonggolnya, diharapkan dapat menurunkan insidensi resistensi antibiotika karena mengandung bromelain, flavonoid, saponin dan tanin, yang memiliki efek antibakteri. Penelitian ini dilakukan untuk menguji potensi antibakteri ekstrak bonggol nanas terhadap MRSA. Penelitian ini merupakan eksperimental murni dengan *post-test controlled group design*. Bonggol nanas diesktraksi dengan metode maserasi. Konsentrasi ekstrak bonggol nanas yang digunakan antara lain 750, 500, 250, 187,5, 125, dan 62,5 mg mL^{-1}. Konsentrasi Hambat Minimum (KHM) dan Konsentrasi Bunuh Minimum (KBM) ditentukan dengan uji dilusi sebanyak lima replikasi. Vancomycin digunakan sebagai kelompok kontrol. KHM diamati secara visual dengan membandingkan kekeruhan suspensi setelah inkubasi pada suhu 37 ^{o}C selama 24 jam. Tiap suspensi dikultur pada media agar nutrien pada suhu 37 ^{o}C selama 24 jam untuk melihat media yang tidak ada pertumbuhan koloni bakteri agar dapat menentukan KBM. Konsentrasi Hambat Minimum (KHM) tidak dapat diamati karena tidak ada perbedaan kekeruhan. Kontrol vancomycin tidak dapat digunakan untuk menentukan KHM. Kultur pada media agar nutrien menunjukkan tidak ada pertumbuhan koloni pada konsentrasi 750 dan 500 mg mL^{-1}. Ekstrak bonggol nanas memiliki potensi antibakteri terhadap *methicillin-resistant Staphylococcus aureus* (MRSA) dengan KBM 500 mg mL^{-1}.

Kata kunci: antibakteri, bonggol nanas, *methicillin-resistant Staphylococcus aureus* (MRSA), uji dilusi, vancomycin

Infectious disease is one of global health problems especially in tropical areas. *Staphyloccocus aureus* is one of the commonest and potentially dangerous human pathogens (Miller and Diep 2008). One of therapies against *Staphylococcus aureus* infection is using beta-lactam antibiotics. However, inadequate and irrational use of the antibiotics causes

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Staphylococcus aureus resistances against beta-lactam antibiotics and emerges incidence of methicillin-resistant *Staphylococcus aureus* (MRSA). Meta-analytical study by Tacconelli *et al*. (2008) shows correlation between frequency of antibiotic use and incidence of antibiotic resistance. Based on the research conducted by Jarvis *et al*. (2012) involving 67,412 hospitalized patients in the United States, the prevalence of MRSA colonization or infection is 66.4 per 1000 patients, as many as 61.8% has been colonized by MRSA and 38.2% were infected by MRSA. MRSA infects mostly the integument system and soft tissue, and is largely nosocomial infection. MRSA infection epidemiologically has higher morbidity and mortality that infection caused by methicillin-sensitive *Staphylococcus aureus* (MSSA) (Gordon and Lowy, 2008). In addition, decrease in incidence of MRSA infection would reduce the treatment cost (Rubin *et al.* 1999). Therefore, it is necessary to search for alternative medicines from natural materials to reduce the incidence of MRSA, one of them is pineapple core extract.

Pineapple is one of the commodities in Indonesia that has been consumed by many people. However, pineapple core is often discarded as waste, while Gautam *et al*. (2010) through experiments came to the conclusion that bromelain activity in pineapple stem tissue is higher than the activity of the enzyme in the pineapple fruit. Pineapple core contains proteolysis enzyme called bromelain that lyse bacterial cell wall (Ali *et al.* 2015). Besides, pineapple core also contains flavonoid, saponin, and tannin that have antibacterial effect.

MATERIALS AND METHODS

Extraction. Pineapple in the study was obtained from pineapple plantation in Ponggok District, Blitar Regency, East Java, Indonesia. The extraction method used was maceration method. A total of 400 grams of pineapple core powder was added 500 mL of ethanol 96% then homogenized at 120 rpm for one hour, then left for 24 h. After 24 h, the solution was filtered using a Buchner filter and repeated three times. The filtrate was evaporated by using a rotary evaporator at 70°C to obtain crude extracts.

Vancomycin Stock Preparation. One vial of the vancomycin 500 milligrams was dissolved with aqua pro injection solution of 10 mL obtained a concentration of 50 mg mL^{-1}. The solution is dissolved with serial dilution to obtain a concentration of 0.005 mg mL^{-1} or 5 µg mL^{-1}. The final step was making vancomycin stock with concentration of 2 µg mL^{-1} by dissolving 4 mL vancomycin 5 µg mL^{-1} in 6 mL aqua pro injection.

Methicillin-Resistant *Staphylococcus aureus* (MRSA) Preparation. Methicillin-resistant *Staphylococcus aureus* (MRSA) colony was obtained from Laboratory of Microbiology, Faculty of Medicine, Universitas Airlangga. MRSA culture with age of 24 h was taken amount of 0.1 mL 0.5 McFarland (1.5×10^8 CFU mL^{-1}) then placed in tube containing sterilized broth dilution.

Anti-bacterial Dilution Susceptibility Test. The method used in the research was broth dilution test with five replications. The first serial is pineapple core extract with concentration of 1500 mg mL^{-1} then diluted until obtained concentration of 375 mg mL^{-1}, but the tube with concentration of 750 mg mL^{-1} was discarded. The second serial is pineapple core with concentration of 1000 mg mL^{-1} then diluted until achieved concentration of 125 mg mL^{-1}. All the test tube was added 1 mL of inoculum thus obtained final concentration of 750, 500, 250, 187.5, 125, 62.5 mg mL^{-1}. The sterility control consists of 1 mL extract and 1 mL sterile medium, the growth control consists of 1 mL inoculum and 1 mL sterile medium, and antibiotic control consists of 1 mL vancomycin stock and 1 mL inoculum. Then, both test and control tubes were incubated at 37 °C for 24 h for determining the MIC. After observation, each suspension was streaked on nutrient agar medium then incubated at 37 °C for 24 h for determining the MBC.

RESULTS

Extraction. 400 grams of pineapple core powder was extracted with maceration method and obtained 150 grams of pineapple core crude extract that has characteristics of viscous and dark brown-colored. The contamination test was negative.

Anti-bacterial Dilution Susceptibility Test. Dilution susceptibility test was used for determining MIC was summarized in the Table 1. From the result, the MIC could not be determined because the extract's dark brown color and high turbidity interfere the interpretation.

After each suspension was cultured on nutrient agar plate, the result was summarized in the Table 2. The result showed no colonies growth in concentrations of 750 and 500 mg mL^{-1}. Thus, the MBC of pineapple core extract against the MRSA is 500 mg

Table 1 Dilution Susceptibility Test for Determining Minimum Inhibitory Concentration (MIC)

Extract Concentration (mg mL^{-1})	Observation Bacterial Growth in sterilized broth dilution				
	Replication 1	Replication 2	Replication 3	Replication 4	Replication 5
750	X	X	X	X	X
500	X	X	X	X	X
250	X	X	X	X	X
187.5	X	X	X	X	X
125	X	X	X	X	X
62.5	X	X	X	X	X
S-	X	X	X	X	X
G+	X	X	X	X	X
A	X	X	X	X	X

(Note: S- : sterility control, G+ : growth control, A : Vancomycin 2 μg mL^{-1}, X : cannot be assessed).

Table 2 Dilution Susceptibility Test Culture for Determining Minimum Bactericidal Concentration (MBC)

Extract Concentration (mg mL^{-1})	Bacterial Growth on Nutrient Agar Plates				
	Replication 1	Replication 2	Replication 3	Replication 4	Replication 5
750	-	-	-	-	-
500	-	-	-	-	-
250	-	+	+	+	+
187,5	+	+	+	+	+
125	+	+	+	+	+
62,5	+	+	+	+	+
S-	-	-	-	-	-
G+	+	+	+	+	+
A	+	+	+	+	+

(Note: S- : sterility control, G+ : growth control, A : Vancomycin 2 μg mL-1, + : viable growth, - : no viable growth).

mL^{-1}. Test tubes with concentration of 250 mg mL^{-1} could not be referred as the MBC because only killed the bacterial colonies only on the first replication, while the other four replications did not.

DISCUSSION

In this research, the MIC could not be determined because there was no significant difference of turbidity between before and after treatment. The use of vancomycin control was hopefully could be used to determine the MIC by comparing the bacterial colonies growth on nutrient agar plate between the control and test tubes. The dose of vancomycin used was based on literature study that vancomycin 2 μg mL^{-1} is the MIC of vancomycin against MRSA (Sakoulas *et al.* 2004). Unfortunately, there was no significant similarity of culture results between antibiotic control and test tubes thus the MIC still could not be determined. While the MBC in this research was 500 mg mL^{-1}. For further investigation especially for determining MIC, we can consider using agar dilution method or microdilution method.

The pineapple core extract used in this research is crude extract. Based on literature studies, pineapple core contains some antibacterial substances; they are bromelain, tannin, saponin, and flavonoid. Bromelain is proteolysis enzyme that disrupt the peptide bond on bacterial cell wall thus lyse the bacterial wall (Ali *et al.* 2015). Pineapple bromelain extract can inhibit *Streptococcus mutans* with concentration of 2 mg mL^{-1}, *Porphyromonas gingivalis* with concentration of 4.15 mg mL^{-1}, and *Aggregatibacter actinomycetemcomitans* with concentration of 16.6 mg mL^{-1} (Praveen *et al.* 2014). Besides, pineapple contains flavonoid that inhibits peptidoglycan synthesis on bacterial cell wall and induces protein denaturation on bacterial membrane cell (Eumkeb *et al.* 2012). Flavonoid also disrupts bacterial cell wall induces bacterial metabolites leakage and H^+ ions from flavonoid will react with polar groups so alters the phospholipid on membrane cell (Retnowati, 2011). Audies (2015) states that pineapple contains tannin, which is a phenol compound and works at the polypeptide wall of

bacteria causes shrinkage walls of bacteria. Based on other literature, pineapple core also contains saponins which can increase the permeability of the bacterial cell membrane so that it can alter the structure and function of membrane, disrupt the surface tension of the cell wall which allows the antibacterial substances enter easily into cells then interfere bacterial metabolism, and cause denaturation of proteins on bacterial membrane (Karlina *et al.* 2013). Retnowati (2011) also stated that the damage to the bacterial membrane cause the release of enzymes and metabolites resulting in decreased metabolism that decreases ATP production resulting in bacterial cell death. Purification of each substance from the crude extract may be considered for further investigation thus the mechanism and concentration of each substance for antibacterial against MRSA can be achieved.

In summary, pineapple core extract has antibacterial potentiality against methicillin-resistant *Staphylococcus aureus* (MRSA) with MBC of 500 mg mL^{-1}, while MIC can be determined in further investigation using agar dilution method or microdilution method.

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