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Antibacterial Activity of Honey Pineapple Peel Extract (*Ananas comosus* [L] Merr.) against Methicillin Resistant *Staphylococcus aureus*

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) infection is a global health problem that needs to be answered by developing a synthetic or natural antibacterial to overcome the problem. The honey pineapple peel contains potentially antibacterial compounds such as bromelain and flavonoid. This study aims to determine the antibacterial activity of honey pineapple peel extract (*Ananas comosus* [L] Merr.) against MRSA. The design of this study was a true experimental study with a post-test control group design. This study examined the antibacterial activity of honey pineapple peel extract at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.56% against MRSA bacteria by measuring minimum inhibitory concentration (MIC) with dilution method and minimum bactericidal concentration (MBC). The results of this study show that MIC was obtained at concentrations of 50 % and MBC at extract with a concentration of 100 %. There is an antibacterial activity of honey pineapple peel extract (*Ananas comosus* [L] Merr.) against MRSA.

Keywords: Antibacterial Activity, Honey Pineapple Peel, MRSA

INTRODUCTION

Infectious diseases are the cause of high morbidity and mortality.(1) One of the drugs to overcome this problem is antimicrobial, namely antibacterial or antibiotics. Antibiotics are the most widely used drugs for infectious diseases caused by bacteria. (2)

Along with the discovery of antibiotics, now many bacteria are experiencing antibiotic resistance. The main cause of antibiotic resistance is its widespread and irrational use, causing bacteria not to die completely, but somestill survive. The surviving bacteria can produce new resistant bacteria through three mechanisms, namely transformation, conjugation and transduction. Several antibiotic-resistant bacteria have been found throughout the world, including Penicillin-Resistant *Pneumococci*, Carbapenem-Resistant *Acinetobacter baumannii*, Multiresistant *Mycobacterium tuberculosis* and Methicillin-Resistant *Staphylococcus aureus* (MRSA).(3)

Infection of MRSA is listed as a high priority based on the WHO priority list for research and development of new antibiotics for antibiotic-resistant bacteria[4]. In 2016 in Southern Europe (Portugal, Spain, Italy, and Greece), these infections reached 25-50%. Several Asian countries have the highest prevalence of MRSA in the world. However, most available data comes only from high-income countries (e.g., Japan, South Korea and Singapore), while limited information comes from other countries. Japan and South Korea have a very high prevalence of MRSA, with >70%. While in Hongkong and Indonesia, there are about 28%.(5)

Vancomycin is the first-line antibiotic for the treatment of MRSA. However, it has a slow onset of bactericidal activity and is poor at penetrating some tissues[6]. Therefore, efforts are needed so that this problem does not continue to grow. These efforts include developing research related to resistance mechanisms, controlling the

use of antibiotics, and developing new antibacterial agents, both synthetic and natural. (7)

Indonesia is the fourth largest pineapple-producing country in the world after Costa Rica, the Philippines and Brazil, and the province of Lampung provides the largest contribution to pineapple production in Indonesia.(8,9) Pineapple skin contains a lot of flavonoids and bromelain.(10) Flavonoids can cause inhibition of nucleic acid synthesis. In addition, flavonoids also inhibit the energy metabolism of bacteria. Therefore, flavonoids are potential antibacterial components.(11) Bromelain is a proteolytic enzyme that can break down protein molecules. Bromelain can break protein bonds in bacteria so that it can inhibit bacterial growth.(12)

Based on research conducted by Omorotionmwan (2019), when compared with pineapple pulp extract, pineapple peel extract has a greater minimum inhibition against *Staphylococcus aureus, Streptococcus faecalis, Escherichia coli, Pseudomonas aeruginous* bacteria, which is 13-16 mm.(13) Another study stated that the activity, specificity and production of the bromelain enzyme were more in the pineapple skin than in the fruit and stem.(14)

Based on the description above, it can be seen that MRSA infection is a global health problem, so efforts are needed to overcome it. One of the efforts that can be made is to develop synthetic and natural antibacterial. From its content, it is expected that honey pineapple skin extract (*Ananas comosus* [L] Merr.) can provide inhibitory effects on MRSA. Therefore, this study looked at the effect of the antibacterial activity of honey pineapple peel extract (Ananas comosus [L] Merr.) on MRSA by measuring minimum inhibitory concentration (MIC) with dilution method and minimum bactericidal concentration (MBC).

METHODS

This type of research is a descriptive experimental study with a post-test control group design. The research was conducted at the Microbiology and Parasitology Laboratory, Faculty of Medicine, University of Lampung. Honey pineapple peel extraction (*Ananas comosus* [L] Merr) was conducted at the Laboratory of Organic Chemistry, Faculty of Mathematics and Natural Sciences (FMIPA), University of Lampung. The culture media used in this study were sheep blood agar, Mannitol Salt Agar (MSA) and Mueller Hinton Agar (MHA). In this study, the honey pineapple peel extract concentration was 100%, 50%, 25%, 12.5%, 6.25%, 3.125% and 1.56%. There was also treatment with vancomycin as a positive control and aquades as a negative control. To determine the number of repetitions in the study, the Federer formula was used, and the results were repeated 3 times.

Samples of 10 honey pineapples were peeled, and the skins were taken, cut about ± 0.5 cm thick, dried and then blended until coarse fibres were formed. The coarse fibre was weighed as much as 500 grams, then put into a 1 litre Erlenmeyer flask and 500 ml of 96% ethanol was added. Furthermore, the fibre was shaken for one hour to achieve homogeneous conditions in a water bath shaker at a speed of 120 rpm (rotation per minute). The solution was macerated for 24 hours at room temperature. Then, the solution was separated using a Buchner filter. The drying residue was aerated and re-macerated up to 3 times. The results were mixed and concentrated with a Rotary Vacuum Evaporator at 50ºC until a thick extract was obtained. This viscous extract has a concentration of 100%. (15) The honey pineapple peel extract formed will be diluted using sterile distilled water with concentrations of 1.56%, 3.125%, 6.25%, 12.5%, 25%, and 50%.

MRSA was obtained from the Department of Microbiology, Indonesia University. Identification of MRSA was carried out in the microbiology laboratory of the medical faculty of the University of Lampung using gram staining, catalase test, and MSA test. This test aims to identify Staphylococcus aureus bacteria. Furthermore, using the cefoxitin disk diffusion test, a susceptibility test was carried out to ensure that the bacteria used were resistant to methicillin.

The MIC was measured by the microdilution method. The inoculum was MRSA on Mueller Hinton Broth with turbidity of 1x108 CFU/ml or standard 0.5 McFarland. A total of 1 ml of inoculum was added to each tube containing 1 ml of honey pineapple peel extract with concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.56%. In addition, a positive control in the form of a 30 g vancomycin solution and aquadest as a negative control was also prepared. All tubes were incubated at 35ºC for 24 hours and then observed and compared with positive controls. MIC is the lowest concentration of extract that can inhibit the growth of bacteria in the tube, as seen from the clear liquid culture.(16,17)

To determine the MBC, the bacteria was planted into Mueller Hinton Agar media from all liquid seeds for the determination of MIC. Then incubated at 37°C for 24 hours. The smallest concentration with no growth of bacterial colonies in the media is considered MBC.(18)

This research was conducted based on ethical clearance from the Medical Research Ethics Commission of Medical Faculty, University of Lampung, with published number NO3204/UN26.18/PP.05.02.00/2019.

RESULT

Gram staining, catalase test and MSA test were performed to identify*Staphylococcus aureus* bacteria (table 1):

The result of the test bacteria was Staphylococcus aureus. Furthermore, the susceptibility test was carried out using the cefoxitin disk diffusion test. The results obtained an inhibition zone with a diameter of 13.95 mm (≤21 mm), which means the bacteria are resistant to the methicillin group of bacteria.(19) From the tests on bacteria that have been carried out, it can be concluded that the bacteria used were MRSA.

The MIC was carried out using the microdilution method, namely by looking at the smallest concentration of pineapple peel extract that was able to inhibit the growth of MRSA bacteria, as seen from the change in the turbidity of the solution in each tube. The results obtained are attached in Table 2.

Table 1. Identification of *Staphylococcus aureus*

No	Test	Observation	Result
1.	Gram Stain	There are purple colonies, coccus	Gram-positive
2.	Catalase test	Copious bubble produce	+ (there is *Staphylococcus sp*
3.	MSA test	There are yellow colony growth	+ (*Staphylococcus aureus* detected)

Table 2. Minimum Inhibitory Concentration (MIC) of honey pineapple extract *(Ananas comosus [L] Merr)* against *Methicillin-Resistant Staphylococcus aureus*

Repetition	Minimum Inhibitory Concentration (MIC)								
	100%	50%	25%	12,5%	6,25%	3,125%	1,56%	K(+)	K(-)
1	+	+	-	-	-	-	-	+	-
2	+	+	-	-	-	-	-	+	-
3	+	+	-	-	-	-	-	+	-

(+): clear liquid culture
(-): cloudy liquid culture
K(+): vancomycin
K(-): aquades

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The results of the MIC test showed clear liquid cultures in honey pineapple peel extract with concentrations of 100%, 50% and positive control in the first, second and third repetitions. The clear liquid culture showed that the extract could inhibit the growth of bacteria. While at concentrations of 25%, 12.5%, 6.25%, 3.125%, 1.56%, and the negative control culture were cloudy in each repetition. This indicates that there is no inhibition at that concentration.

MBC was measured by a subculture of the sample in liquid culture on the agar surface. Positive results indicate the absence of bacterial growth in the media, and negative when there is growth in the culture media. The results of the MBC test are presented in Table 3.

Table 3. Minimum Bactericidal Concentration (MBC) of honey pineapple extract *(Ananas comosus [L] Merr)* against Methicillin-Resistant *Staphylococcus aureus*

Repetition	Minimum Bacterial Concentration (MBC)			
	100%	50%	K(+)	K(-)
1	+	-	+	-
2	+	-	+	-
3	-	-	+	-

(+) : no colony
(-) : there is a colony
K(+) : vancomycin
K(-) : aquades

Based on the results of the MBC test, in the honey pineapple peel extract at 50% concentration in the first, second and third repetition. Bacterial colony growth was found at 100% concentration in the third repetition and in the negative control. While the pineapple peel extract concentration of 100% in the first and second repetitions and the positive control, no bacterial colony growth was found. The absence of bacterial colonies on the media indicates that the extract can kill bacteria. Due to no bacterial colony growth in two out of three repetitions of the 100% concentration of pineapple peel extract, we can conclud that the 100% concentration was the extract with the minimum killing concentration.

DISCUSSION

The MIC was obtained by the dilution method. The incubation results showed clear liquid cultures in honey pineapple peel extract with concentrations of 100%, 50% and positive control. This shows that pineapple peel extract with a concentration of 50% is the minimum inhibitory concentration in this study because it is the lowest concentration that can inhibit bacterial growth in liquid culture.

In a study by Wirhaningtyas et al. (2018), the MIC carried out by the dilution method of pineapple peel extract against *Staphylococcus aureus* was 1.56%.(20) In a study conducted by Punbasayakul et al. (2018), the MIC carried out by the pineapple peel extract dilution method against Staphylococcus aureus was 0.0084 g/ml. (10) In a study conducted by Loon et al. (2018), the minimum inhibitory concentration of pineapple extract carried out by the dilution method against *Staphylococcus aureus* was 1.56%.(21) Based on existing research, pineapple peel extract and pineapple peel against Staphylococcus aureus had a much smaller MIC compared to pineapple peel extract against MRSA. This is influenced by several factors. These factors include the dark color of the extract,

making it difficult to interpret based on clarity. In addition, bacterial colony isolates were made by manual method so that there is a risk of error in the determination of MIC.

To determine the MBC) the media that does not contain bacterial growth is considered as MBC.(18) The pineapple peel extract tested included concentrations of 100%, 50%, positive control and negative control. In this study, bacterial colonies were obtained in the pineapple peel extract with a concentration of 50%, a concentration of 100% in the third replication and the negative control. MBC is the smallest concentration with no bacterial colony growth in the media. There was no bacterial colony growth in two out of three repetitions of 100% concentration of pineapple peel extract, so we can conclude that 100% concentration is the MBC of pineapple peel extract against MRSA.

In a study conducted by Putra et al. (2016), pineapple hump extract was tested for its antibacterial activity against MRSA and obtained the minimum kill concentration at a concentration of 500mg/ml (50%). (22) This indicates that the MBC of pineapple hump extract to MRSA is lower than of pineapple peel extract to MRSA. So it can be said that pineapple hump has better bactericidal properties than pineapple peel against MRSA.

In a study by Omorotionmwan et al. (2019), pineapple peel extract had an MBC of 50% against *Staphylococcus aureus* bacteria[13]. In research conducted by Punbasayakul et al. (2018), the MBC of pineapple peel extract against Staphylococcus aureus bacteria is 0.0675 g/ml.(10) This indicates that the MBC of pineapple peel extract against *Staphylococcus aureus* is lower than that of pineapple peel extract against MRSA.

The results showed that the MBC concentration (100%) was greater than the MIC concentration (50%), or it could be said that the MBC concentration was twice as large as the MIC concentration. This is a natural thing to happen. Antimicrobials are said to be bactericidal if their MBC value is not more than four times the MIC.(23) As previously explained, pineapple peel contains flavonoids. Flavonoids consist of various components, named according to their chemical structure. The flavonoid components found in pineapple skin are gallic acid, epicatechin, catechin, and ferulic acid. (24) The flavonoid components epigallocatechin gallate, galanin, and 3-O-octanoyl-(+)-catechin have bactericidal activity. However, if 3-O-octanoyl-(+)-cathechin is induced by other components of flavonoids, it can cause flavonoids not to kill bacterial cells but only to inhibit their growth.(25)

CONCLUSION

Based on the result and discussion, we can conclude that the MIC of pineapple extract (*Ananas comosus* [L] Merr.), which is able to inhibit the growth of methicillin-resistant *Staphylococcus aureus* was a concentration of 50%. The minimum bactericidal concentration (MBC) of pineapple peel extract (*Ananas comosus* [L] Merr.) which is able to kill the growth of methicillin resistant *Staphylococcus aureus* is a concentration of 100%. So there is antibacterial activity of honey pineapple peel extract (*Ananas comosus* [L] Merr.) against MRSA.

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