In vitro Activity of Rambutan Binjai (*Nephelium lappaceum*) Peel Extract from Indonesia to Methicillin-Resistant *Staphylococcus aureus (MRSA)*

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(Received: July 17, 2018; Accepted: August 28, 2018; Published (web): December 10, 2018)

ABSTRACT: Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most common bacteria causing nosocomial infections with high levels of resistance to available antibiotics. So, it is necessary to search for new compounds to solve this problem. Various studies showed antibacterial activity of rambutan peel but for Rambutan Binjai peel extract that are from Indonesia has never been studied against the MRSA. This study aims to determine the antibacterial activity, the value of minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) using agar diffusion method. The concentration of rambutan peel ethanol extract at as much as 62.5 mg/ml showed the inhibitory diameter i.e 21.3 ± 2.4 mm. MIC and MBC were in the same range, which was between 0.98 (mg/ml) to 1.95 (mg/ml). The activity strength of tetracycline against the extract was at 1:50. This revealed that Rambutan Binjai peel extract had great potency as antibacterial agent to MRSA.

Key words: Methicillin-resistant *Staphylococcus aureus*, Rambutan peel extract, and Nosocomial infection.

INTRODUCTION

Nosocomial infection is an infection acquired during the treatment period or hospital admission process in the absence of signs of previous infection and minimal signs and symptoms 48 h after entry of germs.[1] The main bacteria that cause nosocomial infections are strains of *Staphylococcus aureus.*[2] *S. aureus* infection can generally be treated with penicillin-group antibiotics.[3] In 1940, cases of *S. aureus* bacterial resistance to antibiotics began to be discovered. Then in 1960 the bacterial strain Methicillin-resistant *Staphylococus aureus* (MRSA) was discovered. The spread of this bacterial population was increasing very rapidly in some countries and healthcare facilities.[4] Over the last 40 years, MRSA infections had become endemic in most American hospitals.[5] In 2013, it was known in Southeast Asia that the proportion of resistant *S. aureus* bacteria to methicillin was 10-26%.[6] The second-line antibiotics commonly used for treating *S. aureus* infections that are resistant to methicillin are vancomycin and teicoplanin.[7] However, both of these antibiotics are used intravenously so requiring special monitoring to avoid possible side effects. New treatments as an option for MRSA are also widely used i.e. linezolid (1970) and daptomycin (1980). Second-line drugs used to treat or prevent infectious diseases caused by MRSA are more expensive, and their side effects require reasonable monitoring.[6]

To overcome that problem, recently conducted research uses antibacterial compounds that are from plants. Biodiversity found in Indonesia is a distinct advantage that can provide various benefits as traditional medicine. Rambutan is a plant in the form of trees that grow a lot in the tropics. Consumption of rambutan is quite high, but so far only the flesh of

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Dhaka Univ. J. Pharm. Sci. **17**(2): 197-203, 2018 (December)
DOI: http://dx.doi.org/10.3329/dujps.v17i2.39176

fruit that is consumed, while the other rambutan parts, including the peel is not used and much wasted. Rambutan peel is known to be used traditionally as a medicine for fever and dysentery, but has not been widely proven by scientific research. Rambutan peel contains flavonoids and phenolics.[8] Rambutan peel extract is also known to inhibit the growth of *Escherichia coli, Shigella dysentriae, S. aureus* and MRSA bacteria.[8,9] Based on the antibacterial activity of rambutan peel extract, it is necessary to conduct research on the activity of Rambutan Binjai peel extract found in Indonesia. The study was conducted to determine its antibacterial activity against MRSA. Rambutan Binjai is a superior type of rambutan.

MATERIALS AND METHODS

Bacteria. MRSA used in this research was from the Laboratory of Clinical Patology Cipto Mangunkusumo Hospital in Jakarta, Indonesia as clinical isolate.

Preparation of simplicia and phytochemical screening. The processing of simplicia comprised sorting and simplifying the size of simplicia. Rambutan peel used was a slightly yellowish to dark red. Rambutan used in this research was Rambutan Binjai Indonesia obtained from Subang Regency, West Java. After being cut into smaller pieces, rambutan peel was washed, then left in open space until dry. The analysis of secondary metabolite contained in simplicia was performed by phytochemical screening of plants to find out the secondary metabolite group found in simplicia.[10]

Extraction process and quality analysis of rambutan binjai peel extract. Rambutan peel simplicia was reduced in size with a blender. A total of 501.52 g of rambutan simplicia was macerated with 3L ethanol (96%). The maceration process was done until completely submerged for 3 × 24 hrs. Each 1 × 24 hrs macerate was collected and re-macerated with new ethanol while occasionally stirring.[11] The gained macerate was then accommodated and concentrated with a rotary evaporator until a viscous extract was obtained. Then, the analysis of quality parameters of the extract included organoleptic, species weight, water content, and TLC profile. The water content was determined using distillation method. A total of 10 g extract and some boiling stones were put into a distillation flask. A total of 200 ml of saturated toluene was put into a flask and heated for 15 min. After all the water was distilled, the storage tube was cooled to room temperature and water volume was measured.[11] For TLC profiles chloroform : acetone : methanol : acetic acid (60 : 30 : 10 : 2) solvent was used, then the extract was put onto the silica gel GF_{254} plate as a dot several times using capillary pipe. After that, the result was observed at 254 nm and 366 nm UV, and using cross-section of $FeCl_3$ and $AlCl_3$ spots.

Assay of antibacterial activity of rambutan binjai peel extract. MRSA used was previously assayed by resistance test using ampicillin, oxacillin, amoxicillin and methicillin. Then, the assay of antibacterial activity was performed by agar diffusion method. The stages of assay were sterilization of Mueller Hinton Agar (MHA) medium, manufacturing suspension Mac Farland standard 0.5 and MRSA suspension.[12] A total of 20 µl of bacterial suspension was mixed with 20 ml of MHA that was still melting, then homogenized. To be perforated with a 0.7 cm perforator of 4 holes, then dropped 50 µl of rambutan peel extract solution using DMSO with a concentration of 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/m, in each hole. After that it was incubated for 12-24 h at 37°C aerobically. The inhibit zone formed was measured using a calipers.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The extract was put into MHA media in petri dish to obtain the extract concentration 31.25, 15.62, 7.81, 3.91, 1.95 and 0.98 mg/ml. After the MHA media containing the extract was solid then as much as 10 µl of bacterial suspension (1×10^4 CFU/ml) was spread over the surface of the MHA media. The value of MIC was obtained by observing bacterial growth after 24 h incubation. Then value of MBC was determined by observing bacterial growth after 48 hrs.[13]

Equality test of extract activity against tetracycline. The bacterial suspension was mixed with 50 ml of melted MHA, then homogenized. Making holes in the media using perforator with the bored size 0.7 cm (diameter). Then, 50 µl rambutan peel extract solution at concentrations of 100, 50, and 25 mg/ml was dropped in each hole. At the same media, tetracycline solutions of 2500, 1250, 625, and 312.5 µg/ml were also dropped as much as 50 µl into different holes. After that, media was incubated for 12-24 hrs at 37°C aerobically. The diameter of zone of inhibition was measured using calipers.

RESULTS AND DISCUSSION

Based on the results of phytochemical screening, it was known that Rambutan Binjai peel extract contains flavonoid, polyphenol, tannin, monoterpenoid, sesquiterpenoid and saponin compound (Table 1). The results of this phytochemical screening were slightly different from the test previously performed whereas on alkaloid testing result of this extract was negative.[10] This can happen because the origin of Rambutan used was different. One of the factors that affect the differences in the chemical composition of a compound is the condition of the growth environment such as climate, growing media, and altitude.[14] Different sources and types of rambutan produce different secondary metabolites as well.

Table 1. Phytochemical screening result of simplicia and fruit extract of rambutan binjai peel.

No.	Compounds	Simplicia	Extract
1	Alkaloids	-	-
2	Flavonoids	+	+
3	Poyphenols	+	+
4	Tannins	+	+
5	Monoterpenoids and sesquiterpenoids	+	+
6	Steroids and triterpenoids	-	-
7	Quinone	-	-
8	Saponine	+	+

Note, (+) = detected, (-) = not detected

The condensed extract obtained was 114.84 g, so the yield of extract can be calculated, that was 22.9%. The yield of extract produced from Rambutan Binjai peel depends on various factors such as climate, soil fertility, plant age, and extraction method. For organoleptic examination Rambutan Binjai peel extract had a thick consistency, dark brown, weak odor, and bitter taste. This qualitative observation was performed to know the typical physical properties by observing the shape, color, smell and taste. The density of thick extract of Rambutan Binjai peel was 1.805 g/ml. The density parameter of the extract type is a parameter that indicates the specification of the test extract. This parameter is important due to the density of the type of extract depends on the amount and type of component of a substance dissolved in solvent.[15] The water content of extract was 5.125% (<10%). This result meets the requirement and indicate that the extract is not easily overgrown with fungi.[16] Water content that is too high in the extract can accelerate the growth of microbes in the extract, so it can affect the test results of antibacterial activity. TLC pattern determination of rambutan peel ethanol extract has been shown in Figure 1 and table 2. At a wavelength of 366 nm was seen a fluorescence yellow spot with Rf 0.375. The separated compound was predicted flavonoid due to its fluorescence was getting stronger after sprayed with $AlCl_3$. In the other plate sprayed with $FeCl_3$ was seen dark blue spots with R_f 0.675. This compound was predicted as polyphenolic compound. TLC pattern of rambutan peel extract showed that mobile phase of chloroform: acetone: methanol: acetic acid in the ratio of 60: 30: 10: 2 used has not been able to optimally separate the metabolites. This can be seen from the number of spots that appear only four spots.

Result of resistance assay of bacteria used indicated that the *S. aureus* bacteria was completely resistant to penicillin-group antibiotics (Table 3). From the results of antibacterial activity of rambutan peel extract, it was known that extract had the ability to inhibit the growth and kill bacteria. This was performed from the clear zone formed on the MHA medium. The higher the concentration of extract used, the larges was the inhibition zone diameter

(Table 4). Secondary metabolites suspected to have antibacterial activity in rambutan peel were ellagic acid, corilagin, and geraniin as compounds of ellagitanin group.[17] Antibacterial activity of rambutan peel had been widely tested. The peel of rambutan was known to inhibit the growth of *Staphylococcus aureus*, *Staph. epidermidis*, *Salmonella thypi*, *Enterococcus faecalis* and *Streptococcus mutans.*[4,9,17]

The value of MIC and MBC of rambutan peel extract on MRSA was in the same concentration range i.e 0.98-1.95 mg/ml (Table 5). This showed that the ethanol extract of rambutan peel was bactericidal as the concentration difference between MIC and MBC was very narrow. Plant extracts that can be said to be potential inhibitors of bacterial growth or have good antibacterial activity are extracts that have less than 100 mg/mL of MIC, moderate 100-500 mg/ml, weak 500 -1000 mg/ml.[18] Then the extract is said to be inactive if the MIC value is more than 1000 mg/ml. The rambutan peel ethanol extract had MIC values between 0.98-1.95 mg/ml to be categorized as potential inhibitor (its MIC value<100 mg/ml). It can be said that the Rambutan Binjai peel extract was very potentially active as antibacterial MRSA[18,19] In the previous study there was activity from Thailand's original rambutanpericarp extract as much as 0.4 mg/ml against MRSA.[9] Otherwisethe

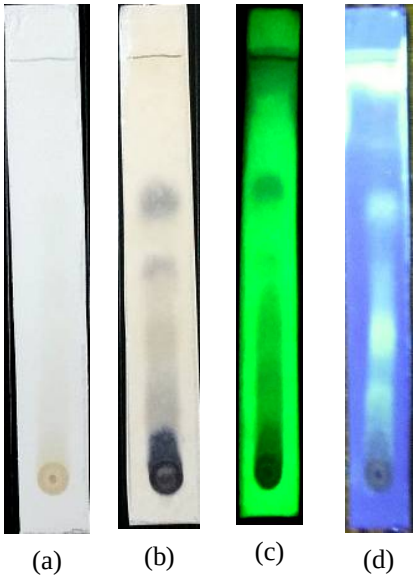

Figure 1. Thin layaer chromatogram pattern of rambutan peel extract on silica gel GF_{254} at (a) visible light (b) visible light with $FeCl_3$ reagent (c) UV light 254 nm (d) UV light 366 nm UV with $AlCl_3$ reagent

Table 2. Thin Layer chromatogram pattern of rambutan peel extract using chloroform : acetone : methanol : acetic acid (60 : 30 : 10 : 2) as moble phase.

spot	Rf	Observation result			
		Visible light	$FeCl_3$	UV 254 nm	$AlCl_3$ + UV 366 nm
1	0.175	-	black	brown	light yellow
2	0.375	-	black	brown	yellowish blue
3	0.500	-	black	-	-
4	0.675	-	blue black	brown	light yellow

Table 3. Result of resistance assay of bacteria used.

Antibiotic	Concentration (µg/ml)	Inhibition zone (mm)	Break point (R <)	Note
Ampicillin	10	0	28	Resistant
Oxacillin	5	0	10	Resistant
Amoxicillin	25	0	28	Resistant
Methicillin	5	0	9	Resistant

Resistance test was conducted by CLSI (2016).

Table 4. Antibacterial activity of rambutan peel extract against MRSA.

Extract concentration (mg/mL)	Inhibition zone (mm)				Positive control	Negative control
	I	II	III	Average		
500	29.5	27.8	28.3	28.5±0.87	+	-
250	25.2	26.1	23.5	24.9±1.07		
125	22.3	25.4	23.1	23.6±1.6		
62.5	19.3	21.5	16.7	21.3±2.4		

(+) bacteria grow, (-) no bacteria grow (n=3).

activity of Rambutan Binjai peel extract was still lower compared with other ethanolic extracts of *Garcinia mangostana, Punicagranatum* and *Quercus infectoria*, with MICs for MRSA isolates of 0.05-0.4, 0.2-0.4 and 0.2-0.4 mg/ml.[20] Activity of extract of *Piper betle* and *Zanthoxylum clavaherculis* was also higher than that of Rambutan Binjai peel extract i.e. at 0.078 mg/ml and 0.032 mg/ml.[21,22] While activity of Rambutan Binjai peel extract was almost the same with that plant *Camellia sinensis* activity against MRSA i.e at 0.85 mg/ml.[23]

Table 5. Minimum inhibitory concentration and minimum bactericidal concentration of rambutan peel extract against methicillin resistant *Staphylococcus aureus.*

Extract concentration (mg/ml)	Incubation 24 hrs	Incubation 48 hrs
31.25	-	-
15.62	-	-
7.81	-	-
3.91	-	-
1.95	-	-
0.98	+	+

(+) bacteria grow, (-) no bacteria grow (n=3).

The result of determination of equality test of Rambutan Binjai peel extract against tetracycline can be seen at figures 3-4 and table 6. Equation of straight line of tetracycline antibacterial activity to MRSA i.e. y = 9,1196x - 9,7084 with correlation coefficient value equal to 0,9963. From the correlation coefficient value it can be seen that there was a close correlation and good linearity between the concentration of tetracycline solution with the resulting inhibitory diameter 0.9 < R2 < 1. Based on the line equation, the equal value of Rambutan Binjai peel extract on tetracycline against MRSA was 1: 0.02. It means to produce the same inhibitory diameter, 1 part tetracycline was proportional to 50 parts extract. This showed that the strength of extract activity on MRSA was quite good. The activity of Rambutan Binjai (Indonesia) peel extract is very potential as an antibacterial to MRSA.

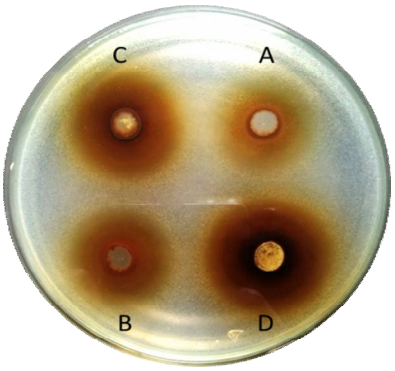

Figure 2. Antibacterial activity of rambutan peel extract with concentration 62.5 mg/ml (A), 125 mg/ml (B), 250 mg/ml (C) and 500 mg/ml (D) against methicillin resistant *Staphylococcus aureus*.

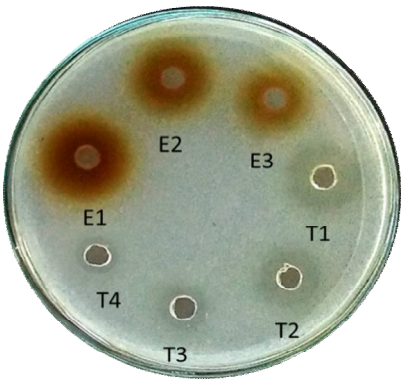

Figure 3. Equality test of extract activity against tetracycline with concentration of extract 100,000 µg/ml (E1), 50,000 µg/ml (E2), 25,000 µg/ml (E3) and concentration of tetracycline 2500 µg/ml (T1), 1250 µg/ml (T2), 625 µg/ml (T3), 312.5 µg/ml (T4) against methicillin resistant *Staphylococcus aureus*

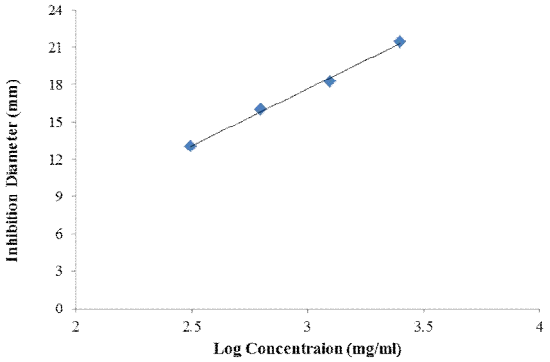

Figure 4. Tetracycline antibacterial activity against Methicillin Resistant *Staphylococcus aureus* with equation y = 9,1196x – 9,7084r^2 = 0,9963

Table 6. Measurement and calculation result of equality test of extract activity against tetracycline to methicillin resistant *Staphylococcus aureus*.

Antibacterial agents	Concentration C (µg/ml)	Log C	Inhibition diameter (mm)
Extract of	100,000	5.000	23.4
Rambutan peel	50,000	4.699	17.8
	25,000	4.398	15.9
Tetracycline	2,500	3.398	21.4
	1,250	3.097	18.2
	625	2.796	16
	312.5	2.495	13

ACKNOWLEDGEMENT

Thanks to the Clinical Pathology Laboratory of Cipto Mangunkusumo Hospital which has provided clinical isolates of MRSA

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