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RESEARCH ARTICLE

Antibacterial activity of ethanol extract and fraction of Rambutan leaf (*Nephelium lappaceum*) against *Pseudomonas aeruginosa* multiresistant

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ABSTRACT

Background: Nosocomial infection is a hospital-acquired infection that can cause various severe diseases. The main bacteria that causes nosocomial infection is *Pseudomonas aeruginosa* multiresistant (PAMR). The Rambutan leaf (*Nephelium lappaceum*) is known to have antibacterial activity because it is suspected to have the content of flavonoids, polyphenols, and tannins. **Aims and Objectives:** This research was conducted to determine the antibacterial activity, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of the extract and the most active fraction. **Materials and Methods:** The research was started by extraction and then fractinationed by liquid-liquid extraction to obtain ethyl acetate, water, and n-hexane fraction. After that, antibacterial activity was carried out by the agar diffusion method, and the value of MIC and MBC was determined by the microdilution method. **Results:** The result showed that ethanol extract, ethyl acetate fraction, and water fraction of the Rambutan leaf had antibacterial activity against PAMR, of which the ethyl acetate fraction was the most active fraction. The value of MIC and MBC of the extract and ethyl acetate fraction was in a concentration 2.5% w/v and 5% w/v against PAMR. **Conclusions:** The antibacterial activity of this extract and fractions against PAMR was probably derived from flavonoids, polyphenols, saponins, and tannins.

KEY WORDS: Nosocomial Infection; *Nephelium lappaceum*; Antibacterial; *Pseudomonas aeruginosa Multiresistant*

INTRODUCTION

Nosocomial infection is hospital-acquired infection that can cause various severe disease, such as pneumonia, urinary tract infections, gastroenteritis, and puerperal sepsis.[1] This can occur due to the lack of hygiene in the hospital environment. Nosocomial infections can be caused by bacteria, viruses, and fungi.[2] One of the main bacteria that had resistance to antibiotics on nosocomial infections is *Pseudomonas aeruginosa*.[1]

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P. aeruginosa multiresistant (PAMR) is *P. aeruginosa,* which has been resistant to various antibiotics, such as seftazidime of 34.7%, sefepime 27.7%, piperasilin-tazobactam 36.9%, imipenem 27.2%, and ciprofloxacin 30.1%.[3,4]

Antibiotic resistance can occur due to the use of antibiotics that are not rational. Clinical treatment using broad-spectrum antibiotics regularly is a common factor causing changes in patterns of bacterial infections and the patterns of bacterial resistance to various antibiotics. Antibiotic resistance leads to increase mortality and morbidity in hospitals. To overcome this, it is necessary to research the antibacterial activity of the active compounds contained in the natural materials.

Indonesia is an archipelagic country with a variety of biodiversity. Various kinds of plants can thrive in Indonesia. One of the plants that is commonly found is Rambutan (*Nephelium lappaceum* L.). Conventionally, people use

Rambutan plants for the treatment of various diseases.[5] There has been much research to determine the antibacterial activity of Rambutan plants.

Research that was conducted by Thitilertdecha *et al*.[6] showed that the methanol extract of the Rambutan peel has antibacterial activity against Gram-negative bacteria such as *P. aeruginosa* and Gram-positive bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis*, and *Enterococcus faecalis.* According to the study of Rajasekaran *et al*.,[7] methanol extract of the Rambutan seed has antibacterial activity against *S. epidermidis* when the minimum inhibitory concentration (MIC) value is 40 g/mL. According to the study of Sekar *et al*.,[8] the crude extract of the yellow Rambutan peel potential as antimicrobial activity against Gram-positive bacteria, *Streptococcus pyogenes* and *S. aureus*. According to the study of Selvia *et al*.,[9] the extract of the Rambutan peel has antibacterial activity against *Escherichia coli* and *S. aureus* when the MIC value is 0.5%. According to the study of Maradona,[10] the Rambutan leaf ethanol extract (*N. lappaceum* Linn) has antibacterial activity against *S. aureus* ATCC 25925 and contains secondary metabolites such as flavonoids, saponins, tannins, and hydroquinone. Wisnuputri[11] concluded that the ethanol extract of the Rambutan peel has antibacterial against methicillin-resistant *S. aureus*.

MATERIALS AND METHODS

Preparation of Plant Extract

Rambutan leaves (*N. lappaceum*) were obtained from Subang and processed in Lembang Bandung. Determination of the Rambutan leaf was carried out in Taxonomy Laboratory, Faculty of Biology, Universitas Padjadjaran.

Preparation of Bacteria

Clinical isolates of PAMR was obtained from the Microbiology Laboratory of Indonesia University.

Bacterial Growth Medium

Mueller-Hinton Agar (MHA) was used as media for culturing bacteria (Oxoid, Basingstoke, UK).

Phytochemical Screening

Simplicia and extract were subjected to preliminary phytochemical qualitative screening for the presence and absence of various secondary metabolites.

Extraction Procedure

Simplicia of Rambutan leaves (500 g) was saturated in ethanol 70% for 24 h thrice. Then, the macerat was concentrated using rotatory evaporator at 60°C and reassembled over the water bath at 50°C till a thick extract was obtained.

Fractionation Procedure

Liquid-liquid extraction was used as the method for fractionation. A thick extract of the Rambutan leaf (20 g) was dissolved in 100 mL aquades. The extract solution was inserted into a separating funnel, and then, the n-hexane solvent was added with a volume ratio of 1:1 with the aquades. Furthermore, it was shaken for a while until the two solutions were mixed into one phase. The mixture was allowed to remain until the water phase and the n-hexane phase were separated into two phases. The n-hexane phase was then accommodated in a beaker glass. This process was repeated 3 times until the n-hexane phase was almost clear. The fractionation was continued by the addition of ethyl acetate into the water phase using a separating funnel, as much as 100 mL or in 1:1 ratio. The same procedure was performed as the n-hexane fraction. Then, each fraction was using a rotary evaporator and water bath until the viscous fractions of water, ethyl acetate, and n-hexane fractions were obtained.

Antibacterial Activity Tested

Agar diffusion with a perforation technique was used as the method for antibacterial activity tested. The extract and fractions were dissolved with dimethyl sulfoxide (DMSO) 3%. The concentrations of the extract and fraction for antibacterial activity test were 5, 10, 20, and 40%. The bacterial suspension was fed into a sterile petri dish, then 20 mL of MHA was added and shaken gently until homogeneous. After the media solidified, holes were made using a perforator aseptically. The soluble extract or fractions were inserted into the holes using a micropipette. After that, it was incubated in an incubator at 37°C for 18–24 h. The antibacterial activity of extract and fractions were shown by a clear zone around the hole that was caused by inhibition of bacterial growth by extract and fractions.[12]

Determination of MIC and Minimum Bactericidal Concentration (MBC)

The microdilution method was used to determine the MIC and MBC value from extract and the most active fraction. The first well of microplate was negative control (only filled 100 µL Mueller-Hinton Broth (MHB), and the second well was an extract or fraction control (50 µL extract or fraction and 50 µL MHB). From the third well until the twelfth well, it was filled with 100 µL MHB, and then, 100 µL extract or the most active fraction were fed into the third well. Then, the extract or the most active fractions were diluted from the third well till the tenth well by piped up 100 µL extract or fraction from the third well to the fourth well and from the fourth well to the fifth well and so on, until the tenth well.

Hence, the third well contained the highest concentration of extract or fraction, and the tenth well was contained the lowest concentration of extract or fraction. After that, 10 µL bacterial suspension was fed into the third until the tenth well.[13]

The eleventh well was the control of DMSO 3% (50 µL MHB, 50 µL DMSO 3%, and 10 µL bacteria suspension). The twelfth well was positive control (100 µL MHB and 10 µL bacteria suspension). Microplate was covered by cellophane plastic and incubated at 37°C for 18–24 h. After that, a subculture from each microdilution well was incorporated into the MHA agar plates and dispersed using a spreader. Then, it was incubated at 37°C for 18–24 h. The MIC value was determined from the lowest concentration of extract or fraction that showed the growth of bacteria. While the MBC value was determined from the culture that did not show the growth of the bacteria.

RESULT

Phytochemical Analysis

Extraction

From extraction, 500 g dried simplicia of Rambutan leaves were obtained 116.97 g thick extract with rendement value 23.394%. The thick extract has rubbery and hard consistency, brownish black, typical smell, and bitter.

Fractionation

From 20 g of viscous extract, three fractions were obtained, i.e., 8.98 g of water fraction with rendement 44.9% (w/w), 0.18 g of n-hexane fraction with rendement 0.9% (w/w), and ethyl acetate fraction as much as 5.53 g with Randement 27.65% (w/w). Organoleptic observation of the three fractions showed that the water fraction was sticky and brown viscous liquid, the n-hexane fraction was thick and green, and the fraction of ethyl acetate, viscous liquid, rather hard and shiny black green color [Table 1].

Antibacterial Activity Tested

The antibacterial activity test was performed using agar diffusion method with perforation technique. Antibacterial activity can be observed from the clear zone formed and measured using calipers.

The results showed that Rambutan leaf extract had antibacterial activity against PAMR starting at concentrations of 5% w/v, 10% w/v, 20% w/v, and 40% w/v [Table 2].

The results showed that the fraction of ethyl acetate and water fraction of Rambutan leaves gave antibacterial activity against PAMR. While the n-hexane fraction did not have antibacterial activity against PAMR [Table 3].

MIC and MBC Value

The results showed that ethyl acetate fraction has the most to inhibit PAMR. PAMR can still grow in extracts and ethyl acetate fraction of the Rambutan leaf with a concentration of 2.5% w/v. Therefore, the concentration of 2.5% w/v was defined as the MIC value for the extract and ethyl acetate fraction of the Rambutan leaf against PAMR. While the MBC value was at concentration of 5% w/v [Table 4].

DISCUSSION

Phytochemical analysis carried to determine of secondary metabolites. The phytochemical screening from simplicia and extract Rambutan leaf contained the presence of some secondary metabolites such as flavonoid, polyphenol, tannins, saponins, monoterpens, sesquiterpens as shown in Table 1. Flavonoid and polyphenols are natural compounds that have antibacterial activity because that compounds from phenolic compounds.[14] The antibacterial activity of this extract and fraction was probably derived from flavonoids and polyphenols.

The extract, ethyl acetate fraction, and water fraction have antibacterial activity against PAMR is shown by the inhibition zone or clear zone. The size of the clear zone was directly proportional to the increase in the extract, ethyl acetate fraction, and water fraction concentration. The higher concentration of the extract and fraction is the

Table 1: The result of phytochemical analysis of Simplicia and extract Rambutan leaf

Compound	Simplicia	Extract
Alkaloid	-	-
Polyphenol	+	+
Flavonoid	+	+
Tannin	+	+
Monoterpene and Seisquiterpene	+	+
Steroid and Triterpenoid	-	-
Quinone	-	-
Saponin	+	-

(+): Detected, (-): Not detect

Table 2: Result of antibacterial activity tested of Rambutan leaf extract against PAMR

Concentration of extract (%)	The averasge of clear zone (mm)
	PAMR
40	17.60
20	13.72
10	10.62
5	9.22

PAMR: *Pseudomonas aeruginosa* multiresistant

Table 3: Result of antibacterial activity tested of fractions against PAMR

Fraction	Concentration (% w/v)	The average of clear zone (mm)
		PAMR
Ethyl acetate	40	20.53
	20	16.80
	10	11.21
	5	9.55
Water	40	15.05
	20	11.78
	10	9.83
	5	8.53
N-hexane	40	-
	20	-
	10	-
	5	-

(-): Not detect, PAMR: *Pseudomonas aeruginosa* multiresistant

Table 4: MIC and MBC value of extract and ethyl acetate fraction on PAMR

Concentration (% w/v)	Bacterial growth of PAMR	
	Ethanol extract	Ethyl acetate
5.00	-	-
2.50	+	+
1.25	+	+
0.62	+	+
0.31	+	+
0.15	+	+
0.08	+	+
0.04	+	+

(+): Detected, (-): Not detect, PAMR: *Pseudomonas aeruginosa* multiresistant, MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

greater inhibition zone, or clear zone was formed. Based on the size of the inhibition zone, ethyl acetate fraction of Rambutan leaves was the most active fraction giving the best antibacterial activity against PAMR. Ethyl acetate fraction was the most active than the other fractions.

Determination of MIC value aimed to find the lowest concentration of extract and ethyl acetate fraction as the most active fraction which can inhibit the growth of bacteria, while the determination of MBC value aimed to find the smallest concentration of extract and the most active fraction that was bactericide. Determination of MIC value was carried out using turbidimetry method by looking at a turbidity of well that indicating the presence or absence of growth of bacteria. However, turbidity was difficult to observe because of the effect of color extracts and fractions that interfered the observations. Therefore, subculture was carried out. From each well microdilution, as much as 10 µL was incorporated into the MHA and dispersed using a spreader, to observe the growth of bacterial colonies. Then, it was incubated for 18–24 h at 37°C.

CONCLUSION

Ethanol extract, ethyl acetate fraction, and water fraction of Rambutan leaf had antibacterial activity against PAMR starting at a concentration of 5%. The value of MIC and MBC both was in the range concentration of 2.5–5% w/v. The antibacterial activity of this extract and fractions against PAMR were probably derived from flavonoids, polyphenols, saponins, and tannins.

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