

Antibacteria Activity Peel and Seed Extracts of Rambutan (Nephelium lappaceum L.) Against MDR Bacteria Causing Urinary Tract Infections

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Antibacteria Activity Peel and Seed Extracts of Rambutan (*Nephelium lappaceum* L.) Against MDR Bacteria Causing Urinary Tract Infections

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Abstract. Multidrug-resistant (MDR) - *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) are the main causes and have become serious problems in urinary tract infections, so antibacterial agents derived from biological materials are needed. ESBL-*E. coli* and ESBL-*K. pneumoniae* bacteria are resistant to extracts from rambutan peels and seeds, but there is no knowledge of the use of different solvents, such as n-hexane, chloroform, or ethanol. The objective of this research was to assess the antibacterial activity of rambutan peels and seed extracts (n-hexane, chloroform, and ethanol) against MDR bacteria that cause urinary tract infections (UTI). The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values for antibacterial activity were calculated using agar well diffusion and dilution procedures. The results demonstrated that the ethanol extract of rambutan peels had inhibitory zones against MDR *K. pneumoniae* and *E. coli* that varied from 9.00 to 14.13 mm. 15.625 mg/mL for MDR *E. coli* and 3.90 mg/mL for MDR *K. pneumoniae*, respectively, the MIC value was determined. The MBC value was 62.50 mg/mL for MDR *K. pneumoniae* and 31.25 mg/mL for MDR *E. coli*. Conclusion: Of the six rambutan peel and seed extracts, the ethanol extract has greater potential as an antibacterial agent. It is advised that more in-vivo studies be done to understand how the antibacterial activity operates. The benefits of research for the science are providing alternative solutions to antibiotic resistance, to further advancing the field of antimicrobial research, and reducing the risk of bacterial infections.

Keywords: Rambutan fruits (*Nephelium lappaceum* L.), *E. coli*, *K. pneumoniae*, MIC, and MBC

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INTRODUCTION

Men and women alike are susceptible to the infectious known medically as urinary tract infection (UTI) (Vasudevan, 2014). Acute, recurrent, or chronic urinary tract infections are the most common microbial diseases, affecting approximately 150 million people each year worldwide. In the United States, up to 11 million people suffer from UTI each year (Zalewska-Piątek, 2020). The most frequent pathogens that cause UTI are gram-positive bacteria like *Staphylococcus saprophyticus* and *Enterococcus* sp. and gram-negative bacteria like *E. coli*,

Proteus mirabilis, and *K. pneumoniae* (Mazzariol et al., 2017). In a study of 421 UTI patients by Jafari-Sales (2020), it was discovered that 165 (39.2%) tested positive for *K. pneumoniae*, including 89 (53.9%) females and 76 (46.1%) males. *Klebsiella pneumoniae* and *E. coli* are members of the family Enterobacteriaceae. *E. coli* is a normal bacterial flora of the intestine and has an important role in the synthesis of vitamin K, conversion of bile pigments, bile acids, and absorption of nutrients, while *K. pneumoniae* is a normal flora of the skin, mouth, and digestive tract. The ability of *K. pneumoniae* to cause disease in sick and healthy individuals is

influenced by its pathogenicity (Vading, et al., 2018).

Antibiotics can be used to treat UTIs because antibiotic misuse results in the emergence of multidrug resistance (Rowe & Juthani-Mehta, 2014). A hazard to world health is antimicrobial resistance. Since over 80% of all antibiotics used in healthcare have been prescribed, primary healthcare has made a significant contribution. Some common bacterial illnesses can be challenging to treat due to the limited number of viable treatment choices caused by antibiotic-resistant bacterial infections (Bryce *et al.*, 2016). Therefore, numerous options are required to treat cases of resistant bacteria, such as the use of natural ingredients. Antibacterial agents can be obtained from various sources, for example, Latex (Prastiyanto *et al.*, 2020b), Mushrooms (Prastiyanto *et al.*, 2020a), marine bacteria (Prastiyanto *et al.*, 2022c, 2022b, 2023), and plant (Prastiyanto *et al.*, 2021a, 2021b, 2022a; Prastiyanto, 2021). One of the natural ingredients that can be used as antibacterial agents is the rambutan (*Nephelium lappaceum* L.).

Indonesia is a country where the tropical fruit rambutan grows. It is a sapindaceous fruit tree grown for ornamental purposes (Wahyuningsih, 2019). Numerous pharmacological effects of this fruit include antidiabetic, anti-hypercholesterolemic, antibacterial, antioxidant, anti-hyperuricemia, and anticancer effects. Rambutan peel is strong in anthocyanins, which give ripe rambutan fruit its red color (Poernomo *et al.*, 2016). Fruit contains sugar, vitamin C, carbs, protein, calcium, iron, phosphorus, macro and micro minerals, and lipids. (Chai *et al.*, 2018). The seeds contain a lot of fat and polyphenols, whereas the leaves have a lot of tannins and saponins. The bark contains tannins, saponins, flavonoids, pectin, and iron (Lestari *et al.*, 2013). Previous research related to the antibacterial activity of rambutan against *S. aureus* (Phuong, *et al.*, 2020), Methicillin-resistant *S. aureus* (Rostinawati *et al.*, 2018), and *E. coli* (Aksonkird *et al.*, 2019), bacillus bacteria (Phuong, *et al.*,

2020), while previous research related to antibacterial activity rambutan fruit seeds against pathogenic

Chromatographic Analyses, virtual screening and pharmacokinetics of yellow Malaysian rambutan (*Nephelium lappaceum* L.) fruit epicarp extracts reveal potential antibacterial compounds (Asgar, 2020). There has been no research on the antibacterial activity of rambutan peel and seed extracts against MDR bacteria that cause UTIs, so more research is needed. Rambutan peel and seed extracts were tested in this work utilizing a number of solvents, including n-hexane, chloroform, and ethanol. The purpose of this study was to evaluate antimicrobial rambutan peel and seed extracts performed in different solvents (n-hexane, chloroform, and ethanol) against MDR bacteria that cause UTIs (*K. pneumoniae*, and *E. coli*). The benefits of research for the science are providing alternative solutions to antibiotic resistance, to further advance the field of antimicrobial research, and to reducing the risk of bacterial infections

METHOD

The research used the post only design experimental method Rambutan peels and seeds were taken from Jl. Tirta Agung, SOS, Taruna Village, Km 01, Pedalangan, Banyumanik Sub-district, Semarang City, with the coordinate of -7.062715.110 in January 2022. Isolate of *K. pneumoniae* and *E. coli* bacteria were taken from the Microbiology Laboratory of the Muhammadiyah University of Semarang.

Extraction of Rambutan Peels and Seeds

Rambutan peels (Figure 1) was saturated in 1500 mL ethanol 70% for 24 h thrice. Then, the macerated was concentrated using rotatory evaporator at 45°C and reassembled over the water bath at 45°C till a thick extract was obtained. This procedure is repeated with other solvents namely n-hexane and chloroform. (Sulistiyaningsih *et al.*, 2017).

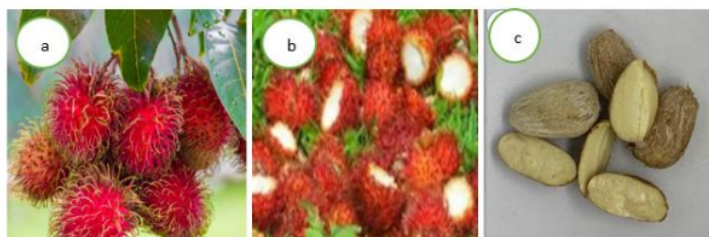


Figure 1. Rambutan (*Nephelium lappaceum* L.): a) rambutan fruit, b) peel and c) seeds of rambutan fruit

This type of research is an invitro experiment. Equipment are Petri dishes, sterile pipettes, sterile test tubes, test tube racks, spirit lamps, autoclaves, incubators, scales, beakers, ovens, tweezers, blank paper discs, sterile cotton sticks, and vernier calipers. Materials are extract of rambutan peel and seed, *E.coli* and *K. pneumoniae* bacterial suspension, distilled water, MCA media (Macconkey Agar), MHA Media (Mueller Hinton Agar) (Oxoid), MHB Media (Mueller Hinton Broth), BHIB Media (Brain Heart Infusion Broth) (Oxoid), NaCl (Merck, Germany), antibiotics and Mc Farland standards.

Extraction of rambutan peel using 70% ethanol, n-hexane and chloroform. The peel of the rambutan fruit is washed, cut into small pieces, then dried in the oven at 45°C for 5 days. The dried rambutan peels were ground into a fine powder and filtered through a 100 mesh sieve. 500 g of rambutan peel powder soaked in 1500 mL of 70% ethanol for 24 hours. After that it was filtered, the

filtrate or macerate was stored and the rambutan peel simplicia was soaked again in 70% ethanol for 24 hours and repeated up to 3 times. The macerate was concentrated using a rotary evaporator at 45°C until slightly thick, then transferred to a water bath to be heated at 45°C until a viscous extract was obtained. The extract obtained was then diluted to a concentration of 1; 10; 100 and 1000 mg/mL. This procedure was repeated with n-hexane and chloroform. The procedure for extracting rambutan fruit seeds with 70% ethanol, n-hexane and chloroform, is the same as the extraction procedure for rambutan peels with 70% ethanol, n-hexane and chloroform, by replacing the rambutan peel with rambutan fruit seeds.

Phytochemical Test of peel and seeds of rambutan fruit. Extracts of the peel and seeds of rambutan fruit with ethanol, n-hexane and chloroform solvents were carried out for each phytochemical test using the procedure in Table 1.

Table 1. Phytochemical Test, Reagent and Positive Result

Phytochemical Test	Reagent	Positive Result
Alkaloids	a) The extract was added with 2N HCl solution, and filtered. Filtrat was added Mayer solution b) Dragendroff's solution	a) white to yellow precipitate b) orange precipitate
Flaonoids	The extract was added with magnesium powder and 2 mL of 2% HCl	An orange to red solution
Tannins	The extract was added with 2-3 drops of 1% FeCl ₃ solution.	black green or blue ink
Terpenoids/ Steroids	The extract was dissolved in 4 mL chloroform then added with 0.5 mL CH ₃ COOH and 2 mL H ₂ SO ₄ through the tube wall	a) The formation of a bluish green color indicates the presence of sterol compounds b) The formation of a brownish or violet ring indicates the presence of triterpenoid compounds
Phenols	The extract was added 1 mL of 10% FeCl ₃ solution.	Formation of a dark blue, blackish blue or greenish black solution
Saponins	The extract was added to 10 mL of hot water, then cooled and then shaken vigorously for 10 seconds.	Constant foam formation

Preparation of Bacterial Suspensions.

Pure cultures of *E. coli* and *K. pneumoniae* bacteria were taken as much as 1 colony each using ose on MCA media and suspended with 5 ml of 0.85% physiological NaCl then shaken until cloudy for 1-3 minutes, compared to standard MC farland solution until the turbidity is the same color.

Dilution (MIC and MBC) Test

The MIC value of each extract was evaluated using a microdilution method on microplates with Muller Hilton broth (MHB) media, followed by a serial dilution approach on 12-well microplates.

Each well received 100 µL of MHB, and 100 µL of extract was added to the first well until it reached the 12th, then 100 µL of bacterial suspension adjusted to the McFarland standard (0.5) was added to each well except the negative control well. The microplates were incubated at 37°C for 18 hours. MBC was a continuation of MIC in which culture samples were taken from MIC results on microplates using inoculation needles. After homogenizing the samples, they were streaked onto broth agar plate (BAP) media and incubated at 37°C for 16-20 hours. The MBC value was calculated by watching bacteria growth on BAP media.

RESULTS AND DISCUSSION

Rambutan Extract Yield

According to the study's findings (Table 2), ethanol extract of the peels, seed chloroform, and seed n-hexane yielded higher yields than the others. The solubility of phytochemical components in peels and seeds in ethanol was larger than in chloroform and n-hexane. The Polarity ethanol high creates significant interactions with the majority of the polar phytochemical chemicals recovered from rambutan skin and seeds, it has a larger extraction potential than semi-polar chloroform and n-hexane, which extract nonpolar phytochemical compounds (Sbihi et al. 2018). The results of this study are in line with Septiani (2021), the average yield obtained from extract of leaves by maceration with 12% chloroform and 27% ethanol. Extraction with solvents is based on the polarity of the substance, polar compounds will only dissolve in polar solvents, such as ethanol, methanol, butanol and water. Nonpolar compounds will also only dissolve in nonpolar solvents, such as ether, chloroform and n-hexane. Ethanol extract, ethyl acetate fraction, and water fraction of Rambutan leaf had antibacterial

activity against *Pseudomonas aeruginosa* Multi-*Drug Resistant* starting at a concentration of 5%. The value of MIC and MBC both was in the range concentration of 2.5–5% w/v (Sulistiyaningsih et al, 2017).

Table 2. The results of rambutan (*Nephelium lappaceum* L.) peel and seed extraction

Part of Plant	Solvent	Yield (%)
Peel	n-hexane	0.95
	Chloroform	1.33
	Ethanol	22.62
Seed	n-hexane	14.4
	Chloroform	18.95
	Ethanol	24.4

Phytochemical screening of rambutan extracts

Data from biological screening, phytochemical analysis is a preliminary test for identifying the existence of particular chemical compounds in plants, such as alkaloids, phenolic compounds (including flavonoids), saponins, steroids, and terpenoids (Jantapaso and Mittraparp, 2022). The findings of the phytochemical analysis of rambutan peel and seed extracts are displayed in Table 3.

Table 3. Results of phytochemical analysis of rambutan peel and seed extracts.

Test	Peel Extract			Seed Extract		
	n-Hexane	Chloroform	Ethanol	n-Hexane	Chloroform	Ethanol
Flavonoids	+	-	+	+	+	-
Alkaloids	-	+	-	-	-	-
Phenolates	-	+	+	-	-	-
Tannins	+	+	+	+	+	+
Saponins	-	-	+	-	-	-

All peel and seed extracts are shown in Table 3 to contain tannin components. Tannins are polyphenolic substances that are insoluble in benzene, chloroform, ether, petroleum ether, and carbon disulfide but soluble in water, glycerol, methanol, hydroalcoholic, and propylene glycol. Tannins are antibacterial and astringent, decreasing the intestinal walls harmed by bacteria or acid, according to Hassan et al. (2020). By harming cell membranes, deactivating vital enzymes, and damaging the genetic material, tannins hinder the growth of bacteria. Results of phytochemical screening were reported in other studies Jantapaso and Mittraparp (2022), phenolic chemicals predominate in the ethanol extract of rambutan peels, but rambutan seed extracts contain phenolic compounds, flavonoids, and saponins but are devoid of alkaloids and tannins.

Antibacterial activity of the extracts against MDR bacteria

Test for agar well diffusion

The antibacterial ability of the peel and seeds of rambutan fruit against MDR *K. pneumoniae* and *E. coli* bacteria obtained from the Microbiology Laboratory of Universitas Muhammadiyah Semarang was assessed. This activity was demonstrated by the existence of an inhibitory zone (Table 4). A qualitative way to assess an antibiotic substance's capacity to prevent the growth of germs is the inhibition zone. According to the study's findings, rambutan peel and seed extracts with different solvents (n-hexane, chloroform, and ethanol) displayed antibacterial activity against MDR bacteria.

Table 4. Lists screening tests for *K. pneumoniae* and *E. coli* resistance to various antibiotics

Types of Bacteria	Antibiotic Resistance Pattern
MDR- <i>K. pneumoniae</i> 1	Ceftazidime, aztreonam, meropenem, ceftriaxone, gentamicin, tigecycline, amikacin
MDR- <i>K. pneumoniae</i> 2	Meropenem, ceftazidime, ceftriaxone, amikacin, tigecycline, gentamicin, aztreonam
25) R- <i>E. coli</i> 1	Ceftazidime, aztreonam, ceftriaxone, meropenem, amikacin, tigecycline
25) R- <i>E. coli</i> 2	Ceftazidime, aztreonam, ceftriaxone, meropenem, amikacin, tigecycline
MDR- <i>E. coli</i> 3	Ceftazidime, aztreonam, ceftriaxone meropenem, amikacin, tigecycline

The inhibition zone diameter of rambutan extract and other common antibiotics as positive controls of MDR *K. pneumoniae* and MDR *E. coli* presented in Table 5.

Table 5. The diameter of the inhibition zone for MDR *K. pneumoniae* and MDR *E. coli* when rambutan extract and various common antibiotics are used as positive controls.

Part of Plant	Type of Solvent	Concentration(mg/mL)	Inhibition zone (mm) against				
			MDR <i>K. pneumoniae</i>		MDR <i>E. coli</i>		
			1	1	1	2	3
Peel	N-hexane	1	NE	NE	NE	NE	NE
		10	NE	NE	NE	NE	NE
		100	NE	NE	NE	NE	NE
		1000	11	NE	11	11	11
	Chloroform	1	NE	NE	NE	NE	NE
		10	NE	NE	NE	NE	NE
		100	NE	11.03±0.35	NE	NE	NE
		1000	NE	19.12±0.20	NE	NE	NE
	Ethanol	1	NE	NE	NE	NE	NE
		10	NE	NE	NE	NE	NE
		100	NE	NE	NE	NE	NE
		1000	12.10±0.29	14.13±0.26	10.00±0.10	9.00±0.21	12.00±0.10
Seed	N-hexane	1	NE	NE	NE	NE	NE
		10	NE	NE	NE	NE	NE
		100	NE	NE	NE	NE	NE
		1000	NE	NE	NE	NE	NE
	Chloroform	1	NE	NE	NE	11.00±0.23	NE
		10	NE	NE	NE	13.00±0.31	NE
		100	NE	NE	NE	13.00±0.25	NE
		1000	NE	NE	NE	NE	NE
	Ethanol	1	NE	NE	11	NE	NE
		10	NE	NE	NE	NE	NE
		100	NE	NE	NE	NE	NE
		1000	NE	NE	NE	NE	NE
Antibiotics	Type of antibiotics	Concentration (µg)		15			
	CAZ	30	15.00 (R)	16.00 (R)	NE (R)	NE (R)	NE (R)
	CTX	30	NE (R)	NE (R)	NE (R)	NE (R)	NE (R)
	MRP	10	17.00 (R)	18.00 (R)	28 (S)	18.00 (S)	28.00 (S)
	ATM	30	26.00 (S)	25.00 (S)	28 (R)	NE (R)	NE (R)
	GEN	10	21.00 (S)	20.00 (S)	NE (R)	NE (R)	NE (R)
	TGC	15	19.00 (S)	18.00 (S)	20.00 (S)	21.00 (S)	20.00 (S)
	AK	30	18.00 (S)	20.00 (S)	19.00 (S)	19.00 (S)	21.00 (S)

Notes: NE = No Effect; R= Resistant; S= Sensitive

Type of Antibiotic: CAZ= Ceftazidime; CTX=Ceftriaxone; MRP= Meropenem; ATM= Aztreonam; GEN= Gentamicin; TGC= Tigecycline; AK = Amikacin.

29 Table 5 reveals that the inhibition zone of rambutan seed chloroform extract against MDR-*E. coli* was 11.00-13.00 mm, whereas the zone of inhibition of rambutan peel chloroform extract against MDR-*K. pneumoniae* was 11.03-19.12 mm. The inhibition zone of ethanol peel was 12.10-14.13 mm against MDR-*K. pneumoniae* and 9.00-12.00 mm against MDR-*E. coli*. The chloroform extract of rambutan seeds had superior

antibacterial activity than n-hexane and ethanol. The ethanol extract of peels had a bigger inhibiti 35 zone than the other two extracts. This is in accordance with 14 results of research by Yunusa et. al (2018), that Rambutan peel demonstrated the highest free radical scavenging activity with inhibitory concentration (IC50) value of 24.99 ± 2.82 and 144.59 ± 1.36 µg/mL for ethanolic.

Determination of MIC and MBC values

Figure 3 shows the MIC values of rambutan peel and seed extracts against MDR-*E. coli* and *K. pneumoniae* bacteria that cause UTI, while the MBC value is presented in Figure 2.

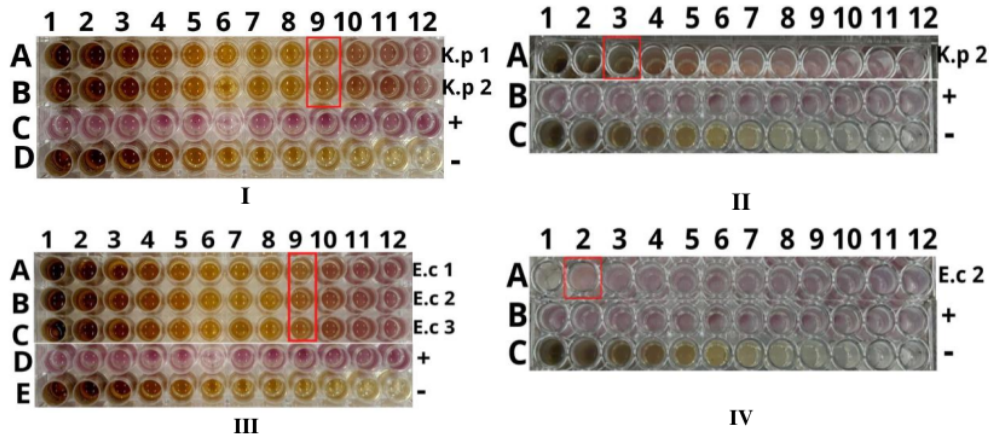
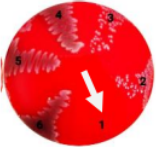

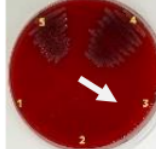
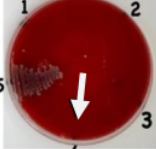


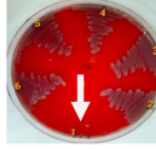


Figure 3. MIC values of rambutan peel and seed extracts. I. Ethanol extracts of rambutan peels against MDR-*K. pneumoniae* 1 and 2; II. Chloroform extracts of rambutan peels against MDR-*K. pneumoniae* 2; III. Ethanol extracts of rambutan peels against MDR-*E. coli* 1, 2, and 3; IV. MDR-*E. coli* 2 resistance to chloroform extracts of rambutan seeds. The letters A, B, C, and D represent bacterial codes, while the numerals 1-12 represent rambutan extract concentrations of 1000; 500; 250; 125; 62.5; 31.250; 15.063; 7.813; 3.9063; 1.954; 0.977; 0.488 mg/mL. 62.5; 31.250; 15.063; 7.183; 3.9063; 1.954; 0.977; 0.488 mg/mL.

Code	<i>K. pneumoniae</i> 1	<i>K. pneumoniae</i> 2	<i>E. coli</i> 1	<i>E. coli</i> 2	<i>E. coli</i> 3
Chloroform extract of rambutan peel	NI	 1000 mg/mL	NI	NI	NI
Ethanol extract of rambutan peel	 62.50 mg/mL	 62.50 mg/mL	 31.25 mg/mL	 125 mg/mL	 62.50 mg/mL
Chloroform extract of rambutan seed	NI	NI	NI	 1000 mg/mL	NI

Notes: NI = No Inhibition

Figure 2. MBC value of rambutan peel and seed extract. I. Chloroform extract of rambutan peel against MDR-*K. pneumoniae* 2; II. Ethanol extract of rambutan peel against MDR-*K. pneumoniae* 1 and 2, and MDR-*E. coli* 1, 2 and 3; III. Chloroform extract of rambutan seed against MDR-*E. coli* 2.

The MIC values of the six extracts were determined in vitro using microplates and the microdilution method for five resistant bacteria (Figure 3). Because of the lower MIC values of 3.90 mg/mL for MDR-*K. pneumoniae* and 15.625 mg/mL for MDR-*E. coli*, the ethanolic peel extracts displayed greater antibacterial action than the other two solvent extracts in this investigation. The chloroform extracts of peels had a MIC value of 250 mg/mL against MDR-*K. pneumoniae* and a MIC value of 500 mg/mL against MDR-*E. coli*.

The MBC values of rambutan peel and seed extracts using different solvents (n-hexane, chloroform, and ethanol) were measured using the microdilution method with a mixture of MIC cultures on microplates and cultivated on BAP medium for five resistant bacteria isolated from urine (Figure 4). The MBC values of the six extracts ranged from 31.25 to 1000 mg/mL, according to the data. Rambutan peel ethanol extracts outperformed other extracts due to lower MBC values of 31.25 mg/mL against *E. coli* and 62.50 mg/mL against MDR-*K. pneumoniae*. This study's findings revealed higher MBC values than earlier research. The MBC values for the six extracts ranged from 31.25 to 1000 mg/mL, according to the data. The ethanol extracts of rambutan peels performed better than other extracts due to reduced MBC values of 31.25 mg/mL against MDR-*E. coli* and 62.50 mg/mL against MDR-*K. pneumoniae*. This study's findings showed higher MBC values than earlier research (Putri, 2021). Rambutan peel extracts have better antimicrobial potential in nanoparticles (26.5 mg/mL) than micro-particles (62.5 mg/mL and 250 mg/mL) in inhibiting the growth of *S. aureus* and *S. mutans* bacteria (Florenly et al, 2022). Potency of Rambutan Plant as Antibacterial, to have medicinal properties, such as anthelmintic and antidiarrheal. Several scientific studies have shown that the leaves, seeds, and peel of the rambutan fruit have antibacterial activity. This activity is shown by extracts or fractions obtained through various methods and different solvents (Bina et al., 2022). The idea of food therapy leads to extensive nutraceuticals research on the potential of exotic fruits such as *N. lappaceum* and *N. ramboutan-ake* to act as supplements (Tsong et al, 2021).

The benefits of research for the science are providing alternative solutions to antibiotic resistance, to further advance the field of antimicrobial research, and to reducing the risk of bacterial infections. Potential economic benefits such as promoting sustainability, may generate

new markets, create jobs in the production and processing sectors, contribute to the economic growth of regions, reduces reliance on synthetic chemicals and potentially minimizes the environmental impact associated with their production and use. The benefits of rambutan skin can be made as a sunscreen gel to avoid exposure to ultraviolet rays.

CONCLUSION

In conclusion, the six rambutan peel and seed extracts, the ethanol extract has greater potential as antibacterial than n-hexane and chloroform extracts. The ethanol extract of rambutan peel had an inhibition zone against MDR *E. coli* and *K. pneumoniae*. It is advised that more in-vivo study be done in order to understand how the antibacterial activity operates.

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