Extraction of Pectin from Durian Rind and Its Minimum Inhibitory Concentration towards *Staphylococcus Aureus* and *Escherichia Coli*

Mufti Ghaffar[1], Harsi Dewantari Kusumaningrum[2] and Nugraha Edhi Suyatma[2]
[1]*Study Program of Food Science, Graduate School, IPB University, Bogor 16680, Indonesia*
[2]*Department of Food Science and Technology, IPB University, Bogor 16680, Indonesia*

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Abstract: Durian is a big tropical fruit grown in Indonesia that leaves many waste from its rind. Recently, some reports shows that durian rind can be extracted for its pectin that showing a potential antimicrobial activity toward pathogen. The aim of this study was to characterized durian pectin isolate from two different extraction methods and to determine the minimum inhibitory concentration (MIC) of durian pectin isolate against pathogenic bacteria. Inhibitory activity of durian pectin isolate against two bacterial strain: *Staphylococcus aureus* and *Escherichia coli*, was determined by using macrodilution methods, and amoxicilline used as positive control. Results showed that water content of the two extraction methods resulted in around 96%. Yield of durian pectin isolate in methods A (5,45 ± 0,10 %) more than extraction B (3,46 ± 0,06 %). MIC of isolates durian pectin in mueller hinton broth (MHB) medium against *E. coli* was 500 mg/ml and *S. aureus* was 110 mg/ml, this concentration showed no sign of bacterial growth, this condition was same as positive control.

1 INTRODUCTION

Indonesia is tropical country that has many fruit can grow, one of that fruit is Durian (Figure 1a). Durian is a popular fruit in Indonesia, a tropical, seasonal climateric fruit belonging to the Bombacaceae family. Durian fruit is quite large, round or oval, green (brownish when ripe), has a thick outer shell, hard, and covered with many thorns shaped like a pyramid. Durian fruit has a short shelf life, it is known to be damaged 36 to 72 hours from the time the durian fruit falls (Manoharan, 2013). Durian grows well in 75-80% humidity conditions with rainfall between 1600 and 4000 ml a year, and with an average temperature of 24-30^{o}C. Requires a tropical climate to grow, and not grow well in areas over 3000 feet (Ashraf et al., 2011).

It is customary for people in several regions in Indonesia to use durian rind as a drinking container after eating durian fruit. It is said that by drinking water from durian rind can eliminate the pungent odor of durian fruit, and can reduce durian motion sickness due to consumption of durian in large quantities. Based on this, there is a possibility that the durian rind contains active components, but has not been much studied. It is reported in some literature that durian rind has a number of therapeutic benefits such as: has anti-diabetic properties, anti-hyperlipidemic effect, anti-proliferative activity, and antimicrobial activity.

Pectin can be extracted from durian rind which has antimicrobial activity. Pectin from durian rind has the ability to inhibit the growth of *Vibrio harveyi* 1526 (MIC = 6.3 and 12.5 mg / mL) in black tiger / tiger shrimp (Pholdaeng and Pongsamart, 2010). Durian rind pectin has anti microbial activity against *E. coli*, *S. aureus* (Lipipun et al., 2002). Films made from durian rind pectin also have antibacterial properties when tested on several bacteria (Ho and Bhat, 2015).

Utilization of durian rind has not been done much by households, or industries that process durian fruit. Pectin can be extracted from durian rind which has antimicrobial properties that have the potential to be developed. This study aims to characterized durian pectin isolate and to determine the minimum inhibitory concentration of durian pectin isolate against pathogenic bacteria *E. coli* and *S. aureus*.

2 MATERIALS AND METHODS

2.1 Materials

Durian was obtained from the Warso Farm durian

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farm, Bogor, Indonesia. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 use as test bacteria were obtained from the food microbiology laboratory of Food Science and Technology Department IPB University. The initial treatment of raw materials begins with durian rind cut and separated mesocarp (the inner part of durian rind) and exocarp (the outer part of durian rind). Mesocarp was cut to a size of ± 0.5 cm to maximize the process of drying and crushing the material. The durian peel was placed into aluminum foil and dried in an oven at 60^{o}C for 48 hours, then crushed to powder and wrapped in adhesive plastic or stored in a desiccator.

2.2 Methods

2.2.1 Durian Pectin Extraction

The extraction method in this study uses the extraction method from Amaliah (2014) and Hokputsa et al. (2004) and compared the characteristics of durian rind pectin isolates obtained. The following was the Amaliah extract method (2014) with a modification (extraction A). The process of extracting pectin from durian rind is carried out through several stages, heating, filtering, concentrating, extracting. Durian rind powder was mixed with distilled water (durian rind powder: aquades = 1: 25 w / v) and stirred until it becomes an acidic solution with a pH of 2 by adding 2 N HCl acid solution and extracted at 85^{o}C for 2 hours. Then filtered using a cloth, then added an acid-ethanol solution (4% HCl in 95% ethanol) with a ratio of 1: 1 v / v and incubated at room temperature for 1 hour. To separate the gel (durian pectin isolate) filtering is done in vacuum conditions. The resulting gel is rinsed twice using 95% ethanol (1: 1, v / v) and shaken for 15 minutes (Amaliyah, 2014).

The following was a method of extraction Hokputsa et al. (2004) with the modification (extraction B). Durian skin powder was mixed with distilled water (durian skin powder: aquades = 1: 25 b / v) and stirred to form an acidic solution with a pH of 4.5 with citric acid solution added and extracted at a temperature of 90-100^{o}C for 20 minutes. Then filtered using a cloth, then added an acid-ethanol solution (4% HCl in 95% ethanol) with a ratio of 1: 1 v / v and incubated at room temperature for 1 hour. To separate the gel / durian pectin solate filtering done by vacuum conditions. The resulting gel is rinsed twice using 95% ethanol (1: 1, v / v) and shaken for 15 minutes. The resulting sludge was a durian pectin isolate (DPI). The DPIs obtained were then characterized in the form of measurements of water content, yield, and pH values.

2.2.2 Measurement Antibacterial Activity from Durian Pectin Isolate

Microorganism that used in this macrodilution method are common contaminating microorganism in food products, namely*: Eschericia coli and Staphylococcus aureus*. Macrodilution is one of the basic antimicrobial testing methods. Before the macrodilution was carried out the sterilization process was done on samples of DPI. The procedure begins by testing an antimicrobial agent DPI with a concentration of 1) 100%, 50%, 25%, 12.5%, 6.25%, 3.125% (*E. coli*) and 2) 100%, 25%, 6,25%, 1,56%, 0,39%, 0,09 % (*S. aureus*) in 2 mL (*E. coli*) and 3 mL (*S. aureus*) microbial growth medium (Mueller Hinton Broth) in a tube. Then each tube was inoculated with a bacterial inoculum prepared in the same medium. The size of the inoculum microbe is around 5 x 10^5 CFU / mL. After mixing well, each tube was incubated at 30^{o}C for 20 hours. Then each tube was inoculated on the Mueller Hinton Agar medium by scratching, because DPI covers the detection of microbial growth with its color. This procedure was repeated three times (Balouiri et al., 2016).

3 RESULTS AND DISCUSSIONS

3.1 Durian Pectin Isolate Characterization

To get durian pectin isolate, the initial preparation process of durian rind raw material was done to separate the mesocarp (Figure 1b) and exocarp. From this part (mesocarp) had yield approximately 29.63 ± 0.02% from all part of durian rind. Mesocarp is a raw material that used to obtain durian rind powder. And the yield of durian rind powder was 10,44 ± 0.06 (Table 1).

Table 1: Yield and water content of mesocarp and durian powder.

Sample	Yield (%)	Water content (%)
Mesocarp	29,63 ± 0.02	-
Durian rind powder	10,44 ± 0.06	1,01 ± 0,17

Characterization of durian pectin isolate (Figure 1c) was performed, giving values of water content, yield and pH value (Table 2). Water content of both methods resulted about 96%% which indicated that durian pectin isolate was composed of large amounts of water. Extraction method A (pH: 2.00, temperature: 85°C, t: 2 hours) has bigger yield (5,45 ± 0,10) than extraction B (pH: 4.5 and temperature: 90-100°C, t : 20 minutes that had yield 3,46 ± 0,06, which indicates extraction A had more yield than extraction B. The more extraction yield are related to differences of pH. The lower the pH, the more yield of pectin will be obtained. The yield of both methods is in the range of the previous report's yield, which ranges from 1.04% - 10% (Arlofa et al., 2015; Pholdaeng and Pongsamart, 2010; Wai et al., 2009).

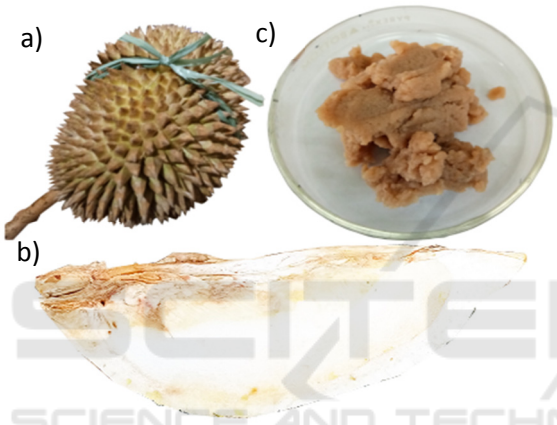

Figure 1: a) Durian fruit, b) Mesocarp of durian fruit, and c) Durian pectin isolate.

Lower pH value result in more yield of pectin can be extracted (Ardiansyah et al., 2014). The principle of extraction of pectin is the overhaul of protopectin which does not dissolve into pectin which can dissolve with acid solvents. In the extraction process with a high level of acidity will increase the hydrolysis of protopectin from durian rind tissue to be dissolved in water faster, so that the yield of pectin is higher. Protopectin does not dissolve easily because it is in the form of calcium and magnesium salts. Hydrolysis of protopectin with acids causes hydrogen ions to replace calcium ions and magnesium ions in the protopectin molecule. This is due to a pH of 1.5 which is more acidic and has more hydrogen ions so that the possibility of substituted calcium and magnesium is greater (Prasetyowati et al., 2009).

Table 2: Yield, pH, and water content from extraction method A and extraction method B.

Para-meter	Extraction A (acid: HCl 2 N, pH: 2, Temperature: 85°C, time: 2 hour)	Ekstraction B (acid: citric acid, pH: 4.5, Temperature: 90-100°C, time: 20 minutes)
Yield (%)	5,45 ± 0,10	3,46 ± 0,06
pH	2,03 ± 0,10	3,75 ± 0,09
Water content (%)	96,26 ± 0,1	96,4 ± 0,04

3.2 Antibacterial Activity of Durian Pectin Isolate

Durian pectin isolates (DPI) obtained through the extraction process were tested for their antibacterial activity. The test results showed that there were two concentrations of DPI and positive control that could inhibit the *E.coli* bacteria as indicated by the clear growth media (Figure 1). Both concentrations are 100% and 50% for E. *coli* and concentration 100% and 25 % for S.*aureus*. From that figure we can get the value of the minimum inhibitory concentration (MIC) of each bacteria (Table 3). Based on the results it was found that the MIC value of DPI in *E. coli* was 500 mg/ml and *S. aureus* was 110 mg/ml.

The difference in antibacterial activity that occurs in Gram-positive (S. *aureus*) and Gram-negative (E. *coli*) bacteria is probably caused by differences in the composition and structure of cell walls in the two types of bacteria. The structure of the cell wall of Gram-positive bacteria is simpler, that is single layer with a low lipid content (1-4%) making it easier for bioactive materials to enter the cell. The structure of the cell wall of Gram negative bacteria is more

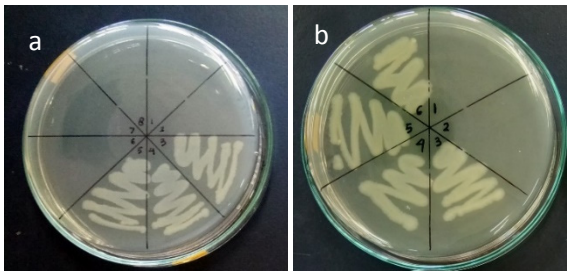

Figure 2: a) Growth *Escherichia coli* inhibited by durian pectin isolate in MHA medium (1: 100%, 2: 50%, 3: 25%, 4: 12.5%, 5: 6.125 %, 6: clear media, 7: amoxicilin as positive control), b) Growth *Staphylococcus aureus* inhibited by durian pectin isolate in MHA medium (1: 100%, 2: 25%, 3: 6,25%, 4: 1,56%, 5: 0,39 %.

Table 3: Microbial growth and minimum inhibitory concentration of durian pectin isolate against *E. coli* dan S. *aureus.*

Bacteria		Antibacterial concentration (%)						MIC (%)
		100	50	25	12,5	6,25	positive control	
E. coli	1	-	-	++	++	++	-	50
	2	-	-	++	++	++	-	50
	3	-	-	++	++	++	-	50
		100	25	6,25	1,56	0,39	positive control	
S. aureus	1	-	+	++	++	++	-	25
	2	-	-	++	++	++	-	25
	3	-	-	++	++	++	-	25

- : no growth or no colony
+ : there is growth or there is colony
++ : many growth or many colony

bioactive materials to enter the cell. The structure of the cell wall of Gram negative bacteria is more complex, three layers, namely the outer layer of lipoprotein, the middle layer of lipopolysaccharide which acts as a barrier to the entry of antibacterial bioactive material, and the inner layer is in the form of peptidoglycan with high lipid content (11-12%) (Magdalena and Kusnadi, 2015).

4 CONCLUSIONS

Durian pectin isolate of both extraction methods resulted in water content aproximately 96 %. Extraction method A (pH: 2.00, temperature: 85ºC, t: 2 hours) had bigger yield (5,45 ± 0,10) than extraction B (pH: 4.5 and temperature: 90-100ºC, t: 20 minutes) that had yield 3,46 ± 0,06 %. Minimum inhibitory concentration of durian pectin isolate against pathogenic bacteria E. *coli* was 500 mg/ml and S. *aureus* was 110 mg/ml.

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