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The Potential Antibacterial Power of Ethanol Extract of Durian Peel (*Durio zibethinus murr*) Against *Enterococcus faecalis*

Aurelia Maulini Rizky[1*], Danica Anastasia[1], Listia Eka Merdekawati[1]

[1]Dentistry Study Program, Faculty of Medicine, Sriwijaya University, Palembang, Indonesia
*Correspondence author email: aureliamaulini@gmail.com

Abstract

Introduction : Durian *(Durio zibethinus murr)* is a fruit with the 4th commodity in Indonesia where the skin has antibacterial properties. Durian peel contains active substances in the form of flavonoids, alkaloids, saponins, tannins, and triterpenoids which have significant antibacterial activity against Gram-positive bacteria such as *Enterococcus faecalis*. This study aimed to determine the antibacterial activity of the ethanol extract of durian peel against *Enterococcus faecalis* bacteria. **Methods**: This was quasi-experimental using post-test only control group design with 4 treatment groups consisting of ethanol extract of durian peel with concentrations of 10%, 15%, 20%, 25% and 2% chlorhexidine as a control group with 6 repetitions. The antibacterial activity was tested using the agar diffusion method Kirby-Bauer discs with Mueller Hinton Agar media. All groups were assessed after 24 hours of incubation period at 37°C. The diameter of the inhibition zone was calculated using a caliper and the data were analyzed using the One Way ANOVA test and the Post Hoc Games Howel test. **Results**: There was a significant difference in the antibacterial activity of the ethanol extract of durian peel against *Enterococcus faecalis* between all groups except in the concentration groups of 25% and 20% as well as 15% and 10%. The higher concentration of extract formed a larger clear zone. **Conclusion:** the ethanol extract of durian peel concentrations of 25%, 20%, 15% and 10% have antibacterial power against Enterococcus faecalis bacteria with an average of 1.69 mm, 1.25 mm, 0.78 mm and 0.42 mm which are classified as weak category.

Keywords: Antibacterial, *Durio zibethinus murr*, *Enterococcus faecalis*

Introduction

Enterococcus faecalis is an anaerobic facultative Gram-positive bacteria which is commonly detected in post-treatment endodontic disease with a prevalence reaching 90% .[1] *Enterococcus faecalis* has virulence factors such as aggregation substance (AS), surface adhesion, sex pheromones, lipoteichoic acid (LTA), extracellular superoxide production (ESP), gelatinase, and cytolysin and can enter the dentinal tubules where not all bacteria able to do so.[1] This virulence factor causes these bacteria to have unique characteristics such as being able to survive in extreme environments at very high temperatures, various pH, and resistance to some irrigation and endodontic medicaments, which often lead to root canal treatment failure.[2]

The success of root canal treatment is determined by several factors, especially the control of microorganisms in the root canals. Control of microorganisms in root canals can be

carried out by mechanical and chemical means. Chemicals that are currently commonly used in root canal treatments and are readily available include chlorhexidine gluconate (CHX).[4] The antibacterial efficacy of CHX can eliminate Gram-positive and Gram-negative bacteria by damaging the permeability of the cell wall thus providing a way for CHX to penetrate bacteria and cause bacteria to die. from Bahlouli et al. proved that alcoholic or non-alcoholic CHX is effective in eliminating oral microbes. CHX has been reported in vitro to kill *Enterococcus faecalis* in 30 seconds.[4] CHX has been shown to be effective against oral microbes, but there are still adverse side effects, such as discoloration of teeth, tongue, restorations and dentures, dysgeusia, gingivitis and allergic reactions. These side effects are the reason for choosing natural ingredients that can eliminate bacteria and are biocompatible in the surrounding tissue.

One of the natural ingredients in Indonesia that has antibacterial properties is durian skin. Durian is the 4th main fruit commodity in Indonesia after bananas, mangoes and oranges. Durian production in Indonesia in 2017 reached 795,200 tons, and specifically in South Sumatra, it was 19,930 tons.[5] The edible part of the durian is the flesh which about 20-35% of the total weight of the fruit. It means that the seed and skin are only wasted and have not been fully utilized. Arlofa et al. (2015), reported that the results of the phytochemical test for durian skin were positive for containing active ingredients in the form of tannins, alkaloids, triterpenoids, saponins and flavonoids. This is in line with research conducted by Pratiwi et al. (2018), they reported that ethyl acetate extract from durian peel is effective in inhibiting the growth of *P. acnes* bacteria because it has active ingredients in the extract, including flavonoids, triterpenoids, steroids, phenolics, and tannins.[12]

The research of Duazo et al. proved that the antibacterial activity of methanol extract of durian peel (*Durio zibethinus*) in concentration of 25%, 50%, 75% and 100% can inhibit the activity of the *Escherichia coli* and *Staphylococcus aureus* bacteria.[7] This is in line with research conducted by Hasrianti et al., that the ethanol extract of 96% durian peel (the white internal part of the durian skin) in a concentration of 10%, 15% and 20% can inhibit *Staphylococcus aureus* bacteria.[8] Permatasari et al. (2015), reported that durian peel extract has the potential to inhibit the activity and growth of supragingival plaque.[9]

Not much research has been done on durian skin as an antibacterial, especially *Enterococcus faecalis*. This study aimed to increase the use of natural ingredients as

antibacterial agents, it is necessary to conduct research on the antibacterial power of the ethanol extract of durian peel (*Durio zibethinus murr*) against *Enterococcus faecalis* bacteria.

Methods

This was a quasi-experimental in-vitro study with a post-test only control group design where the experimental group receives treatment and assessed.

Enterococcus faecalis ATCC 29212 bacteria were cultured with Mueller Hinton Agar (MHA) media for 24 hours at 37^{o}C. Mueller Hinton Agar (MHA) as much as 34 grams was dissolved in 1 liter of distilled water and sterilized using autoclave at a temperature of 121^{o} C for 25 minutes in order to let the temperature drop to 40^{o}C, then immediately pour it into a 15-20 ml petri dish and allow it to solidify. *Enterococcus faecalis* ATCC 29212, was obtained from the Palembang Clinical Laboratory Center which was stored in a scheme with a temperature of -80℃ using an ose needle inserted into a tube containing the Brain Hearth Infusion. The bacterial suspension was added with 0.9% NaCl and measured with a density meter so that the turbidity was in accordance with the standard bacterial concentration of 0.5 McFarland, equivalent to 1.5x10 8 cfu / mL.

The dependent variable in this study was the growth of *Enterococcus faecalis* which can be seen through the diameter of the bacterial inhibition zone. The measurement of the inhibition zone is carried out by measuring the clear diameter around the disc paper using a caliper. Measurements on each petri dish were carried out 3 times by taking two perpendicular lines through the center point of the disc paper and a third line was drawn between the two lines by forming an angle of 45^{o} then divided by three to get the area of the average drag zone on each petri dish.

The independent variable was the ethanol extract of durian peel (*Durio zibethinus murr*) with a concentration of 25%, 20% 15% and 10%. Twelve kilograms of the yellowish-white mesocarp and endocarp part of the skin was prepared to be extracted. The skin was washed, cut into small pieces measuring 2 cm x 2 cm, and dried in an oven at 60℃ for 24 hour. The dried skin then blended into a fine powder of 1 kg. The refined powder was put into a jar with the addition of 96% ethanol and then macerated for 3 x 24 hours. Afterward, filtering was carried out using filter paper to obtained 1 liter of the filtrate. The filtrate was evaporated with a rotary evaporator to obtain a thick 100% extract of durian skin. The extract

was subjected to phytochemical tests to assess alkaloids, flavonoids, tannins, saponins and triterpenoids.

Data analysis was performed using SPSS. The normality test was carried out by using the Shapiro-Wilk test, and continued with the homogeneity test of the data using the Levene's Test. Next, the data were analyzed using the One Way ANOVA test ($p \leq 0.05$) and followed by the Post-Hoc Games Howel test.

Results

The phytochemical test was carried out qualitatively to determine the compounds contained in the ethanol extract of durian skin (*Durio zibethinus murr*). The results depicted in Table 1 showed that the ethanol extract of durian peel contains moderate intensity flavonoids, saponins and tannins, as well as alkaloids and triterpenoids with strong intensity.

Table 1. Results of Phytochemical Screening of Ethanol Extract of Durian Bark.

Chemical Ingredients	Testing Method	Result	Information
Flavonoids	Concentrated Mg^+ and HCl	Saffron	+
Alkaloids	Dragendorff	Orange sediment redness	++
	Mayer	Yellowish white precipitate	++
Saponins	Shaking HCl^+	Formed Foam	+
Triterpenoids	Concentrated CH_4COOH and concentrated H_2SO_4	Purple	++
Tanin	+ FeCl 1%	Greenish Brown	+

+: Medium positive / There is a medium intensity active compound
++: Strong positive / Contains strong intensity active compounds

The treatment groups in this study were ethanol extract of durian skin with concentrations of 25%, 20%, 15%, 10%, and 2% chlorhexidine act as a positive control group. Each group consisted of 6 samples so that the amount of the total sample was 30.

Table 2. The mean inhibitory zone diameter of various concentrations of ethanol extract of durian peel

Treatment Group	Mean ± SD
25% Durian peel ethanol extract	1,69 ± 0,3888
20% Durian peel ethanol extract	1,25 ± 0,2291
15% Durian peel ethanol extract	0,78 ± 0,2106
10% Durian peel ethanol extract	0,42 ± 0,3529
2% Chlorhexidine (control group)	12,37 ± 0,7554

Table 2 shows the mean value of inhibition in all groups, where chlorhexidine 2% has the highest ability (12.37 mm) in inhibiting *Enterococcus faecalis* bacteria, followed by the 25%, 20%, 15%, and 10% ethanol extract group of durian peel. The normality test was carried out using Shapiro-Wilk showing that the data were normally distributed ($p > 0.05$). The homogeneity test using Levene's test shows a value smaller than 0.05 ($p < 0.05$) which means that the data is not homogeneous. Data analysis using One Way ANOVA parametric test was conducted to determine the effect of ethanol extract of durian peel at various concentrations in inhibiting bacterial growth between treatment groups.

The one way ANOVA test results shows that there is a significant difference in the mean diameter of the bacterial inhibition zone between groups, with a p-value $<\alpha$ ($0.000 < 0.05$). A follow-up test to determine which group was significantly different was carried out by using the Games Howel Post Hoc analysis.

Table 3. The results of the Post Hoc Games Howel test between the test and control groups

Group	Durian peel ethanol extract 25%	Durian peel ethanol extract 20%	Durian peel ethanol extract 15%	Durian peel ethanol extract 10%	Chlorhexidine (CHX 2%)
Durian peel ethanol extract 25%		0.221	0.007*	0.001*	0.000*
Durian peel ethanol extract 20%			0.025*	0.007*	0.000*
Durian peel ethanol extract 15%				0.287	0.000*
Durian peel ethanol extract 10%					0.000*

Chlorhexidine 2%					

* Shows a significant difference at the 0.05 level

The results of the Games Howel test showed that there was a significant difference in inhibition ($p < 0.05$) between all groups except for the 25% and 20% groups and the 15% and 10% groups.

Discussions

The results showed that the ethanol extract of durian peel (*Durio zibethinus murr*) with a concentration of 25%, 20%, 15%, and 10% had antibacterial activity which could be seen from the clear zone or the inhibition zone around the disc paper. The 25% ethanol extract of durian peel showed the largest diameter of the inhibition zone than ethanol extracts durian peel with lower concentrations. Concentrations of 25%, 20%, 15%, and 10% were obtained from diluting pure viscous extracts using distilled water to obtain the desired concentrations.

The results of this study indicate that the mean diameter of the inhibition zone is directly proportional to the concentration of the ethanol extract of durian peel. This is in line with previous in vitro studies by Jamal et al. It shows that the 15%, 20%, 25%, 30%, and 35% ethanol extract of durian peel has antibacterial activity against *Bacillus cereus* which is classified as anaerobic facultative Gram-positive bacteria similar to *Enterococcus faecalis* used in this research.[10]

The ethanol extract of durian peel in this study was identified to contain active compounds in moderate intensity including flavonoids, saponins and tannins, as well as alkaloids and triterpenoids with strong intensity. This is in accordance with the research of Anggraeni et al. (2016). They explained that the ethanol extract of durian peel showed inhibition of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* because of those compounds.[11,12] The content of these active compounds provides antibacterial activity by several mechanisms. Flavonoids are working by inhibiting nucleic acid synthesis, inhibiting cell membrane function by forming complex compounds from extracellular and dissolved proteins that damage bacterial cell membranes. It also inhibits energy metabolism, insufficient energy will lead to permanent damage to bacterial cells and cause bacterial cell death.[13,14]

Alkaloids contained in the ethanol extract of durian peel have an antibacterial mechanism by disrupting the peptidoglycan constituent components in bacterial cells, so that the cell wall layer is not formed intact and causes cell death. In addition, alkaloids can cause changes in amino acids which will cause changes in the genetic balance in the DNA chain so that bacteria are damaged and encourage bacterial lysis.[11,25,50]

The triterpenoid reacts with purines in the outer membrane of bacterial cells to form strong polymer bonds, destroying purines. Damage to purines, which are the entry and exit points for compounds, will reduce the permeability of the cell membrane so that it is deficient in nutrients and will inhibit the growth of bacteria or bacterial death.[15,16]

Tannins as antibacterials able to pass through cell membranes because they can precipitate on proteins and shrink cell walls or cell membranes which disrupt cell permeability. The cells unable to carry out living activities so that their growth is stunted and experiences death.[17,18] Other antibacterial components are saponins that can increase the permeability of the bacterial cell membrane so that it can change the structure and function of the membrane. It will cause denaturation of membrane proteins so that the cell membrane will be damaged and lysis.[19,20]

The mean inhibition zone in the durian peel extract group with concentrations of 25%, 20%, 15% and 10% was 1.93 mm, 1.24 mm, 0.81 mm and 0.49 mm, respectively. This is caused by the difference in the number of active compounds contained therein. Surjowardojo P et al. (2015), stated that the greater the concentration of the extract, the greater the diameter of the inhibition because more active components are contained in the extract.[21,21]

The mean inhibition zone in the 2% chlorhexidine was higher than any concentration of ethanol extract of durian peel. This occurs because 2% of chlorhexidine has a positive charge while the bacteria are negatively charged. This can cause cell death due to cytolysis. It can increase the bacterial cell membrane permeability which results in the release of the main intracellular components, including potassium. This will change cell structure and lead to protein cytoplasm coagulation.[22,23,24] Chlorhexidine also has substantivity properties, i.e the ability to release antibacterial effects continuously and gradually, this causes the antibacterial effect of chlorhexidine to increase and produce a long antibacterial effect.[25,26]

Davis and Stout categorized the strength of antibacterial activity as follows: the inhibition zone diameter of 5 mm or less is categorized as weak; 5-10 mm as moderate; 10-20

mm as strong, and 20 mm or more as very strong.[27,28] Based on these criteria, 25%, 20%, 15%, and 10% ethanol extract of durian peel has weak antibacterial activity. It is not yet certain whether the ethanol extract of durian peel can be used as an alternative of root canal irrigation compared with chlorhexidine 2% that was included in the strong category. Therefore, from the results of this study, it can be said that the ethanol extract of durian peel has not been able to compensate for the antibacterial power provided by 2% chlorhexidine.[29,30]

This study still has limitations. It did not carry out quantitative phytochemical tests, so it is not known exactly how many active substances contain and what active substances have the dominant effect on the tested bacteria. In addition, this study was carried out in vitro so that the effect of the ethanol extract of durian peel on clinical use is uncertain.

Conclusion

The 25%, 20%, 15%, and 10% ethanol extract of durian peel has antibacterial activity against *Enterococcus faecalis* that is classified as the weak category. There was a significant difference in the antibacterial activity of the ethanol extract of durian peel against *Enterococcus faecalis* between all groups except for the concentration groups of 25% and 20% and between 15% and 10%.

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