Antibacterial potential of Durian montong peel extract (Durian zibethinus murray) against growth inhibition of Streptococcus mutans

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Abstract

Introduction: Caries is a chronic infection of tooth tissue which is experienced by 50% of the world’s population. Loss of teeth due to caries can cause problems, including mastication disorders. Caries can occur due to the activity of the main bacteria, ie Streptococcus mutans (S. mutans). A solution is needed to inhibit the growth of S. mutans bacteria, one of them with montong durian peel extract (Durian zibethinus murray) which has antibacterial properties such as flavonoids, saponins, and tannins.

Purpose: Proving the antibacterial potential of montong durian peel extract against growth barriers of Streptococcus mutans.

Methods: This research is an in vitro study with a posttest only control group design. Montong durian peel extract was obtained by maceration method, and divided into concentrations of 90%, 45%, 22.5%, 11.25%, 5.625%; with replication of each concentration 6 times. The tests carried out included phytochemical tests, Minimum Inhibitory Concentration (MIC) tests and Minimum Bactericidal Concentration (MBC) tests. The data obtained was then tested statistically with ANOVA.

Results: There was a significant difference between the groups for the MIC test (p = 0.00) and for the MBC test (p = 0.00), with MIC at a concentration of 22.5%, and MBC at a concentration of 45%.

Conclusion: Montong durian peel extract has antibacterial potential that can inhibit growth of S.mutans.

Keywords: Montong durian peel extract; Streptococcus mutans; MIC, MBC; Good health and well-being

1. Introduction

Caries is one of the problems in dental hard tissue. There are four etiologies for caries, namely: host, substrate, microorganism, and time (1). Caries is a problem that is experienced by nearly 50% of the world’s population, around 3.58 billion people in the world have experienced it. Indonesia is a country with a very high percentage of caries. This
can be seen in 2019 the prevalence of caries in Indonesia reached 88.8%. The highest caries prevalence in Indonesia occurs in people with an age range of 55 to 64 years with a rate of 96.8% (2).

Caries has a negative impact on sufferers, such as sufferers will experience pain, discomfort when eating, drinking and talking. If this condition is not treated immediately, it can interfere with productivity and quality of life. What’s more, caries experienced by someone will also interfere with the aesthetics and appearance of that person (3).

Caries can occur due to the role of microorganisms, one of which is bacteria. Several genus of bacteria that can cause caries include Streptococcus, Lactobacillus, Enterococcus, Actinomyces, Propionibacterium, Prevotella, Porphyromonas, and Scardovia. Streptococcus being the most dominant genus with Streptococcus mutans (S. mutans) bacteria as the main cause (4).

Caries can be prevented by routinely cleaning the oral cavity, such as brushing your teeth twice a day in the morning and evening and reducing consumption of materials containing glucose and sucrose. In addition, caries can also be treated with dental restorations using composite materials. However, this material also has a weakness, namely it can cause secondary caries if the filling does not fit properly (5). Therefore, a material is needed that can prevent caries from occurring.

Durian is a fruit that is liked by many people in Indonesia, even up to around 80% of Indonesians like it. The popularity of durian has even reached Southeast Asia, especially Thailand and Malaysia. Because of this, this fruit has earned the nickname King of Fruits (6). Apart from having a distinctive taste, durian also has herbal properties, one of which is antibacterial. It is proven that when extracted from the peel of the fruit, several antibacterial ingredients are found, including flavonoids, saponins, and tannins (7).

One of the durians that many Southeast Asian people like, especially Indonesia, is the montong durian. Durian which has a Latin name Durian zibethinus murray is a durian that comes from Thailand. Special features of Durian zibethinus murray is to have yellow flesh, thick, dry, and little fat. Durian has a sweet taste with a smooth texture of the fruit flesh and has a pungent aroma that is appetizing. Durian has a yellowish green peel color with conical thorns and is small and dense. Durian is difficult to split because of the many sharp thorns. Durians weigh about 3 to 5 kg (8).

Montong durian can be used on the peel. Durian peel contains starch, pectin, essential oils, flavonoids, saponins, cellulose elements, tannins, lignin, and ethanol compounds. Durian fruit peel can be used as a mosquito repellent and as an anti-bacterial, the roots can be used to treat nail infections (9). However, there is still very little research exploring the benefits of this herbal fruit. In addition, a phenomenon was also found at the durian center in the Gunungpati region, namely the volume of durian peel waste piling up. This occurs as a result of the widespread practice of consumers and traders of durians to discard the peel. If not managed properly, This durian peel waste is harmful to the environment, causing it to become unclean and emit an unpleasant odor when the durian peel rots. To solve this issue, the management of durian peel waste must be improved by incorporating the larger community and turning the waste into goods with a marketable purpose, including as an ingredient for herbal medicine. Using the justification provided, the objective of the present investigation is to demonstrate the antibacterial ability of the montong durian peel extract (Durian zibethinus murray) in preventing the growth of the S. mutans bacterium.

2. Material and Methods

2.1. Ethic

Ethical feasibility has been reviewed and accepted by the Ethics Committee of the Faculty of Dental Medicine, Universitas Airlangga before conducting the research with the ethical permit certificate number: 045/HRECC.FODM/1/2023.

2.2. Manufacture of durian peel extract

The sample used montong durian, which was purchased from the Agrotourism Garden, Wonosalam District, Jombang Regency. Extraction uses the maceration technique, in which durian peel samples are selected which are still sterile and then cleaned, cut into small pieces, dried without using sunlight, and mashed. Furthermore, the mashed durian peel was stored in a container and dissolved in 96% ethanol solvent, and left for three days. The extract precipitate was then discarded, and the remaining extract solution was evaporated using evaporator rotary and freeze drying until a viscous extract is obtained, which is then dissolved in distilled water.
2.3. Preparation of BHIB and TYC bacterial media
Preparation of BHIB media by dissolving a ratio of 37 grams of BHIB media powder in 1000 ml of distilled water. Preparation of TYC media by dissolving a ratio of 21 grams of TYC media powder in 1000 ml of distilled water. The two media were then heated until completely dissolved, and sterilized with autoclave at 121ºC for 15 minutes. Then it was cooled at a temperature of 45-50ºC (11).

2.4. Making bacterial culture Streptococcus mutans
Bacteria were taken from bacterial stock of S. mutans from the Research Center of the Faculty of Dental Medicine, Universitas Airlangga using a loop needle, then put it into a test tube containing BHIB media, then incubated for 24 hours at 37ºC. After that, the bacterial culture was calibrated to the equivalent of 0.5 McFarland (11).

2.5. Phytochemical Testing

2.5.1. Flavonoid content testing
Testing for the content of flavonoids was carried out using 2 mL of durian peel extract reacted with Magnesium and HCL of 1 mL. If there was a change in color to red, yellow, or orange, it indicates that it is positive for flavonoids (12).

2.5.2. Saponin Content Testing
Saponin content testing was carried out using 2 mL of durian peel extract, then 10 mL of distilled water was added, then heated for 2-3 minutes, then cooled and shaken, then 1 mL of HCl was added. If the results show the presence of foam, then it is positive for saponins (12).

2.5.3. Tannin Content Testing
Testing for tannin content was carried out by adding 2 mL of durian peel extract to 10 mL of distilled water. Then boil for 3 minutes then cool. Then it was tested with several reagents, the FeCl3 reagent was positive if the result was a blue-black color change. In 1% gelatin reagent, it is positive when a white precipitate forms. In Steasny's reagent, it is positive when a pink precipitate forms (12).

2.6. Testing of anti-bacterial power and calculation of the number of bacteria
Testing of MIC and MBC was carried out using the serial dilution method. In each test tube, 2 ml of BHIB media were given, then in the first test tube, 8 ml of pure maceration solution was given, so that a concentration of 90% was obtained in the first test tube. The first test tube was stirred with a vortex and then 5 ml was taken and put into the second tube which contained 5 ml of BHIB media to obtain a 45% concentration, this was repeated in each test tube at a concentration of 22.5%, 11.25% and 5.625 %. The next was the bacterial suspension S. mutans put into a test tube and incubated for 24 hours at 37ºC then observed for turbidity. 0.1 ml of suspension for each treatment was then poured into a petri dish containing BHIB media, smoothed with a spreader and then incubated for 24 hours at 37ºC. After incubation, perform a colony count of S. mutans (11).

2.7. Data analysis
The normality test was carried out using Shapiro Wilk, and the homogeneity test was carried out with the Levene Test, as well as the One Way Analysis of Variance (ANOVA) for different test.

3. Result and Discussion
The results of the phytochemical test were carried out to verify and measure the antibacterial substances in the durian montong peel extract. (Fig 1). Measurements were made on three compounds contained in it, namely flavonoids, saponins, and tannins.
Figure 1 Bar chart of phytochemical test results of durian montong peel extract.

Based on the bar chart above, the active ingredients contained in the rind of the montong durian are 4.01% flavonoids, 2.61% saponins and 3.84% tannins.

Figure 2 Culture of S. mutans in BHIB and TYC media

The results of the dilution test were carried out using durian peel extract to S. mutans using concentrations of 90%, 45%, 22.5%, 11.25%, and 5.625% and used a negative control group as a comparison (Figure 2). In the dilution test of durian montong peel extract showed that at concentrations of 90% and 45% there was no S. mutans bacteria growth. The growing number of S. mutans bacteria was evident in the 5.625 concentration group, reduced at 11.25% concentration, and least in the 22.5% concentration group. From the results of statistical tests showed that the treatment concentration of 22.5% was MIC (Minimum Inhibition Concentration) while at a concentration of 45% was MBC (Minimum Bactericidal Concentration).
A disorder characterized by the destruction of the teeth's hard tissue is called caries. The affected tooth tissues are enamel, dentin, and cementum. Caries can occur due to a demineralization process that has an impact on the tooth tissue. The etiology of caries consists of four parts, namely: host, substrate, microorganisms, and time. Please note that the four parts are related to one another. So if one of them is not fulfilled, then caries does not occur. Certain bacteria have the ability to ferment a variety of carbohydrates found in foods, including sucrose and glucose, and produce acids, causing the pH of the plaque to drop less than 5 about 3-5 minutes. Demineralization of the enamel of the tooth happens as a result of a sustained pH decline over a predetermined period of time.

Plaque on the tooth enamel signals the start of the caries process. Salivary ingredients such as mucin, traces of oral tissue cells, leukocytes, lymphocytes, food leftovers, and bacteria combine to form plaque. This plaque is initially formed, liquid agar which over time becomes a colony where bacteria grow.

This plaque formation over time will cause demineralization on the enamel surface first. Bacteria attached to the enamel will produce lactic acid. The lactic acid will then decompose the mineral matrix of the teeth, causing them to appear white spot lesion on enamels. As time goes by, then white spot lesion turns into black lesions and form cavities in the enamel. After invasion of the enamel, bacteria will enter the dentin. Bacterial invasion will cause dentine decomposition. Once the dentin is infected, the bacteria travel to the cementum. This condition is dangerous, because it can cause gingival recession. Caries that invades the cementum generally occurs in the elderly.

Streptococcus mutans (S. mutans) is a gram-positive pathogenic bacterium that causes dental caries. The bacteria attach to the tooth surface and then metabolize converting glucose into ATP and lactic acid. Lactic acid causes demineralization gradually to form carious lesions. This bacterium also has a virulence factor for caries, namely the presence of adesin. Adesin plays a part in the bacterial adherence to the enamel's surface. Without the attachment of bacteria to the tooth surface, caries will not occur. S. mutans having a virulence factor is able to survive in an acidic environment. The ability to survive in an acidic environment makes S. mutans highly adaptive to conditions in the oral cavity. Another bacterial virulence of S. mutans is ability to form polysaccharide extracellular hydrophobic structures causing it to survive various defense systems in the oral cavity.

S. mutans will adhere to the tooth surface, especially to dental plaque, which is a multispecies biofilm that forms on the hard surface of tooth tissue. There are two main ways how S. mutans causing pathogens on the hard tissue of teeth: first
it can form organic acids by breaking down carbohydrates in food, so that the environment becomes acidic (acidogenecity); the second, S. mutans able to survive and thrive in an acidic environment (low pH). S. mutans is capable of producing glucosyltransferases (GTFs) enzymes, which produce extracellular polysaccharides from sucrose. This allows other bacteria to attach to the teeth and form cariogenic biofilms, causing demineralization without remineralization of the teeth, until dental caries occurs.

This condition requires a solution, which can be by using natural ingredients that have antibacterial power to inhibit or kill S. mutans bacteria. One of the natural ingredients that can be utilized is the peel of the montong durian. Durian is one type of plant that is very popular in Southeast Asia. Plants that have Latin names Durian zibethinus murray is also one of the plants in this region. Indonesia is a country that is one of the centers of durian diversity. Plants belonging to the family Bombacceae is a seasonal fruit that is often found. Kalimantan Island is one example. Durian ranks fourth as the fruit with the highest production volume in Indonesia with a total production of 700 thousand tonnes per year. The fruit is widely available from September to February, but April to July can be hard to find.

Durian is a type of herbal plant. This is proven by the existence of several benefits of durian in the health sector. Some of the benefits found in durian include being rich in vitamins A, C, and D, especially in the roots and stems, so that it functions as an antioxidant. In addition, durian is also rich in iron in the leaves, so it can improve blood flow. Furthermore, durian also contains macronutrients such as carbohydrates, protein, and fat in fruit, so that it functions as a source of energy, accelerates cell and tissue repair, as well as food reserves in the body.

Based on the phytochemical tests that have been carried out, it was found that durian peel contains active ingredients that are anti-bacterial, namely flavonoids (4.01%), saponins (2.61%), and tannins (3.84%). The ability of durian peel extract to inhibit growth as well as kill S. mutans, because durian skin has active ingredients that act as anti-bacterials above. Flavonoids are chemical compounds having a fifteen-carbon chain with two benzene rings. Positions 3, 5, 7, 2, 3, and 4 are frequent locations for hydroxylation in flavonoids. Flavonoids also have glycosidic bonds which are typically found in positions 3 or 7 with carbohydrates in the form of L-rhamnose, D-glucose, glucorhamnose, galactose, orarabinose. Natural sources of flavonoids can be found in plants.

The active ingredient of flavonoids is one of the most effective main ingredients in inhibiting bacterial growth. The way the flavonoids work is by damaging the bacterial cell walls, through reactions with lipids and amino acids in their alcohol compounds. Furthermore, the protein complex in the cell membrane will experience denaturation, and cell damage occurs.

Flavonoids on durian skin have antibacterial properties, because they have the ability to stop bacterial growth. The content of chemical compounds of flavonoids naturally provides antibacterial defense for durian plants. The mechanism of antibacterial activity of flavonoids is by preventing energy metabolism, harming the cytoplasmic membrane, and preventing the formation of nucleic acids (DNA/RNA). Flavonoids prevent the formation of nucleic acids in DNA/RNA because their ring B group will result in the addition of nucleic acid bases. During the hydroponic bonding or intercalation process, the process of intercalation is the insertion of molecules between base pairs. The phenomenon of intercalation results in inhibition of DNA/RNA nucleic acid synthesis.

Flavonoid chemical compounds are also able to inhibit the function of the cytoplasmic membrane by dissolving cell membrane proteins. In addition, flavonoids are also able to inhibit energy metabolism by inhibiting NADH Cytochrome C Reductase, as cytochrome C is an important component in the formation of energy/ATP in the process of electron transport.

The next anti-bacterial ingredient contained in the peel of the durian fruit is tannin. Tannins are water-soluble chemicals classified into the polyphenol group. Tannins are a class of polyphenolic compounds that are found in leaves, peel and fruits of plants. Tannin compounds are also known as one of the compounds that have antibacterial properties, where tannins are able to damage the bacterial cell structure so that cell death occurs.

Tannins can be found easily in various herbal plants. Currently, tannins have been extensively studied for their antibacterial and antiviral abilities. Tannins work by diffusing into the walls of the phospholipid bilayer of bacterial cells and then damaging them, so that the bacterial cells will undergo lysis. The mechanism for this is because tannins inactivate enzymes and interfere with protein pathways, resulting in an imperfect bacterial cell wall. Imperfect cell walls cause bacterial cells to die due to lysis due to osmotic pressure.

The next compound is saponins. Saponins are chemical compounds that are abundant in various plant species, one of which is durian. This compound is an amphipathic glycoside. The chemical formula of saponins is C27H42O3. Saponins
have a molecular weight of 414.6231 gram/mol. Saponins have a fairly high boiling point, reaching 158°Celsius. Saponins can dissolve in various solvents, such as water, ethanol, and methanol.

Saponin compounds are effective ingredients for inhibiting bacterial growth, especially gram-positive bacteria. The way saponins work is shown through a mechanism that involves a reduction in tension at the bacterial cell wall's surface, where the cell wall of bacteria becomes permeable, and cell leakage occurs causing damage or cell death. In addition, saponins can also cause denaturation of bacterial proteins. As a result of this, the bacteria will become lysed because the protein compounds in the bacteria experience a loss of structure. So that the protein structure in bacteria becomes unstable and interferes with bacterial metabolism.

The results of this study prove that montong durian peel extract containing active ingredients of flavonoids, tannins and saponins can inhibit bacterial growth. Streptococcus mutans, with MIC at a concentration of 22.5% and MBC at a concentration of 45%, according to previous research, that the ethanol extract of montong durian peel can inhibit Staphylococcus aureus gram-positive bacteria growth at a 10% minimum inhibitory concentration. Other studies have also explained that montong durian peel extract can effectively inhibit the growth of both gram-negative and gram-positive bacteria, with the content of its antibacterial compounds.

4. Conclusion
Montong durian peel extract (Durian zibethinus murray) has antibacterial potential in inhibiting growth of Streptococcus mutans.

Disclosure of Conflict of interest
No Conflict of interest to be disclosed.

References


