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Potential of honey pineapple peel (*Ananas comosus* (L) Merr.) from Kediri in Killing Bacteria *Porphyromonas gingivalis*: Periodontitis agent

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Abstract

Background: *Porphyromonas gingivalis* is a bacteria that is commonly found in periodontal pockets. *Porphyromonas gingivalis* has pathological abilities that can damage the supporting tissue of the teeth (periodontitis). Periodontal disease can be treated with mechanical therapy and antibiotics. However, antibiotic therapy can cause negative side effects. One alternative therapy that can be used is the use of natural ingredients. Honey pineapple (*Ananas comosus* (L.) Merr.) is a plant that has many properties, one of which is as an antibacterial

Objective: To determine the potential of honey pineapple peel (*Ananas comosus* (L.) Merr.) from Kediri in killing *Porphyromonas gingivalis* bacteria which cause periodontitis.

Methods: The method used in this study was solid dilution using Mueller Hinton Agar media to obtain the Minimum Bactericidal Concentration (MBC).

Results: The results of the study showed the effectiveness of Kediri honey pineapple peel extract in killing *Porphyromonas gingivalis* bacteria with a concentration of 6.25% having an MBC value of 69.8%, a concentration of 12.5% obtained an MBC value of 90.7%, and at concentrations of 25%, 50%, and 100% obtained an MBC value of 100%.

Conclusion: Honey pineapple peel (*Ananas comosus* (L.) Merr.) from Kediri can kill *Porphyromonas gingivalis* bacteria maximally at concentrations of 25%, 50%, and 100%

Keywords: Honey pineappple peel; Kediri; Killing Bacteria; *Porphyromonas gingivalis*; Periodontitis

1. Introduction

Porphyromonas gingivalis is a black-pigmented, rod-shaped, obligate anaerobic gram-negative bacterium that is often found in periodontal pockets. This bacterium can form a biofilm protected by a layer of Lipopolysaccharide (LPS) which is an endotoxin that can cause damage to the supporting tissues of the teeth (periodontal) [1]. *Porphyromonas gingivalis* bacteria have the ability to tolerate oxygen, allowing these bacteria to survive in the gum pocket area (gingival sulcus) [2]. *Porphyromonas gingivalis* bacteria are also able to invade periodontal tissue cells, including epithelial cells, fibroblasts and osteoblasts, and are able to exploit macrophages and dendritic cells through their fimbriae. This virulence ability can cause damage to periodontal tissue because the bacteria enter deeper cell areas than where the bacteria initially started the invasion [3].

Periodontal disease (periodontitis) is a disease caused by *Porphyromonas gingivalis*. The accumulation of food scraps and plaque containing pathogenic bacteria can trigger a response from the body's immune system [4]. The interaction

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between pathogenic bacteria over a certain period of time causes inflammation in the periodontal tissue, damage to the tooth supporting tissue (periodontal ligament and alveolar bone), formation of pockets, gingival recession, and tooth looseness [2,5]. Periodontal disease can be treated using mechanical treatment (scaling, root planing and surgery) or the use of antibiotics [6]. Long-term use of antibiotics is not recommended because it can cause antibiotic resistance[7]. Utilizing the potential of plants as natural medicinal ingredients is one alternative.

Honey pineapple (*Ananas comosus* (L.) Merr.) is one of the superior fruit commodities originating from Kediri Regency, East Java, Indonesia [8]. The most common type of Kediri honey pineapple is the Queen, which has an oval-shaped fruit similar to a cone, is yellow in color, has a sweet taste (like honey) and is slightly sour [9]. Phytochemical tests of pineapple peel extract have antibacterial activity including flavonoid, tannin and saponin compounds [10]. Other research states that antibacterial activity is caused by the presence of the enzyme compound bromelain which is only found in pineapples [11]. Pineapple peel has nutritional content, namely 17.53% carbohydrates, 4.41% protein, 13.65% reducing sugar, 81.72% water content, and 20.87% crude fiber. Honey pineapple skin also has high antioxidant activity [12].

From the description above, researchers want to know the potential of honey pineapple peel (*Ananas comosus* (L) Merr.) from Kediri in killing bacteria (*Porphyromonas gingivalis*) which causes periodontitis.

2. Material and methods

2.1. Making Pineapple Honey Peel Extract

Kediri honey pineapple fruit is peeled, skinned (peel), cut, dried and then blended until coarse fibers are formed. Pineapple fibers are put into a 1 liter Erlenmeyer flask and 500 ml of 96% ethanol is added. The solution is macerated for 24 hours at room temperature. The solution is then separated using a Buchner filter. The filtered results are mixed and concentrated with a rotary vacuum evaporator at a temperature of 50°C until a thick extract with a concentration of 100% is obtained.

2.2. Determination of Concentration

The method used in determining the concentration of the extract used the dilution method or multilevel dilution with a ratio of 1:2 (w/v). The test tubes were labeled with numbers 1-7 sequentially, filled with 5 ml of sterile distilled water, 10 ml of pineapple peel extract until a pineapple peel extract concentration of 100%, 50%, 25%, 12.5%, 6.25% was obtained. The sixth test tube contained BHI-B as a positive control, and the seventh test tube contained BHIB and bacteria.

2.3. Preparation of *Porphyromonas gingivalis* Bacterial Isolate

Porphyromonas gingivalis bacteria (Isolate stock from the Airlangga University Research Center) were planted in a test tube containing Brain-Heart Infusion Broth (BHIB) liquid media. The test tube was then inserted into an anaerobic jar. Then inserted into an incubator and incubated for 24 hours at 37°C.

2.4. Making Mueller Hinton Agar Media (Solid Dilution)

Weigh 19 grams of Mueller Hinton Agar powder into sterile distilled water. Heat on a stove until boiling. The media is poured into a petri dish of about 25 ml and left to solidify.

2.5. Minimum Bactericidal Concentration (MBC) Determinantion

Honey pineapple peel extract and *Porphyromonas gingivalis* were dropped into Mueller Hinton Agar as much as 0.1 ml on a petri disk. The petri dish was incubated anaerobically in an incubator at 37° C for 24 hours. Then counted using a colony counter using the formula:

$$\textbf{\% Colony} = 100\% - \frac{\text{Number of living colonies}}{\text{Number of colonies on bacterial control}} \times 100\%$$

3. Results and discussion

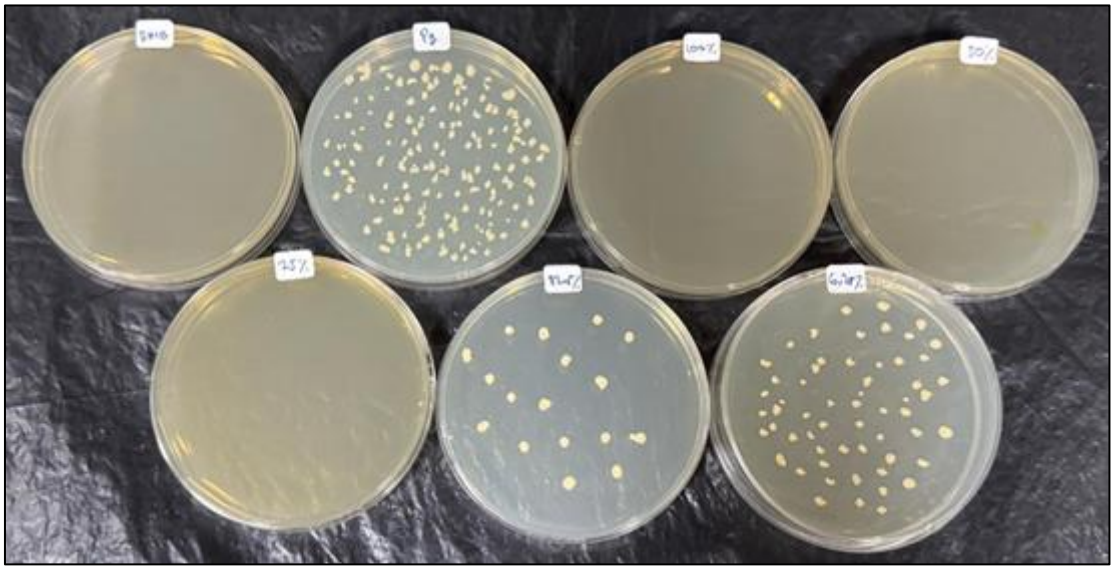

Figure 1 Observation results (a) BHIB (Negative Control); (b) *Porphromonas gingivalis* (Positive Control); (c) *Porphromonas gingivalis* + Extract 100%; (d) *Porphromonas gingivalis* + Extract 50%; (e) *Porphromonas gingivalis* + Extract 25%; (f) *Porphromonas gingivalis* + Extract 12.5%; (g) *Porphromonas gingivalis* + Extract 6.25%

The research results obtained are as follows:

Table 1 Research Results

No	Sample	Number of Living Colonies	MBC	Information
1	*Porphromonas gingivalis* + Extract 100%	0	100%	Not growing
2	*Porphromonas gingivalis* + Extract 50%	0	100%	Not growing
3	*Porphromonas gingivalis* + Extract 25%	0	100%	Not growing
4	*Porphromonas gingivalis* + Extract 12,5%	17	90,1%	Bacteria growth
5	*Porphromonas gingivalis* + Extract 6,25	58	66,1%	Bacteria growth
6	BHI-B (Negative Control)	0	-	Not growing
7	*Porphyromonas gingivalis* (Positive Control)	171	-	Bacteria growth

The honey pineapple peel extract with a concentration of 6.25% produced 58 live colonies with an MBC value of 66.1% and the honey pineapple peel extract with a concentration of 12.5% produced 17 live colonies with an MBC value of 90.1%. The results of this study are in line with previous research conducted by Putri et al., (2023) which stated that pineapple peel extract (*Ananas comosus* (L.) Merr.) has antibacterial properties against gram-negative bacteria *Aggregatibacter actinomycetemcomitans* through a solid dilution test (agar) with a Minimum Bacterial Concentration (MBC) obtained at a concentration of 6.25% [13].

At higher concentrations of 100%, 50%, and 25%, no live colonies were found and had a MBC of 100%. This study is in line with the study conducted by Husniah, Ningtyas and Soleha (2023) which concluded that extracts from honey pineapple peel have antibacterial activity against *Staphylococcus aureus* bacteria with inhibitory power at a concentration of 50% and at a concentration of 100% [14]. Other studies have shown that giving pineapple honey extract with higher concentrations will be more effective in destroying gram-negative bacteria than gram-positive. This is because the cell wall structure of gram-negative bacteria has a thinner pepidoglycan wall than gram-positive, even though it has an outer phospholipid membrane. Pepidoglycan functions to maintain cell stability, maintain cell shape, provide mechanical strength, and protect cells from osmotic pressure. If this wall is damaged, bacterial cells will be more easily lysed [15].

Honey pineapple contains bioactive compounds (tannins) which play a role in suppressing bacterial growth [16]. Tannins produced from honey pineapple peel are one of the natural phenolic compounds that have the ability to inhibit and kill bacteria. Tannin compounds are also able to coagulate cell proteins and bacterial cytoplasmic membranes which ultimately cause lysis [17]. Other sources mention that tannin compounds have the ability to shrink or shrink cell membranes, inactivate enzymes and cell walls so that cell permeability is disrupted. This condition can cause cells to be unable to carry out activities, growth and can cause death [7]

Another content that honey pineapple peel has is the enzyme bromelain. The enzyme bromelain is a proteolytic enzyme that has the ability to hydrolyze proteins so that it acts as an antibacterial [18]. The mechanism of hydrolysis by bromelain is by destroying amino acid bonds at certain locations and dividing the amino acid chain into peptide fragments [24]. In addition, bromelain also changes the structure of bacterial cell walls, breaks protein bonds and changes the properties of the proteins that make up bacterial cell walls so that it can cause leaks and cause cell death [19]. Previous research has shown that the enzyme bromelain has the ability to inhibit the growth of the bacteria *Aggregatibacter actinomycetemcomitans*, *Streptococcus mutans* and *Porphyromonas gingivalis* [5].

Honey pineapple peel also contains flavonoids which consist of various components, and are named according to their chemical structure. Flavonoid components found in pineapple peel include gallic acid, epicatechin, catechin, and ferulic acid [20]. Other flavonoid components such as epigallocatechin gallate, galanin, and 3-O-octanoyl-(+)-catechin have bactericidal activity. However, if 3-O-octanoyl-(+)-catechin is induced by other flavonoid components, flavonoids cannot kill bacterial cells but only inhibit their growth [21]. Other sources mention that flavonoid compounds have the ability to kill bacteria (bacteriocidal) by forming complex bonds with extracellular proteins through hydrogen bonds. Hydrogen bonds with extracellular proteins can cause instability in the structure of bacterial cell membranes. As a result, the permeability of the bacterial cell wall will be disrupted, resulting in bacterial cell death [22].

Another chemical compound that can act as a bacteriocidal on *Porphyromonas gingivalis* is saponin. Saponin can reduce surface tension, thereby increasing the permeability of the cell membrane, which causes damage to the cell membrane. Damage to the bacterial cell membrane causes various important components to be released from the cell, namely proteins, nucleic acids, and nucleotides. This condition will gradually cause lysis of the bacterial cell [23].

4. Conclusion

Honey pineapple peel (*Ananas comosus* (L.) Merr.) from Kediri can kill *Porphyromonas gingivalis* bacteria (the cause of periodontitis), especially at concentrations of 25%, 50%, and 100%.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

In accordance with the guidelines for publication ethics, we, the authors of the manuscript entitled Potential of Honey Pineapple Peel (*Ananas comosus* (L) Merr.) from Kediri in Killing Bacteria *Porphyromonas gingivalis*: Periodontitis agent. Confirm that we have collaborated with institutions or products mentioned in the manuscript that are relevant to the research presented. We are committed to being transparent and will continue to disclose any potential conflicts of interest that may arise in the future.

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