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ORIGINAL ARTICLE

Antibacterial and Antibiofilm Efficacy of Pineapple Hump (*Ananas comosus*) on *Porphyromonas gingivalis in vitro*

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ABSTRACT

Background: Periodontal disease is one of the most prevalent oral health problems in Indonesiathat affects supporting tissues of the teeth. *Porphyromonas gingivalis* plays an important role in the pathogenesis of periodontal disease. Alternative therapy with natural plant extracts, includingpineapple (*Ananas comosus*) hump extract may inhibit the growth of bacteria that cause periodontal disease. **Objective:** To determine the effect of pineapple hump extract on bacterial growth and adhesion of *Porphyromonas gingivalis* biofilms. **Method**: The bacterial inhibition test was performed by the agar well diffusion method, and biofilm density measurements were madeusing the biofilm assay method. **Results:** Pineapple hump extract can inhibit bacterial growth optimally at a concentration of 100%, with an average zone of inhibition of 7.3 mm. The extract at a 50% concentration can eradicate the biofilms in a 6 h incubation time with an average OD of 0.124. The Shapiro-Wilk method confirmed a normal distribution of the data. Both one-way ANOVA and post hoc test showed a significant difference between the inhibitory ability of pineapple hump extract and between concentrations to restrict formation of *Porphyromonas gingivalis* biofilms. **Conclusion:** Pineapple hump extract was able to inhibit bacterial growth anderadicate the adhesion of *Porphyromonas gingivalis* biofilms in vitro.

Key words: periodontal disease, pineapple hump extract, *Porphyromonas gingivalis*

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INTRODUCTION

Periodontal disease is a common disorder of the human oral cavity. As the population increases, periodontal disease has become a significant health problem in the health sector.[1,2] Periodontal disease is an inflammatory disease of the oral cavity caused by microorganisms in the supporting tissues of the teeth.[2] Uncontrolled accumulation of plaque and bacteria is the main etiology of periodontal disease.[3,4] The prevalence of periodontitis in Indonesia is high; data obtained from the 2018 National Basic Health Research indicate a percentage of periodontitis cases in Indonesia of 74.1%.[5]

Porphyromonas gingivalis (*P. gingivalis*) is a bacterial species that plays an important rolein the pathogenesis of periodontal disease.[6] These bacteria are classified as Gram-negative anaerobic bacteria and are found mostly in subgingival plaque, where they cause chronic periodontitis.[7] Plaque control is important in periodontal treatment, and can be performed by gargling with chlorhexidine solution, which has antimicrobial properties that can inhibit the growth of bacteria that cause periodontal disease.[8] However, long-term use of chlorhexidine will cause discoloration of the teeth and the formation of supragingival calculus, while also leaving a bitter taste.[9]

The side effects of chemical-based mouthwashes can be reduced by natural ingredients that are beneficial in the health sector. Medicinal plants have been widely used in the management of infectious and it has been proof against oral pathogens.[10-12] One of these is the hump of pineapple. This fruit contains the enzyme bromelain which an enzyme that has been shown to reduce and break the glutanine-alanine and arginine-alanine bonds.[13,14] Almost all parts of the pineapple (*Ananas comosus*) can be beneficial for health including the flesh, skin, and stems. The bromelain enzyme

contained in pineapple hump has many benefits as anti-inflammatory, anticancer, antimicrobial, and reduces the severity of angina pectoris.[15] The mechanism of antimicrobial action on the bromelain enzyme is by destroying the structure of the bacterial wall. According to previous research, the concentration of bromelain contained in pineapple hump is higher than pineapple flesh.[16] The skin and hump of pineapple commonly regarded as wastes, in fact there is an enzyme bromelain which has an inhibitory effect on the growth of periodontal bacteria.[17,18] Some studies have been tested the antibacterial activity of the pineapple hump extract against oral pathogen bacteria and found that pineapple hump extract had antibacterial effects against *P. gingivalis* and *Enterococcus faecalis*.[19, 20]

No research has yet investigated the effectiveness of pineapple hump extract on the adherence of *P. gingivalis* biofilms. Therefore, the aim of this study was to determine the effectiveness of pineapple hump extract on bacterial growth and adhesion of *P. gingivalis* biofilms.

METHODS

This research was an *in vitro* laboratory experimental study with a post-test-only control design, conducted at the Laboratory of Microbiology Center of Research and Education (MiCORE), Faculty of Dentistry, Trisakti University. An extract of pineapple hump from Balai Penelitian Tanaman Rempah dan Obat (Balittro) plantation was prepared by a maceration method using 96% ethanol as a solvent and was then diluted with distilled water to obtain concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125%. Firstly, the pineapple hump is separated from the flesh. Secondly, it is dried under the sun and then put into a grinder until it becomes powder. Thirdly, the pineapple hump powder was extracted by maceration using a 96% ethanol solution in a ratio of 1:5 for 24 hours. Then, the resulting solution is filtered with a filter paper and then evaporated with a rotary evaporator which aims to obtain a thick extract and free from solvents. Finally, this thick extract was diluted with distilled water to obtain concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125% The well diffusion method was used, so the inhibition zone formed could be measured using a caliper. The biofilm assay methodwas used to measure the density of biofilm by crystal violet staining and measurement with a microplate reader at a wave length of 490 nm.

Phytochemical test on pineapple hump extract

Flavonoid test
A small amount of Mg powder and concentrated hydrochloric acid were added to a 2 mL sample. An orange or pink to red color indicated the presence of flavonoid compounds.

Phenol test
A few drops of hot water and 1–2 drops of 1% $FeCl_3$ were added to a 2 mL sample. A blueor purple color indicated the presence of phenolic compounds.

Saponin test
A 10 mL volume of distilled water was added to a 2 mL sample and shaken. Formation of afoam that lasted for 5 min indicated the presence of saponin compounds.

Terpenoid test
Liebermann-Burchard reagent was added to a 2 mL sample. A green to blue color indicatedthe presence of terpenoid compounds.

Alkaloid test
A 5 mL volume of chloroform and ammonia solution was added to a 2 mL sample and heated,shaken, and then filtered. A 1 mL volume of 2N HCl was added, shaken for 2 min, and left until two layers separated. The acid (top) layer was taken and 1–2 drops of Dragendorff reagent wereadded. A red color indicated the presence of alkaloids.

Tannin test
A2 mL sample was combined with 1% FeCl3. A blackish green or dark blue color indicated the presence of tannin compounds.

***Porphyromonas gingivalis* cultures**
Porphyromonas gingivalis samples were obtained from MiCORE, Faculty of Dentistry, Trisakti University with ATCC 33277 strain. The bacteria were cultured on nutrient broth medium and then incubated for 24 h at 37°C in an anaerobic jar using the Gaspack jar system to maintain anaerobic conditions.

Inhibition test
A 20 µL sample of bacterial culture was spread on BHI until evenly distributed in each of 12 petri dishes. Five wells were made in BHI agar. Three wells contained different concentrationsof pineapple hump extract, one well served as a positive control (chlorhexidine), one well servedas a negative control (sterilised water), and each pineapple hump extract at a concentration of 100%, 50%, 25%, 12.5%, 6.25%, and 3.12%. The wells were filled with 20 µL of test solution using a sterile micropipette, and all concentrations were tested in triplicate.

The samples were incubated at 37°C and observed for 24h. The diameter of the inhibitionzone around the wells was measured using a caliper.

Biofilm assay
This study used 0.2% chlorhexidine as a positive control and sterilised water as a negativecontrol. A 200 µL sample of bacterial culture was added to a 96-well plate and incubated at 37°Cfor 48 h under anaerobic conditions. The medium from the culture was then discarded, leaving the remaining biofilm layer adhered

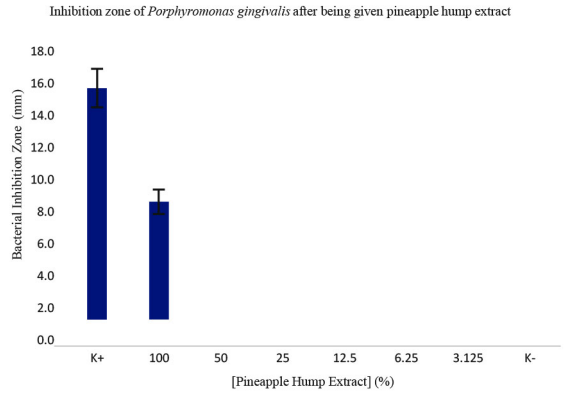

Figure 1. Inhibition zone of pineapple hump extract against *Porphyromonas gingivalis*

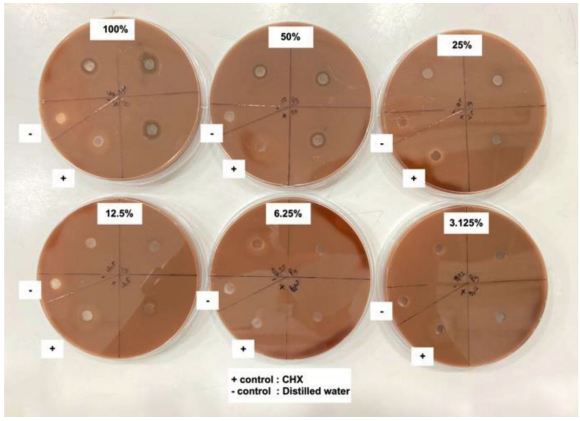

Figure 2. Minimum Inhibitory Concentration of Pineapple Hump for *P.gingivalis* in different concentration 100%, 50%, 25%, 12.5%, 6.25%, and 3.125%. Distilled water were used as the negative control and chlorhexidine (0.2%) was used as the positive control. All treatment were done in triplicate

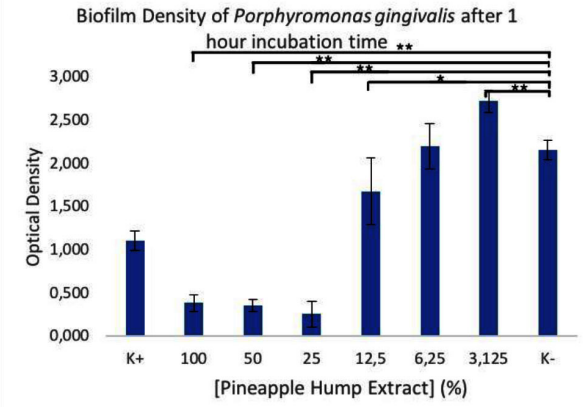

Figure 3. Density of *Porphyromonas gingivalis* biofilm treated with pineapple hump extract atseveral concentrations for 1 h. *p<0.05, **p<0.01

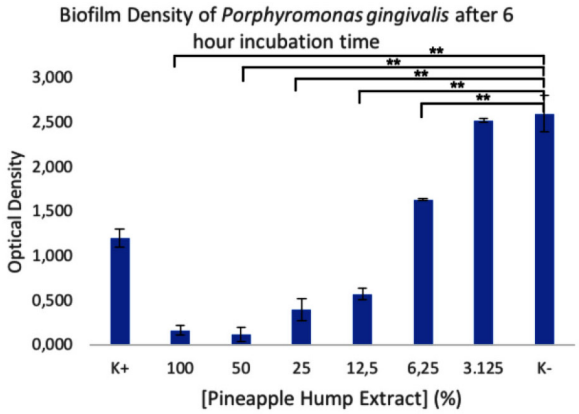

Figure 4. Density of *Porphyromonas gingivalis* biofilm treated with pineapple hump extract atseveral concentrations for 6 h. *p<0.05, **p<0.01

to the bottom surface of the well plate. The well plate was rinsed with phosphate buffered saline (PBS). Pineapple hump extract (up to 200 µL) was added at several concentrations (100%, 50%, 25%, 12.5%, 6.25%, and 3.12%), and the well plates were incubated under anaerobic conditions at 37°C and observed for 1, 6, and 24 h. The extract was then discarded and the well plate was rinsed with phosphate buffer saline (PBS), then heat-fixed.The biofilms were stained for 15 min with crystal violet (0.05% w/v) and then rinsed with PBS. A 200 µL volume of 96% ethanol was added, and the stained biofilm in the well plate was measuredwith a microplate reader at a wavelength of 490 nm.

Statistical analysis

All data collected were analyzed using the Shapiro-Wilk normality test, followed by a one-way analysis of variance (ANOVA) and post hoc least significant difference (LSD). A normality test confirmed a normal distribution of the results ($p > 0.05$). The one-way ANOVA showed a significant difference ($p < 0.05$). The post hoc LSD test, performed to determine the significant differences between the results of the study, confirmed a significant difference ($p<0.05$) .

RESULTS

The phytochemical test results revealed that the pineapple hump extract contained alkaloids, saponins, and flavonoids. The inhibition zone measurements for *Porphyromonas gingivalis* bacteria revealed that a clear zone began to form at an extract concentration of 50%. By contrast, concentrations of 25%, 12.5%, 6.25%, 3.125%, and the negative control showed noinhibition zones. (Figure 1)

The biofilm assay on *Porphyromonas gingivalis* bacteria after a 1 h incubation showed that the pineapple hump extract significantly reduced the amount of *Porphyromonas gingivalis* biofilm at all concentrations and all incubation times ($p < 0.05$). (Figure 3,4,5). The most effective concentration was 25% with a 1 h incubation time.

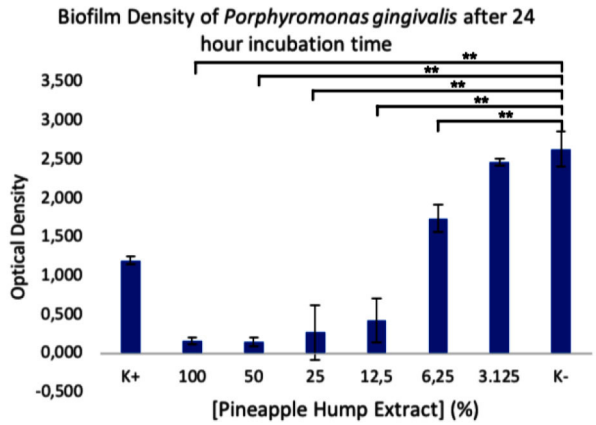

Figure 5. Density of *Porphyromonas gingivalis* biofilm treated with pineapple hump extract atseveral concentrations for 24 h. *p<0.05, **p<0.01

DISCUSSION

Pineapple hump was confirmed to contain alkaloids, flavonoids, and saponins. Alkaloids act as antibacterial agents, as they can interfere with the formation of peptidoglycan in bacterial cells. This prevents the complete formation of the bacterial cell wall layer, resulting in cell death. Flavonoids are polar phenolic compounds that can penetrate into the bacterial peptidoglycan layer, break down the bacterial cell proteins, and damage the cytoplasmic membrane. This causes an efflux of amino acids and nucleotides and leads to cell death.[20] Saponins in pineapplehump can damage the cytoplasmic membrane of bacteria and reduce cell membrane permeability, causing disruption of nutrient uptake into the bacterial cells and resulting in cell death.[21]

The results of the inhibition zone test indicated that pineapple hump extract was more effective than chlorhexidine (the positive control) at inhibiting bacterial growth. Pineapple hump extract also had the potential to inhibit the growth of *P. gingivalis* biofilms. The *P. gingivalis* biofilmtests showed that pineapple hump extract was more effective than chlorhexidine at eradicating biofilms. The results of this study are supported by the results of previous research conducted byChatty (2019), who used an agar well diffusion method to test the inhibition of *P. gingivalis* by pineapple hump extract. The hump extract showed an antibacterial activity against *P. gingivalis* bacteria starting at a concentration of 50%, with an average inhibition zone diameter of 9.85 mm.[20]Another study by Udin *et al*. (2018) tested the antibacterial effect of pineapple hump extract on *Staphylococcus aureus* and found that pineapple hump extract was effective at inhibiting and killing *S. aureus* bacteria, optimally at a concentration of 70%.[22] Mukti *et al.* (2018) tested the antibacterial effect of pineapple hump extract on *Streptococcus mutans* and found a very strong inhibitory effect at a concentration of 100%.[21] Liliany, *et al* (2018) tested the antibacterial effect ofthe bromelain enzyme from pineapple hump extract against *Enterococcus faecalis* and found thatit was effective at inhibiting the growth of *E. faecalis* bacteria at a concentration of 12.5%.[19]

According to Rahmat et al (2016), the concentration of bromelain contained in pineapple hump is higher than pineapple flesh.[16] The bromelain enzyme can destroy the structure of the bacterial wall as the mechanism of its antimicrobial action. This enzyme breaks the protein that builds the bacterial cell wall, thus the bacterial wall becomes weak and the cell will be damaged. The bromelain enzyme is present in all pineapple tissues. About half of the protein in pineapple contains the protease bromelain.[24,25]

CONCLUSION

The results of the present study confirm that the extract of pineapple hump is able to inhibitbacterial growth and eradicate the adhesion of *Porphyromonas gingivalis* biofilms. Further research is still needed to confirm the effectiveness of pineapple hump extract against other pathogenic bacteria. Toxicity tests are also needed to determine any side effects of pineapple hump extract in the oral cavity.

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CONFLICT OF INTEREST

Authors declared that there is no conflict of interest related to this study.

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