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Antibacterial potential of queen pineapple (*Ananas comosus (L.) Merr Var. Queen*) peel extract on the growth of *Lactobacillus acidophilus* bacteria

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Abstract

Based on the Global Burden of Disease Study in 2017, the prevalence of caries in each country reached 20% to more than 50%. Caries is formed due to the role of several factors including host, agent (microorganisms), substrate, and time. Bacteria involved in the origin of caries formation such as *Lactobacillus* group bacteria found in the oral cavity which can cause dental caries, one of which is *Lactobacillus acidophilus*. The use of natural cleansers is highly recommended to remove residual bacteria as an effort to prevent caries. Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) is a fruit that contains very complex compounds that can act as anticarcinogenic and antibacterial. This study aims to determine the Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC) against the growth of *Lactobacillus acidophilus* bacteria. This type of research was a laboratory experimental type with posttest only control group design. This study used the dilution method of the number of samples, namely concentrations of 0.781%, 1.56%, 3.125%, 6.25%, 12.5%, 25%, 50%, 100%, positive controls and negative controls. This study was conducted 3 times repetition. Manual calculation of the number of bacterial colonies that grow on nutrient agar media and expressed in colony forming units (CFU) which will determine the MBC and MIC. The Shapiro-Wilk test showed a p value> 0.05 at all concentrations and normally distributed data, but when tested for homogeneity the data was not homogeneous so that the non-parametric statistical test continued using the Kruskal Wallis test showing a value of $p < 0.05$. Conclusion: Colony count results showed that the concentration of 3.12% can inhibit the visible growth of *Lactobacillus acidophilus* bacteria which is stated as the Minimum Inhibitory Concentration (MIC) and can prevent growth after subculture to antibiotic-free medium at a concentration of 6.25% which is stated as the Minimum Bactericidal Concentration (MBC).

Keywords: Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) Peel Extract; *Lactobacillus acidophilus*; Minimum Inhibitory Concentration (MIC); Minimum Bactericidal Concentration (MBC)

1. Introduction

One of the health problems experienced globally is infectious diseases in the oral cavity such as dental caries. Based on the Global Burden of Disease Study in 2017, the prevalence of permanent dental caries per 100,000 population in each country reached 20% to more than 50%. The proportion of dental problems in Indonesia is one of which is cavities, damaged teeth and pain. Data on the prevalence of caries in Indonesia stated that it was 88.8% with a root caries prevalence of 56.6% [1].

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The oral cavity is a place where more than 700 species of microorganisms grow which have many impacts on dental and oral health [2]. Caries is formed due to the role of several factors including host, agent (microorganisms), substrate, and time. The highest prevalence of root caries is in the age group of 3-5 years and 65 years because at the age of 65 there are salivary changes associated with aging [3].

The formation of caries is caused by the demineralization process produced by microorganisms on the surface of enamel, dentin or cementum supported by the role of continuous time. Caries is associated with a low pH on the tooth surface caused by acids that produced from carbohydrates by oral bacteria [4]. Bacteries are one of the factors in the development of tooth decay [5]. Bacteria involved in the origin of caries formation such as *Streptococcus mutans* and *Lactobacillus* bacteria. *Lactobacillu*s group bacteria found in the oral cavity can cause dental caries, one of which is *L. acidophilus* [6].

L. acidophilus in the oral cavity can produce lactic acid from fermented sugar so that it will cause the pH of the plaque to decrease. A decrease in pH over a long period of time will cause demineralization of the tooth root surface. The bacterial species *L. acidophilus* plays a role in the development and continuation of the dental caries process, while *S. mutans* bacteria play a role in the initiation of dental caries [7]. The use of cavity cleaners is highly recommended to remove residual bacteria as an effort to prevent caries and advanced bacterial growth. The utilization of natural ingredients in this era is increasing rapidly. The use of traditional medicine is considered to have fewer side effects compared to drugs derived from chemicals [8].

Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) is one of Indonesia's foreign exchange sources in the agricultural sector. Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) is a fruit that contains complex compounds consisting of flavonoids, tannins, bromelain and vitamins that can act as anti-bacterial and anticarcinogenic compounds [9]. Queen Pineapple is a fruit plant with the Latin name *Ananas comosus (L.) Merr Var. Queen* belonging to the Bromoliaceace family [10]. In Indonesia, pineapples are widely cultivated in lowland and highland areas, one of which is in the Kediri area, East Java. Pineapple is a plant that grows throughout the year [11].

The effect of pineapple peel extract on antibacterial has been widely studied. The main chemical content in pineapple fruit and pineapple skin is the Bromelain enzyme which functions as an anti-inflammatory, analgesic, anti- bacterial, antifungal, and anticancer [12]. From some research results, it is stated that pineapple peel extract has antibacterial activity against several types of bacteria *Enterococcus faecalis*, *Streptococcus faecalis*, *Bacillus cereus*, *Streptococcus mutans* and *Streptococcus sangui* [13].

In the field of dentistry as evidenced in previous research, pineapple peel is used as an anti-inflammatory after third molar extraction and dentin deproteination material. Formula in mouthwash preparations of pineapple peel extract with antibacterial activity against *Streptococcus mutans* [14]. Previous studies have shown that pineapple peel can provide antibacterial effects against several types of bacteria. According to Juriah pineapple peel acetone extract at concentrations of 50 mg/ml can inhibit the growth of *Klebsiella pneumonia*, *Enterococcus faecalis* and Staphylococcus *aureus* forming an inhibition zone with a diameter of 12 mm [15]. Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) peel extract has antibacterial potential at 3.125% as MIC and 6.25% as MBC against *Enterococus feacialis* with an inhibition zone diameter of 21.58 mm [16].

2. Material and methods

This is a true experimental laboratory study, with a posttest only control group design. The research was carried out at CV ADC Farm in Ngancar, Kediri district for obtain Queen type pineapple fruit. Preparation of samples Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) peel extract was conducted at the UPT Laboratory of Herbal Materia Medica Batu Malang. *Lactobacillus acidophilus* anti-bacterial test was conducted at the Microbiology Research Centre Laboratory, Faculty of Dental Medicine, Universitas Airlangga Surabaya.

The sample of this study is *Lactobacillus acidophilus* which comes from stock in the Microbiology Laboratory Faculty of Dental Medicine, Universitas Airlangga which cultured using Brain Heart Infusion Both (BHIB) in a test tube. The inclusion criteria in this study were fresh Queen Pineapple peel, the ripe old pineapple is yellow in colour with an age of 25 month, bacteria *Lactobacillus acidophilus* which cultured by using Brain Infusion Broth (BHIB) in a test tube. Exclusion criteria in this study are pineapple skin that is not fresh, pineapple skin that is rotten and brown in colour and bacteria other than *Lactobacillus acidophilus*. The sample size in the study was 33 samples and required 3 repetitions of each treatment.

Group 1: Queen Pineapple peel extract 0.781%, Group 2: 1.56% Queen Pineapple peel extract, Group 3: 3.125% Queen Pineapple peel extract, Group 4: Queen Pineapple peel extract 6.25%, Group 5: 12.5% Queen Pineapple peel extract, Group 6: 25% Queen Pineapple peel extract, Group 7: Queen Pineapple peel extract 50%, Group 8: 100% Queen Pineapple peel extract, Group 9: as a control containing BHIB media and bacteria *Lactobacillus acidophilus*, and Group 10: as an indicator of BHIB.

The independent variable in this study is Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) peel extract. The dependent variable in this study is *Lactobacillus acidophilus* bacteria on nutrient agar media expressed in colony forming units (CFU). The controlled variables are how it works, tools, and materials.

The tools have been calibrated to have good reliability and validity, and are commonly used as research instruments, such as mask and handscoon, petridish, test tubes and test tube racks, spreader, spirtus brender and lighter, incubator, osse needle, spluit, micropipet, sterile object glass, anaerobic jar, extract pot, extract stirrer, rotabavor, digital scales, multipurpose knife. The research material that was used in this study include Queen Pineapple extract (*Ananas comosus (L.) Merr Var. Queen*) peel extract, *Lactobacillus acidophilus* bacteria suspension, BHIB Media and 96% ethanol solvent.

The data obtained were then collected, grouped and presented in tabular form and then analyzed using statistical tests. The normality test is Shapiro-Wilk, the data is declared normal if p>0, 05. The homogeneity test is Levene's test, the data is declared homogeneous if p>0, 05. The non-parametric test used was the Kruskal-Wallis test (Dahlan, 2014).

3. Results and discussion

There are 10 samples consisting of treatment groups and control groups. Table 1 shows the MIC and MBC test.

Table 1. MIC and MBC test

No.	Control Media	Bacterial Control	100%	50%	25%	12.5%	6.25%	3.125%	1.56%	0.78%
1	-	176	-	-	-	-	-	13	59	88
2	-	156	-	-	-	-	-	14	56	95
3	-	172	-	-	-	-	-	12	39	78
4	-	168	-	-	-	-	-	13	42	82
5	-	162	-	-	-	-	-	14	52	97

This study used the research used 8 concentrations of Queen Pineapple (*Ananas comosus (L) Merr Var. Queen*) peel extract including concentrations of 0.78%, 1.56%, 3.125%, 6.25%, 12.5%, 25%, 50% and 100%, along with positive control and negative control. In each concentration, 3 replications were done. The MIC of Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) peel extract from 3 replicates was at a concentration of 3.12% and MBC was at a concentration of 6.25%.

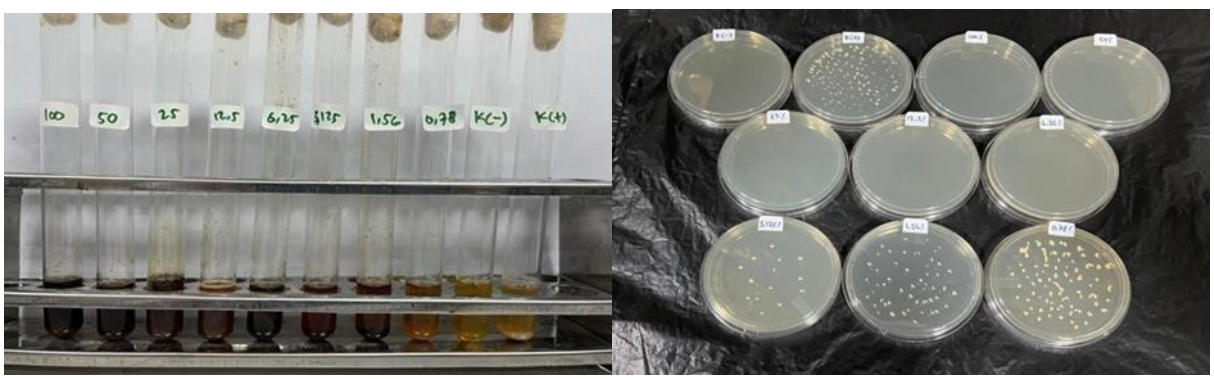

Figure 1 Dilution test of Queen Pineapple (*Ananas comosus (L) Merr Var. Queen*) peel extract againts *Lactobacillus acidophilus* bacteria at each concentration. At the next stage, the streaking method was carried out on nutrient agar.

Used to ensure the growth of colonies, it is checked by planting bacteria on nutrient agar media using the spreading method at the concentration, positive control and negative control on nutrient agar media.

Figure 2 Growth results of *Lactobacillus acidophilus* on nutrient agar media. From the results of the research that has been done, it shows that there is no inhibition zone formed, which means that there is still growth of *Lactobacillus acidophilus* bacteria at concentrations of 0.781%, 1.56% and the negative control group. At concentrations of 3.1%, 6.25%, 12.5%, 25%, 50% and 100% there is an inhibition zone formed, which means that at these concentrations there is inhibition of Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) peel extract against the growth of *Lactobacillus acidophilus*.

Table 2 Average bacterial growth

Group	Average	Standar d deviation
Media Control	0	0
Bacterial Control	0	0
100%	0	0
50%	0	0
25%	0	0
12.5%	0	0
6.25%	0	0
3.125%	13.2	0.8
1.566%	49.6	8.3
0.78%	88.0	8.2

The results of the analysis using descriptive statistics presented in table 2, it is known that the average bacterial growth at a concentration of 0.78% is 88%. At an extract concentration of 1.56% there was a decrease in bacterial growth to an average of 49.6%. At a concentration of 3.125%, the average bacterial growth was 13.2%. While starting at a concentration of 6.25%, there was no bacterial growth. Further statistical data analysis uses the One Way Anova test. The assumptions that must be met in this test are normally distributed data and homogeneous data variants. Normality test using Shapiro- Wilk test while the Homogeneity test uses Levene's test.

The data normality test results using the Shapiro Wilk test. The assumption of data normality is fulfilled if the significance value is more than 0.05 (5%). Based on the Shapiro test results in table 5.3, it is known that at a concentration of 3.125%. 1.56%, 0.78% and bacterial control fulfil the assumption of data normality because the resulting significance value is greater than 0.05. While at concentrations of 100%, 50%, 25%. 12.5%, and 6.25% did not appear because the values at these concentrations were all 0.

The results of the data homogeneity test using Levene's Test. The significance value resulting from the test is 0.000. This value is smaller than 0.05 so it can be concluded that the data is not homogeneous. Because the assumption of homogeneity of data variance is not met, the One Way Anova test is replaced with the Kruskall wallis test.

To determine which group have significant differences, the statistical test was continued using the Tukey HSD test. The results of Tukey's HSD post Hoc statistical test show that Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) peel extract with a concentration of 0.78% has a significant difference with all concentrations. This means that the inhibition of Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) peel extract at a concentration of 0.78% is the lowest inhibition zone value against the growth of *Lactobacillus acidophilus* bacteria. Similarly, concentrations of 1.56%, and 3.125% also had significant differences with all groups. The highest inhibition occurred at a concentration of 3.125%. While at concentrations of 6.25%, 12.5%, 25%, 50%, 100% have the same killing power where these groups do not have the growth of *Lactobacillus acidophilus* bacteria.

The oral cavity consists of various microorganisms. In the oral cavity there are more than 700 species of bacteria that colonise the biofilm and then form plaque some bacteria from the plaque ecosystem cause infections in the oral cavity. Microorganisms are one of the main causes of abnormalities from endodontic failure [17]. The most resistant species

and often found in abnormalities after treatment is *Lactobacillus acidophilus*. *Lactobacillus* species is an aerobic facultative bacterium that is easy to grow, quickly colonising the dentinal tubules [18].

This research is a laboratory experimental study conducted to determine the antibacterial potential of Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) peel extract against *Lactobacillus acidophilus* bacteria by knowing the MIC and MBC against *Lactobacillus acidophilus* bacteria. The research was carried out by dilution method and then the number of bacterial colonies was calculated.

The results showed that the Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) peel extract has antibacterial potential against *Lactobacillus acidophilus*, this is shown through the presence of MIC and MBC. The results of colony counts show that the concentration of 3.12% can inhibit bacteria which is expressed as the Minimum Inhibitory Concentration (MIC) and can prevent growth after subculture to antibiotic-free medium at a concentration of 6.25% which is expressed as the Minimum Bactericidal Concentration (MBC).

This is in line with the discussion that Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) peel extract contains substances that have the potential to anti-bacterial such as bromelain compounds, flavonoids, saponins and tannins. Bromelain which has antibacterial effectiveness on aerobic and anaerobic bacteria. Antimicrobial properties contained in flavonoid compounds are shown by the ability to inactivate attachment to microbes. Tannin compounds cause shrinkage of the bacterial cell wall which causes disruption of cell permeability which will result in inhibition or death of bacteria. The mechanism of action of saponin compounds as antibacterials can basically cause leakage of proteins and enzymes from the cell. Saponin compounds can damage membrane permeability, damage to the cell membrane leads to disruption of survival in bacteria.

The results showed that Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) peel extract can inhibit the visible growth of *Lactobacillus acidophilus* bacteria and prevent the growth after subculture to antibiotic-free medium of it.

4. Conclusion

The results of research on the antibacterial potential of Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) peel extract against the growth of *Lactobacillus acidophilus* bacteria can be concluded that the Minimum Inhibitory Concentration (MIC) of Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) peel extract was 3.12% and the Minimum Bactericidal Concentration (MBC) was 6.25%. So it can be stated that Queen Pineapple (*Ananas comosus (L.) Merr Var. Queen*) peel extract can inhibit the growth of *Lactobacillus acidophilus* bacteria in the oral cavity.

Compliance with ethical standards

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Disclosure of Conflict of interest

The authors of this manuscript do not have any financial or personal conflicts of interest.

Statement of ethical approval

The study received ethical approval by Faculty of Dentistry, Bhakti Wiyata Institute of Health Sciences, Kediri, East Java, Indonesia (133/FKG/EP/XII/2022)

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