Analysis of the effect of extracted yellow kepok banana peels 
(*Musa paradisiaca l.*) on the size and morphology of 
*Enterococcus faecalis*

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**Abstract.** The treatment of pulp and periapical diseases often experience failure due to persistent infections in the pulp and periapical teeth. The dominant bacteria in pulp and periapical infections is *Enterococcus faecalis*. The use of antimicrobials has shifted to natural materials that have low toxicity, cheaper and have long-lasting properties. Yellow kepok banana peels have many antibacterial compounds such as flavonoids, tannins, alkaloids and easily found in Indonesia. The objective of this research was to determine the description of changes in the morphology and size of *Enterococcus faecalis* after giving the extract of Yellow kepok banana peels (*Musa paradisiaca L.*) in vitro. The samples of the Yellow kepok banana peels extract with concentrations of 100%, 90%, 80%, 70%, 60% and control treatment were placed on wells with a coverslip. Each well was given BHI-B and *Enterococcus faecalis*. Coverslips at the 5 concentrations were coated using gold and photographed using a Scanning Electron Microscope (SEM). The photo results were then observed for the bacteria length and morphology. Data were analyzed with descriptive statistics. The average length of the bacteria given the extract increased, compared to the bacteria not given the extract. Meanwhile, the average width of the bacteria given the extract showed an uncertain change. The treated bacteria showed morphological changes in the form of blebs. In conclusion, extract of yellow kepok banana peels (*Musa paradisiaca L.*) provides an overview of the occurrence of changes in morphology and size of *Enterococcus faecalis* in vitro.

1. **Introduction**

Pulp and periapical diseases are one of the teeth and mouth problems that often occur in Indonesia with the largest prevalence of 7 out of 10 diseases in outpatients [1]. Pulp disease in serious condition can cause tooth necrosis. Necrotic teeth need root canal treatment (RCT) to maintain the teeth function without having to be extracted [2]. However, not all RCTs are successful and it has varying success rates ranging from 40-93% [3]. The main cause of RCT failure is the persistence of infection after
treatment, inhibiting the healing of the apical area [4]. The infection occurring indicates that there are still bacteria in the root canal so that the use of sterilization material is very important and dominant in determining the success of RCT [3][4].

After RCTs were carried out, 10% of the bacteria remained in the root canals [5]. The dominant bacteria in the root canal is Enterococcus faecalis (E. faecalis), which is a bacteria that can cause infection mostly found in root canals and cause RCT failure [6].

One of the stages of RCT is the use of medicaments used to prevent bacterial growth and kill any remaining bacteria from the preparation and irrigation results. The most often used medicament material today is calcium hydroxide (Ca(OH)$_2$) [6]. Calcium hydroxide has good biocompatibility to tissues, has pH of 12 which can change the environmental situation to alkaline so that it can provide antimicrobial effects, has strong antimicrobial properties and stimulates the formation of hard tissues [7]. However, E. faecalis is resistant to calcium hydroxide [8].

To overcome the resistance of E. faecalis bacteria, we can start to switch to natural or herbal ingredients because they are considered cheap, durable, easy to obtain, and are expected to have toxicity [9]. Indonesia is one of the countries with abundant crop yields and one of them is banana. Indonesia itself is the largest banana producer in the world [10]. One of the most commonly found bananas in Indonesia is yellow kepok banana (Musa paradisiaca L.). Banana peels are usually considered waste even thought, in yellow kepok banana, it contains important compounds, namely flavonoids, alkaloids, tannins, saponins and quinones [11].

Antibacterial activities can be observed directly using a light microscope, but morphological and size observations require the use of a high-precision microscope, namely Scanning Electron Microscope (SEM). SEM is an electron microscope using high-level wave scanning to image a sample at a high resolution with a magnification of up to 1,000,000x [12].

Based on this description, the researcher intended to conduct this research to determine the antibacterial activity of yellow kepok banana peels extracts against changes in morphology and size of Enterococcus faecalis in vitro using SEM.

2. Methods
2.1 Sample preparation and extraction procedures
Raw yellow kepok banana peels were separated from the banana flesh, washed and then dried in the sun until being dried completely (not runny) then blended until it became a powder. 500 grams of dry raw yellow kepok banana peel powder was extracted by maceration method using ethanol as a solvent and evaporated for 5 hours to produce 30 ml of extract. Yellow kepok banana peel extract showed a brownish yellow color. Before using the antibacterial test, a contamination test was carried out on the yellow kepok banana peel extract to ensure that the extract was free of contaminants [13].

2.2 Bacteria preparation
E. faecalis was obtained from the Laboratory of Microbiology, Faculty of Medicine, Brawijaya University. Bacterial isolates have been tested for identification of gram stain, catalase test, hemolysis test and oxidase test. E. faecalis bacteria were cultured in liquid media in 0.9% NaCl tube and diluted using BHI – B to a concentration of 106 CFU/ml. Yellow kepok banana extracts were diluted at a concentration of 60%, 70%, 80%, 90% and 100% using distilled water. Each banana peel extract, water, chlorhexidine gluconate positive control of 2% was added to the well which already contained E. faecalis bacterial suspension with the coverslip-filled well basis and then incubated for 18-24 hours. The bacterial suspension that had been treated was then prepared so that it could be observed using Scanning Electron Microscope (SEM). The preparation consisted of 2% glutaraldehyde fixation, graded dehydration using ethanol 30%, 50%, 70%, 80% and 90% and the administration of amyl acetate, then dried and coated using gold or carbon with a vacuum evaporator. Specimens that had been plated with gold were then observed in SEM. Based on the results of the SEM photos, measurement of the length and width of the bacteria and morphological observation were performed.
2.3. Data analysis
The data obtained were then analyzed using descriptive analysis techniques on quantitative and qualitative data. Data are presented in tables and graphs to show the picture obtained from the data obtained.

3. Result and Discussions
The preliminary test was carried out at the extract concentrations of 100%, 50%, 25%, 12.5%, 6.25% and 3.125% to determine the concentration range of the yellow kepok banana peel extract which can inhibit the growth of *E. faecalis*.

![Figure 1. Results of preliminary test](image)

The results of the preliminary test showed that the extract concentrations of 3.125%, 6.25%, 12.5%, 25%, 50% still showed a quite dense growth of the *E. faecalis* bacteria colony while, at a concentration of 100%, there was a decrease in the number of bacterial colony growth. The extracts were condensed in the concentration range of 100%, 90%, 80%, 70%, and 60%. The control materials used were Chlorhexidine gluconate of 2% as a positive control and water as a negative control. SEM observations of the treated bacteria can describe the conditions of the post-induction bacterial morphology with the extract. The SEM observations were carried out at 6000x magnification.

![Figure 2. *E. faecalis* in SEM observations. (A) Negative control, (B) Positive control (C) After 60% extract administration, (D) After 70% extract administration, (E) After 80% extract administration, (F) After 90% extract administration, (G) After 100% extract administration.](image)
The results of SEM photos on bacteria were measured using Microsoft Visio 2016. Each photo was counted according to the scale on the photo and observed whether there was any formation of blebs. The results of the observations are written in Table 1.

Table 1. Observation in *E. faecalis*

| Changes     | Negative Control | Positive control | 60%  | 70%  | 80%  | 90%  | 100% |
|-------------|------------------|------------------|------|------|------|------|------|
| Length      | 0.5805           | 2.1839           | 0.6551| 0.7292| 0.7264| 0.9523| 1.0453|
| Wide        | 0.5162           | 1.9602           | 0.5827| 0.5891| 0.6868| 0.5238| 0.5714|
| Blebs       | (-)              | (+)              | (+)  | (+)  | (+)  | (+)  | (+)  |

Based on Table 1, it is found that there is a change in size and the formation of blebs after the administration of yellow kepok banana peel extract. The length of bacteria occurring increased in all concentrations of yellow kepok banana peel extract compared to the negative control, especially at a concentration of 100%. This change in the length of the bacteria was accompanied by an increase in the concentration of yellow kepok banana peel extract. The width of the bacteria changes was uncertain and not following the increase in the concentration of the yellow kepok banana peel extract used. Meanwhile, the blebs were found in all samples treated with yellow kepok banana peel extracts.

The results of the SEM observations indicate that there are differences in shape and size as seen from the magnification scale used at each concentration of yellow kepok banana peel extract. Starting at a concentration of 60%, it is obtained an enlargement of the size and the formation of blebs. This is consistent with the statement of Cushnie *et al.* (2016) that anti-bacterial compounds will provide morphological and ultrastructural changes in bacteria, one of which is the increase in size and the formation of blebs [14].

The flavonoid, alkaloid and tannin contents in banana peels illustrate the changes occurring in *E. faecalis* bacteria. The contents enter the bacteria by a diffusion process so that they enter the cell through the bacterial cell membrane. The flavonoid and tannin contents cause damage to the bacterial cell membrane where this content will blind to the peptidoglycan synthesis contained in the cell membrane. This peptidoglycan is responsible for the shape and resistance of bacteria because *E. faecalis* bacteria are gram-positive bacteria which have a thick peptidoglycan layer. The disruption of peptidoglycan will certainly have an impact on the condition of these bacteria because peptidoglycan, which is the main constituent of cell walls, weakens, automatically leading to weakened bacteria resistance. Besides, the
disruption of the cell walls also causes the permeability of the cell walls disrupted so that the activity of growth and development of bacteria is also disrupted [15]. Flavonoid can affect bacterial DNA by binding to the bacterial cell nucleus so that they damage the lipid structure of DNA and the cell nucleus will be lysis [16]. Flavonoids are toxic to bacteria because they can cause changes in organic components and interfere with nutrient transport by damaging the permeability of bacterial cell walls, interfering with microsome synthesis and lysosomes, releasing transduction energy to the cytoplasmic membrane and inhibiting bacterial motility [17].

Alkaloids are also toxic to bacteria so that they are antibacterial and antiprotozoal [18]. Alkaloids damage the nuclear structure of cells by inhibiting DNA replication from these bacteria. Alkaloids that interfere with the bacterial DNA replication process will experience death. Tannin, which is a compound from yellow kepok banana peels, also has an anti-bacterial effect. This effect of tannins causes a spasmyolytic effect on bacteria. The spasmyolytic effect allows the bacterial cell wall to shrink so that it interferes with the permeability of bacterial cells. Due to disrupted cell wall permeability, the synthesis process of bacteria will also be disrupted, inhibiting the growth and even taking a life of the bacterial cells [15]. Besides, tannins can cause protoplasmic coagulation of bacterial cells [19].

The increase in length occurs due to PBP3 inhibition where PBP3 cross-links the peptidoglycan on the septal wall but not on the lateral wall, causing more increased inhibition on the septal wall where the bacteria divide than on the bacteria lateral wall [14]. PBP3 is a type of protein that helps wall synthesis. This protein is found in the cell membrane or cytoplasm.

Blebs emerge due to high cytoplasmic membrane pressure while inhibition of peptidoglycan synthesis occurs. This causes a shrinkage effect and blebs since peptidoglycan is less able to withstand the pressure of the cytoplasmic membrane. The blebs are sometimes osmotically sensitive so that it can cause cells to lysis easily because the cytoplasm easily comes out from bacterial cells. Thus, bacteria with a bulb formation show an activity causing the bacteria to die [14].

4. Conclusion
Based on the results of the study, it can be concluded that an increase in the bacteria size, specifically the length of *E. faecalis*, and a change in the bacteria morphology in the form of blebs were consistent with the addition of the concentration of yellow kepok banana peel extract. From these results, it is learned that the extract of yellow kepok banana peels (*Musa paradisiaca* L.) provides an overview of changes in the morphology and size of *E. faecalis* bacteria in vitro.

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