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# Antibacterial Activity Test of Ethyl Asetate Fraction of Gletang Growth (*Tridax procumbens L.*) on The Growth of *Enterococcus faecalis*

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## ABSTRACT

**Background:** Intractable root canal infection remains a serious obstacle in endodontic therapy and is a frequent cause of treatment failure, with *Enterococcus faecalis* (*E. faecalis*) as a key pathogen. This Gram-positive bacterium can persist under harsh conditions within the root canal system. Chlorhexidine is commonly used as a chemical irrigant, but its long-term application is associated with undesirable side effects, prompting the search for natural antibacterial alternatives. One promising candidate is the gletang plant (*Tridax procumbens L.*), which contains bioactive compounds with potential antimicrobial effects. **Objective:** This study aimed to evaluate the antibacterial activity of the ethyl acetate fraction of *Tridax procumbens L.* at various concentrations against *E. faecalis*. **Method:** A laboratory experimental design with post test only control group was used. Antibacterial testing was performed using the agar diffusion method with the gletang ethyl acetate fraction at 2%, 4%, 6%, and 8%, chlorhexidine as a positive control, and 96% methanol as a negative control. Data were analyzed univariately in tables and bivariately using Kruskal–Wallis and Mann–Whitney tests. **Results:** The fraction inhibited *E. faecalis* growth, with the largest mean inhibition zone of 3.6 mm at 8% and the smallest at 2%, 1.6 mm. Overall activity across all concentrations was classified as weak; however, statistical analysis ( $p=0.003$ ,  $p<0.05$ ) confirmed a significant inhibitory effect. **Conclusion:** The ethyl acetate fraction of the gletang plant (*Tridax procumbens L.*) at 8% is effective as an antibacterial against *Enterococcus faecalis*, with an average inhibition zone diameter close to that of the positive control. The higher the concentration of the ethyl acetate fraction, the higher the bacterial inhibition.

**Keywords:** *Enterococcus faecalis*, inhibition zone, *Tridax procumbens L.*

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## INTRODUCTION

Root canal treatment is a treatment for pulp disease that involves removing the necrotic pulp from the root canal and replacing it with a filling material. It aims to keep the damaged tooth functioning properly and does not need to be extracted. Root canal treatment is carried out with three main stages: biomechanical preparation, sterilization, and hermetic root canal filling or obturation, also known as the endodontic triad.<sup>1</sup> PSA can fail, one of the main causes is microorganisms that remain after treatment or reappear after PSA. Microorganism Root canal treatment is a treatment for pulp disease that involves removing the necrotic pulp from the root canal and replacing it with a filling material. It aims to keep the damaged tooth functioning properly and does not need to be extracted. Root canal treatment is carried out with three main stages: biomechanical preparation, sterilization, and hermetic root canal filling or obturation, also known as the endodontic triad.<sup>1</sup> PSA can fail, one of the main causes is microorganisms that remain after treatment or reappear after PSA. Microorganisms are considered the main cause of persistent pulp-periapical abnormalities and play an important role in the infectious course of pulp and periapical diseases. Microorganisms commonly found in infected root canals include *Enterococcus faecalis*, *Streptococcus anginosus*, *Bacteroides Gracilis* and *Fusobacterium nucleatum*. However, the bacteria that are known to be the most resistant and most commonly found are *Enterococcus faecalis* bacteria.<sup>2,17</sup> *Enterococcus faecalis* bacteria are found nine times more in infections after root canal treatment because it has pathogenic properties associated with oral infections and can cause root canal infections, marginal periodontitis, and abscesses. *Enterococcus faecalis* can survive in the root canal by passing through the dentinal tubules, forming a smear layer, and binding to the dentinal plug at the apex of the tooth.<sup>3,17</sup>

*Enterococcus faecalis* bacteria have the ability to avoid instruments and irrigation materials used during biomechanical preparation, as well as being able to catabolize energy sources and can survive in various environments.<sup>4,17</sup>

Irrigation solutions commonly used in root canal treatment include *chlorhexidine*, sodium hypochlorite, and ethylene diamine tetraacetic acid. *Chlorhexidine* is a broad-spectrum antibacterial solution that can be used as an irrigation solution because it has electrostatic properties on the surface of bacteria, although it must be combined again with other irrigation materials to be effective. Some studies show that 2% *chlorhexidine* is recommended as a final rinse because of its outward spectrum of antimicrobial activity, substance and ability to inhibit collagen degradation in addition, *chlorhexidine* does not irritate periapical tissues, is less toxic than other solutions and does not smell strong however, this ability depends on pH and the presence of organic components used while sodium hypochlorite also has antibacterial activity but, when using high concentrations will cause toxicity because it can damage periapical tissues and irritate tissues and smell bad.<sup>5,15,16</sup> Irrigation solutions should have no toxic properties and minimal tissue damage to reduce treatment failure. Therefore, irrigation solutions may be replaced with more biocompetent materials, such as natural materials.

One of the plants that has natural properties is gletang (*Tridax procumbens L.*). Gletang (*Tridax procumbens L.*) is easily found as a wild weed, this plant can be used as a traditional medicine in either fresh form, or dried.<sup>6,16</sup> The whole gletang plant (*Tridax procumbens L.*) has antimicrobial activity against various bacterial species. In addition, every part of this gletang plant is useful because it has pharmacological activities such as hepatoprotective effects, immunomodulating properties, antidiabetes, antimicrobial, anti-inflammatory, antioxidant, diarrhea, and dysentery. This gletang plant is rich in chemical



contents such as flavonoids, saponins, tannins, and terpenoids as major secondary metabolites.<sup>7,8,16</sup>

**MATERIALS AND METHODS**

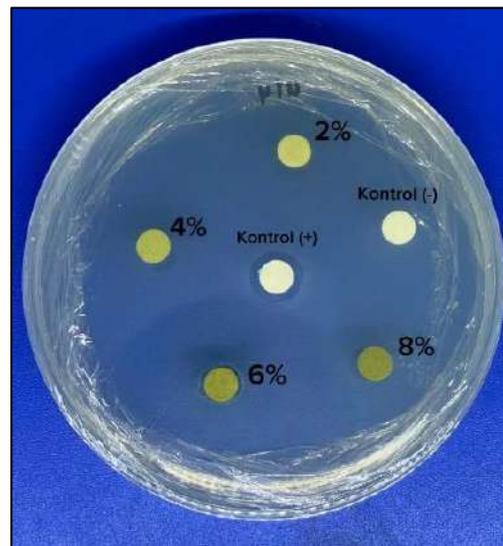
This research is a laboratory experimental study aimed to determine the antibacterial activity of the ethyl acetate fraction of gletang plant (*Tridax procumbens L.*) against the growth of *Enterococcus faecalis* bacteria. This study used all parts of gletang plants, including leaves, stems, flowers, and roots, obtained from Jalan By Pass, Padang city, West Sumatra. The processing of gletang plants begins with wet sorting to separate from dirt and other plants that are not needed, washing using clean and running water, drying by drying samples at room temperature without exposure to sunlight, and placed on a white cloth that absorbs water for 14 days. After that, the dried gletang plant samples were cut into small pieces and mashed using a blender to obtain 2 kg and then extracted.

The extraction process chosen is the maceration method. Maceration is a cold extraction method used for simplisia that has a soft texture. Methanol was chosen as the solvent in the maceration process because it is universal and can attract all types of active substances, both polar and semi-polar, so that the active compounds will be dissolved in it and the toxicity levels are relatively low.<sup>9</sup> Extraction was carried out by immersing the sample in an Erlenmeyer tube until it was completely submerged, then stirring every 3x24 hours until a clear colored maserat was obtained. The mixture was evaporated using a vacuum rotary evaporator, and a thick extract weighing 41.2247 kg was obtained. The results of the thick extract obtained were carried out by the separation process using the column chromatography fractionation method. This research was ethical approval from the Ethics Committee of Baiturrahmah University with

registration number: 010/KEPK-FKGUNBRAH/18/12/2024.

**RESULTS**

The results of measuring the average diameter of the inhibition zone of the ethyl acetate fraction of gletang plant (*Tridax procumbens L.*) against the growth of *Enterococcus faecalis* bacteria can be seen in the following table.



**Figure 1.** Antibacterial activity test results of the ethyl acetate fraction of the gletang plant (*Tridax procumbens L.*) at concentrations of 2%, 4%, 6%, and 8% against the growth of *Enterococcus faecalis* bacteria.

**Table 1.** Mean antibacterial activity of ethyl acetate fraction of gletang plant (*Tridax procumbens L.*) against the growth of *Enterococcus faecalis* bacteria

| Fraction     | Repetition of inhibition zone (mm) |     |     |      | Mean ±SD |
|--------------|------------------------------------|-----|-----|------|----------|
|              | 1                                  | 2   | 3   | 4    |          |
| 2%           | 0                                  | 2.8 | 0   | 3.8  | 1.6      |
| 4%           | 0                                  | 2.7 | 2.8 | 2.75 | 2.0      |
| 6%           | 2.7                                | 3.8 | 3.8 | 3.8  | 3.5      |
| 8%           | 2.8                                | 3.9 | 3.8 | 3.9  | 3.6      |
| CHX 0,2%     | 4.0                                | 4.1 | 4.9 | 4.9  | 4.4      |
| Methanol 96% | 0                                  | 0   | 0   | 0    | 0        |

It can be seen that the 8% concentration has the largest inhibition zone diameter of 3.6



mm and the smallest at 2% concentration of 1.6 mm. The 4% and 6% concentrations produced inhibition zone diameters of 2.0 mm and 3.5 mm, respectively. The positive control of *chlorhexidine* obtained the diameter of the inhibition zone of *Enterococcus faecalis* bacterial growth of 4.4 mm, while the negative control of 96% methanol had no antibacterial activity.

**Table 2.** Result of normality test with *Shapiro-Wilk*

| Group | Sig   |
|-------|-------|
| 2%    | <0.05 |
| 4%    | <0.05 |
| 6%    | <0.05 |
| 8%    | <0.05 |
| K+    | >0.05 |

Note: \* (p>0.05)

In the normality test, a p-value of <0.05 was obtained, indicating that the data were not normally distributed.

**Table 3.** The result for the variance homogeneity with *Levene's test*

| Levene's test | Sig   |
|---------------|-------|
| 8,699         | <0.05 |

Note: \* (p>0.05)

The results of the variance homogeneity test with *Levene's test* obtained a p value <0.05, so it can be said that the data in this study are not homogeneous. Based on the normality and homogeneity tests, where the data is proven by the distribution of data is not normal and not homogeneous, then the *Kruskal-Wallis* nonparametric test is carried out with the provisions if the Sig value <0.05.

**Table 4.** Result of *Kruskal-Wallis* test

| Variables   | Sig   |
|---|-------|
| Zone of inhibition of <i>Enterococcus faecalis</i> bacteria | <0.05 |

Note: \*Significant (p<0.05)

Based on the results of the *Kruskal-Wallis* non-parametric test, the sig value was 0.003<0.05, which means that the treatment tested had a significant effect on the inhibition zone of *Enterococcus faecalis* bacteria.

**Table 5.** Result of *Mann-Whitney* test

| Group | Comparison concentration between group | Sig   |
|-------|--|-------|
| K-    | 2%                                     | >0.05 |
|       | 4%                                     | <0.05 |
|       | 6%                                     | <0.05 |
|       | 8%                                     | <0.05 |
|       | K+                                     | <0.05 |
| 2%    | 4%                                     | >0.05 |
|       | 6%                                     | >0.05 |
|       | 8%                                     | >0.05 |
|       | K+                                     | <0.05 |
| 4%    | 6%                                     | >0.05 |
|       | 8%                                     | <0.05 |
|       | K+                                     | <0.05 |
| 6%    | 8%                                     | >0.05 |
|       | K+                                     | <0.05 |
| 8%    | K+                                     | <0.05 |

Notes: \*Significant (p<0.05)

Based on the *Mann-Whitney* test results, there is a significant difference between K- and K+, 4% concentration with 8% concentration because the sig value is <0.05. Furthermore, at 2% concentration with 4%, 6%, and 8% concentrations, 4% concentration with 6% concentration, and 6% concentration with 8% concentration, there was no significant difference.

**DISCUSSION**

Based on the results of Table 1, the highest antibacterial activity was observed at a concentration of 8% (3.6 mm) to inhibit *Enterococcus faecalis* bacteria, and a small concentration that can inhibit *Enterococcus faecalis* bacteria is at a concentration of 2%.



According to Davis and Stout (1971), the strength of antibacterial power is divided into several criteria: weak (inhibition zone <5 mm), moderate (inhibition zone 5-10 mm), strong (inhibition zone 10-20 mm), and very strong (inhibition zone >20 mm). Based on these criteria, the antibacterial power of the ethyl acetate fraction of gletang plants (*Tridax procumbens* L.) against *Enterococcus faecalis* bacteria with concentrations of 2% (1.6 mm), 4% (2.0 mm), 6% (3.5 mm), and 8% (3.6 mm) was included in the weak category in inhibiting the growth of *Enterococcus faecalis* bacteria.<sup>20</sup> The size of the ability to inhibit the growth of the tested bacteria is influenced by the concentration of an ingredient that functions as an antibacterial. According to Imansyah *et al.* (2022), the difference in the diameter of the inhibition zone of each concentration is due to differences in the amount of active substances contained in that concentration; the greater the concentration, the greater the compound that acts as an antibacterial in the gletang plant fraction.

Ethyl acetate fractionation of gletang plants (*Tridax procumbens* L.) in inhibiting *Enterococcus faecalis* bacteria is higher at a concentration of 8%.<sup>19,20</sup> This is due to the concentration of bioactive compounds that are sufficient to provide a synergistic effect on the antibacterial mechanism of action, this is in line with the research results of Sharma *et al.*, 2020 antibacterial activity of secondary metabolites such as flavonoids and terpenoids depends on concentration. At low concentrations, the amount of active compounds is not enough to effectively inhibit bacterial growth. Conversely, at high concentrations, these metabolites reach an effective threshold that allows significant inhibition of microorganisms.

Based on the results in Table 4, obtained a significant ( $0.003 < 0.05$ ), meaning that the growth of *Enterococcus faecalis* bacteria is influenced by the ethyl acetate fraction of gletang plants (*Tridax procumbens* L.) with concentrations of 2%, 4%, 6% and

8%.<sup>19,20</sup> This is due to the influence of several factors, including the results of the test of secondary metabolite compounds on the ethyl acetate fraction, which is known to contain flavonoids, terpenoids, saponins, and tannins with antibacterial properties.<sup>18</sup>

The results in Table 5. showed that at 2 %, 4 %, 6 %, and 8% concentrations, 4% and 6% concentrations, and 6% and 8% concentrations, there was no significant difference. This is due to the difference in active substances contained in the concentration of the fraction. The ethyl acetate fraction is a semi-polar solvent that can dissolve semi-polar compounds in cell walls. The ethyl acetate fraction is also known as a medium-polar solvent that is volatile, non-toxic, and non-hygroscopic. In line with research conducted by Kumar *et al.* (2020), the ethyl acetate fraction of *Tridax procumbens* L. contains active compounds such as flavonoids, tannins, saponins, alkaloids, steroids, terpenoids, and polyphenols that have potential as antibacterials. Tannins inhibit bacteria by denaturing bacterial cell proteins, inhibiting the function of cell membranes to interfere with the intercellular transport process, and disrupting bacterial growth by inhibiting nucleic acid synthesis.<sup>13</sup> Saponins work as antibacterials by inhibiting the function of microbial cell membranes, forming complex compounds with cell membranes through hydrogen bonds, thus destroying the permeability properties of cell walls, causing cell detachment and cell death.<sup>14</sup>

## CONCLUSION

The higher the concentration of the ethyl acetate fraction, the better the bacterial inhibition.

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# *Cinnamomum zeylanicum* Essential Oil as an Antibacterial Agent and Dental Caries Prevention

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## ABSTRACT

**Background:** The prevalence of dental caries in Indonesia reaches 88.8%. Chlorhexidine, although effective, has side effects such as tooth discoloration. *Cinnamomum zeylanicum* is rich in cinnamaldehyde and eugenol, with antibacterial activity that makes it a natural alternative. **Objective:** To assess the effectiveness of *Cinnamomum zeylanicum* essential oil against *Streptococcus mutans* inoculated in Wistar rats (*Rattus norvegicus*) in preventing dental caries. **Method:** This study used 20 male Wistar rats, divided into five groups: negative control, positive control, and three treatment groups with *Cinnamomum zeylanicum* essential oil concentrations of 6.25%, 10%, and 12.5%. *Streptococcus mutans* (*S. mutans*) was inoculated orally in 0.2 ml using a pipette for three consecutive days. During the study, rats were fed a diet mixed with a sucrose solution (600 mg of sucrose dissolved in 1 liter of Aquadest) at a 1:500 ml-to-pellet ratio to induce caries. After 20 days of treatment, data were collected using a bacterial inhibitory zone assay by disk diffusion and salivary pH measurement. Data analysis was performed using one-way analysis of variance (ANOVA), Tamhane's post-hoc test, and the Wilcoxon signed-rank test. **Results:** One-way ANOVA test showed no significant differences in the inhibitory zone ( $p > 0.05$ ). Post-hoc tests showed that all groups were not significant ( $p > 0.05$ ). The Wilcoxon signed-rank test showed significant differences in salivary pH before and after treatment ( $p < 0.05$ ). **Conclusion:** *Cinnamomum zeylanicum* essential oil has potential as an antibacterial agent and natural alternative in preventing dental caries against *S. mutans*, and is able to change the salivary pH to alkaline.

**Keywords:** Antibacterial agent, *cinnamomum zeylanicum*, dental caries prevention, essential oils

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## INTRODUCTION

Based on data from the 2018 National Basic Health Research (Riskesdas), the prevalence of dental caries in the Indonesian population was 88.8%, with the highest prevalence among people aged 15–24. The national Decayed, Missing, and Filled Teeth (DMF-T) index was reported to be 7.1, while the community Oral Hygiene Index–Simplified (OHI-S) was 1.4 (Riskesdas, 2020). According to World Health Organization (WHO) criteria, a DMF-T index greater than 6.6 indicates severe dental caries, whereas an OHI-S score of 1.4 indicates fair oral hygiene. However, the national oral health targets set for 2020 aimed to achieve a DMF-T index of less than 1 and an OHI-S index below 1.2, indicating a substantial gap between current conditions and desired outcomes.<sup>1,2</sup>

Dental caries is a multifactorial disease that affects the hard tissues of the teeth. It is primarily caused by bacterial activity, particularly *Streptococcus* species, with *Streptococcus mutans* (*S. mutans*) recognized as the main etiological agent in plaque formation and caries progression. Caries is a pathological process characterized by the progressive demineralization and destruction of enamel, dentin, and cementum, resulting from acid production by microorganisms during carbohydrate metabolism, ultimately causing structural damage to tooth tissues.<sup>3,4</sup> In recent decades, preventive approaches in oral health care have been increasingly emphasized over curative treatment. Preventive strategies play a critical role in reducing the incidence of dental caries, especially given that excessive consumption of sweet and sticky foods, inadequate motivation, improper toothbrushing techniques, and infrequent dental visits (ideally recommended every six months) remain major contributing factors.<sup>5,6</sup>

Chlorhexidine is widely used in dentistry as an anti-caries mouthwash due to its broad-spectrum antimicrobial activity. At a concentration of 0.2%, chlorhexidine is effective

in inhibiting plaque formation and reducing *S. mutans* colonies. In clinical dental practice, chlorhexidine is also used as a cavity cleanser at an optimal concentration of 2%. At this concentration, chlorhexidine has been shown to effectively reduce *Enterococcus faecalis* within dentinal tubules and root canals when applied for approximately two minutes during endodontic treatment. Despite its effectiveness, prolonged use of chlorhexidine is associated with undesirable side effects, including tooth staining and discoloration.<sup>7-10</sup>

Indonesia is well known for its abundant production of essential oils, which have gained increasing attention, particularly as natural and alternative therapeutic agents. One such essential oil is derived from cinnamon (*Cinnamomum* spp.). Approximately 300 species of cinnamon exist worldwide, with at least 12 species found in Indonesia, including *Cinnamomum zeylanicum*.<sup>11-14</sup> The essential oil of *Cinnamomum zeylanicum* contains several bioactive compounds, such as cinnamaldehyde, linalool, caryophyllene, and eugenol. Cinnamaldehyde constitutes approximately 60–70% of the essential oil extracted from cinnamon bark and exhibits strong antibacterial and antifungal properties. Linalool, a terpenoid compound with a pleasant aroma, contributes to fragrance intensity, while caryophyllene provides a warming and spicy sensation when consumed.<sup>13-15</sup> Given its widespread use and antibacterial potential, cinnamon essential oil is a promising natural alternative for cavity cleaning. Essential oils were selected in this context due to their ability to inhibit plaque growth associated with dental caries and their potential to protect tooth structures from caries-related damage.<sup>16-19</sup>

Eugenol, a major constituent of cinnamon essential oil, exhibits strong antimicrobial activity and functions as an effective antiseptic agent. Eugenol has been reported to eliminate various bacterial species, including antibiotic-resistant strains, such as *S. mutans*. Its mechanism of action involves



penetration of the cytoplasmic membrane and disruption of bacterial cell wall permeability. Additionally, the hydrophobic nature of eugenol facilitates its passage through lipopolysaccharides, leading to structural alterations in bacterial cell walls, particularly in Gram-negative bacteria. Several studies have demonstrated that eugenol possesses anti-caries properties, consistent with its antimicrobial and antiseptic effects, which inhibit *S. mutans* activity and plaque formation on tooth surfaces.<sup>20-22</sup>

Furthermore, the novelty of this study lies in using an in vivo experimental model to evaluate the antibacterial and anticaries potential of *Cinnamomum zeylanicum* essential oil. Most previous studies investigating the antimicrobial effects of essential oils against cariogenic bacteria have predominantly employed in vitro methods, which, although valuable, do not fully represent the complex biological conditions of the oral environment. Using male *Wistar* rats as an in vivo model, this study provides a comprehensive assessment of the antibacterial efficacy of *Cinnamomum zeylanicum* essential oil under physiological conditions, including bacterial colonization, host response, and oral ecological dynamics. Therefore, the findings of this study are expected to contribute a novel, and clinically relevant evidence supporting the potential application of *Cinnamomum zeylanicum* essential oil as a natural alternative for dental caries prevention.

## MATERIALS AND METHODS

This study was a true experimental laboratory study using a post-test-only control-group design. The instruments used in this study included rat cages (five cages, each containing four rats), a digital scale, rat feeding and drinking containers, sucrose solution bottles, micropipettes, 1 ml syringes, microtubes, Petri dishes, gloves, masks, and laboratory coats. The materials used included *Cinnamomum zeylanicum* essential oil, *S. mutans* bacteria, a

mixed diet containing sucrose, pilocarpine, 96% ethanol, ketamine, and xylazine.

This research was conducted at the Experimental Animal Laboratory of the Faculty of Dentistry, Universitas Hang Tuah, and the Oral Biology Laboratory of the Faculty of Dentistry, Universitas Hang Tuah. This study received ethical approval from the Ethics Committee of the Faculty of Dentistry, Universitas Hang Tuah, Surabaya, with ethical clearance certificate number S.Ket/120/KEPKFKGUHT/XI/2024.

## Preparation of Experimental Animals

This study used male *Rattus norvegicus* weighing 180–200 grams, with a total of 20 rats. The animals were housed in cages measuring 37 cm x 30 cm and 50 cm x 37 cm, with four rats per cage. *Rattus norvegicus* were divided into five groups. The negative control group (K-) received no treatment. The positive control group (K+) received chlorhexidine treatment. The first treatment group (P1) received a mixture of *Cinnamomum zeylanicum* essential oil in drinking water at 6.25%. The second treatment group (P2) received a mixture of *Cinnamomum zeylanicum* essential oil in drinking water at a concentration of 10%, and the third treatment group (P3) received a mixture of *Cinnamomum zeylanicum* essential oil in drinking water at a concentration of 12.5%. All groups were subjected to bacterial induction with *S. mutans* and fed a sucrose-containing diet. *Rattus norvegicus* were acclimated to the new environment for seven days. The rats were fed 15–25 g/day in small containers, while drinking water was provided in bottles with a drinking tube, filled with boiled water. This treatment was administered for 20 days.<sup>23</sup>

## Preparation of *Cinnamomum zeylanicum* Essential Oil

The preparation of *Cinnamomum zeylanicum* essential oil in this study involved drying the cinnamon bark for 48 hours without direct exposure to sunlight. The dried bark was then blended until finely ground and placed into



a Soxhlet apparatus containing 700 ml of 96% ethanol as the solvent. The highest concentration (12.5%) was prepared first by diluting an appropriate volume of *Cinnamomum zeylanicum* essential oil with 96% ethanol to obtain the desired final volume. Subsequently, 10% and 6.25% concentrations were prepared by serially diluting the higher concentration with the same solvent. The mixture was heated to the boiling point of 78.3°C. The extraction process lasted for 10 hours. After extraction, the extract was mixed with propylene glycol and flavored with menthol.<sup>13-15</sup>

### Induction of *S. mutans* in Experimental Animals

*S. mutans* were administered orally to the experimental animals at a volume of 0.2 ml using a pipette, and the procedure was repeated continuously for three days.<sup>23</sup>

### Administration of Sucrose-Containing Diet

The experimental animals were fed a cariogenic diet containing sucrose as the cariogenic agent. A total of 600 mg of sucrose was dissolved in 1 liter of distilled water. Subsequently, 1 kg of pellet feed was mixed with the sucrose solution at a ratio of 1 kg pellets to 500 ml of sucrose solution and mixed thoroughly. The moisture content of the pellets was then removed using microwaves.<sup>23</sup>

### Collection of Saliva Samples

Saliva was collected from the experimental animals' oral cavities using a micropipette, specifically from the sublingual region. A volume of 50 microliters of saliva was collected from each rat. Before saliva collection, a pilocarpine injection was prepared by mixing 15 ml of distilled water with 5 ml of pilocarpine, then administered subcutaneously at a dose of 0.75 ml per 100 g of body weight.<sup>24</sup>

### Salivary pH Measurement

Each saliva sample was immediately tested by two experienced examiners. Salivary

pH was first measured using pH paper. pH-indicating paper (Whatman® indicator paper) was used in the current study. Droplets of randomly selected saliva were dropped onto a disinfected stainless-steel plate with a micropipette. An examiner placed a piece of pH paper on the droplet for one second, and the color change was immediately compared to the manufacturer's color-coded chart. The other examiner duplicated this procedure. No communication between the examiners was allowed during all tests. Salivary pH was measured before and after treatment on the 20th day in *Rattus norvegicus*.<sup>24</sup>

### Antibacterial Inhibitory Activity

This method is an antibacterial testing procedure in which a substance is placed on agar media and inoculated with the test microorganism. After incubation, the inhibition zone around the disc was measured to determine the effectiveness of the tested substance. The antibacterial activity test in this study used the disk diffusion method and included the following groups: without extract (K-), chlorhexidine (K+), and extract concentrations of 6.25% (P1), 10% (P2), and 12.5% (P3). Measurements were taken after 24 hours of incubation. *S. mutans* colonies were counted in colony-forming units per milliliter (CFU/ml) using a colony counter.<sup>25,26</sup>

### Data analysis

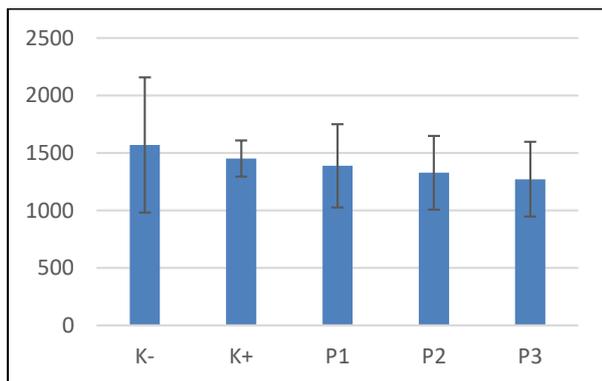
The data were analyzed using the Shapiro–Wilk test for normality and Levene's test for homoscedasticity in IBM SPSS Statistics (version 28; SPSS, Chicago, Illinois, United States). Since the data were normally distributed and showed homoscedasticity ( $p > 0.05$ ), one-way ANOVA and post hoc Tamhane multiple comparisons were performed to detect substantial differences across concentrations of *Cinnamomum zeylanicum* essential oil. pH of saliva before and after treatment was evaluated using the Wilcoxon signed-rank test. A statistically significant difference was set at

$p < 0.05$ . Descriptive statistics were used to assess the mean, standard deviation, and 95% confidence interval for the antibacterial activity test and salivary pH before and after treatment.

**RESULTS**

**Table 1.** Mean, standard deviation, and 95% confidence interval of antibacterial activity test.

| Group | n | Mean $\pm$ SD (CFU/ml) | 95% CI             |
|-------|---|------------------------|--------------------|
| K-    | 4 | 1.570.00 $\pm$ 587.95  | (634.43, 2505.56)  |
| K+    | 4 | 1.450.68 $\pm$ 15.04   | (1200.79, 1700.56) |
| P1    | 4 | 1.387.88 $\pm$ 361.34  | (812.91, 1962.85)  |
| P2    | 4 | 1.328.22 $\pm$ 320.44  | (818.32, 1838.12)  |
| P3    | 4 | 1.271.70 $\pm$ 352.49  | (753.77, 1789.63)  |



**Figure 1.** Graph showing the mean and standard deviation of the antibacterial activity test

**Table 2.** Mean, standard deviation, and 95% confidence interval of salivary pH before and after treatment.

| Group  | n | Before treatment | After treatment |
|--------|---|------------------|-----------------|
| K-     | 4 | 7.50 $\pm$ 0.57  | 6.85 $\pm$ 0.19 |
| 95% CI |   | (6.58, 8.41)     | (6.54, 7.15)    |
| K+     | 4 | 6.65 $\pm$ 0.25  | 7.25 $\pm$ 0.50 |
| 95% CI |   | (6.24, 7.05)     | (6.45, 8.04)    |
| P1     | 4 | 6.95 $\pm$ 0.10  | 7.75 $\pm$ 0.50 |
| 95% CI |   | (6.79, 7.10)     | (6.95, 8.54)    |
| P2     | 4 | 7.00 $\pm$ 0.16  | 7.75 $\pm$ 0.50 |
| 95% CI |   | (6.74, 7.25)     | (6.95, 8.54)    |
| P3     | 4 | 6.75 $\pm$ 0.19  | 7.25 $\pm$ 0.50 |
| 95% CI |   | (6.44, 7.05)     | (6.45, 8.04)    |

Based on Table 1 and Figure 1, the highest antibacterial inhibitory activity was observed in the positive control group (K+), with a value of 1.450.68. In contrast, the lowest antibacterial inhibitory activity was found in the P3 group, with a value of 1.271.70. Salivary pH was higher after treatment than before in all experimental groups (Table 2). Data analysis using a One-Way ANOVA showed no significant differences in the inhibitory zone ( $P=0.823$ ). Further analysis using the Wilcoxon signed-rank test showed significant differences in salivary pH before and after treatment ( $P=0.001$ ), with higher pH in the K+, P1, P2, and P3 after-treatment groups. Based on the results of Tamhane’s post hoc test, all experimental groups showed p-values greater than 0.05 ( $p > 0.05$ ), indicating that there were no statistically significant differences in the antibacterial activity against *S. mutans* among the treatment groups (Table 3).

**Table 3.** Post Hoc Tamhane test results

| Research Group | K- | K+    | P1    | P2    | P3    |
|----------------|----|-------|-------|-------|-------|
| K-             |    | 1.000 | 1.000 | 0.999 | 0.996 |
| K+             |    |       | 1.000 | 0.999 | 0.991 |
| P1             |    |       |       | 1.000 | 1.000 |
| P2             |    |       |       |       | 1.000 |
| P3             |    |       |       |       |       |

**DISCUSSION**

Dental caries is one of the most common oral health problems worldwide. The primary etiological agent of dental caries is *S. mutans*, which plays a major role in biofilm formation and enamel demineralization. In recent years, preventive strategies for dental caries using natural materials with antibacterial properties have gained increasing attention. One such natural material is *Cinnamomum zeylanicum* essential oil, which contains bioactive compounds such as cinnamaldehyde, eugenol, and linalool, all of which have demonstrated antibacterial activity against *S. mutans*.<sup>27,28</sup>



This study aimed to evaluate whether *Cinnamomum zeylanicum* essential oil could be used as an antibacterial agent against *S. mutans* to reduce dental caries. The study used male *Wistar* rats aged 8–10 weeks, weighing 180–200 g, and in healthy physical condition, as indicated by clear eyes, active movement, and normal feces. The rats were inoculated with *S. mutans*, fed a cariogenic diet containing sucrose, and administered *Cinnamomum zeylanicum* essential oil in their drinking water starting on day three, followed by antibacterial testing using the disk diffusion method and saliva pH measurement.

Descriptive analysis showed that the group inoculated with *S. mutans* without any treatment (K–) had an antibacterial inhibition mean of  $1.570.00 \pm 587.95$ , which was higher than that of the groups receiving *Cinnamomum zeylanicum* essential oil in their drinking water. A lower inhibition value indicates greater suppression of bacterial growth or activity, suggesting that *S. mutans* was more effectively inhibited, thereby reducing its ability to proliferate or form dental plaque. In other words, the lower the antibacterial inhibition value, the more effective the substance is at reducing the risk of caries. Without antibacterial inhibition, bacterial growth occurs more readily, increasing the risk of dental caries because *S. mutans* plays a critical role in plaque formation.<sup>29</sup> The groups treated with cinnamon essential oil at concentrations of 6.25% (P1) and 10% (P2) also demonstrated better antibacterial inhibition than the untreated group. However, their effectiveness was lower than that observed in the 12.5% concentration group (P3;  $p < 0.05$ ). These findings indicate that cinnamon essential oil exhibits antibacterial activity even at low concentrations, with greater effectiveness at higher concentrations, suggesting a concentration-dependent effect.<sup>30</sup>

The antibacterial activity of cinnamon essential oil is attributed to its active compounds, including cinnamaldehyde, eugenol, and linalool. Cinnamaldehyde, the major component,

disrupts bacterial cell membrane integrity, inhibits protein synthesis, and interferes with bacterial metabolism, thereby suppressing bacterial growth. It has also been reported to reduce biofilm biomass and metabolic activity of *S. mutans* at sub-MIC concentrations, increase cell surface hydrophobicity, reduce bacterial aggregation, inhibit acid production, and enhance acid tolerance. Eugenol and linalool further contribute to the antibacterial efficacy of cinnamon essential oil.<sup>28</sup>

In the absence of intervention, *Streptococcus mutans* grows freely under favorable environmental conditions, particularly in carbohydrate-rich environments such as sucrose. Although the treated groups showed antibacterial inhibition, the differences were not statistically significant compared with K–, except for P3, which showed better results descriptively but not statistically.<sup>31</sup> Cinnamon essential oil at concentrations of 6.25% and 10% had antibacterial effects comparable to 0.2% chlorhexidine. The antibacterial effects of P1 and P2 were attributed to cinnamaldehyde and eugenol, although at lower concentrations than in P3. Previous studies have shown that the effectiveness of chlorhexidine may be limited by its concentration, while cinnamon essential oil at 6.25% and 10% can produce comparable antibacterial effects.<sup>32</sup> Descriptively, P2 exhibited greater antibacterial inhibition than other groups. The reduced effectiveness in P1 may be due to lower concentrations of active compounds that were insufficient to achieve optimal antibacterial effects. Previous studies have reported a linear increase in the antibacterial activity of essential oils with increasing concentration.<sup>33</sup> Chlorhexidine (0.2 %) primarily exerts a bacteriostatic effect by disrupting bacterial cell membranes and causing leakage of intracellular components. In contrast, 12.5% cinnamon essential oil contains higher concentrations of cinnamaldehyde and eugenol, which effectively disrupt bacterial cell membranes, interfere with enzymatic function, and increase cellular leakage. These findings

confirm that higher concentrations of cinnamon essential oil are more effective at inhibiting *S. mutans*, thereby helping prevent dental caries.<sup>34-</sup>

<sup>36</sup> Cinnamaldehyde, a hydrophobic compound, disrupts bacterial lipid membranes more effectively at higher concentrations. Additionally, higher concentrations of eugenol increased cell membrane permeability, resulting in greater leakage of bacterial cellular components.<sup>37</sup>

These findings are consistent with studies by Waty (2022), which reported that cinnamon essential oil at 12.5% showed a descriptively significant antibacterial effect against *S. mutans* compared with lower concentrations. Similar conclusions were reported by Nugraha, Astuti, and Tunggadewi (2021), who demonstrated that higher concentrations of cinnamon essential oil resulted in stronger antibacterial effects.<sup>14</sup> Eugenol is generally considered safe for oral consumption at doses of 5–10 mL, without dilution. However, prolonged use or high doses without dilution may exhibit toxic effects, particularly on vital tissues, and excessive oral exposure may lead to periodontal tissue damage due to diffusion from the cavity. Therefore, cinnamon essential oil should be used within safe limits and in diluted form. Overall, *Cinnamomum zeylanicum* essential oil shows strong potential as a natural antibacterial agent for caries prevention.<sup>31,38,39</sup>

The increase in salivary pH observed in the treatment groups (K+, P1, P2, and P3) may be closely associated with the bioactive compounds present in *Cinnamomum zeylanicum* essential oil, particularly eugenol and cinnamaldehyde. These compounds exhibit strong antimicrobial activity against *Streptococcus mutans* by disrupting bacterial cell membranes, altering cell wall permeability, and inhibiting bacterial metabolic processes responsible for acid production. By suppressing *S. mutans* acidogenic activity, the production of organic acids is reduced, thereby preventing a decline in salivary pH. An alkaline oral environment plays a critical role in caries prevention, as elevated pH decreases enamel

solubility and inhibits the demineralization of hydroxyapatite crystals. The reduced acidogenic potential of *S. mutans* under the influence of cinnamon essential oil limits the diffusion of hydrogen ions into the enamel and promotes remineralization. Therefore, the ability of *Cinnamomum zeylanicum* essential oil to modulate salivary pH through its antimicrobial constituents provides a plausible biological mechanism for its protective effect against enamel demineralization and progression of dental caries.<sup>40-42</sup>

*Cinnamomum zeylanicum* essential oil has potential as a natural antibacterial agent for preventing dental caries, inhibiting the growth of oral bacteria in *Rattus norvegicus* infected with *Streptococcus mutans*, and altering salivary pH to an alkaline state.

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# Enhancing Dental Health Knowledge Through The Combination of Storytelling and Interactive Activities at TK Sepuluh November

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## ABSTRACT

**Background:** Dental health in pre-school children is an important aspect that should be introduced early. However, cognitive limitations and short attention spans at this age require educational methods appropriate to their developmental stage. *Pojok Sikat Gigi* is an activity that consists of various health promotion and preventive strategies carried out at TK Sepuluh November Surabaya, targeting teachers, parents, and students. One strategy is dental health education through storytelling, using hand puppets and interactive activities. **Objective:** This study aimed to determine the effectiveness of dental health education with storytelling and interactive activities on improving pre-school students' knowledge of dental health. **Method:** 34 students aged between 5-7 were the sample in this research. This was a pre-experimental research with a pre- and post-test design. The pre-test score was measured through a Q&A session before education. The post-test score was measured by asking students to write down their answers on a piece of paper, guided by the researcher who read the same questions. The pre- and post-test knowledge scores were compared using the Wilcoxon statistical test to assess whether there was a significant difference after the educational intervention. **Results:** The mean pre-test score was 2.24, and the post-test score was 5 (maximum score was 5). The Wilcoxon test value was  $\text{sig}=0.00<0.05$ , meaning that there was a significant difference between the scores before and after education. **Conclusion:** The storytelling method and interactive activities are effective in improving pre-school students' knowledge of dental health.

**Keywords:** Dental health education, health promotion, pre-school children, preventive dental health, storytelling

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## INTRODUCTION

Oral health in pre-school children is an important part of their growth and development that needs to be introduced early on. Recent national survey data also indicate that dental caries prevalence remains high, reaching 82.8% in the 2023 Indonesian Health Survey, confirming that oral health problems in children are still a major public health issue.<sup>1</sup> At this age, children are in the habit-forming phase, which means education about dental hygiene greatly influences their future behavior. However, pre-schoolers have limitations in terms of cognitive aspects, the ability to understand abstract information, and a relatively short attention span. This condition requires an educational approach that is not only informative but also fits the child's stage of development, engaging, and easy to understand.<sup>2,3</sup>

The researchers and their team designed a program called the Toothbrushing Corner, a series of dental health promotion activities targeting teachers, parents, and especially students, to create synergy between teachers and parents and improve the dental and oral health status of pre-school children. This program includes various promotive and preventive strategies consisting of:

**Table 1.** *Pojok Sikat Gigi Programs*

| Target   | Activities   |
|----------|--|
| Teachers | Workshop on creating dental health co-curricular and basic skills for maintaining pre-school children's dental health  |
| Students | <ul style="list-style-type: none"> <li>• Oral health education using storytelling with hand puppets and interactive activities</li> <li>• Practicing brushing teeth together</li> <li>• Oral health screening</li> <li>• Topical fluoride application for cavity prevention</li> </ul> |
| Parents  | Children's dental health talk show with an expert  |

Different evaluation methods were used to determine the effectiveness of each activities. This study was conducted especially to evaluate one of the activities: dental health education using hand puppets and interactive activities. This study aimed to determine the effectiveness of storytelling and interactive activities in improving pre-schoolers' knowledge of dental health at TK Sepuluh November Surabaya.

The target of this study was preschool-aged children. Preschool-aged children are in the preoperational stage according to Piaget's theory of cognitive development, where learning processes are more effective when conducted through concrete, visual, imaginative, and enjoyable experiences.<sup>2</sup> At this stage, children are not yet able to understand abstract concepts in depth; therefore, delivering dental health education through conventional methods such as lectures tends to be ineffective. Therefore, educational methods are needed that can attract children's attention, engage their emotions, and stimulate active participation so that health messages can be well understood and remembered.<sup>3</sup>

Storytelling using hand puppets is an educational approach that is considered appropriate for pre-schoolers. Hand puppets serve as visual and symbolic media that can facilitate communication between educators and children. Through simple and interesting stories, messages about the importance of brushing teeth, avoiding excessive consumption of sugary foods, and maintaining dental and oral health can be conveyed indirectly, making it easier for children to accept and imitate them. In addition, the emotional interaction built through puppet characters can increase children's engagement and memory of educational material.<sup>4,5</sup>

Interactive educational media, such as educational games, audio-visual media, and participatory question-and-answer activities, also have great potential for improving the effectiveness of dental health education. Interactive media allow children to learn through direct experience (learning by doing), engage

more than one sense, and encourage children to actively participate in the learning process. This interactivity can increase children's motivation to learn and help them internalize healthy behaviors from an early age.<sup>6,7</sup>

Through stories and interactive activities, children can associate new information with familiar characters, situations, and plots, thereby reinforcing messages about dental health. The use of media such as hand puppets, picture books, or interactive games also helps create a fun learning environment and reduces boredom, allowing children to focus and engage more actively.<sup>5,7</sup>

The effectiveness of storytelling and interactive activities approaches in the context of dental health programs needs to be scientifically tested through structured research. Data-based evaluation is needed to ensure that this method truly has an impact on improving children's knowledge while also serving as the basis for developing more optimal educational strategies in early childhood education institutions.

## MATERIALS AND METHODS

This study was conducted at TK Sepuluh November, Surabaya. 34 out of 37 students Kindergarten B students were randomly selected as respondents through a sample size calculated using the Slovin formula. All respondents obtained parental consent through the signing of an informed consent. The type of research used was pre-experimental with a pre-test and post-test design without a control group. Pre-test scores were measured through a question-and-answer session before the education, where the researcher asked questions to the students and recorded their answers directly. Post-test scores were obtained by asking students to write their answers on individual sheets of paper, guided by the researcher, who read the same questions aloud. Dental health education was divided into three sessions :

1. Story telling with a puppet doll for an introduction to toothache and its treatment (10 minutes)
2. Interactive session with a big activity book and tooth shaped doll equipped with removable velcro fasteners for teaching children about foods that are good and bad for their teeth (15 minutes)
3. Interactive session with 2D toothbrush simulation (15 minutes)



**Figure 1.** Dental Health Education with Story Telling and Interactive Activities

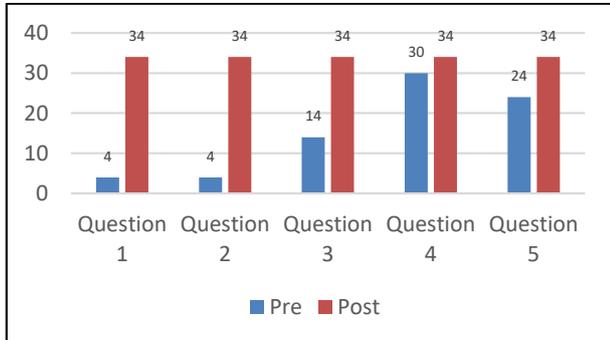
**Table 2.** Questionnaire

| No | Questions   |
|----|---|
| 1  | How many baby teeth are there?                    |
| 2  | How many adult teeth are there?                   |
| 3  | How many times a day should you brush your teeth? |
| 4  | What foods are good for dental health?            |
| 5  | What foods are bad for dental health?             |

Each correct answer received a score of 1, the minimum score was 0, and the maximum score was 5. The pre- and post-test knowledge scores were compared using the Wilcoxon statistical test to assess whether there was a significant difference after the educational intervention.

**RESULT**

The frequency of each student's right answers on the pre-test and post-test can be seen in the table below (Picture 2).



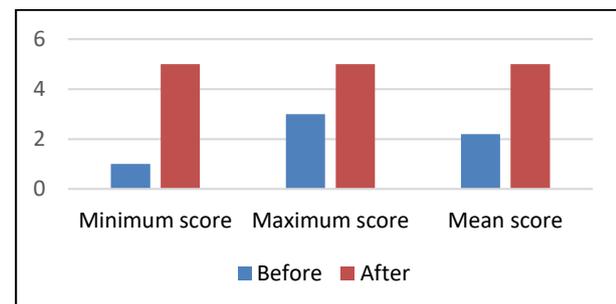
**Figure 2.** The frequency of each student's right answer

The results in Figure 2 show that most students had the correct answers in question 4, followed by questions 5, 3, 2, and 1.

The pre- and post-test scores were analyzed statistically, and the results are as follows (Tables 3, 4, 5):

**Table 3.** Descriptive Statistics

|                    | N  | Minimum | Maximum | Mean   | Std. Deviation |
|--------------------|----|---------|---------|--------|----------------|
| Before             | 34 | 1.00    | 3.00    | 2.2353 | .55371         |
| After              | 34 | 5.00    | 5.00    | 5.0000 | .00000         |
| Valid N (listwise) | 34 |         |         |        |                |



**Figure 3.** Comparison of minimum, maximum, and mean score of the level of knowledge between before and after education

The statistical test results showed that there was an increase in the average knowledge

level score from before education to after education. Before education, no students answered all questions correctly, as evidenced by a minimum score of 1 and a maximum score of 3, whereas after education, all students were able to answer the questions correctly with the same minimum and maximum scores of 5.

**Table 3.** Normality Test

|        | Statistic | df | Statistic | df | Sig. |
|--------|-----------|----|-----------|----|------|
| Before | .370      | 34 | .722      | 34 | .000 |
| After  | .         | 34 | .         | 34 | .    |

The normality test showed a sig value of  $0.00 < 0.05$ , meaning that the data were not normally distributed. Therefore, the parametric statistical assumption could not be fulfilled, and the statistical test was continued with the Wilcoxon signed-rank test.

**Table 4.** Wilcoxon Signed Rank Test

| Test Statistics        | Before-After Storytelling & Interactive Activities Education |
|------------------------|--|
| Z                      | -5.276 <sup>b</sup>  |
| Asymp. Sig. (2-tailed) | .000   |

a. Wilcoxon Signed Ranks Test  
b. Based on negative ranks.

The Wilcoxon signed-rank test results showed a sig value of  $0.00 < 0.05$ , indicating a significant difference in pre-schoolers' dental health knowledge scores before and after education using storytelling and interactive activities.

**DISCUSSION**

The variation in students' correct answer scores across dental health questions may be influenced by differences in the level of familiarity, concreteness of concepts, and prior exposure to oral health information. In this study, most students answered Question 4 correctly, followed by Questions 5, 3, 2, and 1. Alrashdi et al (2022), in their study about oral health promotion in pre-schoolers said that children



tend to demonstrate better understanding of dental health concepts that are more concrete, familiar, and closely related to daily habits.<sup>8</sup> Question 4, which showed the highest correct responses, may represent knowledge that children encounter regularly through parental guidance, school activities, or health education programs. Repetition of routine behaviors strengthens memory retention and facilitates recall.<sup>9</sup>

The results of this study show a significant increase in dental health knowledge among pre-school students after being educated through storytelling using hand puppets and interactive activities. The average knowledge score increased from 2.24 on the pre-test to 5.00 on the post-test, with the Wilcoxon test showing a significance value of  $p=0.000$  ( $p<0.05$ ). This indicates that the educational intervention provided was effective in improving pre-schoolers' understanding of dental and oral health.

This significant increase in knowledge can be explained through the cognitive development approach of pre-school children. According to Piaget's theory, preschool-aged children are in the preoperational stage, where the learning process is more optimal when done through concrete, visual, and imaginative experiences. Damjanovic et al (2025) stated in their study that pre-school learning improves when teachers use tangible materials and hands-on tools, consistent with Piaget's view that children learn through active engagement with real or similar objects.<sup>10</sup>

The storytelling method using hand puppets is able to present health messages in a symbolic and narrative form that is easily understood by children, so that the information conveyed is not abstract.<sup>11</sup> Stories allow children to associate dental health messages with interesting characters and storylines, thereby improving their memory and understanding.<sup>4,8,10</sup>

In addition, the use of interactive activities in dental health education plays an important role in the success of this intervention.

Interactive activities involve the active participation of children through question-and-answer sessions, games, and demonstrations, so that the learning process is not one-sided.<sup>12,13</sup> This approach is in line with the concept of learning by doing, where children learn through direct experience. Recent research shows that interactive media can improve focus, learning motivation, and knowledge retention in early childhood compared to conventional methods such as lectures.<sup>7,8,14</sup>

The results of this study are in line with various previous studies that report that storytelling methods and educational play media are effective in improving dental health knowledge and attitudes in early childhood.<sup>4,8,11</sup> Storytelling with hand puppets in particular are considered capable of building emotional engagement between children and educators, so that health messages are conveyed persuasively without giving the impression of lecturing. This emotional interaction contributes to increased interest and attention in children during the educational process.<sup>11,13,15</sup>

The finding that all respondents obtained maximum scores on the post-test shows that this method is very suitable for pre-school students. However, these results require critical examination. Although this study provides empirical evidence that dental health education through storytelling and interactive activities can be an effective alternative strategy for promoting dental health in early childhood education settings, several limitations should be critically considered when interpreting the findings. The pre-experimental research design without a control group limits the ability of the study to compare the effectiveness of this method with other educational methods. Without comparison to a group that did not receive storytelling and interactive activities, it is difficult to exclude alternative explanations. In addition, knowledge was measured immediately after the intervention, so it does not yet describe the sustainability of understanding and long-term behavioral changes in children.<sup>16-18</sup> In early

childhood education, increases in knowledge do not always translate directly into sustained oral health practices, as behavior formation requires repetition, reinforcement, and environmental support over time.<sup>19,20</sup>

However, this study provides empirical evidence that dental health education through storytelling and interactive activities can be an effective alternative strategy for promoting dental health in early childhood education settings. This approach also supports the concept of the Toothbrushing Corner program, which emphasizes synergy between schools, children, and a pleasant learning environment. In the future, this method has the potential to be further developed with long-term evaluation and combined with parental involvement to strengthen the formation of healthy behaviors from an early age.

## CONCLUSION

The storytelling method and interactive activities were effective in improving pre-school students' knowledge of dental health in KB-TK Sepuluh November Surabaya.

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# Oral Health Care Management in Atypical Oral and Cutaneous Bullous Pemphigoid

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## ABSTRACT

**Background:** Bullous Pemphigoid (BP) is the most common subepidermal bullous autoimmune disease, typically presenting as tense bullae. Atypical presentations of bullous pemphigoid often mimic TEN or impetigo, creating diagnostic ambiguity. Effective management of these cases requires integrated strategies to address systemic health factors and extensive oral mucosal involvement. **Objective:** This report highlights the Oral Health Care Management of atypical oral and cutaneous manifestations of Bullous Pemphigoid. **Case:** A 58-year-old woman with a history of Diabetes Mellitus and heart disease was referred with extensive bullae, erosions, and "honey-like" crusts involving over 30% of her body surface area. Her condition worsened despite two weeks of treatment with acyclovir, which was administered for suspected herpes. The presentation was highly suggestive of TEN and impetigo. **Case Management:** Management of the oral and perioral lesions focused on infection prevention and pain relief. Debridement was performed using sterile gauze soaked in normal saline and 0.2% chlorhexidine. This was followed by the application of Aloe vera extract gel/spray. Comprehensive systemic therapy, including corticosteroids and immunosuppressants, was coordinated by a multidisciplinary team to control the autoimmune disease and its comorbidities. **Conclusion:** The presence of comorbidities, such as diabetes, further complicates systemic management and heightens the risk of secondary infections, requiring meticulous wound care. A multidisciplinary diagnostic algorithm, supported by supplementary examinations, is crucial for differentiating atypical BP from TEN and impetigo infection. Adequate management of associated oral and perioral manifestations is an integral component of comprehensive patient care.

**Keywords:** Bullous pemphigoid, impetigo, multidisciplinary approaches, oral health care, toxic epidermal necrolysis

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## INTRODUCTION

Bullous Pemphigoid (BP) is the most common subepidermal bullous autoimmune disease, accounting for up to 80% of subepidermal immune-bullous cases and primarily affecting elderly patients, generally between the ages of 60 and 80.<sup>1,2</sup> Pathogenetically, BP is caused by IgG autoantibodies targeting hemidesmosome components in the Basement Membrane Zone (BMZ), especially the BP180 (collagen XVII) and BP230 antigens, leading to the separation of the epidermis from the dermis and the formation of tense bullae. The classic clinical manifestation is an eruption of tense bullae accompanied by severe pruritus on normal or erythematous skin, which significantly impacts the patient's quality of life and mortality.<sup>3,4</sup>

Although the clinical presentation of BP is often characteristic, the diagnosis of BP can be difficult due to variable clinical phenotypes.<sup>2</sup> Atypical presentations can resemble much more severe and life-threatening acute bullous diseases, such as Toxic Epidermal Necrolysis (TEN), which histopathologically involves full epidermal keratinocyte necrosis, in contrast to the subepidermal clefting in BP.<sup>5</sup> Furthermore, ruptured bullae often undergo secondary superinfection, which in this case resulted in yellowish crusts (honey-like appearance) similar to the characteristic features of impetigo. The distinction between atypical BP, TEN, and Impetigo is critical because the management protocols and prognosis for these conditions differ significantly. Confirmation of the diagnosis cannot rely solely on clinical features.<sup>6</sup>

A systematic diagnostic algorithm is necessary to overcome the diagnostic challenges caused by atypical and overlapping clinical manifestations. The definitive diagnosis of BP requires confirmation through supplementary examinations, including a biopsy for histopathology (showing a subepidermal cleft with a rich eosinophil infiltrate) and Direct Immunofluorescence (DIF) on perilesional skin

(showing linear deposition of IgG and/or C3 at the BMZ), which is the gold standard for autoimmune bullous diseases.<sup>1,5</sup> Additionally, serological tests such as ELISA detecting anti-BP180/BP230 antibodies have high sensitivity and specificity.<sup>2</sup> The presence of systemic comorbidities, such as uncontrolled Diabetes Mellitus (DM) in this case, further complicates disease management and increases the risk of infectious complications, demanding an interprofessional approach.<sup>7</sup>

Oral mucosal involvement, although less common than skin lesions, has been reported in several patients with BP. Oral lesions in the form of bullae, which can inevitably rupture into painful erosions, may lead to difficulty in eating (dysphagia) and speaking, and are a high potential source of infection. Therefore, for Oral Medicine specialists, the management of oral manifestations, including pain management, prevention of secondary infection, and supportive care, is a crucial component of comprehensive treatment.<sup>6,8</sup> This case report aims to present a case of BP with highly atypical cutaneous and oral manifestations, highlight the challenge of distinguishing it from TEN and Impetigo, and specifically emphasize the strict diagnostic algorithm and outline the comprehensive management of oral manifestations as an essential contribution to the management of BP patients with complex clinical presentations.

## CASE AND CASE MANAGEMENT

A 58-year-old female was referred to the Emergency Department of Dr. Ramelan Naval Hospital with extensive bullae and crusting over her entire body. The patient had previously been treated with Acyclovir (5x800 mg) for two weeks at another healthcare facility for suspected herpes, but showed no improvement, leading to a worsening condition and referral to a higher-level facility for follow-up. The patient had a history of Diabetes

Mellitus (routine therapy: vidagliptin 50 mg, Gliclazide 40 mg) and heart disease (bisoprolol 5 mg).

Vital sign examination showed a decreased level of consciousness (GCS 3/25), blood pressure 135/41 mmHg, pulse 99x/minute, body temperature 36.5° Celsius, respiration rate 40 bpm, and oxygen saturation 96%. Extraoral clinical examination revealed bullae, erosions, and "honey-like appearance" crusts, which were yellowish-brown, tense, and painful, involving

over 30% of the total body surface area, including the face, superior and inferior labial areas, perioral area, hands, and feet, resembling the features of Toxic Epidermal Necrolysis (TEN) and impetigo (Figure 1). Intraoral examination was limited because the patient had difficulty opening her mouth; however, the accessible areas (labial mucosa and gingiva) appeared normal. The initial working diagnosis was suspected Bullous Pemphigoid with differential diagnoses of impetigo and TEN.



**Figure 1.** Clinical manifestations of the patient at visit 1, (a) showing "honey-like appearance" crusts on the face, including the labial and perioral regions, and (b) reddish-brown to blackish erosion on the foot. (c) Tense bullae on the fingers and wrist skin.

Supplementary examinations performed included a complete blood count, liver and kidney functions, random blood

glucose, HbA1c, urine culture, blood culture (Table 1), and bacterial culture from crusted lesions (Table 2).

**Table 1.** Results of the supportive examinations included a complete blood count, liver function, kidney function, random blood glucose, HbA1c, urine culture, and blood culture

| Date                  | Tested Markers           | Result  | Unit                      | Reference Range | Comment         |
|-----------------------|--------------------------|---------|---------------------------|-----------------|-----------------|
| 28 Sep 2025           | Leukosit (WBC)           | 19.88   | $\times 10^3/\mu\text{L}$ | 4.00-10.00      | High            |
|                       | Neutrofil%               | 90.6    | %                         | 50.0-70.0       | High            |
|                       | Limfosit%                | 4.5     | %                         | 20.0-40.0       | Low             |
|                       | IMG%                     | 2.9     | %                         | 0.00-0.99       | High            |
|                       | Hemoglobin               | 10.6    | g/dL                      | 12-15           | Low             |
|                       | Hematokrit               | 32.4    | %                         | 37.0-47.0       | Low             |
|                       | Trombosit (Platelet)     | 516     | $\times 10^3/\mu\text{L}$ | 150-450         | High            |
|                       | PCT (Plateletcrit)       | 4.7     | $\times 10^3/\mu\text{L}$ | 0.108-0.282     | High            |
|                       | Albumin                  | 2.21    | mg/dL                     | 3.50-5.20       | Low             |
|                       | Glukosa Darah Acak (GDA) | 580     | mg/dL                     | <200            | Critically High |
|                       | Kreatinin                | 2.5     | mg/dL                     | 0.6-1.5         | High            |
|                       | BUN                      | 90.2    | mg/dL                     | 10-24           | High            |
|                       | Na (Sodium) serum        | 126.6   | mEq/L                     | 135-147         | Low             |
|                       | K (Potassium) serum      | 7.7     | mmol/L                    | 3.0-5.0         | Critically High |
|                       | PO2                      | 185.1   | mmHg                      | 80.0-100.0      | High            |
|                       | Urine ALB                | 150     | g/L                       | $\leq 0.02$     | High            |
|                       | Urine RBC (Eritrosit)    | 48.8    | /hpf                      | 0-3             | High            |
| Urine WBC (Lekosit)   | 7.7                      | /hpf    | 0-5                       | High            |                 |
| Urine CAST (Silinder) | 11.1                     | /hpf    | 0-2                       | High            |                 |
| Urine BACT (Bakteri)  | 7.9                      | /hpf    | 0-2                       | High            |                 |
| 29 Sep 2025           | Albumin                  | 2.09    | mg/dL                     | 3.50-5.20       | Low             |
|                       | K (Potassium) serum      | 7.17    | mmol/L                    | 3.0-5.0         | Critically High |
|                       | Cl (Chloride) serum      | 112     | mEq/L                     | 95-105          | High            |
| 30 Sep 2025           | Albumin                  | 2.28    | mg/dL                     | 3.50-5.20       | Low             |
| 08 Oct 2025           | Leukosit (WBC)           | 15.19   | $\times 10^3/\mu\text{L}$ | 4.00-10.00      | High            |
|                       | Neutrofil#               | 13.97   | $\times 10^3/\mu\text{L}$ | 2.00-7.00       | High            |
|                       | Neutrofil%               | 92      | %                         | 50.0-70.0       | High            |
|                       | Limfosit%                | 6.6     | %                         | 20.0-40.0       | Low             |
|                       | Monosit%                 | 1.3     | %                         | 3.0-12.0        | Low             |
|                       | Hemoglobin               | 10.6    | g/dL                      | 12-15           | Low             |
|                       | Hematokrit               | 33.2    | %                         | 37.0-47.0       | Low             |
|                       | MCHC                     | 31.9    | g/dL                      | 32-36           | Low             |
|                       | Trombosit (Platelet)     | 97      | $\times 10^3/\mu\text{L}$ | 150-450         | Low             |
|                       | MPV                      | 13.3    | fL                        | 6.5-12.0        | High            |
|                       | P-LCR                    | 51.1    | %                         | 11.0-45.0       | High            |
|                       | Albumin                  | 2.17    | mg/dL                     | 3.50-5.20       | Low             |
|                       | 09 Oct 2025              | Albumin | 2.52                      | mg/dL           | 3.50-5.20       |
| 10 Oct 2025           | Albumin                  | 3.4     | mg/dL                     | 3.50-5.20       | Low             |

**Table 2.** Bacterial culture results of the oral and perioral lesion swab specimen and antibiotic susceptibility test

| Category                  | Parameter          | Result   | Interpretation/Clinical Note                          |
|---------------------------|--------------------|--|---|
| Specimen Details          | Specimen Type      | Swab   |   |
|                           | Completion Date    | October 5, 2025  |   |
| Bacterial Isolate         | Organism Found     | Serratia marcescens  | Identified as potential causative agent of infection. |
|                           | Intrinsic Property | Intrinsically resistant  |   |
| Antibiotic Susceptibility | Sensitive (S)      | Ciprofloxacin, Gentamicin, Meropenem, Trimethoprim-Sulfamethoxazole  | Effective antibiotics for treatment.                  |
|                           | Intermediate (I)   | Tigecycline<br>Amikacin, Amoxicillin, Ampicillin, Cefazolin, Cefepime, Cefotaxime, Ceftazidime, Ceftriaxone, Nitrofurantoin, Piperacillin- | Antibiotics deemed                                    |

The management of this case involved several disciplines, including internal medicine, dermato-venereology, and oral medicine. This case report highlights the management of oral and perioral lesions, which aimed to prevent secondary infections and reduce pain symptoms in the affected areas.

Debridement, which is the initial stage of Oral Health Care (OHC) action, was performed by compressing sterile gauze soaked in 0.9% NaCl (normal saline) until the crusts softened and could be peeled off naturally. This was followed by a sterile gauze compress soaked in 0.2% chlorhexidine gluconate solution. Subsequently, aloe vera extract gel was applied to the labial and perioral mucosa, and aloe vera extract spray was used for difficult-to-reach intraoral areas. This series of procedures was performed routinely three times a day and showed significant progress in the oral and perioral areas after 4 consecutive days of Oral Health Care (OHC) actions (Figure 2).

Topical therapy for areas other than the oral and perioral regions, as well as comprehensive systemic therapy consisting of antibiotics, corticosteroids, and antihistamines,

was coordinated by the internal medicine and dermato-venereology specialists to control the autoimmune disease and comorbidities. The medications included albumin 25%, Fusidic Acid cream 20 mg/gram, methylprednisolone 125 mg injection, mebhydrolin napadisylate, meropenem 1 g, and gentamicin sulfate 0.3% eye pointment.



**Figure 2.** Progress of the patient's oral and perioral lesions after four consecutive days of Oral Health Care (OHC) procedures.

## DISCUSSION

Bullous Pemphigoid (BP) is the most common subepidermal bullous autoimmune disease, classically characterized by tense

bullae and intense pruritus.<sup>1,2</sup> This case presents a significant diagnostic challenge because of its atypical clinical manifestations. The patient presented with extensive erosions and crusting involving both the skin and oral mucosa, resembling epidermal sloughing. The widespread clinical features, combined with a history of prior treatment with acyclovir (for suspected herpes simplex), made it visually very difficult to distinguish from Toxic Epidermal Necrolysis (TEN), a life-threatening drug hypersensitivity reaction, especially in the early phase.

The primary diagnostic challenge is differentiating BP from TEN. Clinically, TEN is characterized by a painful, dark erythematous rash followed by keratinocyte necrosis and total epidermal detachment.<sup>5</sup> Although atypical BP variants (BP-like TEN) can mimic this picture, a definitive diagnosis cannot rely on clinical presentation alone. A definitive diagnosis is established through a combination of examinations. Several previous case reports have documented a similar phenomenon in which Bullous Pemphigoid (BP) presents with massive epidermal sloughing resembling Toxic Epidermal Necrolysis (TEN). For comparison, a study by Patel et al. (2020) reported a case of BP that was initially diagnosed as TEN due to extensive mucosal involvement and a positive Nikolsky sign; however, the diagnosis of BP was only established after histopathological examination revealed a subepidermal cleft rather than the full-thickness necrosis characteristic of TEN. The similarities found in this case emphasize that while TEN is a primary medical emergency that must be ruled out, clinicians should not overlook the possibility of an autoimmune subepidermal bullous disease when the culture results or response to initial therapy do not align with the typical clinical course of TEN.

The gold standard supplementary examinations are histopathology-anatomy (HPA) and direct immunofluorescence (DIF). However, a tissue biopsy could not be

performed on this patient because their haematological and albumin status had not reached the minimum acceptable level for the procedure, thus requiring albumin therapy and blood transfusion. If the patient's condition becomes suitable for a biopsy, the expected HPA result to strengthen the BP diagnosis is a subepidermal cleft with a rich eosinophil inflammatory cell infiltrate (typical of BP), which would rule out the full keratinocyte necrosis characteristic of TEN.<sup>4</sup> Furthermore, the definitive finding expected from immunopathology is the linear deposition of IgG/C3 at the Basement Membrane Zone (BMZ) via Direct Immunofluorescence (DIF) and increased anti-BP180 autoantibody levels on ELISA, which reliably confirm the BP diagnosis.<sup>9</sup>

This case is further complicated by clinical features resembling Bullous Impetigo. Bullous impetigo, caused by toxins from *Staphylococcus aureus*, produces superficial bullae that rupture and leave honey-colored crusts. The golden-yellow crusts observed in the patient's perioral and cutaneous areas were manifestations of superinfection on the BP erosions. Therefore, urine, blood, and oral/perioral lesion swab cultures were also performed in this case.<sup>10,11</sup>

Oral mucosal involvement in BP, although less common than in Pemphigus Vulgaris, is reported to occur in approximately 10–40% of cases. In this patient, extensive and severe oral involvement caused trismus and significant pain, which is not only an indicator of disease severity but also a critical management challenge. Severe oral involvement significantly reduces the patient's quality of life. Comorbidity risk factors, such as Diabetes Mellitus (DM), also play an important role in increasing the patient's susceptibility to secondary infections and delaying healing. Furthermore, the history of DM also necessitates evaluating for drug-induced BP, given that certain anti-diabetic medications (such as DPP-4 inhibitors) have been identified as significant BP triggers.<sup>2,8,12</sup> The role of Diabetes Mellitus as a comorbidity in

this case warrants specific attention. Recent literature indicates a strong correlation between the use of dipeptidyl peptidase-4 inhibitors (DPP-4i) and an increased risk of BP. According to a meta-analysis by Kridin and Bergman (2019), patients with DM face a significant relative risk of developing drug-induced BP, which frequently manifests with more severe mucosal involvement compared to the classic variant. Comparison with the current case suggests that evaluating the patient's diabetic medication history is crucial, as discontinuing the triggering agent serves as a primary management step that often accelerates clinical remission more effectively than relying solely on immunosuppressants.

The use of systemic and topical medications in this atypical BP case was designed to achieve three main goals: control the autoimmune response, treat and prevent secondary infection, and provide vital patient support. Given the complexity of the clinical presentation, disease severity, and risk of infectious complications, this case justifies a comprehensive multidisciplinary management approach.<sup>12</sup> A medical team involving specialists in Dermato-Venereology, Internal Medicine, and Oral Medicine is highly necessary. Systemic management to control the autoimmune disease was coordinated by the Internal Medicine and Dermato-Venereology specialists. Methylprednisolone (125 mg) injection was administered as a high-dose corticosteroid to suppress the abnormal immune response underlying BP.

Corticosteroid use is the mainstay of therapy for controlling autoimmune diseases.<sup>13</sup> Additionally, Albumin 25% was administered as supportive therapy. Albumin use was crucial because the patient's hematological and albumin status had not yet reached the minimum threshold for a biopsy; thus, albumin was administered to stabilize and support before the patient's definitive diagnostic procedure could be performed.<sup>14</sup>

Infection control was a priority, considering the patient's clinical presentation resembled impetigo with golden-yellow (honey-like appearance) crusts in the perioral and cutaneous areas, suspected to be a bacterial superinfection of the BP erosions. Based on the bacterial culture results from the oral lesion swab, *Group A Streptococcus (GAS)*, the etiology of impetigo, was not found. However, the culture test showed the presence of *Serratia marcescens*, a bacterium that causes Ventilator-Associated Pneumonia (VAP). Meropenem is a broad-spectrum antibiotic used to treat severe systemic bacterial infections, including those causing pneumonia, which is a high risk in hospitalized patients with extensive skin lesions and comorbidities such as Diabetes Mellitus. The choice of this antibiotic was reinforced by the patient's bacterial culture and antibiotic sensitivity test results. Meanwhile, Fusidic Acid cream (20 mg/gram) served as a topical antibiotic for the treatment of local skin infections in areas other than the oral and perioral regions. The patient was given mebhydrolin napadisylate and gentamicin sulfate 0.3% eye ointment to manage symptoms and protect sensitive areas. Mebhydrolin napadisylate functions as an antihistamine and is part of the comprehensive systemic therapy to reduce the itching (pruritus) that usually accompanies BP.<sup>16-18</sup>

Gentamicin sulfate 0.3% eye ointment, an antibiotic eye ointment, was used to prevent or manage potential infection and inflammation in the vulnerable eye due to widespread bullous disease. Integrated management of systemic therapy (autoimmune control) and local/symptomatic care (infection and pain control) is key to improving patient prognosis.<sup>16</sup>

Specific oral management, including daily debridement and 0.2% chlorhexidine gluconate irrigation for infection control, as well as the application of Aloe vera extract gel/spray for pain palliation and mucosal protection, is essential to support adequate nutritional intake. Aloe vera extract gel/spray forms a protective layer over labial, perioral,

and intraoral mucosal erosions and ulcerations. This protective layer relieves pain and burning sensations.<sup>19</sup>

This combination of antiseptics and anti-inflammatory covering agents is key to creating an optimal healing environment and supporting the success of primary systemic therapy for BP. Integrated management of systemic therapy (to control the autoimmune disease) and local care (to control infection and symptoms) is the key to improving patient prognosis.<sup>4,20</sup>

Atypical Bullous Pemphigoid (BP) mimicking the features of TEN and impetigo poses a significant diagnostic challenge that cannot be overcome by clinical appearance alone. A multidisciplinary diagnostic algorithm supported by supplementary examinations is necessary to differentiate BP from other differential diagnoses. Therefore, integrated comprehensive management, involving specialists in Dermato-Venereology, Internal Medicine, and Oral Medicine, is crucial. The management of oral and perioral manifestations through Oral Health Care (OHC) procedures is an essential and integral component of overall care to support patient recovery.

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# Physical Characterization of Micro-Porosity in Decellularized Gambier Leaf for Potential Use as a Plant-Based Scaffold

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## ABSTRACT

**Background:** Scaffolds represent biomaterials designed to provide structural support for cellular adhesion and growth factor sequestration, emulating the extracellular matrix (ECM) to promote tissue regeneration. Plant-based tissues have garnered attention as viable scaffold alternatives owing to their architectural homology with human extracellular structures. Gambier leaves (*Uncaria gambir*) stand out for their inherent porous, trabecular morphology, where microporosity is pivotal in facilitating cell attachment, proliferation, and differentiation. **Objective:** This study aims to elucidate the microporous characteristics of decellularized gambier leaves via scanning electron microscopy (SEM). **Method:** Fresh leaves were meticulously cleaned, cryopreserved at  $-20^{\circ}\text{C}$ , and fashioned into five circular discs employing a biopsy punch. Decellularization entailed submersion in 10% sodium dodecyl sulfate (SDS) for five days, succeeded by distilled water lavage. Subsequent cyclic treatment with Tween-20 and NaClO solutions, applied every 24 hours, continued until optical translucency was achieved. Processed tissues underwent thorough washing, overnight fixation, serial ethanol dehydration, hexamethyldisilazane (HMDS) treatment, 50 nm gold sputter-coating, and SEM evaluation across three magnifications. **Results:** Microscopy revealed surface wrinkling and partial architectural collapse in multiple specimens, likely due to dehydration-induced artifacts. Conversely, a single intact sample exhibited pronounced microporosity, as evidenced by pore diameters of  $0.689\ \mu\text{m}$  and  $0.5512\ \mu\text{m}$ . **Conclusion:** These observations affirm the microporous potential of decellularized gambier leaves for cellular anchorage and nutrient permeation, bolstering their candidacy as plant-derived scaffolds in tissue engineering. Nonetheless, inter-sample variability underscores the need for refined decellularization/dehydration methods and expanded quantitative assessments to ensure reproducible structural integrity.

**Keywords:** Decellularization, gambier leaf, microporosity, plant-based scaffold, SEM

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## INTRODUCTION

Pathological bone damage arises from an imbalance between osteoblast and osteoclast activities, disrupting the equilibrium between bone formation and resorption. This imbalance ultimately leads to structural deterioration of bone tissue.<sup>1</sup> Advances in technology and medical science have enabled the development of Bone Tissue Engineering (BTE), which integrates knowledge of bone structure, biomechanics, and regenerative processes to facilitate functional tissue repair.<sup>2</sup> BTE relies on three essential components: growth factors, cells, and biomaterial scaffolds.<sup>3</sup>

Scaffolds serve as three-dimensional templates that support cell attachment, proliferation, and differentiation while mimicking the role of the Extracellular Matrix (ECM) in maintaining and restoring tissue function.<sup>4</sup> Based on their source, scaffolds can be broadly categorized into synthetic and natural materials. Natural scaffolds derived from animal tissues typically contain key ECM components, such as collagen and elastin. These ECM proteins exhibit specialized properties tailored to specific tissues. For example, scaffolds for partially damaged liver tissue are rich in laminin, fibronectin, and collagen type IV.<sup>5,6</sup>

In recent years, researchers have explored using plant tissues as alternative three-dimensional acellular scaffolds. Plant-derived scaffolds retain their structural, chemical, and mechanical characteristics even after cell removal through decellularization.<sup>7</sup> This process removes cellular components while preserving an ECM-like framework to support cell attachment and tissue regeneration.<sup>8</sup> The remaining matrix functions as a biological substrate that influences cellular metabolism, including proliferation, morphogenesis, and differentiation.<sup>9</sup> Previous studies by Adamski et al. (2018) and Harris et al. (2021) highlighted the differences between detergent-based and detergent-free decellularization approaches.<sup>10,11</sup> Detergent-based methods have become the

gold standard because they effectively remove cells while preserving tissue architecture across plant species with varying structures and compositions. Plant-derived scaffolds also provide a wide range of natural vascular architectures, making them adaptable to specific regenerative applications.<sup>10</sup> One local plant with significant regional availability in South Sumatra is gambir.<sup>12</sup>

The gambir plant (*Uncaria gambir* Roxb.) contains a variety of bioactive compounds widely used in traditional and modern medicine.<sup>12,13</sup> Including catechu tannic acid (tannin), catechin, pyrocatechol, fluorine, waxes, and oils.<sup>14,15</sup> Catechins, a major constituent of gambir leaves, have demonstrated potential to stimulate osteoblastogenesis and promote osteoclast apoptosis, thereby reducing osteoclastogenesis and limiting bone resorption. Structurally, gambir leaves possess sponge-like mesophyll layers and interconnected air spaces.<sup>16</sup> This architecture resembles porous scaffolds and trabecular bone, facilitating vascularization, nutrient exchange, cell adhesion, and tissue ingrowth.<sup>16,17</sup>

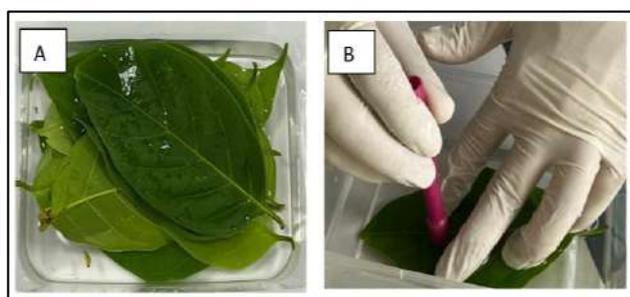
Effective scaffold function requires materials with key attributes, including biocompatibility, biodegradability, adequate mechanical strength, and suitable structural characteristics.<sup>8</sup> Porous scaffolds are especially important in bone regeneration because their interconnected pores and large surface area support osteogenic processes.<sup>18</sup> Microporosity enhances the specific surface area, improves permeability, promotes osteogenic protein binding, and accelerates the release of degradation products, all of which facilitate cell adhesion, proliferation, differentiation, and biomineralization.<sup>19</sup> Microporosity can be evaluated using Scanning Electron Microscopy (SEM).<sup>20</sup> Generally, pores  $\geq 100 \mu\text{m}$  with interconnected networks are considered optimal for cell proliferation, vascularization, and tissue integration.<sup>2</sup>

To the best of our knowledge, no research has specifically investigated the

utilization of gambir leaves as a plant-based scaffold for bone regeneration. This gap in the literature highlights the need to explore the microstructural characteristics of gambir leaves following decellularization, particularly their microporosity, as an early step in assessing their feasibility as scaffolds for bone tissue engineering. Therefore, this study aimed to characterize the microporosity of decellularized gambier leaves using Scanning Electron Microscopy (SEM) as an initial assessment of their potential application as plant-based scaffolds for bone tissue engineering.

## MATERIALS AND METHODS

This was an *in vitro* laboratory design with a post-test-only control group approach. Gambier leaves (*Uncaria gambir* Roxb.) were collected from plantations in Babat Toman Village, Musi Banyuasin Regency, South Sumatra, Indonesia. The selected leaves met the following criteria: light green, approximately five months old, measuring 8-13 cm in length, and structurally intact. Leaves that were wilted or showed signs of pest damage were excluded from the study. Freshly harvested leaves were rinsed with distilled water and stored at  $-20^{\circ}\text{C}$  until further processing. Before decellularization, the leaves were cut with a biopsy punch while submerged in distilled water to maintain tissue stability (Fig. 1).



**Figure 1.** (A). Native gambier leaf (B). The gambier leaf samples are being cut using a biopsy punch

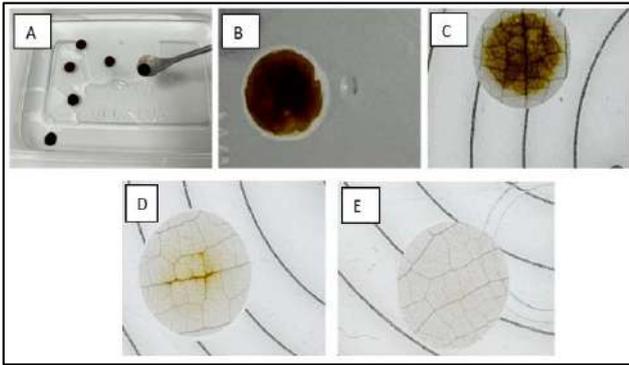
Decellularization was performed using a detergent-based protocol. Leaf samples were immersed in 10% sodium dodecyl sulfate (SDS) for five days at room temperature ( $20\text{--}25^{\circ}\text{C}$ ) on a low-speed orbital shaker. Samples were then rinsed thoroughly with distilled water to remove residual SDS<sup>11</sup>. Subsequently, the leaves were immersed in a mixed solution of 1% Tween-20 (v/v) and 10% sodium hypochlorite ( $\text{NaClO}$ , v/v). This solution was replaced every 24 hours until the leaves became fully transparent. After complete decellularization, samples were washed with deionized water.<sup>11</sup>

A total of five decellularized gambier leaf samples ( $n=5$ ), prepared as circular disc-shaped specimens, were used in this study. No experimental grouping or treatment comparison was used because all samples underwent the same preparation protocol for physical characterization.

The decellularized samples were fixed in 4% formaldehyde prepared in Phosphate-Buffered Saline (PBS) and incubated overnight. Samples were then rinsed with PBS and dehydrated through a graded ethanol series (30%, 50%, 80%, and 95%) for 15 minutes at each concentration. Following dehydration, the samples were treated with Hexamethyldisilazane (HMDS) and air-dried. Dried samples were sputter-coated with a 50 nm gold layer and examined using *Scanning Electron Microscopy* (SEM) at magnifications of  $600\times$ ,  $1200\times$ , and  $2400\times$ .

## RESULT

The decellularization and sample preparation procedures were carried out at the Integrated Biology Laboratory at Raden Fatah State Islamic University, Palembang, and SEM examination was then performed at the Engineering Laboratory at Sriwijaya University. The outcomes of the decellularization process are presented in Figure 2.



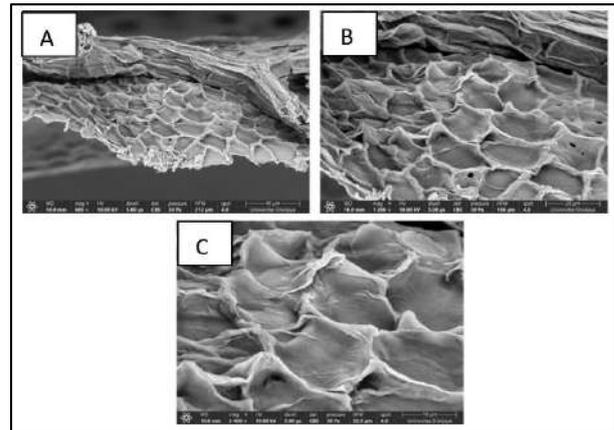
**Figure 2.** Gambier leaves post-decellularization. (A). After 5 days of immersion in SDS (B-D). Color changes occurred during immersion in the bleach solution (E). After 5 days in the bleach solution, the leaf samples were completely colorless.

Figure 2 shows the gambier leaves following SDS-based decellularization. After decellularization, visual observation revealed that the natural green pigmentation had completely disappeared, and the leaf appeared translucent. The internal tissue architecture remained intact, with the vascular channels clearly visible and exhibiting no tearing or structural damage.

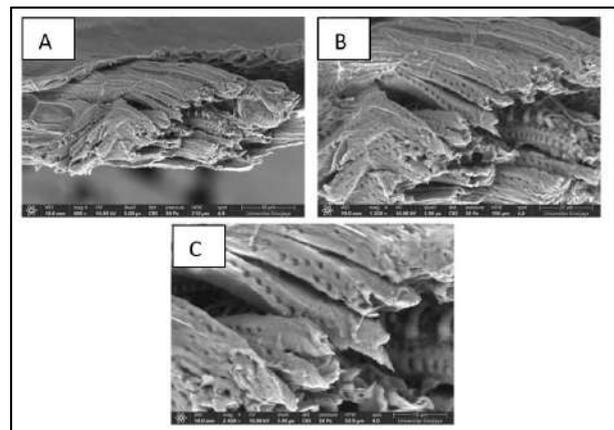
SEM analysis was conducted at three different magnification levels on transverse sections of decellularized gambier leaves. The observations in Figures 3 and 4 revealed surface wrinkling within the internal structure of the leaves after decellularization. Uneven sample edges were observed in all specimens, likely caused by mechanical cutting during sample preparation.

Microporous features were identified in representative well-preserved samples. At 1200 $\times$  magnification (Figure 4B), distinct microporous features were observed in localized regions of the decellularized leaf section. Two micropores were measured directly from the SEM image using the analysis software, with diameters of 0.689  $\mu\text{m}$  and 0.5512  $\mu\text{m}$ . These measurements represent localized pore dimensions observed at specific regions of interest and do not reflect mean pore size values across all samples. Due to the exploratory and descriptive nature of this study, a quantitative

pore size distribution analysis was not performed.



**Figure 3.** The SEM images of sample 1 with 600 $\times$  magnification (A), 1200 $\times$  (B), and 2400 $\times$  (C).



**Figure 4.** The SEM images of sample 3 with 600 $\times$  magnification (A), 1200 $\times$  (B), and 2400 $\times$  (C). Localized microporous features were visible within the leaf matrix at higher magnifications.

## DISCUSSION

This study examined the microporosity of gambier leaves after decellularization. This process successfully changed the leaf color from green to translucent, as shown in Figure 5. An SDS-based ionic detergent method was used to remove cellular membranes and nuclei by solubilizing lipid components and denaturing proteins.<sup>9</sup> This technique is widely recognized as the gold standard for decellularization in both plant and mammalian tissues.<sup>6,7</sup> Because SDS is cytotoxic, an extensive washing procedure

using 10% NaClO and Tween 20 was performed to remove detergent residues and remaining cellular debris.<sup>11</sup>

Following this process, decellularized gambier leaves preserved vascular-like structures consistent with xylem architecture, as shown in Figure 3E. The vascular architecture remained intact and clearly defined. These channels play an essential role in facilitating nutrient transport, gas exchange, and the removal of metabolic by-products during subsequent cellular regeneration. Preservation of such structural complexity indicates that a properly optimized decellularization protocol can maintain the intrinsic morphological characteristics of plant tissues.<sup>10</sup>

To assess microstructural changes after decellularization, SEM analysis was performed on samples prepared using the *Hexamethyldisilazane* (HMDS) drying method. This approach followed the protocol described by Melis Toker et al. (2020).<sup>20</sup> HMDS served as an alternative to the *Critical Point Drying* (CPD) technique, functioning by gradually replacing ethanol and removing residual moisture through evaporation. This method has demonstrated drying performance comparable to CPD for preparing plant-derived samples for SEM analysis.<sup>21</sup>

The markedly smaller pore sizes observed in the present study compared to those reported by Ali Salehi et al. (2020) may be attributed to several factors. First, the pore architecture in plant-derived scaffolds is highly dependent on species-specific leaf anatomy.<sup>22</sup> Gambier leaves possess a relatively dense mesophyll structure with tightly packed parenchymal cells, which may inherently limit the formation of large interconnected pores following decellularization.<sup>9</sup> Second, micropore measurements in this study were obtained from localized regions of interest within decellularized leaf sections rather than from clearly identifiable vascular channels. As the SEM analysis was focused on surface-level morphology, the observed pore dimensions likely represent

microstructural features of the leaf matrix rather than larger transport channels. This localized observation approach may have contributed to the detection of submicron-scale pores. While these submicron pores are insufficient for cell penetration or vascular ingrowth, they may still enhance surface area, protein adsorption, and initial cell attachment, which are critical during the early stages of tissue regeneration.

Furthermore, structural shrinkage or partial collapse of the extracellular matrix may have occurred during decellularization and dehydration processes, particularly during ethanol dehydration and HMDS drying, resulting in reduced pore dimensions, as illustrated in Figures 3 and 4. This deformation is likely attributable to the drying protocol: using 100% HMDS at two drops per sample may have induced excessive shrinkage of the cell walls. Similar phenomena were reported by Moritz Schu et al. (2021), who found that drying with 10% HMDS can cause cracking in biological specimens.<sup>21</sup> These observations suggest that drying conditions influence the structural appearance of the samples.

Beyond preparation effects, the inherent morphology of gambier leaves also contributed to the SEM visualizations. Based on previous anatomical descriptions, gambier leaves are reported to possess a rigid anatomical framework composed of several distinct layers: the upper epidermis, a double palisade mesophyll layer, vascular bundles, a spongy mesophyll with abundant air spaces and oil droplets, and the lower epidermis.<sup>9</sup> Their rigidity is largely due to the thick sclerenchyma tissue, which consists of dead cells with heavily lignified secondary walls that provide mechanical strength and protection. The thickness and density of this layer may limit SEM visualization primarily to surface and near-surface features, thereby restricting observation of deeper microporous structures.<sup>23</sup>

Additional challenges emerged during sample processing, particularly due to the lack of established HMDS concentration and dosage

guidelines for gambier leaf tissues. Despite careful handling, noticeable shrinkage occurred. Suboptimal sample manipulation further contributed to the wrinkling observed in the SEM micrographs. Moreover, the delicate structure of the leaves, combined with extensive internal air cavities, likely increased the samples' susceptibility to collapse during drying.<sup>24</sup>

Finally, this study acknowledges technical limitations related to specimen preparation for SEM. The irregular cut surfaces seen in several images may have resulted from variability during manual trimming of leaf specimens before imaging. Since the cutting process was performed mechanically, inconsistencies in blade pressure, angle, or sample stabilization could introduce artifacts unrelated to the true morphology of the decellularized specimens. Moreover, operator-related factors during sample handling and mounting may have contributed to minor microstructural distortions, particularly when performed by individuals with limited experience in leaf-tissue preparation for SEM. These artifacts must be considered when interpreting morphological features, as they may affect the surface appearance without reflecting true structural changes caused by decellularization.

Taken together, the findings in the present study suggest that decellularized gambier leaves may require additional structural modifications, such as mechanical perforation, chemical treatment, or combination with secondary biomaterials, to achieve pore sizes optimal for bone tissue engineering applications. Future research should focus on optimizing drying protocols, particularly the HMDS concentration and volume, to minimize sample shrinkage and structural distortion. Refining the decellularization process, including varying detergent concentrations or adding additional washing steps, may also improve microstructural preservation. Further evaluation of the mechanical properties, pore interconnectivity, and biocompatibility is necessary to assess the

feasibility of gambier leaves as a potential scaffold for bone tissue engineering.

## CONCLUSION

This study successfully characterized the surface morphology of decellularized gambier leaves and identified microporosity with diameters of 0.689  $\mu\text{m}$  and 0.5512  $\mu\text{m}$  in one of the analyzed samples. Another samples displayed pronounced surface wrinkling, which limited detailed interpretation of their microstructural features. To the best of our knowledge, this is the first study to document microporosity measurements in decellularized gambier leaves, providing essential baseline data for future structural optimization of this plant-derived material.

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# Saliva Accuracy Analysis as a Non-Invasive Method for Determining Blood Type

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## ABSTRACT

**Background:** A phobia is an excessive, irrational, and persistent fear of something that makes it difficult for someone to carry out certain activities. Psychological disorders, such as a phobia of blood and injections, indicate the need for forensic identification methods that do not require blood samples. Blood type identification plays an important role in forensic cases, especially in matching the blood type of the evidence of victims or perpetrators. **Objective:** To analyze the accuracy of saliva as a noninvasive method for determining blood type in students of the Faculty of Dentistry, Baiturrahmah University, class of 2021. **Method:** This was a quantitative observational analytic study with a cross-sectional design. The study population comprised 80 students selected using a simple random sampling technique. Saliva samples were analyzed using the absorption inhibition method and compared with available blood type data. The data were analyzed using SPSS version 23.0, and Fisher's exact test was performed as an alternative to the chi-square test. **Results:** Blood types A, B, and AB have 100% compatibility in secretor individuals, while blood type O has 0% compatibility because there are no antigens A and B in the saliva. Statistical tests showed a significant level of accuracy between blood type examination through saliva and blood type in the data ( $p=0.000$ ). **Conclusion:** Overall, saliva blood type examination has the same level of accuracy as the conventional method.

**Keywords:** Blood type, forensic identification, phobia, saliva

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## INTRODUCTION

Psychological disorders are defined and treated as medical problems; they are a collection of abnormal conditions involving physical or mental.<sup>1</sup> Anxiety and fear that is irrational, excessive, and persistent towards something, so that a person is unable to do any activity, is called a phobia.<sup>2</sup> The word "phobia" comes from the Greek term "phobos," which means to run (fight), fear, and panic (panic-fear), and great fear or terror.<sup>3</sup> Blood phobia is a fear or avoidance of situations directly or indirectly related to blood, wounds, and so on. Injection phobia is a fear or avoidance of various types of injections, such as taking samples by injecting into certain parts of the body. The significant impact of psychological disorders such as blood phobia and injection phobia shows the importance of developing forensic identification methods that do not involve blood sampling.<sup>2</sup>

Forensic identification methods are divided into primary examinations, namely, dactyloscopy and Deoxyribonucleic Acid (DNA) and secondary examinations, namely medical characteristics, photography, and the victim's property.<sup>4</sup> Blood type identification is very important in several forensic cases, especially in relation to blood type matching of evidence from victims or perpetrators.<sup>5</sup> Blood type is a genetic substance that is inherited.<sup>6</sup> Blood type identification is generally done based on the ABO blood type system.<sup>7</sup> Human blood types in the ABO system are divided into four types, namely A, B, O, and AB.<sup>8</sup> In general, blood type determination using causes among fear among individuals, especially children. In addition to blood, blood type antigens are also secreted in various other body secretions, such as saliva, which can determine blood type.<sup>9</sup> Saliva is a complex biological fluid secreted by the major and minor salivary glands.<sup>10</sup>

Blood type examination through saliva can only be performed on individuals with

secretor status. Secretor groups are individuals who secrete blood type antigens identical to their red blood cells in body fluids, such as saliva. Conversely, the non-secretor type is an individual with blood type A, B, or AB who does not secrete ABO blood type antigens in the body fluids.<sup>11,12</sup> To find out whether someone is a secretor or a non-secretor, can be determined through a secretor test.<sup>12</sup> Blood type examination using the inhibition absorption method is a procedure to determine blood type indirectly, which utilizes materials other than blood, such as saliva, sperm, gastric fluid, and other body fluids.<sup>9</sup> Blood type examination using the inhibition absorption method has the same principle as the ABO blood type examination, which is commonly performed, but uses a different method.<sup>13</sup>

Research from Lakade's (2018) in India showed that 100% of subjects from saliva samples were secretors, and ABO blood type determined from saliva swab samples showed 100% correlation with blood type determined from extraction socket blood. The results of research from Kimberly Alsbrooks & Klaus Hoerauf's (2022) in the United States, showed that the prevalence rate of needle fear was 20-30% in the 20-40 year age group and of participants who experienced injection phobia, 52.2% stated that they avoided blood collection, followed by 49% who refused blood donation, and 33.1% who refused vaccination. Therefore, this can encourage the use of saliva as a non-invasive method in routine blood tests, especially in children who suffer from blood and injection phobias.<sup>9,14</sup>

Based on the background description above, researchers are interested in analyzing the accuracy of using saliva as a non-invasive method for determining blood type. This research was conducted on students of the Faculty of Dentistry, Baiturrahmah University, class of 2021, who had never previously conducted research on determining blood type through saliva, and several students suffered from symptoms of blood and injection phobia.



This research is expected to broaden the scientific horizons of dentistry, particularly forensic odontology, by utilizing saliva as a non-invasive means of blood typing. Practically, this study underpins the development of safer, more efficient, and more comfortable diagnostic procedures for patients who struggle with conventional sampling methods.

## MATERIALS AND METHODS

This type of research is a quantitative observational analytic approach with a cross-sectional design. This study was conducted on 80 students of the Faculty of Dentistry, Baiturrahmah University, class of 2021, consisting of 71 women and 9 men. The inclusion criteria for this study were 2021 Faculty of Dentistry students who were willing to participate in the study, had signed an informed consent form, and knew their blood type. The exclusion criteria included subjects with diseases affecting saliva (e.g., xerostomia and Sjögren's syndrome).

The minimum sample size for this study was 80, determined using the Slovin formula. Slovin's formula was employed to obtain a representative sample with a 5% error margin. This method was chosen because the subject population size was predetermined, ensuring efficient and scientifically valid saliva. The dependent variable was the blood type. This study was conducted in May until December 2024 at the LLDIKTI Laboratory, Region X, West Sumatra. Participants who consented to participate in this study were individuals who already knew their blood type. This research was approved from the Baiturrahmah University Ethics Committee (No.A.004/KEPKFKGUNBRAH/XI/2024).

The research procedure began with preparing the tools and materials, namely an oven, test tube rack, test tube, centrifuge, hot plate, dropper, beaker, measuring cup, incubator, saliva, antisera reagent (Anti A, Anti B, Anti D), distilled water, 5% erythrocyte

suspension, sterile gauze-wrapped cotton (as handscounbe cover), handscoon, face mask, and plastic clip (as a test tube container when collecting saliva).

All tools and materials used in this study were sterilized. Saliva samples were collected in clean and dry test tubes, labeled (Name-Blood Type), and diluted with distilled water (1:2). The samples were heated in a boiling water bath for 10 minutes, cooled, centrifuged at 3000 rpm for 10 minutes, and then the supernatant (the upper centrifugation result which is a liquid separated from the sediment) was discarded. Antisera reagents (Anti-A, Anti-B, Anti-D) in a 1:4 dilution were added to each test tube (1 drop/tube) according to blood type. The sample was shaken well and incubated at 37°C for 10 min. The subject population size was predetermined to ensure an efficient and scientifically valid sampling process. Sampling used a simple random sampling technique according to the calculation results using the Slovin formula. The independent variable in this study was shaken well, incubated at 37°C for 10 minutes, then 5% erythrocyte suspension (1 drop/tube) was added, shaken well, and incubated again for 15 minutes at the same temperature (37°C). Furthermore, the samples were examined for agglutination. If one of the test tubes did not exhibit agglutination, this indicated a positive secretor status, whereas agglutination in all test tubes indicated a negative secretor status. Tubes that did not experience agglutination were considered to show the ABO blood type.<sup>9,13,15</sup> Data were analyzed using SPSS to describe variable characteristics, followed by Fisher's exact test as an alternative to the chi-square test to assess the accuracy between saliva-based and conventional blood type results.

The findings were processed and presented in tables and percentages.



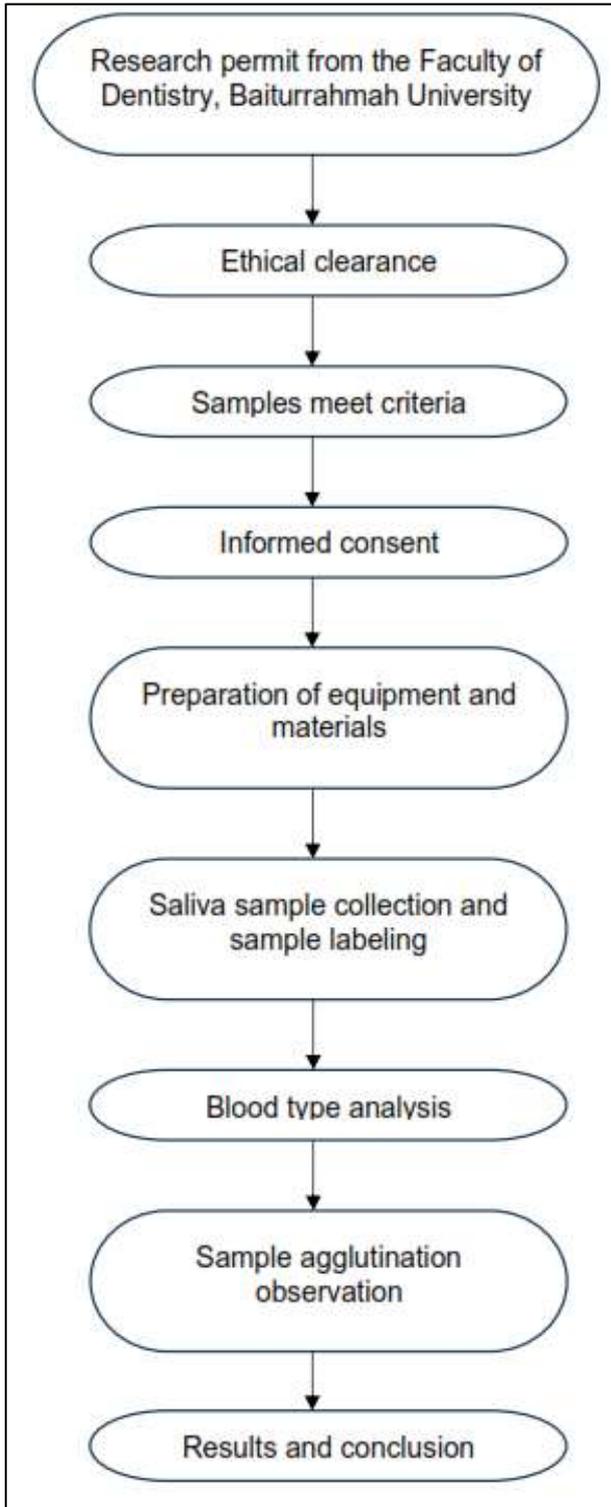


Figure 1. Flowchart Research Methods

RESULTS

Table 1. Characteristics of Research Samples

| Characteristics of Research Samples | f  | %     |
|-------------------------------------|----|-------|
| Age                                 |    |       |
| 20 years old                        | 3  | 3.75  |
| 21 years old                        | 48 | 60    |
| 22 years old                        | 25 | 31.25 |
| 23 years old                        | 2  | 2.5   |
| 24 years old                        | 2  | 2.5   |
| Gender                              |    |       |
| Male                                | 9  | 11.25 |
| Female                              | 71 | 88.75 |
| Blood type                          |    |       |
| A                                   | 18 | 22.5  |
| B                                   | 23 | 28.75 |
| AB                                  | 10 | 12.5  |
| O                                   | 29 | 36.25 |
| Total                               | 80 | 100   |

Table 1 shows that from the 80 research samples, the most dominant age is 21 years old, which is 48 people (60%), while the least age is 23 and 24 years old, with only two people (2.5%) each. The most dominant gender is female, which is 71 people (88.75%), while the least gender is male, which is 9 people (11.25%). The most dominant blood type was O (29 people, 36.25%), while the least dominant was AB (10 people, 12.5%).

Table 2. Status of Secretor and Non-Secretor Groups

| Groups       | f  | %     |
|--------------|----|-------|
| Secretor     | 49 | 61.25 |
| Non-Secretor | 31 | 38.75 |
| Total        | 80 | 100   |

Table 2 shows that among the 2021 intake of students of the Faculty of Dentistry, Baiturrahmah University, the majority were included in the secretor group (49 people, 61.25%), while the rest were included in the non-secretor group (31 people, 38.75%).



**Table 3.** Secretor Status Based on Blood Type

| Blood Type | Secretor Status |    | Total | %   |
|------------|-----------------|----|-------|-----|
|            | Yes             | No |       |     |
| A          | 18              | -  | 18    | 100 |
| B          | 22              | 1  | 23    | 100 |
| AB         | 9               | 1  | 10    | 100 |
| O          | -               | 29 | 29    | 0   |
| Total      | 49              | 31 | 80    |     |

Table 3 shows the secretor status based on blood type of the students of the Faculty of Dentistry, Baiturrahmah University, class of 2021. For blood type A, all samples had secretor status, totaling 18 individuals (100%). In blood type B, 22 people (100%) had secretor status, while one person was a non-secretor. Samples with blood type AB mostly have secretor status, namely 9 people (100%), while 1 person is a non-secretor. Conversely, all samples with blood type O were classified as non-secretors, totaling 29 people (0%).

**Table 4.** Blood Type Test Conformity Results

| Blood Type | Blood Type Data | Blood Type Results (Saliva) |              |      |
|------------|-----------------|-----------------------------|--------------|------|
|            |                 | Secretor                    | Non-secretor | %    |
| A          | 18              | 18                          | -            | 100% |
| B          | 23              | 22                          | 1            | 100% |
| AB         | 10              | 9                           | 1            | 100% |
| O          | 29              | -                           | 29           | 0%   |
| Total      | 80              | 49                          | 31           |      |

Table 4 shows the results of blood type examination suitability using saliva, which has a good level of accuracy, the same as the available blood type data (conventional method). Blood types A, B, and AB have a 100% suitability rate in secretor individuals. In contrast, blood type O had a 0% suitability rate, where all blood type O samples were non-secretors and were not detected in the saliva.

**Table 5.** The results of statistical tests using Fisher's exact test

| Saliva       | Blood Type Data |    |    |    | Total | p-value |
|--------------|-----------------|----|----|----|-------|---------|
|              | A               | B  | AB | O  |       |         |
| A            | 18              | 0  | 0  | 0  | 18    | 0.000   |
| B            | 0               | 22 | 0  | 0  | 22    |         |
| AB           | 0               | 0  | 9  | 0  | 9     |         |
| Not detected | 0               | 1  | 1  | 29 | 31    |         |
| Total        | 18              | 23 | 10 | 29 | 80    |         |

Table 5 shows the results of statistical tests using the Fisher's exact test obtained a *p value* of 0.000 ( $p < 0.05$ ), which means that there is a significant relationship between saliva and blood type data in students of the Faculty of Dentistry, Baiturrahmah University, class of 2021.

## DISCUSSION

This study was conducted on students of the Faculty of Dentistry, Baiturrahmah University, a 2021 class with diverse characteristics. Blood type can be identified from various body fluids, which are then determined by the individual's status as a secretor or a non-secretor. Secretors are individuals who secrete fluids, such as saliva, tears, and sweat. Conversely, non-secretor individuals are individuals who do not secrete blood type antigens in their body fluids.<sup>15</sup>

The results of the study in Table 2 are in line with the study conducted by Alqadri (2016) in Padang City. From 54 samples, it was found that the secretor status was present in 42 samples (78%), while the non-secretor status was present in 12 samples (22%). Similar to a study conducted by Tejasvi (2021) in India, from 60 samples, 52 samples (86.66%) were included in the study.



The secretor group and 8 samples (13.33%) were included in the non-secretor group. Overall, the results of several studies emphasize that the secretor group tends to be more dominant than the non-secretor group because the distribution of the secretor and non-secretor groups in the human population is 85% in the secretor group and 15% in the non-secretor group, which means that most of the human population has the *SeSe* or *Sese* (secretor) gene that encodes a certain fucosyltransferase enzyme found in the epithelium of secretory tissues, such as saliva, tears, and sweat. This enzyme allows body fluids to produce the same blood group antigen as the antigen on red blood cells, but in a water-soluble form. Meanwhile, the non-secretor group has the *se* gene, which is unable to produce antigens in soluble form, so there are no blood group antigens in their body fluids.<sup>11,15</sup>

This study only calculated the secretor group, while the non-secretor groups were not included. Based on the data presented in Table 3, blood groups A, B, and AB have a 100% compatibility rate in secretor individuals.

Meanwhile, in blood group O, all samples had a non-secretor status, which means that there were no blood group O subjects with secretor status. Research conducted by Klarmann (2010), quoted from Saboor (2014) showed that in blood group O, the limitations of this method are caused by low antigen concentrations, such as vWF Ag (von Willebrand factor antigen) and FVIII Ag (Factor VIII antigen) levels, which play a role in hemostatic function.<sup>16</sup> The low levels of vWF Ag and FVIII Ag in body fluids, which are influenced by genetic factors, make it difficult to detect secretor status in blood group O. The results of this study make it clear that each blood group has a different antigen distribution pattern, so it is important to understand the biochemical characteristics of each blood group in order to improve the effectiveness of saliva as a non-invasive method.<sup>17</sup>

Table 4 shows that blood type examination using the saliva method has a good level of accuracy, similar to that of the conventional method. Blood types A, B, and AB in secretor individuals had 100% conformity with conventional method blood type data, while blood type O had 0% conformity with conventional method blood type data because all blood type O samples were non-secretors that were not detected using the saliva method. The results of this study differ from those of the study conducted by Rajawat et al. (2023) in India, in which, out of 300 samples, the majority of those with secretor status were blood types A and AB, with accuracy levels of 87.14% and 94.28%, respectively. Blood type B had an accuracy level of 73.3%, and blood type O had an accuracy level of 85.04%. Likewise, the study conducted by Lakade (2018) in India showed that all samples were secretors (100%), and the ABO blood type determined from dried saliva samples showed a 100% correlation with the blood type determined from the extraction socket blood.<sup>9,13</sup>

The results of this study were influenced by the absence of antigens A and B in body fluids, including saliva. Most individuals with blood type O have 0% compatibility because blood type O does not have antigen A or B on the surface of red blood cells, so they cannot be secretors of antigen A or B.<sup>18</sup> In addition, secretor status is also associated with various medical conditions, such as ankylosing spondylitis (inflammation of the spine), gastric ulcers, ovarian cysts, and several types of cancer, including squamous cell carcinoma.<sup>9,19</sup>

The results of the statistical test using Fisher's exact test as an alternative to the chi-square test obtained a p-value of 0.000 ( $p < 0.05$ ), which means there is a significant level of accuracy between blood type testing through saliva and blood type data in students of the Faculty of Dentistry, Baiturrahmah University, class of 2021. These results are in line with the study by Rajawat et al. (2023) in India, which showed a significant relationship between ABO

blood type, Rh factor, and secretor status. These findings confirm that blood type testing using saliva has good potential to detect blood type accurately, especially in secretor individuals.<sup>13,20</sup>

## CONCLUSION

Blood type examination using saliva has an accuracy level as good as the conventional method. Blood types A, B, and AB have 100% compatibility in secretor individuals, while blood type O is not detected by the saliva method because there are no antigens A and B in the saliva. Statistical tests showed a significant level of accuracy between blood type examination through saliva and blood type in the data.

Further research is recommended to develop a more sensitive detection method for blood type O, for example, by using anti-H reagents, considering the limitations of saliva as a non-invasive method for detecting the secretor status of this blood type. In addition, studies with a wider population are needed to understand the prevalence and characteristics of secretors and non-secretors, because determining ABO blood type through saliva in individuals, this method has limitations and can only be examined using conventional methods. Examination using conventional methods is also recommended to increase the accuracy of the available blood type data.

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# Smart Odontogram on Preventive Dentistry: A Managerial and Policy Review at Nala Husada Dental Hospital Surabaya

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## ABSTRACT

**Background:** Digital transformation in dental healthcare has brought forward Smart Odontogram systems that offer structured, electronic, and real-time documentation of dental conditions. This tool is particularly valuable for early detection, monitoring, and prevention of oral diseases. However, its success relies heavily on management readiness and policy. **Objective:** This study aimed to review the implementation of the Smart Odontogram application from a managerial and policy perspective in supporting preventive dentistry. **Methods:** This study used a mixed-methods approach. The sample consisted of 12 selected individuals using purposive sampling. Inclusion criterion was involvement in Smart Odontogram implementation for at least six months. Primary data were obtained from interviews and questionnaires, focusing on organizational readiness, human resource competency and training, and managerial challenges and policies. **Results:** The hospital management has implemented both the hardware and software of Smart Odontogram, but not much (33.3%) integrated with Electronic Medical Record (EMR) system. Only one participant reported a lack of mentoring (8,3%), and only a few received basic training on Smart Odontogram (16,7%). Only one experienced an oversight of data input (8,3%), and a few reported about the lack of quality of data system control (16,7%). Most participants considered Smart Odontogram to be a significant support of preventive dentistry, particularly in the early identification of caries and periodontal diseases (83,3%). **Conclusion:** The implementation of Smart Odontogram, supported by sound managerial strategies and policies, significantly enhanced preventive dentistry at Nala Husada Dental Hospital.

**Keywords:** Hospital management, preventive dentistry, smart odontogram

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## INTRODUCTION

The development of digital technology has shifted the paradigm of dental medical record-keeping from manual to digital systems. Electronic Health Record (EHR) systems facilitate efficient data management, real-time access to patient histories, and improved collaboration among dental professionals. The adoption of EHR in dentistry has demonstrated significant improvements in patient outcomes by minimizing medical errors, optimizing treatment planning, and enabling better coordination among healthcare providers. Research indicates that digital records improve diagnostic accuracy and procedural efficiency, leading to enhanced patient satisfaction and reduced appointment duration. Furthermore, EHR systems support the integration of decision-support tools that provide evidence-based recommendations for treatment planning.<sup>1</sup>

One of the newest approaches is the Smart Odontogram application, which provides graphical, real-time, integrated recording of dental conditions within an electronic medical record system. Brahmanda et al. (2022), revealed that the inclusion of Artificial Intelligence (AI) elements in this application enables identification of dental conditions through an intraoral camera and deep learning, thereby reducing human error that often occurs in manual recording.<sup>2</sup> The Smart Odontogram application combines automatic detection of dental conditions using an intraoral camera and deep learning algorithms. This study of 15 dentists yielded very positive results; the majority of users reported the system as efficient, effective, and satisfactory in facilitating digital dental status recording. With high accuracy and intuitive operation, this system supports preventive practices such as early caries identification and treatment evaluation from one visit to the next by improving recording consistency and minimizing human error.

Integration of Smart Odontogram into an AI-based Electronic Medical Record (EMR)

system in the Journal of Personalized Medicine. This study demonstrated that the digital data structure added to the odontogram improved recording accuracy and supported the Clinical Decision Support System (CDSS). With structured longitudinal data, this system effectively standardizes diagnoses and streamlines preventive planning processes, such as fluoride varnish scheduling and caries prevention. This implementation has demonstrated significant potential for promoting data-driven, preventative dental practices.<sup>3</sup>

Furthermore, integrating Smart Odontogram into a health information system provides several operational benefits. Smart Odontograms were shown to improve time efficiency, recording accuracy, and clinical user satisfaction. They also facilitate clinical team communication and patient education regarding dental status in a visual and objective manner, as implemented at the Ninsaúde Clinic.<sup>4</sup>

Electronic dental medical record system that complies with the standards of the Indonesian Ministry of Health. One of its modules includes a digital odontogram designed in a format that complies with national regulations and supports educational and preventive functions in dental clinical practice. The development of this system strengthens the potential of Smart Odontogram in systematically identifying dental caries and supporting preventive interventions based on patient needs.<sup>5</sup>

The success of Smart Odontogram implementation is heavily influenced by managerial readiness, including strategic planning, staff training, resource allocation, and support for internal hospital policy. Hensley (2020) stated that implementing complex digital systems requires cross-departmental involvement and effective coordination between Information Technology (IT) units, clinics, and quality management.<sup>6</sup> This emphasizes the critical importance of managerial aspects in implementing digital health technology. Challenges such as information system

adaptation, training, and record quality assurance are key concerns in the Smart Odontogram implementation process.

The development of digital technology through the implementation of Smart Odontograms brings significant opportunities for improving preventive dentistry recording and services at Nala Husada Dental Hospital. However, its utilization is still not optimal, its implementation is not maximal as documentation of odontogram recording improves data accuracy in medical records. It has not become a strategic tool in preventive dentistry practices that rely on long-term documentation, recording, and early management of dental caries; therefore, it has not assisted in technology-based patient education. This is because there are still several obstacles that need to be followed up, especially related to digital infrastructure readiness, system integration, human resource competency, and internal policy support. Based on these conditions, it is important to conduct research that explores managerial factors and internal policies so that this system is fully and effectively implemented.

## MATERIALS AND METHODS

This study used a mixed methods approach to determine and understand the implementation of the Smart Odontogram system in supporting dental disease prevention at Nala Husada Dental Hospital from a managerial policy perspective. This study used purposive sampling methods. This technique was used because the study required informants with competence and direct experience in implementing Smart Odontogram. The sample consisted of 12 respondents selected because they had direct involvement in the use or implementation of Smart Odontogram for at least six months (inclusion criteria). Respondents included elements of management, Information Technology (IT) staff, dentists, and clinical supervisors. This technique was chosen to

ensure that the data obtained came from individuals who had relevant experience and could provide in-depth information regarding the operation and implementation of Smart Odontogram.

The data in this study were primary data obtained from questionnaires and interviews. Data collection was conducted through in-depth interviews using semi-structured guidelines. Focus group discussions were conducted to determine perceptions of use, barriers, and benefits. Direct observation of the use of the Smart Odontogram system in clinical practice and patient data input was conducted. When completing the questionnaire, there were four indicators asked: organizational readiness, human resource competency and training, managerial challenges and policies, and contributions to preventive dentistry. Each indicator had three questions. This study will be conducted over a three-month period, from April to June 2025. Data Analysis Techniques: Analysis was conducted using a questionnaire in the following stages: (1) data collection, (2) identification, (3) data analysis, and (4) managerial and policy interpretation of the data. Data analysis in this study used two approaches: quantitative data from questionnaire results with a frequency distribution of respondent characteristics (age, position, length of system use), perception scores, benefits, ease of use, barriers, and the percentage of users who stated Smart Odontogram was easy to use. Qualitative data analysis was conducted through interviews, Focus Group Discussions (FGDs), observations of Smart Odontogram use, and document audits (SOPs, policies, and digital system evaluations). Before distribution, the questionnaire underwent validity and reliability tests. Validity was determined if:  $r \text{ count} > r \text{ table}$  ( $p < 0.05$ ), while reliability was tested using Cronbach's Alpha. The questionnaire instrument was considered valid if Cronbach's Alpha  $\geq 0.70$ . After the quantitative and qualitative analyses were completed, the data were combined to generate a comprehensive understanding of user



perceptions (quantitative), causes, barriers, and implementation context (qualitative) through FGDs.

**RESULTS**

This study selected four indicator approaches: Organizational Readiness, Human Resource Competency and Training, Managerial and Policy Challenges, an Contribution to Preventive Dentistry. The research findings are as follows:

**Table 1.** Organizational Readiness

| Aspects               | Yes | %    | No | %    |
|-----------------------|-----|------|----|------|
| Hardware availability | 12  | 100  | 0  | 0    |
| Software availability | 12  | 100  | 0  | 0    |
| Integrated with EMR   | 4   | 33.3 | 8  | 66.7 |

The survey results, most respondents stated that management had provided hardware and software to support Smart Odontogram, but it had not been fully integrated with the hospital's electronic medical records system. The majority of respondents (100%) stated that Smart Odontogram hardware and software were available and functional, but only about a third (33.3%) reported partial integration with the electronic medical records system. This means that technical readiness is quite high, but system readiness and integration policies still need to be strengthened by the hospital management and IT team.

Interview results with IT staff Nala Husada Dental Hospital showed that Smart Odontogram system can be used well in the service unit, but it is not yet fully connected to the patient's electronic medical record, and also have to open two separate systems to view clinical data and odontograms. From a technical perspective, full integration is not yet possible because there are no technical guidelines for database integration. The network infrastructure

also still needs to be upgraded so that the system can synchronize in real time. This indicates that there is still a gap between infrastructure readiness, managerial system readiness, and data integration policies. The organization is technically ready, but it needs to strengthen integration policies and cross-unit collaboration (IT team, service, and quality) so that the implementation of Smart Odontogram can optimally support preventive dentistry.

**Table 2.** Human Resources Competence and Training

| Aspects                 | Yes | %    | No | %    |
|-------------------------|-----|------|----|------|
| Basic Training          | 8   | 66.7 | 4  | 33.3 |
| Mentoring               | 1   | 8.3  | 11 | 91.7 |
| Never received training | 2   | 16.7 | 10 | 83.3 |

Table 2 shows that some dentists and clinic staff have not received adequate and ongoing training regarding the use of the Smart Odontogram system. Training was only provided at the initial implementation stage, and no periodic updates. Survey results showed that the majority of dentists and clinical staff at Nala Husada Dental Hospital (66.7%) had received basic training at the initial implementation of the Smart Odontogram system. However, only a small proportion (8.3%) received regular mentoring or competency updates, while 16.7% of respondents had never received any training at all. This indicates that user training is still basic and not sustainable, potentially reducing the effectiveness of digital information system implementation in the long term.

In line with the interview results, the Smart Odontogram system can be used well in the service unit, but it is not yet fully connected to the patient's electronic medical record, and two separate systems must be opened to view clinical data and odontograms. The IT staff stated that, from a technical perspective, full integration was not yet possible because there were no technical guidelines for database



integration. The network infrastructure also still needs to be upgraded so that the system can synchronize in real time. This indicates that there is still a gap between infrastructure readiness, managerial system readiness, and data integration policies. The organization is technically ready, but it needs to strengthen integration policies and cross-unit collaboration (IT, service, and quality) so that the implementation of Smart Odontogram can optimally support preventive dentistry.

Respondents lacked confidence in using Smart integrative features of Smart Odontogram due to a lack of technical assistance. They only learned about Smart Odontogram during practice, and there is no official module yet. Furthermore, there is a gap in user competency regarding the technology. The IT team is often asked to assist users because not everyone understands how to input complex data. Digital skills vary across medical personnel groups. There is no ongoing policy for improving competency through training.

Several policies have not yet supported the use of Smart Odontograms as a preventive tool. This indicator is related to a. Smart odontogram usage policy b. Data input checking c. Data quality control systems. This survey results as shown in Table 3.

**Tabel 3.** Managerial and Policy Challenges

| Aspects                       | Yes | %    | No | %    |
|-------------------------------|-----|------|----|------|
| Smart odontogram usage policy | 5   | 41.7 | 7  | 58.3 |
| Data input checking           | 1   | 8.3  | 11 | 91.7 |
| Data quality control system   | 2   | 16.7 | 10 | 83.3 |

According Table 3 shows that there are no officers who check the completeness and accuracy of data input. Supportive policies (41.7%) indicated that less than half of the organizations felt that institutional policies explicitly supported Smart Odontogram as a preventive support tool. This means that many policies have not been formulated or

implemented concretely. Respondents feel there is no clear picture of the condition of the policy/supervision. Data input checking officers (8.3%) indicated that specific tasks for data verification and validation are not yet routine or not all units have such officers. The optimal quality control of data input (16.7%) was very low, indicating that the data Quality Assurance (QA) system was not yet running effectively. Input errors, incomplete data, or inconsistencies may still occur frequently.

In-depth interviews with several key informants, including medical records officers and IT officers, revealed that institutional policy support for the implementation of Smart Odontogram was suboptimal. Informants stated that there were no specific regulations governing the system's use, staff responsibilities, or data evaluation mechanisms. Interviews with the staff of the Medical Records Unit showed that the policy regarding Smart Odontogram does exist, but it has not been outlined in an Standart Operational Procedure or binding regulations. Therefore, its implementation still depends on the initiative of each unit. Interviews with the IT team revealed that there was no dedicated officer to double-check the inputted data. There is no dedicated officer was assigned to verify the completeness and accuracy of the data. The checking process remains manual and unstructured. In terms of quality assurance, interviews indicated that the QA system for Smart Odontogram data was not yet effective. Overall, the results of in-depth interviews confirmed that the root of the problem lies in weak policy support, minimal supervisory structure, and the suboptimal role of supporting human resources.

Despite the challenges, clinicians agree that Smart Odontogram can support promotive and preventive efforts through systematic early identification of caries and periodontal status and visual-based patient education. The results are shown in Table 4.



**Tabel 4.** Contribution to Preventive Dentistry

| Aspects  | Yes | %    | No | %    |
|--|-----|------|----|------|
| Early identification of caries/periodontal disease | 10  | 83.3 | 2  | 16.7 |
| Visual based patient education                     | 9   | 75.0 | 3  | 25.0 |
| Improved compliance with follow up visit           | 8   | 66.7 | 4  | 33.3 |

Table 4 shows that the implementation of Smart Odontogram plays a significant role in supporting promotive and preventive activities in dental health services: 83.3% of respondents stated that this system helps systematically identify caries and periodontal disease early. 75% of respondents considered smart odontogram effective in visual-based patient education, improving their understanding of dental conditions. 66,6% of respondents stated that there was an increase in patient control compliance due to clearer and easier-to-understand visualization of dental status. Smart Odontograms have proven to be a clinical documentation tool that supports promotive and preventive strategies through systematic early caries/periodontal detection, interactive visual education, and increased patient compliance with follow-up care. It can be concluded that this system will function optimally when integrated with data quality control and training of digital medical record officers.

When asked about the role of Smart Odontograms in supporting early detection of dental disease, most dentists considered Smart Odontograms effective in systematically identifying early caries and periodontal disease, allowing for faster preventive measures.

The following interview results indicate that Smart Odontograms are very helpful, especially for identifying areas of caries or gum recession that might have been missed with manual records. This system visually displays

the dental status, allowing us to immediately focus on problem areas. Similarly, when asked about education, the following responses were obtained: Patients understand more easily when they are shown the condition of their teeth directly on the screen. The colors and symbols on the system help them identify which teeth have cavities and which have been treated. This helps make doctor-patient communication more effective. Regarding changes in patient behavior related to compliance, patients were more willing to come for checkups because they could see the progress of their dental condition on the recording. They want to see whether their teeth are improving or not. This interview emphasized the importance of data quality monitoring and improving human resource competency so that the benefits of Smart Odontograms in improving patient compliance can be optimal and sustainable.

**DISCUSSION**

Infrastructure readiness is a key factor influencing smooth implementation. Wang et al. (2022), stated that system interoperability is crucial in implementing odontogram-based digital medical records.<sup>3</sup> Smart Odontogram without system integration risks becoming a silo of data rather than a tool for comprehensive patient management.

Table 1 shows that Smart Odontogram hardware and software are available and functional at Nala Husada Dental Hospital, but the system is only partially integrated with electronic medical records. This indicates that system integration and supporting policies still need to be strengthened by management and IT teams.

The software to record electronic medical records must meet certain standards and the Indonesian constitution. To increase the legality of dental medical records and communication to each health provider in Indonesia, the Ministry of Health Indonesia created this guidebook. Writing excellent medical records requires meeting



specific standards. The guidebook is on how to write and compose dental medical records in a good and precise form, including using symbols in odontogram. Thus, we have the same symbol based on the guidebook from the Ministry of health.<sup>7</sup>

An analysis of dentists' behavior in completing odontograms shows that, in terms of performance expectations, the use of odontograms in electronic medical records is perceived as simplifying the recording process, thus speeding up the work. In terms of business expectations, the odontogram filling system in EMR is considered easy for users to understand and operate. Meanwhile, in terms of social influence on the implementation of electronic medical records, a supportive work environment and supportive leadership have been shown to positively encourage the use of EMR systems.<sup>8</sup>

Despite the evident benefits, several barriers to the widespread adoption of EHRs in dentistry were identified: Financial Costs: Initial setup costs for EHR software and hardware are high, leading to reluctance among solo practitioners. User Training and Technical Issues: Lack of formal training programs results in low digital literacy among older dental professionals. Resistance to Change: Dentists accustomed to paper-based records often express reluctance to transition to digital systems.<sup>9</sup>

Smart Odontogram user training remains elementary and discontinuous. While most staff members receive initial training, some do not. Only a small number receive regular support. This lack of ongoing training could potentially reduce the long-term effectiveness of digital system implementation (Table 2).

The importance of human resource training is crucial, who emphasized that the successful implementation of a digital health information system depends heavily on competency-based training and regular updates for users, enabling them to adapt to evolving system features and procedures. In a managerial context, this indicates the need for a

strategy to continuously strengthen human resource capacity through institutional policies that regulate regular training, digital competency certification, and mechanisms for evaluating training effectiveness. This ensures the sustainability of Smart Odontogram implementation and supports the hospital's long-term goal of strengthening IT-based preventive dentistry services.<sup>10</sup>

The main challenges facing human resources, including healthcare and non-healthcare workers, include high workloads, potential human error, lack of technical training, and suboptimal EMR systems. Therefore, strategies are needed to improve the accuracy of EMR data through structured, ongoing, and relevant training, which is consistent with system development. Practice-based training and mentoring for doctors, nurses, and medical records officers should be implemented to strengthen user competencies. Furthermore, continuous evaluation and refinement of the EMR system, including strengthening integration between service units, must ensure faster and more accurate access to medical data and support the continuity of healthcare services.<sup>11</sup>

Medical record training is crucial to the successful implementation of health information technology. According to Piper et al. (2021), comprehensive training improves staff knowledge and skills in managing medical records. This increased competency not only reduces recording errors but also improves the accessibility and security of patient data.<sup>12</sup> The use of electronic medical records also benefits doctors and healthcare workers by simplifying information access and aiding clinical decision-making. Electronic medical records also facilitate patient data maintenance, making them more effective because if a medical record is damaged or lost, a backup of the data is stored within the medical record application.<sup>13</sup>

The success of healthcare system digitalization is greatly influenced by the sustainability and digital literacy training of medical personnel. Without continued training,

users are less likely to adapt to system updates. Furthermore, the WHO (2021), in its Global Strategy on Digital Health, recommends that every healthcare institution adopt a continuous capacity-building approach, including technical, managerial, and clinical training, to ensure optimal long-term digital system functionality. A training schedule for Smart Odontogram users, including refresher courses on new features, should be incorporated into work programs, education, and training agendas. Develop competency-based training modules integrated with the academic system for clinical students. Implement an internal mentoring system (senior and junior doctors) to maintain competency in system use.<sup>14</sup>

The research results from the managerial and policy aspects (Table 3) indicate that the monitoring mechanisms and supporting policies for Smart Odontogram remain weak. There are no dedicated staff members who routinely check data completeness and accuracy, while institutional policies supporting this system are still perceived as minimal. Only a small number of units have data verification staff, and the quality of data input control is very low. This indicates that the data quality assurance system is not yet effective, resulting in a high risk of input errors and incomplete data.

Legal and ethical issues are also significant. Privacy regulations vary between jurisdictions, creating uncertainty regarding the lawful collection, storage, and sharing of health records. Moreover, the inclusion of dental data may increase the risk of re-identification, as certain dental features are highly distinctive, which is why dental records are commonly used in forensics. This raises important questions about who should be entitled to access complete patient records and how secondary use of such data should be governed. Patients may be reluctant to consent to data sharing if the risks are not clearly communicated, and clinicians may hesitate to participate if the legal frameworks remain ambiguous. Security breaches further intensify these concerns,

potentially undermining public trust and jeopardizing the widespread adoption of EHR systems.<sup>15</sup>

The success of a digital system is highly dependent on policy support and governance of data quality. This is in line with research that states that the absence of supervision leads to low quality and consistency of data in electronic systems.<sup>16</sup> System and data quality affect the level of satisfaction and success of EMR users. Hospitals with strong data training and supervision policies have higher implementation success rates. These results indicate that policy support and user training have begun to take shape, but data quality oversight remains a weak point in the implementation of digital systems at Nala Husada Dental Hospital's long-term history of preventive care.<sup>17</sup>

Therefore, Nala Husada Dental Hospital needs to create policies to strengthen formal policies regarding the integration of Smart Odontogram into the hospital's quality system and electronic medical records, and to create ongoing and competency-based training programs for all system users. Data quality assurance supervision and periodic audit mechanisms were used to ensure that the quality of odontogram data remained valid and could be used in preventive dental analysis.

Smart Odontograms have been proven to be not only a clinical documentation tool but also support promotive and preventive strategies through systematic early detection of caries/periodontal disease, interactive visual education, and increased patient compliance with follow-up care. This system will function optimally when integrated with data quality control and training of digital medical record staff. Artificial Inteligen (AI) assisted caries detection tools show potential for clinical applications, with high overall accuracy and specificity. However, its sensitivity varies greatly depending on tooth position and caries type; more advanced AI can improve the performance of AI-assisted caries detection in clinical practice.<sup>18</sup>

Intraoral photo images play an important role in early screening and clinical diagnosis of oral diseases, detecting dental caries, dental calculus, and gingivitis, based on the overall characteristics of the tooth surface and gingival margin.<sup>19</sup> The use of information technology in dental health has several roles. First, it supports clinical decisions in the context of oral health. Second, websites, mobile applications, and other information technology resources can be used to promote better oral health. Third, information technology helps individuals access information about oral disease symptoms and preventative measures that help them maintain their oral health.<sup>20</sup>

The evaluation of the quality of dental records, including the charting process and overall clinical documentation, indicates that the quality of medical records and the accuracy of data entry play crucial roles in providing high-quality dental health services. Accurate and complete records not only assist dentists in determining the correct diagnosis and treatment plan but also enable ongoing monitoring of the patient's condition. Therefore, good charting quality contributes directly to the effectiveness of care management, including early identification of changes in dental conditions, allowing for faster, more precise, and more structured preventive measures against oral diseases. This makes good medical records a key component in supporting the practice of preventive dentistry and maintaining overall clinical service standards.<sup>21</sup>

Based on the findings of this study, several recommendations can be made to integrate Smart Odontogram with the electronic medical records system and hospital management dashboard comprehensively to support interoperability and data-driven decision-making. Human resource training and capacity building must be conducted regular training and mentoring for dentists and dental nurses regarding the use of Smart Odontogram. Internal regulations and standard operating procedures must be developed, and internal

policies that require the use of Smart Odontogram in all clinical procedures must be strengthened. Quality indicators for recording must be established as part of the performance evaluation of healthcare workers. Data audit and monitoring conduct regular audits of the completeness and accuracy of data in Smart Odontogram as part of the hospital's quality management system. Encourage the use of data from Smart Odontogram for the development of patient education programs, early screening for caries or periodontal disease, and annual preventive management reports to increase utilization for promotion and prevention. Developing advanced research based on digital data from Smart Odontogram to generate scientific evidence regarding the effectiveness of Preventive.

## CONCLUSION

The Smart Odontogram has strong potential to support preventive dentistry at Nala Husada Dental Hospital; however, its implementation remains constrained by managerial and policy challenges. Adequate digital infrastructure, system integration, continuous staff training, and internal regulatory support are essential to ensure its effectiveness. The system can improve systematic dental records, enhance patient visual education, and support data-driven preventive intervention. However, without strong policies and oversight, its role may be limited to documentation rather than functioning as a strategic preventive tool.

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# Tobacco Leaf Extract as Denture Cleaning Paste on Transverse Strength of Heat-Cured Acrylic Resin Dentures

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## ABSTRACT

**Background:** Removable dentures are prosthetic devices used to replace missing teeth. Acrylic resin is the most commonly used material for denture base fabrication. Plaque accumulation on improperly cleaned denture bases can lead to denture-associated stomatitis. Tobacco leaves have potential as a denture cleaning paste due to their antibacterial and antifungal properties. However, their phenol content may degrade polymer bonds and reduce the transverse strength of heat-cured acrylic resins. **Objective:** This study aims to investigate the effect of using 25% and 50% tobacco leaf (*Nicotiana tabacum* L.) extract paste as a denture cleaning agent on the transverse strength of heat-cured acrylic resin. **Method:** This study used an experimental laboratory design with a post-test-only control group. Thirty rectangular block-shaped samples (60x12x3 mm) were divided into one control group (K) and two treatment groups (n=10), which were brushed with 25% (T1) and 50% tobacco leaf extract paste (T2), respectively, for 28.2 minutes. Transverse strength was tested using a universal testing machine with the three-point bending test method. **Results:** The transverse strengths of groups K (126.926 MPa), T1 (103.136 MPa), and T2 (83.001 MPa) were determined. The parametric test results indicated significant differences between groups K and T2 ( $p=0.001$ ) and between T1 and T2 ( $p<0.001$ ). However, no significant difference was observed between groups K and T1 ( $p=0.052$ ). **Conclusion:** This study found a decrease in the transverse strength of heat-cured acrylic resin after brushing with tobacco leaf extract paste.

**Keywords:** Denture cleaning paste, heat-cured acrylic resin, tobacco leaf, transverse strength

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## INTRODUCTION

Removable dentures are prosthetic devices used to replace missing teeth on the dental arch, allowing patients to insert and remove them independently. The use of dentures is essential as they play a role in the rehabilitation of masticatory function, speech, and aesthetics.<sup>1</sup> However, its benefits are not widely recognized. According to the 2023 Indonesian Health Survey (SKI), the prevalence of tooth loss in Indonesia was 21% of the total population, yet only 6.8% used dentures. More than 95% of denture users rely on acrylic resin as the base material.<sup>2</sup>

Acrylic resin is a polymer composed of repeated methyl methacrylate units, polymerized by heating. Its advantages include color harmony with the replaced and surrounding tissues, reparability and reattachment, simplicity in manipulation and polishing techniques, and affordability. However, its drawbacks include susceptibility to fractures, porosity formation, liquid absorption, and allergenicity.<sup>3,4</sup>

Maintaining denture cleanliness is essential for ensuring oral and dental health. Uncleaned denture bases, particularly in areas in direct contact with the mucosa, can lead to plaque buildup, which can cause denture stomatitis.<sup>5</sup> However, denture cleaning products remain limited in availability.<sup>6</sup> Consequently, investigations into natural materials with potential applications as denture cleaning agents continue to be developed.

Tobacco leaves are a natural material with potential as a denture cleaner. They contain saponins, alkaloids, terpenoids, and flavonoids that exhibit antifungal and antibacterial properties.<sup>7</sup> Tobacco leaves can be utilized as a denture cleaner in the form of tobacco leaf extract paste. Cleaning dentures with paste and a toothbrush (a mechanical method) has the advantage of improving plaque removal efficiency and requiring relatively little time.<sup>8</sup> Previous studies have shown that 25% and 50%

tobacco leaf extract paste can inhibit the growth of *Candida albicans* on thermoplastic nylon plates.<sup>9</sup>

Phenolic compounds in tobacco leaves may trigger hydrolysis reactions, leading to polymer degradation.<sup>10</sup> The breakdown of polymer chains in acrylic resins has the potential to reduce its mechanical properties, including transverse strength. Transverse strength reflects the durability of the denture base during mastication, as it is subjected to compressive, shear, and tensile forces simultaneously.<sup>11</sup> This study aimed to examine the impact of 25% and 50% tobacco leaf extract paste as a denture cleaning agent on the transverse strength of heat-cured acrylic resin.

## MATERIALS AND METHODS

This study was an experimental laboratory with a post-test-only control group design. The samples were rectangular in shape with dimensions (60×12×3) mm, based on the specifications of the American Standards for Testing and Materials.<sup>12</sup> The sample criteria included conformity in shape and size, non-porosity, and smooth, shiny, and flat surfaces. Thirty samples were divided into three groups: control group (K), which was not subjected to brushing treatment; (T1), which used 25% tobacco leaf extract paste; and (T2), which used 50% tobacco leaf extract paste (n=10).

### Tobacco Leaf Extract Preparation

The preparation of the tobacco leaf extract began by drying the tobacco leaves in an oven at 50°C until dry. The dried tobacco leaves were then blended and sieved to ensure uniform sample fineness. Next, the tobacco leaf simplicia was extracted using the maceration method, with a mass-to-solvent ratio of 1:4 (96% ethanol). This process was carried out at room temperature for three days in a sealed inert container. Subsequently, the extract was filtered and evaporated using a rotary evaporator to obtain a 100% tobacco leaf extract.<sup>13</sup>



### Paste Preparation

Tobacco leaf extract paste was prepared by mixing the placebo with powdered tobacco leaf extract. The placebo consisted of calcium carbonate (29%), magnesium carbonate (26%), sterile distilled water (25%), *propylene glycol* (8%), *glycerin* (6%), TEA (*triethanolamine*) (4%), and *Oleum Menthae Piperithae* (2%).<sup>14</sup> The powder was prepared by drying the extract mixed with *maltodextrin* in an oven at 40°C until dry. The 25% tobacco leaf extract paste was prepared by mixing 25 grams of tobacco leaf extract powder with 75 grams of placebo, the 50% tobacco leaf extract paste was prepared by mixing 50 grams of tobacco leaf extract powder with 50 grams of placebo (Fig. 1). The mixture was blended using a mortar and pestle until homogeneous and stored in a sealed container.



Figure 1. Tobacco leaf extract paste

### Heat-Cured Acrylic Resin Plates Preparation

The preparation of the heat-cured acrylic resin plate involved creating a mold space from red wax with dimensions of 60 × 12 × 3 mm. The cuvette was coated with vaseline and filled with gypsum at the bottom. The pattern wax sheet was placed horizontally on wet gypsum. After the gypsum hardened, its surface was coated with vaseline, and the upper cuvette was filled with gypsum and pressed with a beagle press. Once the gypsum hardened completely, the beagle press was placed in a pot of boiling water to form the mold space.



Figure 2. Heat-cured acrylic resin plate

Acrylic resin powder and liquid (ADM brand, America) were mixed (at a 3:1 ratio) until the dough stage, then placed into the mold space lined with Cold Mould Seal (CMS) and covered with cellophane plastic. The mixture was pressed slowly using a hydraulic bench press (1500 psi), followed by final pressing after removing the cellophane. The curing process was carried out by immersing the cuvette in boiling water at 100°C. Once the water reached a boil, the cuvette was immersed for approximately 20 minutes, then the sample was allowed to cool to room temperature. The final steps included deflasking, finishing, and polishing (Fig. 2).<sup>13</sup>

### Brushing Treatment of Heat-Cured Acrylic Resin Plates

The samples were immersed in distilled water for 24 hours before treatment. Heat-cured acrylic resin plates were brushed onto the polished surface using an electric toothbrush with soft, thin bristles measuring 0.15 mm. Brushing was conducted at a 20.000 strokes per minute for 28.2 minutes for groups T1 (25% paste) and T2 (50% paste). After brushing, the samples were rinsed and dried (Fig. 3).



Figure 3. Brushing treatment of heat-cured acrylic resin plates

**Transverse Strength Test**

The transverse strength test was conducted using a Tarno Grocki Universal Testing Machine with a 100 kN capacity, employing the three-point bending method with a support span of 30 mm and a load speed of 5 mm/min (Fig 4). The results were calculated using the transverse strength formula, expressed in MPa. One-way ANOVA test was conducted to analyze the data, followed by the Games-Howell post-hoc test



Figure 4. Transverse strength testing

**Statistical Analysis**

A one-way ANOVA was conducted to analyze the data, followed by the Games-Howell post hoc test ( $p < 0.05$ ).

**RESULTS**

Based on the data (Fig. 4), the highest mean transverse strength was observed in group K at  $126.926 \pm 26.582$  MPa, while the lowest mean transverse strength was found in group T2 at  $83.001 \pm 6.010$  MPa. Group T1 had a mean transverse strength of  $103.136 \pm 9.394$  MPa (Table 1). Subsequently, a parametric test, One-Way ANOVA, was conducted to identify

differences in mean values between groups (Fig. 5).

**Table 1.** Mean and standard deviation values of the transverse strength of heat-cured acrylic resin

| Group   | N  | Mean (MPa) | Standard deviation |
|---|----|------------|--------------------|
| Control (K)   | 10 | 126.926    | 26.582             |
| Concentrated tobacco leaf extract denture cleaning paste 25% (T1) | 10 | 103.136    | 9.394              |
| Concentrated tobacco leaf extract denture cleaning paste 50% (T2) | 10 | 83.001     | 6.010              |

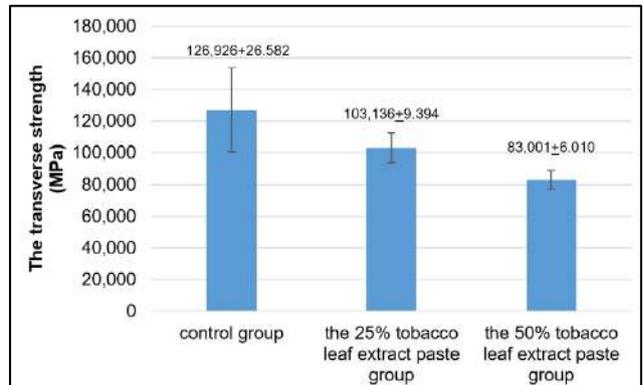


Figure 5. The transverse strength of heat-cured acrylic resin.

The results of the one-way ANOVA test showed significant differences among the groups, followed by the Games-Howell post hoc test. The results of this test indicated no significant difference between the K and T1 groups ( $p = 0.052$ ). However, significant differences were observed between the K and T2 groups, as well as between the T1 and T2 groups ( $p < 0.05$ ).

**DISCUSSION**

Transverse strength is a mechanical property of heat-cured acrylic resins that describes its ability to withstand masticatory



forces without experiencing permanent deformation. This property arises from the concurrent actions of compressive, tensile, and shear forces. Inadequate transverse strength in a denture base limits its ability to resist excessive masticatory load, resulting in an elevated risk of fracture.<sup>11,15</sup> According to the American Dental Association Specification No. 12, the minimum transverse strength required for denture bases made of heat-cured acrylic resin is 65 MPa.<sup>16</sup>

The K group exhibited the highest average transverse strength compared to the other groups, with a value of  $126.926 \pm 26.582$  MPa, followed by the T1 group at  $103.136 \pm 9.394$  MPa and the T2 group at  $83.001 \pm 6.010$  MPa. The decrease in tobacco leaf extract paste concentration is linked to a reduction in denture base strength, as the content of active ingredients diminishes at lower concentrations.<sup>7</sup>

The results indicated a significant difference between the K and T2 groups, as well as between the T1 and T2 groups. This difference is attributed to the phenolic compound content in tobacco leaf extract paste, which can reduce the strength of heat-cured acrylic resin. This finding aligns with previous research on denture cleansers, such as soaking in black tea (*Camellia sinensis*), which showed that the phenolic compounds therein can decrease the surface strength of heat-cured acrylic denture bases.<sup>17</sup> In this study, the Games–Howell test was used because the homogeneity-of-variance test indicated that the data did not meet the homogeneity of variance assumption. Further analysis (post-hoc) was conducted using the Games–Howell test. This test was chosen because it does not assume equal variances across groups and is more appropriate for data with heterogeneous variance.

Tobacco leaves contain flavonoids from the phenol group, nicotine from the alkaloid group, steroids from the saponin group, and terpenoids from essential oils. Phenol has a lower molecular weight than heat-cured acrylic resin, enabling it to penetrate the acrylic resin

matrix and break its polymer bonds.<sup>18</sup> When phenol is absorbed into the acrylic resin matrix, an ion-exchange reaction may occur. Phenol, being acidic, is more prone to oxidation because it releases H<sup>+</sup> ions, as the carbon (C) atom in the phenol benzene ring (C<sub>6</sub>H<sub>5</sub>OH) has a stronger ability to attract electrons from oxygen (O) atoms than hydrogen (H) atoms. The release of H<sup>+</sup> ions leads to the breakage of phenol into phenoxide ions (C<sub>6</sub>H<sub>5</sub>OH<sup>-</sup>) as an anion and H<sup>+</sup> ions as a cation. Additionally, acyl groups (RCO<sup>+</sup>) and methoxide ions (CH<sub>3</sub>O<sup>-</sup>) are released from esters. In the presence of phenol compounds, the phenoxide ions will react with acyl groups, while the H<sup>+</sup> ions will interact with methoxide ions. This process alters the chemical structure of the acrylic resin, leading to cavities.<sup>13,19–22</sup> Besides ion exchange reactions, hydrolysis reactions may also occur.

Heat-cured acrylic resin is a polymer composed of repeating methyl methacrylate units that form Polymethyl Methacrylate (PMMA) with the chemical formula (C<sub>5</sub>O<sub>2</sub>H<sub>8</sub>)<sub>n</sub> through free radical addition polymerization. This resin has low polarity. In contrast, phenol is a highly polar compound with acidic characteristics. Polyester undergoes hydrolysis in acidic conditions, producing carboxylic acids and alcohols. The degradation of polyester disrupts the chemical bonds within heat-cured acrylic resin.<sup>10</sup>

Heat-cured acrylic resin is generally insoluble in water or other liquids present in the mouth, but can dissolve in aromatic hydrocarbon solvents. Phenol is an aromatic hydrocarbon compound that consists of a hydroxyl group (-OH) and an aromatic hydrocarbon ring. This dissolution reduces hardness and affects mechanical properties. Based on the standards set by the International Organization for Standardization (ISO), the solubility of heat-cured acrylic resin must not exceed 1.6 µg/mm.<sup>3,23,24</sup>

The significant difference in transverse strength values may also be due to the abrasive materials in the paste (calcium carbonate and

magnesium carbonate). The paste contains abrasive substances that may cause surface abrasion of the acrylic resin. This condition can increase surface roughness and ultimately damage the physical properties of acrylic resin.<sup>21</sup>

Porosity is a physical characteristic of acrylic resins. Porosity allows heat-cured acrylic resin to absorb liquids.<sup>7</sup> Heat-cured acrylic resin can absorb liquid up to 0.69 mg/cm<sup>2</sup>. The resin can absorb liquids because its base material, Polymethyl Methacrylate (PMMA), is polar due to ester groups in its structure. These polar groups are hydrophilic, allowing the resin to absorb liquids.<sup>18,25</sup>

Water absorption occurs through diffusion, where solution particles penetrate the polymer-based material. Water molecules slowly diffuse into the polymer matrix until saturation is reached.<sup>13</sup> Diffusion is the process of transferring molecules from areas of high concentration to areas of low concentration.<sup>26</sup>

According to the matrix degradation theory, acrylic resins absorb water molecules, which then enter the intermolecular spaces between the polymer chains. These water molecules disrupt the polar interactions between the chain. The ester groups in heat-cured acrylic resins create polar interactions; however, when water molecules enter the spaces between polymer chains, these interactions are disrupted. This weakens the attractive forces between the polar molecules of the polymer, causing the polymer chains to separate, increasing the distance between them, and making the matrix looser or swollen, a process known as matrix expansion. This expansion is followed by matrix softening, which is a decrease in polymer strength.<sup>28</sup>

The tobacco leaf extract-paste at concentrations of 25% and 50% reduced the transverse strength of heat-cured acrylic resin. However, the decrease remained above the minimum transverse strength required for denture bases made of heat-cured acrylic resins.

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