ORIGINAL ARTICLE

Protective Effect Of Mauli Banana Stem Gel Against Dentin Loss Through Inhibition Of Collagen Degradation

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ABSTRACT

Introduction: Dentin is composed of nanocrystals of hydroxyapatite and collagen molecules. Dentin demineralization refers to the depletion of minerals and hydroxyapatite in peritubular dentin, primarily due to acidic environments. Dentin demineralization will initiate matrix opening and degradation by matrix metalloproteinases (MMPs). To the researchers' knowledge, there have been no studies proving the benefits of MBSG against dentin loss through inhibition of collagen degradation in the carious models. Hydroxyproline serves as a marker for the deterioration of collagen. This study aimed to investigate the protective effect of MBSG against dentin loss through inhibition of collagen degradation. **Materials and Methods:** Forty-two teeth samples were divided into six groups (n = 7 teeth in each group): the control group, the demineralized and degraded dentin treated with 25%, 37.5%, 50 %, 62.5% MBSG, and Chlorhexidine. The hydroxyproline was quantified using a spectrophotometer. Dentine loss was measured using a profilometer. Therefore, the data were analyzed using a one-way ANOVA test and the post-hoc Bonferroni. **Results:** The hydroxyproline levels in all doses of the MBSG groups were considerably reduced compared to the control group ($p < 0.05$). The higher the dose of MBSG, the lower the hydroxyproline levels. The efficacy of MBSG on dentin loss at the three highest doses was superior to that of Chlorhexidine. **Conclusion:** These results indicate that MBSG can prevent dentin loss through inhibition of collagen degradation by suppressing hydroxyproline production.

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Keywords: Dentin, Hydroxyproline, Musa acuminata, Tooth Demineralization, Dental caries

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INTRODUCTION

Dental caries is caused by the demineralization of the tooth structure, and it has an incidence rate of 36% (1,2). In 2018, the prevalence of dental caries among Indonesians was 88.8% (3). The average number of damaged teeth per person in South Kalimantan-Indonesia was 6.83%. This includes 1.31% of teeth with caries, 5.52% of teeth with indications for extraction, and 0.12% of teeth with indications for restoration (4). Suryanto's research at the East Kelayan Health Centre in Banjarmasin City, Indonesia revealed a significant lack of understanding regarding the treatment of dental caries (5). Furthermore, it was observed that the majority of individuals with dental caries experienced severe damage, necessitating intensive treatment.

Dental caries is a progressive dental disease caused by the interaction of bacteria and carbohydrate fermentation in tooth tissue, which causes acidic conditions and a

decrease in pH (6). Continuous occurrence of this condition will result in demineralization of hard tooth tissues, including enamel, dentin, and cementum, leading to damage to their organic composition. Demineralization can reach dentin if the enamel is completely demineralized. Collagen breakdown will occur in demineralized dentin (7). The main component of dentin collagen is hydroxyproline, which is an amino acid produced by the hydroxylation of proline (8). Collagen degradation can be detected by measuring the amount of hydroxyproline released (9). Collagen degradation can lead to dentin loss. Matrix metalloproteases (MMPs) and cysteine cathepsins are responsible for dentin collagen degradation (7). Hence, in order to avert dentin loss, it is imperative to have a medicinal substance that can effectively impede the activity of MMPs and cysteine. Chlorhexidine (CHX) has demonstrated its ability to inhibit MMPs and cysteine cathepsin (10,11). CHX has the capability to impede the degradation of dentin collagen. However, prolonged usage of CHX might result in the occurrence of discoloration, ulceration, and a diminished sense of taste (12). Therefore, alternative ingredients are needed with optimal function and minimal side effects, such as herbal compounds.

Mauli banana (Musa acuminata) is a plant from South Kalimantan often used for traditional wound healing. Mauli banana stems (MBSG) contains tannins (67.59%), saponins (14.49%), alkaloids (0.34%), and flavonoids (0.25%) (13). MBSG content is proven to inhibit MMPs and cysteine cathepsin, hence preventing collagen degradation and dentin loss. Proanthocyanidins (PA) tannins from grape seed extract are collagen cross-linkers and MMPs inhibitors (14). Saponins can inhibit MMP-2 and MMP-9 (15). Alkaloids can also inhibit cathepsin L and V (16). Flavonoids can inhibit cathepsin B and cathepsin L which are a class of cysteine cathepsins (17). Prior research has demonstrated that MBSG effectively enhances the mineral content of demineralized dentin(18). However, its potential to inhibit collagen degradation remains uncertain. This study aimed to investigate the protective effect of MBSG against dentin loss through inhibition of collagen degradation based on hydroxyproline.

MATERIALS AND METHODS

Experimental design

This research is true experimental post-test only design with control group design. The research sample used forty-two teeth mandibular Incisor bovine teeth, which had intact crowns and were not carious. The study sample was divided into six groups: seven samples were treated with Mauli Banana Stem gel 25% (MBSG1), seven samples were treated with Mauli Banana Stem gel 37.5% (MBSG2), seven samples were treated with Mauli Banana Stem gel 50% (MBSG3), seven samples were treated with Mauli Banana Stem gel 62.5% (MBSG4), seven samples were treated with Chlorhexidine 2% (CHX) (Consepsis-Ultradent-USA) as a positive control, and seven samples were untreated (Control) as a negative control. The approval and ethical clearance from the Health Research Ethics Commission, Faculty of Dentistry, University of Lambung Mangkurat with No.040/KEPKG-FKGULM/EC/IV/2022.

Preparation of Mauli banana stem extract gel (MBSG)

Mauli banana stems were obtained from SMK-PP Banjarbaru (South Kalimantan, Indonesia) and subjected to a determination test. Mauli banana stems were taken 10 cm from the root hump and, after that, cleaned, cut, and dried for three days using an oven at 40–60oC. The Mauli banana stem was subsequently processed into extract through maceration with 70% ethanol. The acquired extraction results were evaporated using a rotating vacuum evaporator at 40–50oC until the extract reached a viscous consistency. The extract was then tested for the absence of ethanol by adding potassium dichromate (19,20). The Mauli banana stem gel used in this study is a concentration of 25%; 37.5%; 50%; and 62.5%. The extract of the Mauli banana stem as 2.5, 3.75, 5 dan 6.25 grams was combined with Propylene 0.5 grams, Glycerin 1 grams, Na-CMC 0.5 grams, Nipagin 0.001 grams, and Aquades 10 grams to create a gel formulation (12).

Dentin blocks preparation

The dentin block preparation is referring to the preparation of the carious model. We used forty-two bovine incisors that were immersed in saline solution and then shaped into blocks to obtain dentin samples measuring 10 x 7 x 4 mm. The dentin, which had been shaped into blocks, was preserved in a saline solution to inhibit contraction. The dentine block's surface was leveled with a carborundum disk, polished with felt paper, and moistened with diamond spray (21). The dentin block was coated with nail polish, and an area of 4 x 3 mm remained before the demineralization stage. Demineralization was carried out by soaking in lactic acid pH 4.5 for 72 hours, then washing with deionized water and drying with absorbent paper (21,22). The teeth subsequently treated with MBSG1, MBSG2, MBSG3, MBSG4, and CHX therapy, which were applied for 1 minute. Afterward, the teeth were immersed in artificial saliva (McDougall method) containing collagenase enzyme (Type I Himedia-India) at 1 mg/ml for 24 hours (10,23).

Hydroxyproline measurement

The sample was dissolved in 100 mL of distilled water and hydrolyzed using an autoclaving at 120°C for 20 minutes. The sample was then mixed slowly with 0.056 M Chloramine-T reagent and then oxidized at room

temperature for 25 minutes. Each sample was added with 1 M Ehrlich aldehyde reagent and then incubated for 20 minutes at 65°C to produce chromophores. The absorbance of each sample was measured at 550 nm by spectrophotometry (T80+) and then converted to hydroxyproline concentration. The amount of hydroxyproline was determined by plotting the values against a standard calibration curve (21,24).

Measurement of dentin loss

The dentin blocks were maintained in a wet condition until analysis to prevent shrinkage of the organic layer. Excess water was slowly removed with filter paper, and nail polish was also removed. Measurements were made using a profilometer (Surfcom 2900SD3-USA) from a reference area to an open area and to other reference areas. Five profile measurements were carried out at 0.5 mm intervals, and an average value was obtained with a length of 2.5 mm. The difference in height between the reference area and the eroded area was quantified in µm (11,21).

Statistic analysis

The data were analyzed using the SPPS 26.0 in Windows computer program. The normality test was conducted using the Shapiro-Wilk test, followed by Levene's test to assess the homogeneity of variance. The data analyzed were normal and homogeneous, so the one-way analysis of variance (ANOVA) test and the post hoc Bonferroni test were employed.

RESULTS

Figure 1 shows the hydroxyproline levels in the various study groups. Hydroxyproline levels were significantly reduced at all doses of MBSG groups compared to the control group ($p < 0.05$). The CHX group had a significantly higher reduction compared to the control group ($p < 0.05$). There was a significant difference between the four doses of MBSG administration and CHX ($p < 0.05$). The higher the dose of MBSG, the lower the hydroxyproline level.

Figure 2 shows the degree of dentine loss in the various groups. Compared to the control group, dentine loss was significantly decreased at all doses of MBSG and CHX ($p < 0.05$). The higher the MBSG dose, the lower the dentin loss. There was no significant difference in dentine loss between the MBSG3 group and CHX group ($p > 0.05$).

DISCUSSION

MBSG contains phytochemicals such as tannin (67.59%), saponins (14.49%), alkaloids (0.34%), and flavonoids (0.25%) (13). Previous research indicated that MBSG concentrations of 25%, 37.5%, 50%, and 62.5% satisfied the criteria of homogeneity, spreadability, and

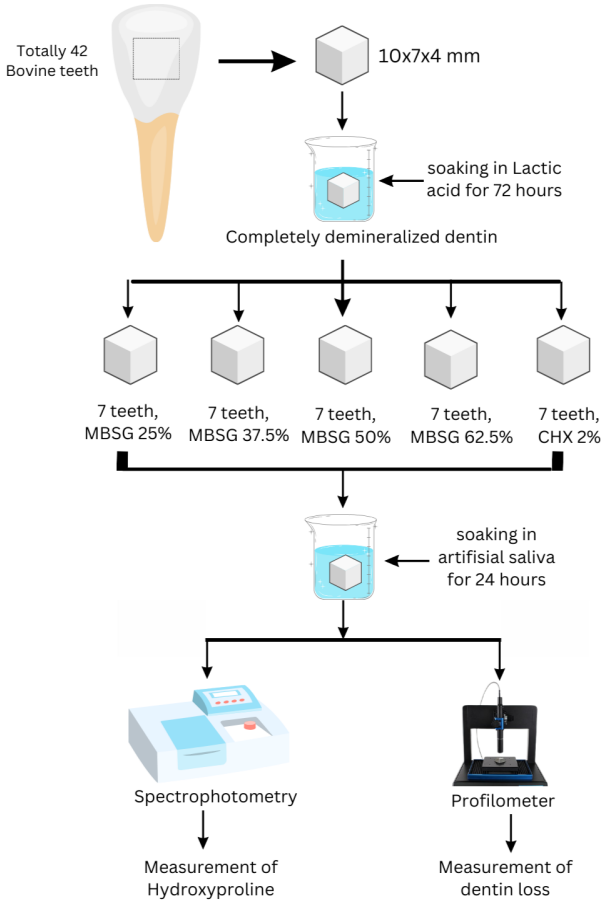

Figure 1: Schematic illustration of experimental design and procedures.

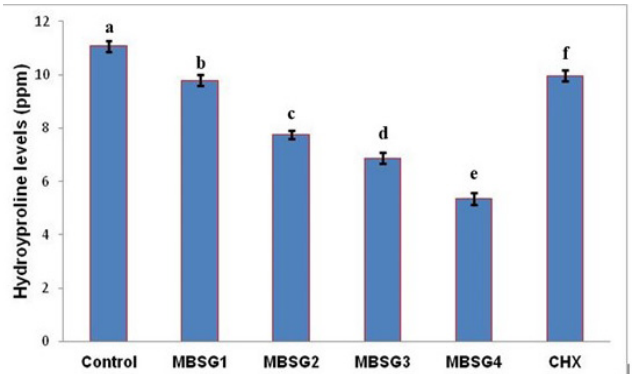

Figure 2: Effect of Mauli Banana Stem (MBSG) gel on Hydroxyproline. The value were the means ± SD. Columns not sharing the same letter were significantly different at $p < 0.05$.

pH (12). The homogeneity test of MBSG confirmed the uniformity of the gel. The homogeneity test of MBSG indicated that the gel was uniform. This suggests that the combination of active substances and components in the gel mixture was thoroughly combined. The spreadability of MBSG varied between 3.75 and 4.6 cm, making it suitable for semi-solid topical treatments. The pH test results of MBSG varied between 5.5 and 7.9, which is within the acceptable pH range for the mouth (12).

The dentin's organic matrix is composed of 90% type I fibrillar collagen and 10% non-collagenous proteins

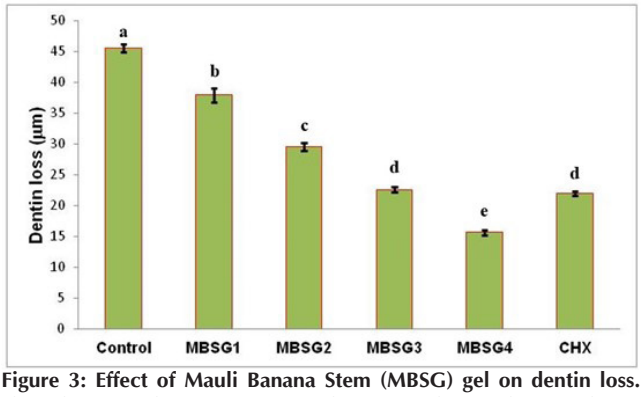

Figure 3: Effect of Mauli Banana Stem (MBSG) gel on dentin loss. The value were the means ± SD. Columns not sharing the same letter were significantly different at $p < 0.05$.

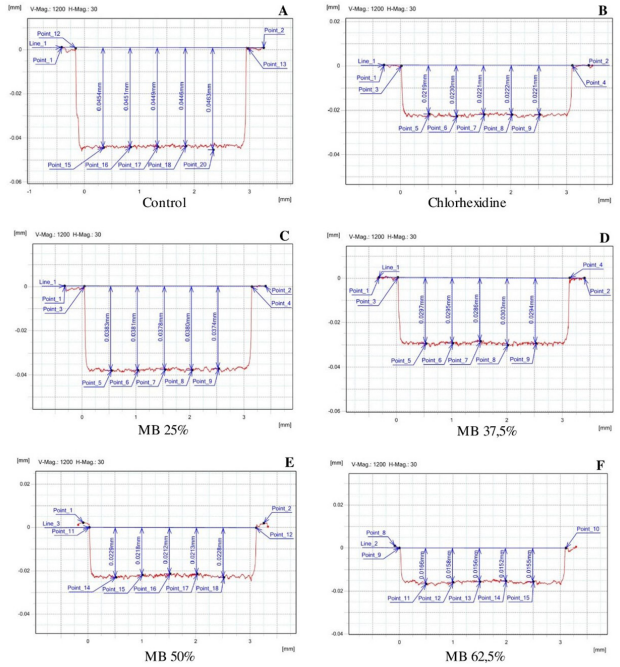

Figure 4: Dentin loss measurements used a Surfcom 2900SD3-USA profilometer.

25). Dentin collagen mainly consists of hydroxyproline, an amino acid synthesized through the hydroxylation of proline (8). Collagen degradation can be detected by measuring the amount of hydroxyproline released (9). MMPs is a group of zinc metallo-endopeptidases secreted by odontoblasts cells. These MMPs are responsible for breaking down collagen in the demineralization process (8,26). MMPs found in dentin are MMP-1 and MMP-8 (collagenous), MMP-2 and MMP-9 (gelatinase), and MMP-3 (stromelysin) (27). Substances classified as MMPs inhibitors can inhibit collagen degradation in dentin demineralization. In this study, there was a significant decrease in hydroxyproline levels in the groups given various doses of MBSG compared to the control group. Our research revealed positive correlation between the dosage of MBSG and its ability to prevent collagen breakdown. This indicates that MBSG can inhibit collagen degradation. The functional activity of MBSG is played by its active compounds, namely tannins, saponins, alkaloids, and flavonoids (13). Tannins such as Proanthocyanidin (PA) and flavonoids such as epigallocatechin-3-gallate (EGCG) can act as crosslinking collagen (9,26). The crosslinking mechanism between PA and collagen is believed to occur through the creation of insoluble compounds. This is achieved by forming hydrogen bonding between the amide carbonyl groups of the protein and the phenolic hydroxyl groups in addition to hydrophobic and covalent bonds (26). The application of crosslinking agent on demineralized dentin can also prevent the release of triple-helical collagen, which can inhibit MMPs from cleaving collagen molecule polypeptides, thus inhibiting collagen degradation (9). PA can inhibit 90% of MMPs (26). EGCG, alkaloids, and saponins can inhibit MMP-2 and MMP-9 (15,26,28). Flavonoid can inhibit MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9 (27,29).

MMPs and cysteine cathepsins synergize in degrading collagen in the caries process (25). Cysteine cathepsins includes Cathepsins-B, -C, -F, -H, -K, -L, -V, -O, -S, -W, and -X (17). Cathepsins-B can increase MMPs activity by shifting the balance between MMPs and TIMPs through inactivation of the MMP-specific tissue inhibitors TIMP-1 and TIMP-2 (30). Cysteine cathepsins initiates the demineralization process in an acidic environment. Upon reaching a neutral pH, MMPs are released and combine with cysteine cathepsins to degrade collagen (17). To inhibit dentin collagen degradation, materials that can inhibit cysteine cathepsins are needed, such as tannins, flavonoids and alkaloids, which are the content of MBSG. PA can inhibit about 70-80% of cysteine cathepsins-B and cathepsins-K (25). Flavonoid can inhibit cathepsin-B dan cathepsin-L (17). Alkaloids also can inhibit cathepsin-L dan cathepsins-V (16).

PA has an additional role in preventing collagen breakdown by enhancing the collagen's ability to resist collagenase. This is achieved by sealing the cleavage point of the collagen matrix (25). PA can stabilize collagen through hydrogen bonding between amide carbonyl and phenolic hydroxyl group (25). PA can also displace water between collagen microfibrils and create some new hydrogen bonds between fibrils, resulting in assembled fibrils that can protect the three helical collagen. A higher concentration of PA can form a denser collagen matrix (25).

The phytochemical compounds in MBSG have active sites such as phenolic ReOH, carboxylic acids (ReCOOH) and N-containing sites (amino acids, amidos and basic NH properties). The active sites can create hydrogen bonds with similar sites in the protein-collagen molecule. This prevents Ch-collagenase from attacking the cleavage sites in the collagen molecule. Furthermore, the Ch-collagenase protein can also undergo hydrogen bonding, which disrupts its molecular structure and results in enzyme inactivation (31). Prior research has demonstrated that CHX functions as an anti-proteolytic agent, effectively inhibiting MMPs activity in carious

dentin or exerting inhibitory effects on collagenase (32,33).

Dentin loss is complex process that involves demineralization and proteolytic degradation. Dental carious causes the demineralization of dentin. The absence of minerals in the organic matrix will lead to exposure to proteolytic enzymes, destroying the organic content (34,35). All doses of MBSG were found to prevent dentine loss effectively. The higher the dose, the greater the ability to inhibit dentin loss. These findings demonstrate that MBSG can repair dentin by improving mineralization and inhibiting proteolytic action. Tannins can inhibit proteases, form bonds with dentin collagen, and act as cross-linking inductors to prevent dentin matrix loss (36). Tannic acid, a tannin, can affect the mechanical properties of mineralized dentin by increasing stiffness and providing stabilizing collagen to prevent dentin loss (36). The primary method of preventing dentin loss in this study is to inhibit the degradation of collagen, as demonstrated by the decreased levels of hydroxyproline. The third dose of MBSG had a similar ability to CHX.

MMPs are responsible for breaking down collagen, and MMP inhibitors can inhibit collagen degradation in dentin demineralization (8,26). Even though MBSG and CHX have the ability to inhibit MMPs, the limitation of this study is that MMPs were not measured. The study's findings suggest that MBSG may be an anti-caries agent, however more research is required to determine whether MBSG can promote dentin remineralization.

CONCLUSION

This study may conclude that MBSG can prevent dentin loss through inhibition of collagen degradation by suppressing hydroxyproline production. This suggests MBSG has the potential to be an anti-carious agent, but more investigation is required, such as examining whether MBSG can promote dentin remineralization.

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