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Demineralized dentin characteristics after application of Mauli banana stem gel

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ABSTRACT

Background: *Demineralization of dentin is a condition of dissolving minerals in peritubular dentin due to exposure to acids that release hydroxyapatite ions. To prevent dentin demineralization, a therapeutic agent that can inhibit the dissolution ability of hydroxyapatite ions is needed. One therapeutic agent that can be used is the Mauli banana (MB) stem gel.* ***Purpose:*** *To observe the characteristics of dentin demineralization after the use of MB stem extract gel.* ***Methods:*** *Mandibular incisor bovine teeth were demineralized with lactic acid pH 4.5 for 72 hours and then treated with 25%; 37.5%; 50%; and 62.5% MB gel and Chlorhexidine 2% for one minute. All samples were soaked in artificial saliva with 1 mg/ml saliva of collagenase enzyme for 24 hours. The characteristic of dentin demineralization was observed by using scanning electron microscope/electron dispersive X-ray spectroscopy (SEM-EDX).* ***Results:*** *The SEM image in the control, Chlorhexidine, 25%; 37.5%; 50%; and 62.5% MB gel groups showed dentinal tubules of about 3.67–4.94 µm; 3.55–4 µm; 4.18–5.6 µm; 2.28–2.86 µm; 3.29–3.81 µm; and 2.42–3.17 µm in size. The EDX test found carbon (C), nitrogen (N), oxygen (O), sodium (Na), phosphorous (P), chlorine (Cl), and calcium (Ca) in all groups. The one-way Analysis of Variance (ANOVA) test results showed significant differences in the levels of C, N, O, Cl, and Ca between all groups, while the Na showed no significant differences.* ***Conclusion:*** *The MB can inhibit the demineralization of bovine dentin based on the decrease in the size of the dentinal tubules and increasing the C, O, P, and Ca.*

Keywords: *bovine teeth; dental caries; dentin demineralization; Mauli banana stem gel; SEM-EDX*
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INTRODUCTION

Dental caries is a hard tissue disease caused by the demineralization of tooth structure.[1,2] The data from Basic Health Research in 2018 states that 93% of children between five and six years old in Indonesia experience tooth decay.[3] The Basic Health Research in 2018 also recorded a high prevalence of caries in Indonesia (88.%), including South Kalimantan alone (46.90%).[3,4] This figure shows that caries are still a major global problem in dental and oral health. Dentin caries occur through a series of demineralization processes initiated by the dissolution of hydroxyapatite crystals, which are the inorganic components of dentin.[5] Therapy for the demineralization process focuses on inhibiting the dissolution of hydroxyapatite ions so that dentin loss due to demineralization can be prevented.[5–7] Collagen degradation markers can be observed through the levels of elements, such as carbon (C), calcium (Ca), oxygen (O), sodium (Na), and phosphorus (P).[7,8] Currently, the gold standard for preventing dentin loss due to demineralization is administration of 2% Chlorhexidine (CHX), which is available in the form of mouthwash and gel.[5,9,10]

CHX has been shown to be capable of inhibiting the activity of protease enzymes through a chelating mechanism. In this mechanism, metal ions such as zinc (Zn) and Ca are bound by CHX, preventing the collagen degradation process and stopping the demineralization process.[11] However, continuous use of CHX in the long term can cause allergic reactions, have toxic effects, cause stains on teeth, and cause the resistance of microorganisms

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to antibiotics.[6,10,12] Therefore, it is necessary to develop other alternative materials, including herbal ingredients. Herbal ingredients have many bioactive components that can be used to inhibit the demineralization of teeth. One of the herbal ingredients that has been shown to affect caries and wound healing is the Mauli banana stem.[13,14]

Mauli banana (MB) stem is a South Kalimantan native plant with numerous benefits, including anti-caries activity. The research of Apriasari et al.[14] stated that MB extract has effective antibacterial activity against *Streptococcus mutans*. In addition, research by Carabelly et al.[15] also showed that this plant was able to reduce the viability of dual-species biofilms that cause dental caries. The main substance contained in MB is tannin, and many of the tannins found in bananas are condensed tannins or proanthocyanidins.[16] Cinnamic acid, isoleucine, saponin, alkaloids, and flavonoids are among the other substances found in MB.[14,15] The bioactive components of MB are known to inhibit the demineralization process in dental caries. This research was conducted using bovine teeth, as they have the same functions and biological characteristics as human teeth, which are 70% inorganic matter, 20% organic matter, and 10% water.[1] Therefore, this research was conducted to observe the characteristics of dentin demineralization after the use of MB gel.

MATERIALS AND METHODS

This research is an experimental study with a post-test only design with a control group design that has been declared ethically feasible by the Health Research Ethics Commission, with No.036/KEPKG-FKGULM/EC/IV/2022. The research sample used mandibular incisor bovine teeth, which had intact and non-carious tooth crowns. The research sample was divided into six groups, which were treated with Mauli banana stem gel with the concentration of 25% (MB1); 37.5% (MB2); 50% (MB3); 62.5% (MB4), CHX with the concentration of 2% (Consepsis, USA) (CHX), and a group of with no treatment as a control.

MB stems were taken from Vocational High School: Development Agriculture (SMK-PP) Banjarbaru and tested for determination. The MB was taken 10 cm from the root corm, then cleaned, cut, and dried for three days using an oven at 40–60^{o}C. The MB was then used as an extract by maceration method using 70% ethanol. The extract obtained was evaporated using a rotary vacuum evaporator at a temperature of 40–50^{o}C until the resulting extract became thick and tested free of ethanol by adding potassium dichromate.[2,4] The MB extract was then mixed with propylene, glycerin, Na-CMC, nipagin, and aquades and formulated into a gel.[17]

The bovine incisor teeth were soaked in saline solution before being prepared, and then they were formed into dentin blocks of 10x7x4 mm. Dentin that has been formed into blocks is stored in a saline solution to avoid shrinkage. The surface of the dentin blocks was leveled with a carborundum disk and polished with felt paper, using diamond spray to keep them wet.[18] The dentin blocks were smeared with nail polish to coat them, and an area of 4x3 mm remained before the demineralization stage. Demineralization was carried out by soaking the teeth in lactic acid pH 4.5 for 72 hours, and then the teeth were washed with deionized water and dried with absorbent paper.[12,18] The teeth were then treated with MB1, MB2, MB3, MB4, and CHX therapy for one minute before being immersed in artificial saliva (McDougall method) with added collagenase enzyme (Himedia, Mumbai, India) at 1 mg/ml saliva for 24 hours.[19,20] For the negative control group, the teeth were immersed in artificial saliva with collagenase enzyme at 1 mg/ml saliva for 24 hours.

Samples were observed using scanning electron microscopy (SEM) (Hitachi TM3000, Japan) connected to energy dispersive X-ray (EDX) spectroscopy. Prior to observation, the sample was coated with double-sided carbon tape for 30 minutes. The sample was then placed in the SEM holder, inserted into the SEM, and then observed at 2000x magnification. Subsequently, the samples were tested using the EDX program (Quorum QR15, Japan) and the data results were taken based on the weight percentage (% weight). The data were tested statistically using the SPSS Version 16 (SPSS Inc., Chicago, IL, USA). The test was carried out using the one-way Analysis of Variance (ANOVA) test and continued with the LSD test. All p-values of <0.05 were considered statistically significant.

RESULTS

The results of SEM observations at 2000x magnification are shown in Figure 1. The SEM image of the bovine teeth in the control group (Figure 1A) shows the appearance of the dentinal tubules covered by the smear layer and measuring around 3.67–4.94 µm. The SEM image of the dentin of the CHX group of bovine teeth (Figure 1B) shows a picture of the dentinal tubules covered by deposits with a size of about 3.55–4 µm; the deposits are CHX products deposited on the surface. The SEM image of the dentine of the bovine teeth in the MB group (Figure 1C-1F) shows the appearance of the dentinal tubules, which are covered by clustered globular deposits, which are products of MB. The dentinal tubules covered by deposits on MB stem gel 25% group measured approximately 4.18–5.6 µm (Figure 1C), the MB stem gel 37.5% group measured approximately 2.28–2.86 µm (Figure 1D), the MB stem gel 50% group measured about 3.29–3.81 µm (Figure 1E), and the MB stem gel 62.5% group measuring approximately 2.42–3.17 µm (Figure 1F).

The results of the sample observation on SEM-EDX contained the elements C, N, O, Na, P, Chlorine (Cl), and Ca. The elemental values were taken based on the mean and standard deviation (mean ±SD) of the weight percentages

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(% weight). The elemental values (mean±SD) based on the LSD test are shown in Figure 2.

Based on the results of the EDX test (% weight), the elements contained in the dentine of bovine teeth were tested using a one-way ANOVA. The one-way ANOVA test results showed significant differences in the elements C, N, O, P, Cl, and Ca with a p-value of 0.00, while the element Na showed no significant differences with a p-value of 0.92. The LSD test was performed after the one-way ANOVA test. The results of the LSD test (Figure 2) showed that the elements C, O, P, and Ca present in the MB groups were significantly different from the control and CHX groups. Element C levels in the MB4 group were significantly increased compared to those in the control and CHX groups.

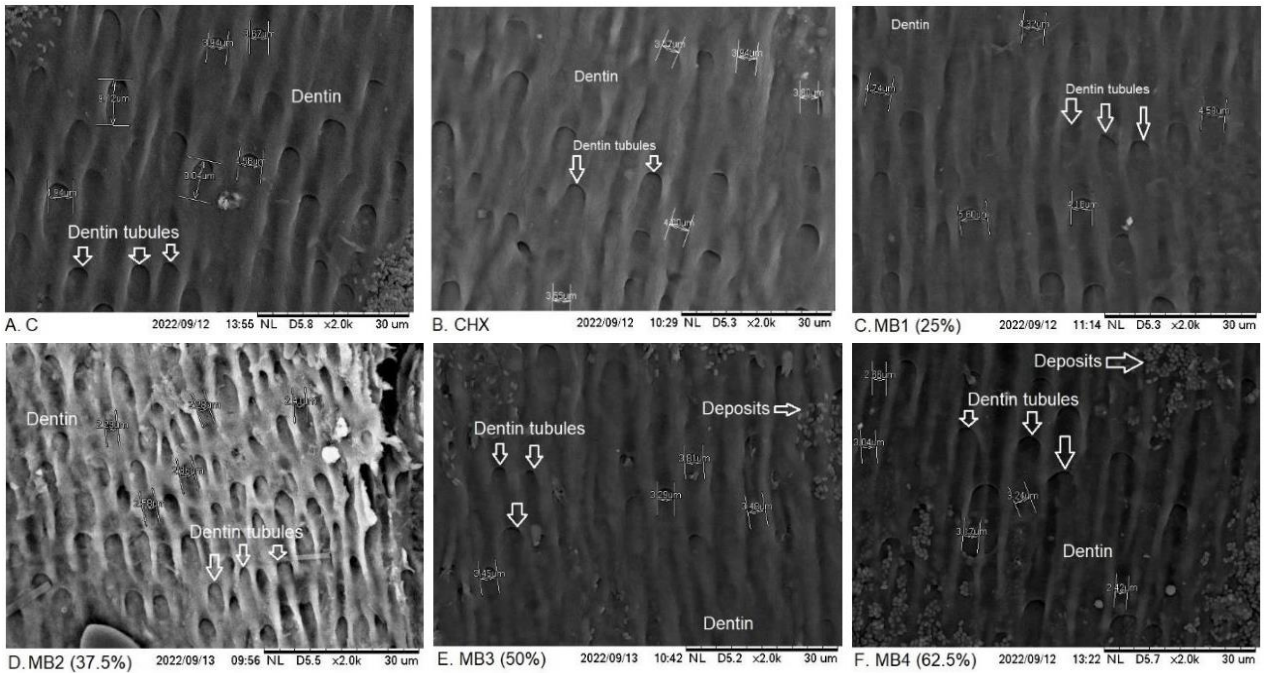

Figure 1. Scanning electron microscopy (2000x magnification). (A) Control group. (B) CHX group. (C) MB stem gel 25% group. (D) MB stem gel 37.5% group. (E) MB stem gel 50% group. (F) MB stem gel 62.5% group.

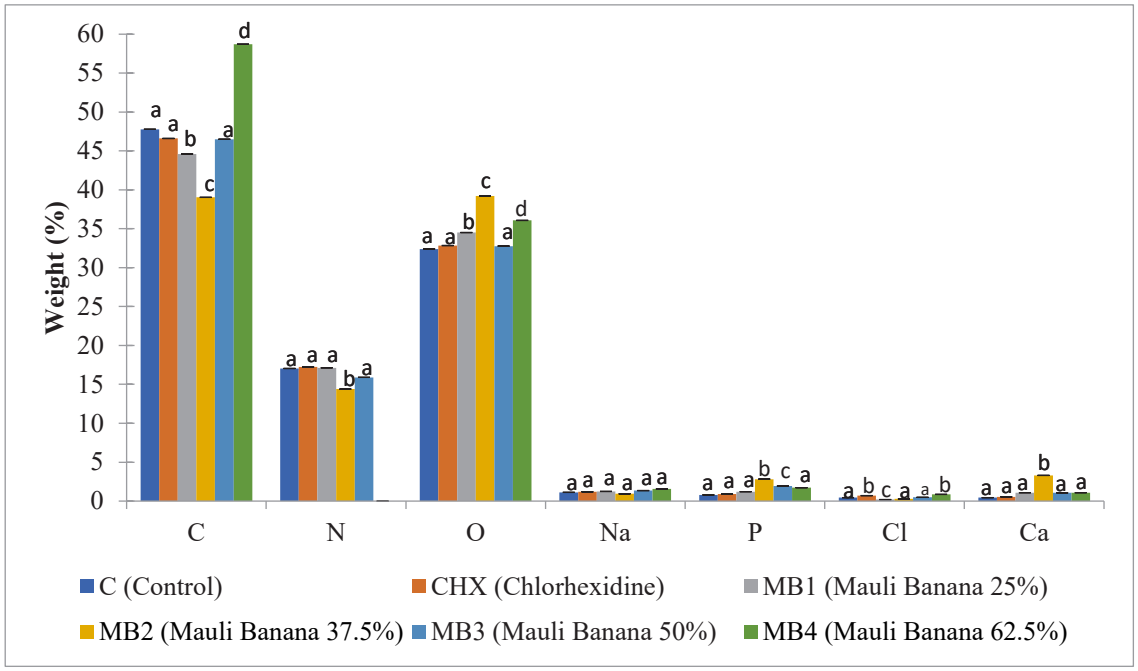

Figure 2. The elemental values (mean±SD) based on the LSD test. C = carbon; N = nitrogen; O = oxygen; Na = sodium; P = phosphorus; Cl = chlorine; Ca = calcium. Identical letters represent no statistical differences ($p > 0.05$).

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Meanwhile, in the MB1 and MB2 groups, the levels were significantly lower than in the control and CHX groups. Element O, which was present in the MB1, MB2, and MB4 groups, was significantly increased compared to the control and CHX groups. Element P, which was present in the MB2 and MB3 groups, was significantly increased compared to the control and CHX groups. Element Ca, which was present in the MB2 group, was significantly increased compared to the control and CHX groups. Element Cl, which was present in the MB4 group, was significantly increased compared to the control, but there was no significant difference in the CHX group. Meanwhile, levels of the element Cl in the MB1 group were significantly lower than in the control and CHX groups. Elements N and Na, which were present in the MB group, were not significantly different from the control and CHX groups.

DISCUSSION

Demineralization is a process of dissolving mineral ions from the hard tissues of the teeth, and it results in the loss of the integrity of the tooth structure. During the demineralization process, hydroxyapatite crystals will dissolve first, and then collagen will be exposed and dissolve.[6] In this study, SEM images showed dentinal tubules covered by a smear layer, which is a layer of debris formed as a result of the cavity preparation process. The entire dentinal tubular orifice is still closed.[1] Administration of MB gel with concentrations of 25%, 37.5%, 50%, and 62.5% had an effect on the tooth surface after demineralization treatment. This effect is seen in the SEM image, which shows a deposit in the form of a globular formation on the dentin surface. The SEM observations in this study showed that the deposits formed by MB on the dentine surface made the size of the dentinal tubules in the MB 37.5% (Figure 1D), the MB 50% (Figure 1E), and the MB 62.5% (Figure 1F) groups smaller than the control group (Figure 1A), and CHX group (Figure 1B). However, this was not the case with the MB 25% group (Figure 1C), where the size of the dentinal tubules was larger than the CHX group, but still resembled the control group. The increased porosity of the dentinal tubules indicates a lot of dentine mineral loss.[5]

The images of SEM showing globular deposits on several dentin surfaces show the reaction products of the MB. Even though in the research procedure, after the dentin surface was applied, the MB stem extract was then cleaned and dried with cotton swab, not all of it disappeared, because it had reacted with the dentin surface to form a protective layer or physical barrier that made it more resistant to acid from outside. This is in line with the study of Amin et al.[21] on SEM images showing a layer of precipitate of insoluble complexes on the enamel surface. The reaction product of the grape seed extract is seen as an amorphous lump. Spherical globular agglomerates of different sizes were observed on the enamel surface. Several crystals adhered to each other and precipitated in the demineralized area. Similar SEM images were also found in propolis-treated dentin which also showed that, after application of propolis to the dentin surface, a physical barrier was formed on the dentin surface, making it more resistant to acid.[5,21]

Dentin consists of inorganic material (50%), organic components (30%), and water (20%).[22,23] In the present study, MB can inhibit the demineralization of tooth dentin's demineralisation based on C, O, P, and Ca value. This study significantly increased the number of elements such as C, O, P, and Ca in this study compared to the control group and CHX group. C is used as a marker for the organic components of dentine. The organic components of dentine were primarily based on carbon-based molecules, and C is the most abundant element in organic dentin.[24] Inorganic dentin consists of hydroxyapatite crystals, consisting of Ca, P, and O, with traces of Na and Cl.[1,22] P and Ca are required for the demineralisation and remineralization processes.[22,25] The MB could not significantly increase the amounts of Na and Cl. N is considered a marker protein, especially the amino parts of peptide bonds in the organic components of dentine. N is the second-most abundant element after C in the organic components of dentin,[24] but the MB in the study could not significantly increase the amount of N.

MB has a tannin content of 67%, and the tannins found in bananas are condensed tannins or proanthocyanidins.[13,16,26] Proanthocyanidins effectively strengthen the collagen fiber matrix, which is the organic matrix of dentin, and keep collagen fibers from degradation.[27] Proanthocyanidins can inhibit demineralization by reducing collagen degradation by dentine proteases, inhibiting proteolytic activity, and protecting dentin from enzymatic degradation.[27,28] Proanthocyanidins can also be used in the dentin remineralization process to maintain and protect the collagen matrix.[27] The research of Bueno et al.[29] stated that proanthocyanidins can reduce the amount of mineral loss in dentine.

The demineralization process in the control group occurred due to the exposed collagen matrix and caused the dentin collagen to dissolve. This demineralization process will also continue because no compounds bind P and Ca on the dentin surface, so low P and Ca elements were found in the control group.[30,31] Hence, this research concluded that MB could inhibit the demineralization of bovine dentin. According to the SEM test, the dentinal tubules in the MB 37,5%, 50%, and 62,5% groups were smaller than those in the control and CHX groups. According to the EDX test, there was a significant increase in the number of elements such as carbon, oxygen, phosphorous, and calcium in the MB groups.

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