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The effect of roselle (*Hibiscus sabdariffa* l.) flower petal extract on smear layer removal of crown dentin

Christian Khoswanto [1,*], Fatina Jilan Rahman [2] and Hendrik Setiabudi [1]

[1] *Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.*
[2] *Undergraduate Student of Dental Medicine Education Program, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.*

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Abstract

One of the initial stages carried out before making a restoration is the tooth preparation. The debris resulting from the preparation process of the tooth structure is called the smear layer. Dentin conditioner is needed to remove the smear layer so that the crown dentin surface is clean and the restoration can adhere well. Roselle (Hibiscus sabdariffa L.) are a herbal ingredient that has acidic properties found in its flower petals. This research was conducted to determine the effect of roselle flower petal extract on the removal of the smear layer on crown dentin. Eight human maxillary first premolars from orthodontic extraction patients were used as samples. This pre-experimental study was conducted with a test group consisted of control group conditioned with sterile distilled water and treatment group with 2,5%, 5%, and 10% of roselle flower petal extract. The research data was obtained through observing the results of scanning Electron Microscope (SEM). Roselle flower petal extracts 5% and 10% were able to clean the smear layer better than extracts with a concentration of 2.5% and the control group. This may be due to the acidic pH of the solution and demineralization process caused by the presence of citric acid and other organic acids in roselle flower petal extract.

Keywords: Dentin conditioner; *Hibiscus sabdariffa l. petal* extract; Citric acid; Smear layer; Scanning Electron Microscope (SEM)

1. Introduction

During the tooth preparation process, debris resulting from the preparation of the tooth structure, known as the smear layer, can cover the dentin tubules. This layer consists of damaged and fragmented hydroxyapatite and denatured collagen. If not removed, this layer can cause microleakage, bacterial penetration, and pulp inflammation. Dentin conditioner is used to remove the smear layer so that the dentin surface is clean and the restoration can adhere well to the dentin surface [1-3].

Class V caries cases according to the classification of G.V. Black is a lesion found on the cervical aspect of the buccal or lingual surface of the tooth which can involve the dentin. Glass Ionomer Cement (GIC) is effective for the treatment of cervical lesions due to its ability to chemically adhere to the tooth structure. This is supported by the thermal expansion coefficient of the GIC which is similar to the tooth structure, which contributes to the quality and durability of the restoration, especially in the case of loss of enamel margins which is common in cervical lesions. The dentin conditioning procedure for bonding GIC to the tooth substrate is carried out to increase the adhesion between the resin and the tooth substrate. The use of dentin conditioner can increase the bond strength of Glass Ionomer Cement (GIC) to dentin and reduce the level of microleakage because reducing the concentration of hydroxyapatite will increase dentin permeability [4-7].

* Corresponding author: Christian Khoswanto.

The dentin conditioning procedure can be carried out by applying a weak organic acid at a low concentration such as polyacrylic acid (10% to 20%) for 10 to 20. Polyacrylic acid can condition the tooth surface by removing the smear layer and exposing the smear plugs. Ten percent of polyacrylic acid is able to clean the tooth surface so that maximum adhesion can be achieved. According to El-Askary 10% polyacrylic acid has a pH of 1.87 [8-11].

Another weak organic acid that is also used as a dentin conditioner is citric acid. Citric acid is a weak organic acid that can cause the opening of dentin tubules, removal of the smear layer, and demineralization of the dentin surface [12]. A 3-10% citric acid solution was found to be effective for removing the smear layer [8]. A 2.5% citric acid solution has a pH of 1. A 5% citric acid solution has a pH of 1.9% [13-14]. In one study, a pH of 1.8 was obtained from a 10% citric acid solution.[15] The use of citric acid with a concentration reduction of up to 5% does not reduce the level of cleanliness of the smear layer compared to the use of a higher concentration.[16] The number of living cells after application of citric acid as a dentin conditioner with concentrations of 40, 50, and 60% shows that citric acid is relatively safe, with less dentin resorption and blood coagulation [6].

The use of natural ingredients in dentistry is an alternative and can minimize the side effects of dentin conditioner ingredients. Roselle flowers (Hibiscus Sabdariffa L.) are a herbal ingredient that has acidic properties and has been widely used in dentistry to reduce inflammation, as an antimicrobial, antiseptic, antioxidant, antifungal, antibacterial, antiviral, and analgesic. The main advantages of using natural ingredients are easy availability, cost-effectiveness, and low toxicity. Roselle petals contain organic acids, especially citric acid, ascorbic acid, malic acid, and tartaric acid. A high percentage of organic acids in roselle extract such as citric acid and malic acid (13% based on dry weight) are found in the petals [17-18].

The percentage of organic acids contained in roselle flower petal extract is citric acid with a percentage of 12-20%, malic acid 2-9%, tartaric acid 8% and ascorbic acid between 0.02 and 0.05%.[18] The presence of citric acid in roselle extract is also supported by the finding of citric acid detected in water extract from Hibiscus sabdariffa L. flower petals.[19] The acid content in it causes roselle flower petal extract to have a low pH. According to Morales-Cabrera (2013), roselle flower petal extract has a pH of between 1.5 to 2.4 depending on the variety. In one study, it was found that the pH of roselle flower petal extract showed a decrease from 2.18 to 1.98 with an increase in the concentration of roselle flower petal extract in the order of 5%, 10%, 15%, and 20% [20-21].

This research was conducted to find out the effect of roselle flower petal extract with concentrations of 2.5%, 5%, and 10% on the removal of the smear layer in crown dentin through Scanning Electron Microscope (SEM) observations.

2. Material and methods

2.1. Materials and tools

The tools used in this research were: scales, blender, low-speed micromotor, contra angle low-speed handpiece, contra-angle high-speed handpiece, micro brush, plasticine, micropipette, Fraser bur, fissure bur, scanning electron microscope, vacuum evaporator, freeze dry and water bath machine.

The materials used in this research are: Maxillary first premolar tooth aged 9-20 years collected from orthodontic treatment extraction patients with an age range of 9-20 years in Drg. Rita Sariwati and Drg. Agus Dental Clinic, roselle flower petal extract, and sterile distilled water. The roselle petals used comes from roselle plantation in East Java, which was then determined and extracted with concentrations of 2.5%, 5%, and 10% with water solvent using a water bath and continued with freeze dried process and dissolved to form a solution at BIOME Laboratory Surabaya.

2.2. Methods

2.2.1. Preparation of roselle flower petal extract

Dried roselle flower petals are ground for 3 seconds using a blender. The experimental concentration of roselle flower petal extract was determined at 2.5 g/100 mL of water, 5 g/100 mL of water and 10 g/100 mL of water. The crushed roselle flower petals were weighed according to the provisions: 2.5g, 5g and 10 g. Extraction was carried out in a water bath at a constant temperature of 50^{o}C for 30 minutes [22]. Results from the water bath continued with a freeze-dried process for 24 hours and were then weighed at 0.75g, 1.5 g, and 2.2 g, which were then dissolved in sterile distilled water so that they were obtained in a solution with a concentration of 2.5%, 5%, and 10% in 30 ml. The final result is roselle flower petal extract in liquid form. The pH of roselle flower petal extract was measured using a pH meter (2.5% roselle flower petal extract : 6.29 ; 5% roselle flower petal extract: 6.31 ; 10% roselle flower petal extract : 6.33).

2.2.2. Preparation of the Tooth samples

Eight human maxillary first premolars with an age range of 9-12 years that were not cracked, abrasion, or caries were used as samples. The tooth samples were randomly divided into four groups with two teeth in each group. Apex and the crown of the tooth were separated using frasser bur. Then, each tooth was prepared with a class 1 cavity in the middle 1/3 of the buccal surface of the crown of the tooth in the shape of a circle with a diameter of 2 mm using fissure bur. Each tooth that is already prepared, is cut into blocks to size 4x4x5mm using a wheel diamond bur in the mesial-distal and service-incisal direction with the cavity located right in the middle of the block.

2.2.3. Treatment procedure of the Tooth samples

The sample was fixed in plasticine blocks and irrigated with 2 µl of sterile distilled water and dried with a chip blower. Samples were divided into 4 groups, the control group with the application of 2 µl distilled water (Group I), the treatment group with an application of 2 µl 2,5% roselle flower petal extract (Group II), 5% roselle flower petal extract (Group III) and 10% roselle flower petal extract (Group IV). Each tooth in the treatment group was applied using a micropipette to the cavity and then smeared using a micro brush on the surface of the preparation. Afterwards, left in contact for 20 seconds. After 20 seconds, the cavity was rinsed with 2 µl of sterile distilled water and dried with a chip blower.

2.2.4. SEM Coating and Examination

The sample is placed in a closed container, before being coated for Scanning Electron Microscope (SEM) examination in the Institute of Life Science, Engineering and Engineering (LIHTR) Airlangga University Surabaya. Each tooth sample was attached to the holder using magnetic tape with the surface of the sample being observed facing upwards, then coated with gold using a Luxor ^{Au} sample coating machine. The coating process takes approximately 20 minutes. After that, the sample was inserted into the SEM and photographed at 3500x, 5000x, and 10000x magnification [23].

3. Results

The effect of roselle flower petal extract on the removal of the smear layer from crown dentin was analyzed by observing the cleanliness of the preparation results between groups given different extract concentrations and the control group.

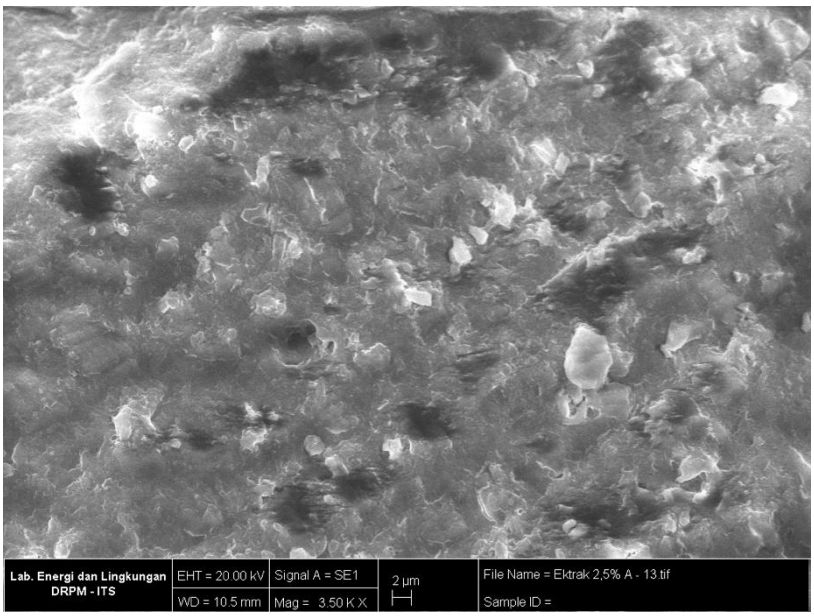

Figure 1 Scanning Electron Microscope (SEM) results from the treatment of 2,5% roselle flower petal extract on crown dentin observed under 3500x magnification

From the scanning electron microscope results of the application of 2.5% roselle flower petal extract to the dentin surface, observed at 3500x magnification, a smear layer was visible and evenly distributed on dentin surface, quite numerous and thick. Only a few dentinal tubules are visible in the image (Fig.1).

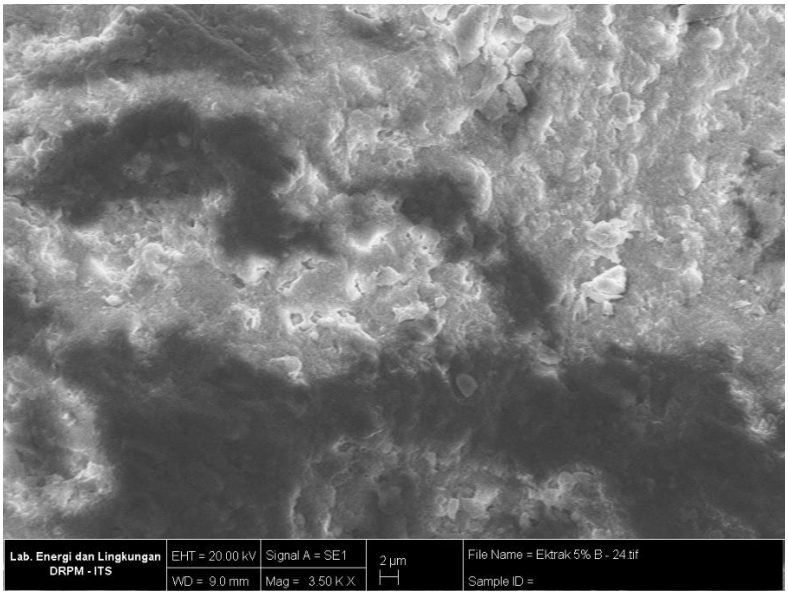

Figure 2 Scanning Electron Microscope (SEM) results from the treatment of 5% roselle flower petal extract on crown dentin observed under 3500x magnification

Application of roselle flower petal extract with a concentration of 5% showed that the dentin surface was still covered with a moderate amount of smear layer in several areas and some of the dentinal tubules were visible (Fig.2).

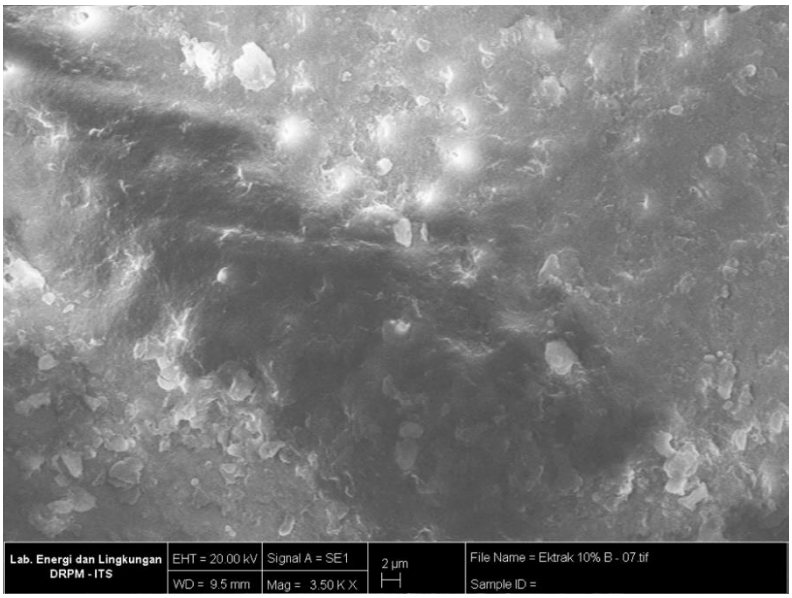

Figure 3 Scanning Electron Microscope (SEM) results from the treatment of 10% roselle flower petal extract on crown dentin observed under 3500x magnification

In the SEM results from the application of 10% rosella flower petal extract with a magnification of 3500x, the image shows that there is still a smear layer in several areas, but it is also found that the surface area is covered with smaller amount of smear layer. Only a few dentin tubules are visible in the image (Fig.3).

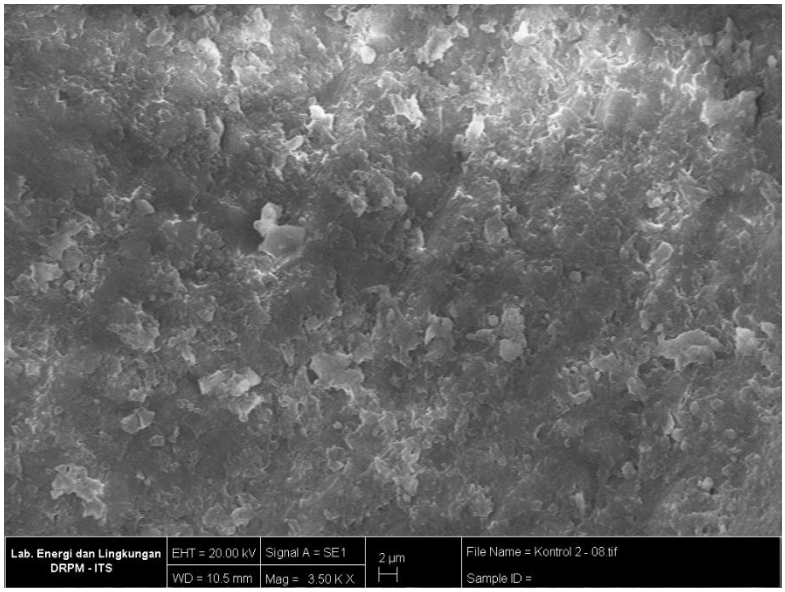

Figure 4 Scanning Electron Microscope (SEM) results from control group using sterile distilled water on crown dentin observed under 3500x magnification

And the results of applying sterile distilled water as a control group in this study as seen in Figure 4, showed that the surface of the dentin was covered in a larger and thicker smear layer spread over the entire area of the dentin surface. Not too many dentin tubules are visible in this image.

4. Discussion

This pre-experimental study was conducted to determine the effect of roselle flower petal extract on the removal of the smear layer from crown dentin by applying roselle flower petal extract with three different concentrations, 2.5%, 5%, and 10% on upper first premolars with class I preparation on the middle of the buccal surface of the tooth with 2 mm depth. The group with the application of sterile distilled water was used as the control group. Tooth preparation will cause the formation of a smear layer which can disrupt the bond between the restoration material and the dentin surface, so the application of dentin conditioner is needed to remove this layer. One of the dentin conditioner materials used in dentistry to remove the smear layer is citric acid. Citric acid with a concentration of 10% with pH 1.8 was found to be an effective approach to remove the smear layer [15-16].

Roselle is a herbal ingredient that has acidic properties and has been widely used in dentistry to reduce inflammation, as an antimicrobial, antiseptic, and antibacterial. A high percentage of organic acids in roselle such as citric acid and malic acid (13% based on dry weight) are found in the petals. Apart from that, there are several other organic acids also contained in roselle flower petal extracts, such as hibiscus acid, malic acid, tartaric acid, and ascorbic acid. Therefore, in this study, researchers used roselle flower petal extract with water solvent to observe the potential of its acidic properties in removing the smear layer from the dentin surface [17-18].

In the Scanning Electron Microscope (SEM) results observed under 3500x magnification from the control group using sterile distilled water, the surface of the dentin was covered in a large and thick smear layer spread over the entire area of the dentin surface. And not many dentin tubules can be seen on the results. On the results obtained from SEM examination with 3500x magnification for administering roselle flower petal extract with a concentration of 2.5%, there was a smear layer on almost the entire surface of the dentin. The smear layer appears large and thick and only a few dentin tubules are visible because they are covered by the smear layer. In the results of applying roselle flower petal extract with a concentration of 5%, smear layers were still found in several areas but in moderate amounts. Some dentin tubules also began to appear in the SEM photography results. In the results of administering 10% roselle flower petal extract, there is still a smear layer in several areas, but it is also found that the surface area is covered with a smaller amount of smear layer. Only a few dentin tubules are visible.

The effect of roselle flower petal extract on the smear layer removal began to be seen in the SEM results with the application of roselle flower petal extract at a concentration of 5% where the layer was still present on the dentin surface, but in smaller amounts and several dentin tubules can be seen. Clearer results were obtained from the

application of 10% roselle flower petal extract, where the dentin surface is coated with a smaller amount of smear layer but it only in some area. Only few dentin tubules are visible according to the SEM data obtained at a 3500x magnification.

The removal of the smear layer from the dentin surface may occur due to the citric acid content in roselle flower petal extract. When citric acid comes into contact with the smear layer on the surface of the crown dentin, it will react with hydroxyapatite crystals by releasing hydrogen ions and binding with calcium (cations). Then, the citrate ion (anion) will replace the phosphate ion (anion) in the hydroxyapatite crystal structure. This results in the dissolution of hydroxyapatite crystals which are the inorganic components of the smear layer. This process is known as a demineralization process [10]. Citric acid also has properties as a chelating agent. This property will clean the smear layer because of its ability to form complex bonds (chelate) with the calcium content in the smear layer [24]. The COOH group will dissociate, forming H^+ for H_3O^+ and allowing the COO^- anion to cause calcium chelation. Because chelates are soluble, calcium and phosphate ions can be released from the enamel surface, resulting in the loss of minerals from the teeth [25-26].

However, from the results of SEM observations, the smear layer was still not completely removed. This is possibly caused by the citric acid content in roselle flower petal extract which is only 13% of the dry weight of the petals so it is unable to clean the smear layer completely [3]. Based on Shin research, it was found that the pH of roselle flower petal extract showed a decrease from 2.18 to 1.98 with an increase in the concentration of roselle flower petal extract in the order of 5%, 10%, 15% and 20 % [21]. In contrast to the results obtained in this study, the pH obtained from roselle flower petal extract was 2.5%, 5%, and 10% respectively with results of 6.29, 6.31, and 6.33. However, this pH value is still classified as an acidic pH, making it possible to cause demineralization of the non-organic components of the smear layer.

The ability of roselle flower petal extract to clean the smear layer is thought to be also supported by the presence of various other organic acid compounds contained in it, including hydroxy citric acid, hibiscus acid, malic acid, and tartaric acid [18]. In comparing the results from the control group with the administration of sterile distilled water and the treatment group with the application of 2,5%, 5%, and 10% roselle flower petal extract, it was found that there was a different effect among the application of roselle flower petal extract and the control group on the presence of a smear layer on the dentin surface. Roselle flower petal extracts 5% and 10% were able to clean the smear layer better than extracts with a concentration of 2.5% and the control group as seen from the results of SEM photography. In the future research, it would be worth investigating the effect of roselle flower petal extract in different concentrations on the crown dentine surface with the hypothesis that a greater concentration of roselle flower petal extract will have a better smear layer removal effect.

5. Conclusion

The removal of the smear layer from the dentin surface in this study caused by roselle flower petal extract application was obtained through SEM observations. Roselle flower petal extracts 5% and 10% were able to clean the smear layer better than extracts with a concentration of 2.5% and the control group. This may be due to the demineralization process caused by the presence of citric acid and other organic acids in roselle flower petal extract. Further research is needed to explore and determine the effect of different concentrations of roselle extract and the influence of other organic acid compounds on smear layer removal.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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