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Effectiveness of Green Okra Fruit Extract (*Abelmoschus esculentus*) 12.5% in Removing Smear Layer of Root Canal Walls

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ABSTRACT

Smear layer is a thin layer formed due to friction of endodontic instruments during preparation. The smear layer must be removed with root canal irrigation material, namely 2.5% NaOCl. However, 2.5% NaOCl causes toxic effects when pushed into the periapical, so natural materials are needed as an alternative, one of which is green okra fruit extract. The purpose of this study was to analyze the ability of green okra fruit extract to remove the smear layer on the root canal wall. This study was an experimental laboratory study with a posttest only control group design. There were two test groups used, namely 2.5% NaOCl solution (group A) and 12.5% green okra fruit extract (group B), each as many as 5 pieces. The samples used were maxillary premolar teeth cut at the cervical level of the tooth. The root sample was embedded in a red wax block, then the root canal was prepared using conventional techniques up to K-file #50. The root canal was irrigated with 2.5% NaOCl solution and 12.5% green okra fruit extract at each instrument change, then re-irrigated with distilled water and dried. The part observed with Scanning Electron Microscope (SEM) was the middle third of the root canal. The results of SEM photography were calculated based on the Hülsmann scoring system and data analysis was performed using the Mann Whitney U Test statistical test. The results of the study and data analysis showed p = 0.606 which indicated no significant difference in the two sample groups. Both groups had a mode score of 3 which means that the smear layer covered most of the root canal walls, there were no or only a few open dentinal tubules, so it can be concluded that 12.5% green okra fruit extract and 2.5% NaOCl. the solution has the same effectiveness in removing the smear layer on the root canal walls.

Keywords: green okra extract, root canal wall, smear layer

INTRODUCTION

Root Canal Treatment (RCT) is one of the endodontic treatments in dentistry that involves removing all pulp tissue infected by bacteria and shaping the root canal to ensure proper filling, thereby preventing bacterial re-entry into the canal.[1] There are three main phases in root canal treatment: biomechanical preparation, disinfection, and root canal obturation. The success of RCT depends on the ability to eliminate microorganisms in the infected root canal. The biomechanical preparation phase

(cleaning and shaping) results in the formation of a smear layer.[2]

The smear layer is a layer formed from instrumentation debris, consisting of both organic and inorganic components, including dentin particles, remnants of vital or necrotic pulp tissue, and bacterial components.[3] Some microorganisms within the root canal use the smear layer and debris as a host for their growth.[4] The smear layer reduces dentin permeability, hinders the penetration of intracanal disinfectants and medicaments, and blocks the sealer from entering the dentinal tubules. Removing the smear layer is a crucial factor in the success of root canal treatment. The smear layer on the root canal walls can be removed using root canal irrigants. A commonly used irrigant in dentistry is 2.5% sodium hypochlorite (NaOCl).[5,6]

Sodium hypochlorite at a 2.5% concentration has strong tissue-dissolving properties, effectively removing necrotic tissue and biofilm. Sodium hypochlorite eliminates the smear layer through saponification, amino acid hydrolysis, and chloramination reactions.[7] However, NaOCl irrigation solutions have some drawbacks. If pushed into the periapical area, they can cause soft tissue irritation and cell damage. At high concentrations, NaOCl is toxic and less effective at cleaning the smear layer in the root canal.[4] Additionally, prolonged exposure can lead to pain, burning sensations, and even necrosis in soft tissues such as the lips, labial mucosa, and periapical tissues.[8]

The use of natural substances can serve as an alternative to chemical agents for removing the smear layer from root canal walls. One such alternative is green okra (Abelmoschus esculentus) extract, which is widely cultivated in Jember. The results of a phytochemical test on green okra extract revealed that it contains active compounds with antibacterial potential, including alkaloids (6.88%), terpenoids (2.95%), flavonoids (5.01%), saponins (4.02%), and tannins (3.81%).[9] KanThese active compounds are essential for root canal irrigation solutions due to their antibacterial properties. Research has shown that 12.5% green okra extract exhibits a minimum bactericidal concentration (MBC) against *S. aureus* and *S. viridans*.[10]

The saponin content in okra acts as a surfactant (detergent), enabling it to dissolve both organic and inorganic components of the smear layer. Saponins work through the saponification reaction by reducing surface tension, allowing water to easily wet the root canal walls and facilitate the detachment of the smear layer.[11] Green okra also contains oxalic acid, which functions as a chelating agent capable of removing the inorganic components of the smear layer.[9] Thus, it is expected that 12.5% green okra extract will be effective in removing the smear layer from root canal walls.

MATERIALS & METHODS

This type of research is an experimental laboratory research with a post-test only control group design. The treatment groups in this study consisted of a group irrigated with 2.5% NaOCl (Group A) and a group irrigated with 12.5% green okra fruit extract (Group B). This study began by submitting ethical clearancekto the Head of the Faculty of Dentistry, University of Jember.

The making of green okra fruit extract was done using the maceration method. 14 kg of green okra fruit was washed first. The green okra fruit was then cut into thin slices and dried by airing it at room temperature for 12 days. After drying, it was ground with a blender and sieved with a 40 mesh sieve until it became a fine powder, weighed as much as 950 grams and then macerated with 96% ethanol solution (ratio 1: 5) for 3 days. Stirring was done 3 times a day as much as 150x clockwise rotation for each stirring. The maceration results were filtered using filter paper and then concentrated using a rotary evaporator until a 100% concentration extract was obtained. Dilution with distilled water was carried out to obtain a 12.5% concentration extract

The samples used were 10 maxillary premolar teeth divided into 2 groups, 5

samples of 2.5% NaOCl (Group A) and 5 samples of 12.5% EBOH (Group B). The tooth crown was cut to the cemento enamel junction (CEJ) and its working length was measured. The root samples were embedded in a red wax block and extirpation was performed on the root canal. Then the root canal preparation was carried out using conventional techniques up to k-file number 50. The K-file is rotated using a watch-winding motion, turned clockwise 60°-90°, then rotated back counterclockwise.[12] At each instrument change, Group A was irrigated with 2.5% NaOCl, while Group B was irrigated with 12.5% green okra extract (EBOH) using 0.5 ml for 30 seconds, followed by final irrigation with sterile distilled water.

Each sample was cut into three sections (cervical third, middle third, and apical third). The middle third of the root was selected and further sectioned mesiodistally (M-D) for imaging using a Scanning Electron Microscope (SEM) at 5000x magnification. The SEM imaging was conducted at the Laboratory of Technology and Life Sciences (LITHR), Airlangga University.

Each sample had 8 SEM images representing the entire observed surface of the root canal walls. Each image was divided into 10 grids, and each grid was scored. The smear layer cleanliness assessment was based on the scoring system by Hülsmann (1997). The evaluation was performed by three observers, who assigned scores to each grid to obtain a score frequency distribution (mode), indicating the cleanliness level of each group. The collected data were analyzed using the non-parametric Mann-Whitney U test.

STATISTICAL ANALYSIS

Analysis of the research data used Mann-Whitney U Test.

RESULT

The effectiveness of 12.5% green okra fruit extract in cleaning the smear layer on the middle 1/3 of the root canal wall is seen from the level of cleanliness of the smear layer on the surface which is photographed with SEM (Scanning Electrone Microscope). The cleanliness of the smear layer can be observed from the open dentinal tubules on the surface of the root canal wall and the loss of the smear layer between the dentinal tubules. The smear layer of the root canal wall irrigated with 2.5% NaOCl and 12.5% green okra fruit extract solution can be seen from the results of SEM photos with a magnification of 5000x as in Figure 1.

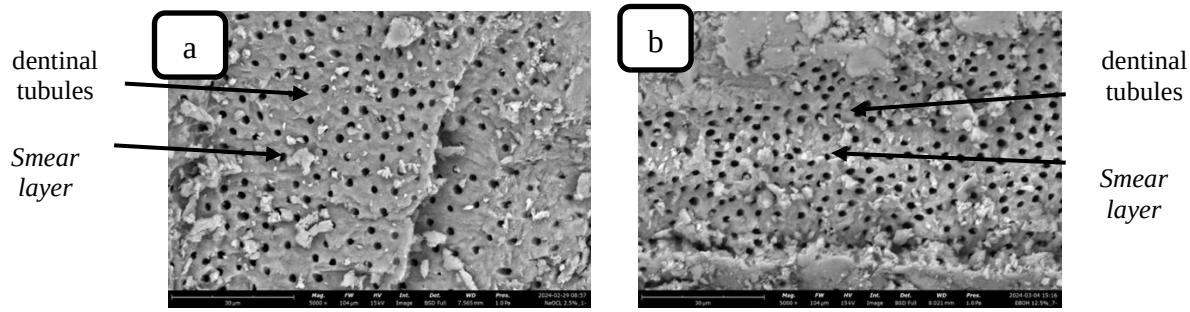

Figure 1.SEM images of (a) root canal wall surface after irrigation with 2.5% NaOCl and (b) root canal wall surface after irrigation with 12.5% green okra fruit extract.

The SEM observation results produced 8 photos for each sample. The photos represent the entire surface of the sample observed. The smear layer cleanliness assessment is based on the scoring system by Hulsmann (1997). Each sample was observed by 3 observers by calculating the smear layer score for each box in each sample photo totaling 10 boxes. The most frequent score (mode) in each sample is the smear layer frequency distribution of the sample as shown in Table 1.

Table 1.Frequency distribution of smear layer in each sample group

Group	N	Observation Result Score					Mode
		S1	S2	S3	S4	S5	
A	5	3	3	3	2	3	3
B	5	3	3	4	3	2	3

Information:
Group A = NaOCl 2.5%
Group B = Green okra fruit extract 12.5%
N = Number of Samples
S = Sample

Table 1 shows that the smear layer cleanliness mode of the root canal wall in group A and group B scored 3 (Smear layer covers most of the root canal wall, no or only a few open dentinal tubules). The basis for assessing root canal cleanliness is seen from the number of open dentinal tubules. The more dentinal tubules covered by debris and smear layer, the higher the score. The less smear layer covering the dentinal tubules, the cleaner the root canal is. This shows that 2.5% NaOCl and 12.5% green okra fruit extract have the same effectiveness in cleaning the smear layer of the root canal wall.

The research result data was tested using Mann Whitney U Test and obtained a significance value of p = 0.606 (p> 0.05). This shows that there is no significant difference between the two groups, namely the group irrigated with 2.5% NaOCl and the group irrigated with 12.5% green okra fruit extract solution (Table 2).

Table 2.Mann-Whitney test results

	Group
Mann-Whitney U	10,500
Z	-0.516
Asymp.Sig. (2-tailed)	0.606
Exact Sig. [2*(1-tailed Sig.)]	0.690[b]

DISCUSSION

The smear layer is a thin layer formed due to the friction between endodontic instruments and the root canal walls during preparation. The smear layer can serve as a host for microorganisms and protect bacteria from the action of irrigants and medicaments.[13] Therefore, irrigation with a solution capable of removing the smear layer is necessary .[14] SEM imaging in this study was conducted in the middle third of the root canal because this section produces more smear layer compared to the cervical and apical thirds.[15] HThe results of the study showed that Group A received a score of 3 (the smear layer covered most of the root canal walls, with no or only a few open dentinal tubules). A similar result was also observed in Group B, indicating that both materials have the same effectiveness in removing the smear layer from the root canal walls.

The ability to clean the root canal is limited by the effect of the irrigation action itself, which relies on a back-and-forth flow. The back-and-forth flow during irrigation causes debris that has formed an emulsion to flow out along with the irrigation solution.[11] A large amount of smear layer requires thorough cleaning, but in this study, root canal irrigation was still performed using a bevel needle. The use of a bevel needle results in less effective distribution of the irrigation solution.[16] The irrigation procedure in this study should have used a side−vented irrigation needle, which can penetrate the apical third due to its small size. Its lateral hole prevents the irrigation solution from being pushed into the apical foramen.[17]

So Sodium hypochlorite (NaOCl) 2.5% is commonly used as a root canal irrigation solution. Sodium hypochlorite works through saponification, amino acid neutralization, and chloramination reactions.[7] The saponification reaction lowers surface tension, allowing water to easily wet the root canal walls and facilitating the removal of debris from the root canal walls.[11] Sodium hypochlorite also neutralizes amino acids, converting them

into water and salts (neutralization reaction). The released hydroxyl ions cause a pH drop and denature membrane proteins.[18] Sodium hypochlorite has antiseptic properties due to the formation of hypochlorous acid and the release of chlorine compounds, which are bactericidal. The bactericidal activity of sodium hypochlorite occurs because, when NaOCl is added to water (H_2O), it forms hypochlorous acid (HOCl), which contains active chlorine. This active chlorine compound is a strong oxidizing agent that, when combined with amino protein groups, forms chloramines.[19]

Hypochlorous acid (HOCl) and hypochlorite ions (OCl^-) cause amino acid degradation and hydrolysis. The chloramination reaction between chlorine and the amino group (NH) forms chloramines, which disrupt cell metabolism. Several tests have proven that chlorine has antibacterial properties because it can oxidize -SH groups found in essential enzymes, disrupting metabolic functions within bacterial cells.[19] Based on its properties, NaOCl is more effective at cleaning the dentin walls of the root canal because it dissolves both necrotic and vital tissue. Its saponification reaction lowers surface tension.[20]

However, sodium hypochlorite is less effective in cleaning the smear layer, which consists partly of inorganic dentin debris. Sodium hypochlorite can only dissolve organic material and lacks the ability to remove inorganic substances, making its smear layer cleaning capacity less than optimal. In practice, NaOCl is often combined with another irrigant, EDTA.[4] This aligns with research by Eko (2020), which found that the combination of EDTA and NaOCl removes the smear layer more effectively than NaOCl alone.[3] Another study by Surbakti (2022) also demonstrated that EDTA and NaOCl together effectively eliminate the smear layer, as evidenced by the minimal smear layer observed in SEM examinations.[16]

Green okra (Abelmoschus esculentus) extract contains saponins, which act as surfactants (detergents) and can dissolve the smear layer.[21] Saponins function through the saponification reaction, reducing surface tension so that water can easily wet the root canal walls and detach debris from them.[11] Additionally, saponins in green okra can disrupt bacterial cell membrane stability, increasing permeability. Damage and increased permeability in bacterial cells inhibit their growth and ultimately lead to cell death. The surfactant property of saponins is due to the presence of both non-polar (hydrophobic) and polar (hydrophilic) groups.[22]

The non-polar (hydrophobic) group in saponins binds to fats, forming a stable emulsion that can be carried away by water, helping to remove the organic layer. The polar (hydrophilic) group cleans the inorganic Ca^{2+} bound to the root canal walls, making surfactant molecules more easily absorbed by water, reducing surface tension, and enhancing the wetting of the root canal walls.[23] The ability of saponins to clean the smear layer in root canals was demonstrated in Ninggar's (2016) study, which found that a 15% starfruit leaf extract containing saponins effectively removed the smear layer from root canals.[24]

Green okra extract also contains oxalic acid, which functions as a chelating agent, making it effective in removing inorganic substances from root canals.[9] *Chelating agents chemically bind to calcium ions and form soluble calcium compounds. The chemical reaction for chelating agents dissolving the inorganic smear layer is as follows:*

$$Ca_{10}(PO_4)_6(OH)_2 + 8H^+ \rightarrow 10Ca^{2+} + 6(HPO_4)^{2-} + 2H_2O$$

Chelating agents bind to calcium ions in the root canal walls, causing calcium to detach and altering the calcium ratio within the root canal walls. This results in a decrease in dentin hardness, making the root canal dentin softer. Softened dentin facilitates root canal preparation as calcium and other inorganic smear layer components dissolve along with the irrigation solution.[25]

According to El-Askary, as cited in Saskia (2015), acidic substances that contact the root canal walls decompose hydroxyapatite, releasing Ca^{2+} and HPO_4, which dissolve in water, leading to demineralization. The more acidic the substance, the more hydroxyapatite is dissolved.[26]

Green okra extract also contains antibacterial active compounds such as alkaloids, terpenoids, flavonoids, saponins, and tannins.[27] These compounds can dissolve organic tissue, potentially aiding in the removal of the smear layer. Based on these properties, green okra extract is expected to be effective in eliminating the smear layer from root canal walls. However, the findings of this study indicate that 12.5% green okra extract was less effective in removing the smear layer. This may be due to the concentration used (12.5%). The saponin content at this concentration may not have reached the critical micelle concentration (CMC). Surfactants form micelles when they reach a certain concentration called the CMC. When CMC is reached, the surfactant's ability to lower surface tension becomes constant.[28] A constant surface tension results in a consistent ability to clean the root canal walls, highlighting the need for further research with higher extract concentrations.

CONCLUSION

In conclusion, green okra extract (12.5%) dissolves the smear layer through saponification, exhibits antibacterial properties, and can dissolve inorganic components due to its acidic content. In contrast, NaOCl 2.5% cleans the smear layer through saponification, amino acid neutralization, and chloramination reactions but lacks the ability to dissolve inorganic components. Therefore, 12.5% green okra extract has greater potential to clean the smear layer compared to NaOCl 2.5%, which requires a combination with 17% EDTA as a chelating agent. Further studies are needed to determine the effectiveness of higher extract concentrations.

Declaration by Authors

Ethical Approval: Approved

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