

Cleaning effect of 25% green okra extract on root canal wall smear layer: an experimental study

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ABSTRACT

Introduction: Ethylenediaminetetraacetic acid (EDTA) removes the inorganic components of the smear layer, whereas sodium hypochlorite (NaOCl) removes its organic components. These two irrigants are commonly used in combination to clean the smear layer effectively. However, both materials have limitations, necessitating exploration of alternative agents that can remove both components of the smear layer. Green okra extract, which contains saponins and oxalic acid, may serve as a potential alternative. This study aimed to analyze the effect of 25% green okra extract on smear layer removal. **Methods:** This study employed a post-test-only control-group design. Extracted permanent premolar teeth were sectioned at the cemento-enamel junction, and the root portions were embedded in wax blocks. We prepared the root canals using a conventional technique. Five samples were irrigated with a combination of 17% EDTA and 2.5% NaOCl (Group A), while five samples were irrigated with 25% green okra extract (Group B). Each sample was sectioned into three parts, and the middle third was examined using a scanning electron microscope (SEM). We scored the smear layer based on observed surface characteristics and used the mode to represent the smear-layer distribution in each group. **Results:** The mode score for Group A was 3, indicating a partially covered root canal surface with a smear layer and a few open dentinal tubules, whereas the mode score for Group B was 4, indicating that the root canal wall was covered with a smear layer and no open dentinal tubules were observed. The Mann-Whitney U test showed no statistically significant difference in smear layer removal between the two groups ($p = 0.221$). **Conclusion:** The smear layer cleaning effect of 25% green okra extract was not significantly different from that of the combination of 17% EDTA and 2.5% NaOCl. Therefore, 25% green okra extract is a potential alternative root canal irrigant for smear layer removal.

Keyword

Green okra extract, irrigation solution, root canal wall, smear layer, scanning electron microscope

Potensi ekstrak okra hijau (*Abelmoschus esculentus*) konsentrasi 25% terhadap pembersihan smear layer dinding saluran akar gigi: studi eksperimental laboratoris

ABSTRAK

Pendahuluan: Sebagai irigasi saluran akar, EDTA 17% menghilangkan komponen anorganik smear layer, sedangkan NaOCl 2,5% menghilangkan komponen organik. Kedua bahan ini digabungkan untuk membersihkan smear layer. Kedua bahan tersebut memiliki kekurangan, sehingga diperlukan bahan alternatif yang lebih aman untuk membersihkan kedua komponen smear layer yaitu 25% ekstrak okra hijau yang mengandung saponin dan asam oksalat. Tujuan penelitian ini adalah untuk menganalisis pengaruh ekstrak okra hijau 25% terhadap pembersihan smear layer. **Metode:** Studi ini menggunakan desain post-test-only control-group. Gigi premolar permanen yang telah diekstraksi dipotong sebatas semen-enamel, lalu bagian akar ditanam dalam balok malam merah. Dilakukan preparasi saluran akar dengan teknik konvensional, dimana lima sampel diirigasi dengan kombinasi EDTA 17% dan NaOCl 2,5% (kelompok A), lima sampel dengan 25% ekstrak okra hijau (kelompok B). Sampel dipotong menjadi 3 bagian, dan bagian sepertiga tengah kemudian diamati dengan Scanning Electron Microscope. Hasil pemotretan dinilai, dan skor yang paling sering muncul (modus) mewakili distribusi smear layer sampel. **Hasil:** Modus kelompok A adalah 3, yaitu permukaan smear layer tertutup sebagian, dengan beberapa tubulus dentin terbuka, sedangkan kelompok B adalah 4, berarti bahwa dinding saluran akar ditutupi dengan smear layer, dan tidak ada tubulus dentin yang terbuka. Uji statistik menggunakan Uji U Mann-Whitney menunjukkan $p=0,221$, artinya tidak ada perbedaan signifikan antara kedua kelompok. **Simpulan:** Efek pembersihan ekstrak okra hijau 25% pada smear layer setara dengan kombinasi EDTA 17% dan NaOCl 2,5%. Oleh karena itu, ekstrak okra hijau 25% berpotensi menjadi alternatif bahan irigasi saluran akar untuk menghilangkan smear layer.

Kata kunci

Ekstrak okra hijau, larutan irigasi, dinding saluran akar, smear layer, scanning electron microscope

INTRODUCTION

Debris forms during root canal preparation as a result of the interaction between the preparation instruments and the canal walls, leaving an irregular, amorphous smear layer. It contains both organic and inorganic components, including viable bacteria, dentin debris, and remnants of pulp tissue.¹⁻³ The smear layer along the root canal walls may facilitate bacterial survival and colonization by serving as a bacterial reservoir and protecting bacteria from irrigants and intracanal medications.⁴⁻⁶

Moreover, the smear layer prevents obturation materials from adhering to the canal walls, increasing the possibility of interfacial leakage between the filling material and the dentinal surface, which may eventually result in treatment failure.⁷ Therefore, root canal irrigation techniques should be used to eliminate the smear layer.⁸⁻¹¹ Irrigating fluids capable of dissolving both organic and inorganic matter are necessary for effective canal cleansing.¹² The most commonly used irrigants in dentistry are 17% ethylenediaminetetraacetic acid (EDTA) and 2.5% sodium hypochlorite (NaOCl).¹

A 17% EDTA solution binds to calcium ions in dental hard tissues, acting as a chelating agent that removes the smear layer and demineralizes dentinal tubules.¹³ This demineralization significantly reduces the mineral concentration of dentin, which is associated with decreased microhardness and, consequently, a weaker structure.^{14,15} This solution effectively removes the inorganic components and associated debris but cannot dissolve organic matter. Additionally, it can cause moderate to severe tissue inflammation and excessive dentinal erosion.¹⁶ EDTA has limited antibacterial properties; therefore, it is often used in combination with sodium hypochlorite (NaOCl) to achieve both antibacterial activity and effective debris removal from the root canal system.^{17,18}

Sodium hypochlorite is commonly used as a root canal irrigant at a concentration of 2.5% to break down organic material in the smear layer. It is affordable, has strong antibacterial properties, and provides lubrication during instrumentation.^{17,19} However, if accidentally extruded into the surrounding tissues, it can cause serious chemical damage, including tissue necrosis, rapid hemolysis, ulceration, inflammation, severe pain, and swelling.²⁰ Furthermore, NaOCl cannot completely remove the smear layer because it primarily affects organic matter and does not dissolve inorganic substances, resulting in incomplete cleaning of the root canal.^{1,21}

Root canal irrigation is crucial for endodontic treatment because it facilitates tissue dissolution and debris removal. An effective irrigant should dissolve both organic and inorganic materials and reach all areas of the root canal.²² An ideal irrigating solution should not only possess antimicrobial properties but also effectively remove the smear layer from the canal walls. Clinicians commonly use a combination of 17% EDTA and 2.5% NaOCl for this purpose, although these substances have certain limitations.¹⁸ There is growing interest in identifying alternative agents for smear layer removal, such as green okra (*Abelmoschus esculentus*).²³

Green okra contains bioactive compounds such as alkaloids, flavonoids, tannins, and terpenoids, which contribute to its antibacterial properties.^{24,25} Studies have shown that a 50% green okra extract can inhibit the growth of *Enterococcus faecalis*, while lower concentrations of 6.25% and 12.5% have also demonstrated inhibitory effects.²⁶ Additionally, these lower concentrations can inhibit *Streptococcus viridans*.²⁷ At higher concentrations (12.5%, 25%, 50%, and 100%), the extract has demonstrated antibacterial effects against *Staphylococcus aureus*²⁸ and *Streptococcus mitis*.²⁹

Green okra contains oxalic acid and saponins. Saponins act as natural surfactants, helping remove debris from root canal walls by reducing surface tension and facilitating the breakdown and removal of organic and inorganic materials.³⁰⁻³² Oxalic acid, similar to EDTA, binds to calcium ions and helps remove inorganic matter from root canals.⁸ A study found that 2.5% NaOCl and 12.5% green okra extract were equally effective smear layer removal agents, with no significant difference between them.³³ Another study indicated

that 12.5% green okra extract removed the smear layer similarly to a combination of 17% EDTA and 2.5% NaOCl, with no significant difference. These findings suggest that green okra may be a promising alternative for smear layer removal.²³

Given this background, this study aimed to evaluate the effectiveness of 25% green okra extract in removing the smear layer from root canal walls. The 25% concentration was selected as a logical stepwise increase from the previously evaluated 12.5% concentration to investigate the dose-response relationship and determine whether a higher concentration could improve smear layer removal efficacy. We hypothesized that increasing the extract concentration may enhance its cleaning efficacy. This formulation was proposed as a novel natural endodontic irrigant that may integrate the beneficial properties of both EDTA and NaOCl into a single irrigation solution. This study aimed to analyze the effect of 25% green okra extract on smear layer removal.

METHODS

This laboratory-based experimental study used a post-test-only control-group design. The research was conducted from May to June 2024 at the Bioscience Laboratory of the Dental Hospital, the Integrated Laboratory of Dentistry at the University of Jember, and the Laboratory of Technology and Life Sciences (LITHR) at Airlangga University. We obtained green okra extract by maceration. The sample consisted of ten extracted permanent premolar teeth. The teeth were sectioned at the cemento-enamel junction using a high-speed diamond disk, leaving the root portions intact. The root samples were embedded in wax blocks and divided into two treatment groups, with five samples in each group: Group A received a combined irrigation solution containing 17% EDTA and 2.5% NaOCl, whereas Group B received an irrigation solution containing 25% green okra fruit extract. Group A served as the comparison group, and Group B was the test group.

The samples were prepared for root canal preparation using conventional techniques with K-files ranging from size 8 to size 40, using watch-winding movements. Irrigation of the root canal was performed after each change of instrument.³³ Irrigation was performed using a 0.1-mL Endo-irrigation needle (Maxiprobe), and the irrigation solution was left in the root canal for 30 seconds,³⁴ according to the treatment group. The irrigation solution was then removed using an empty disposable syringe, and the canals were dried with paper points.

The samples were sectioned longitudinally into two parts and then divided into three sections.²³ The middle third of the root was selected for imaging using a scanning electron microscope (SEM) at 7000× magnification. Images were obtained from eight areas to represent the entire surface under investigation. Each image was divided into 10 grids, and three observers scored each grid independently. The most frequently occurring score (mode) for each grid represented the smear layer distribution in the sample. We assessed smear-layer cleanliness based on the presence of open dentinal tubules on the root canal wall and the removal of the smear layer from the dentinal tubules, according to the scoring system described by Hülsmann.^{35,36}

The scoring criteria were as follows: Score 1, absence of a smear layer, with the orifices of the dentinal tubules uncovered; Score 2, a small amount of smear layer, with some dentinal tubules open; Score 3, the root canal wall partially covered by a smear layer, with no or only a few dentinal tubules open; Score 4, the entire root canal wall covered by a uniform smear layer, with no dentinal tubules uncovered; and Score 5, the entire root canal wall covered by a uniform, very thick, and abundant smear layer. The data were ordinal; therefore, we used a nonparametric statistical test, the Mann–Whitney U test, to compare Group A and Group B.

RESULTS

The level of smear layer removal was evaluated by assessing the extent to which the dentinal tubules were covered by smear debris and the number of tubules that were visibly open on the surface of the root canal wall. SEM images of the specimens treated with 17% EDTA and 2.5% NaOCl and those treated with 25% green okra extract are presented in Figure 1, showing differences in smear layer removal.

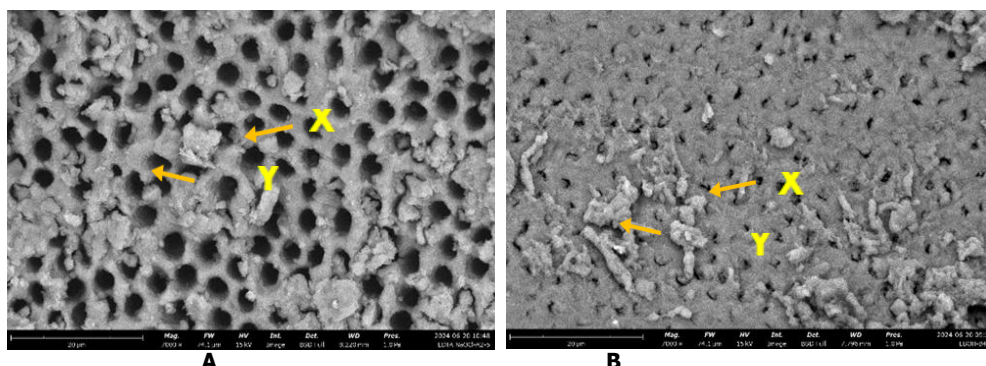


Figure 1. Scanning electron microscopy (SEM) images of the root canal wall surface after irrigation with (A) a combination of 17% EDTA and 2.5% NaOCl and (B) 25% green okra extract, both at 7000 $\times$ magnification. (X) Open dentinal tubules; (Y) smear layer.

Group A demonstrated a mode value of 3, whereas Group B showed a mode value of 4. These findings suggested that specimens irrigated with a combination of 17% EDTA and 2.5% NaOCl may have shown greater smear layer removal and cleaner canal walls than those treated with 25% green okra extract. Figure 2 illustrates the distribution of smear layer scores based on the most frequently observed value (mode). Statistical analysis using the Mann–Whitney U test yielded a p-value of 0.221 ($p > 0.05$), indicating that the difference between the two groups was not statistically significant (Table 1).

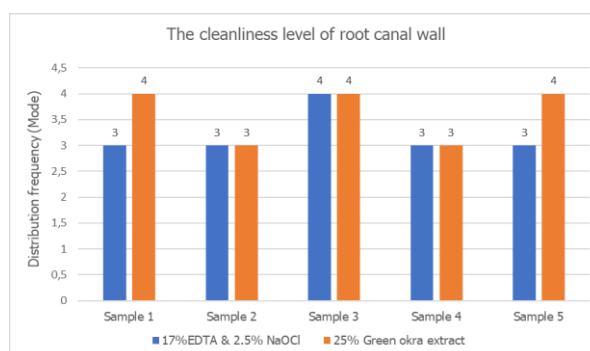


Figure 2. Frequency distribution of smear layer cleanliness scores (mode) for root canal walls irrigated with 17% EDTA and 2.5% NaOCl and with 25% green okra extract.

Table 1. Test statistic

Smear layer removal mode	
Mann-Whitney U	7.500
Wilcoxon W	22.500
Z	-1.225
Asymp. Sig. (2-tailed)	.221
Exact Sig. [2*(1-tailed Sig.)]	.310 ^b

DISCUSSION

Root canal irrigation is essential in endodontic treatment because it helps remove debris, necrotic tissue, and other materials from the canal walls.^{37,38} Research has shown that 25% green okra extract can help remove the smear layer from root canals. Nissa et al.²³ conducted a similar study but used green okra extract at a concentration of 12.5%. The difference between the two studies was the mode values obtained. In the present study, the mode of root canal wall cleanliness after irrigation with green okra extract ranged from 3 to 4, whereas in Nissa et al.'s study, it ranged from 2 to 3. This difference may be associated with increased okra extract concentration, which increases viscosity due to its mucilage content. Higher concentrations of *Abelmoschus esculentus* mucilage are known to increase solution viscosity, potentially affecting the flow characteristics and contact of the irrigant with the root canal walls. Consequently, increasing the extract concentration from 12.5% to 25% may not necessarily improve smear layer removal efficacy.³⁹

Differences were also observed in the group irrigated with a combination of NaOCl and EDTA. In the present study, mode values of 3 and 4 were obtained, whereas in Nissa et al.'s study, the mode values were 1 and 2. Compared with Nissa et al.'s study, the present study showed higher mode values. The depth of needle penetration during irrigation and the anatomical characteristics of the root canal, which affect irrigant distribution, penetration, and contact with the root canal walls, may also influence outcome variation.^{40,41} Both investigations evaluated the middle third of the root; however, differences in the specific location of the observation field within this segment could still have affected the smear layer scores. This may be because the condition of the root canal wall surface varies along its length.⁴² This distinction may help explain the differences in mode values between the two studies.

The differences in mode values of root canal wall cleanliness among the irrigation solution groups in the present study indicated different cleanliness levels were most frequently observed in each group. These differences may be attributed to the chemical characteristics and mechanisms of action of each irrigation solution in removing the organic and inorganic components of the smear layer. Okra extract contains various bioactive compounds, making it a potential alternative irrigation solution.²³ Saponins, a key component of green okra extract, help reduce surface tension by acting as surfactants.²⁹ This property may allow the irrigation solution to penetrate more effectively and facilitate the removal of debris from the tooth surface.³⁵

The amphiphilic structure of saponins accounts for their cleansing properties. They consist of a hydrophilic, sugar-based component that is readily soluble in water and a hydrophobic, steroidal or triterpenoid component that is soluble in lipids.^{3,43} Due to this dual property, saponins can accumulate at interfaces, reduce surface tension, and facilitate the formation of emulsions.²¹ The hydrophobic portion binds to lipid-containing particles, whereas the hydrophilic portion interacts with water molecules and forms hydrogen bonds, thereby increasing solubility. This process forms stable emulsions that the irrigant can remove. The non-polar groups facilitate the removal of organic materials, such as saliva, bacterial remnants, and pulp tissue remnants, whereas the polar groups facilitate the removal of inorganic components, such as hydroxyapatite particles and dentin fragments.^{43,44}

Saponins enhance the wettability of the irrigation solution by reducing surface tension, allowing it to penetrate more deeply into dentinal tubules and irregular canal surfaces that may otherwise exhibit limited wettability. Lower surface tension enhances capillary penetration, improving interaction with the canal walls and increasing the solution's cleaning and disinfection capacity.⁴⁵ As a result, the flushing action of irrigation may more effectively remove and transport debris and remnants of the smear layer.²¹ In addition to reducing surface tension, saponins exhibit detergent-like properties by emulsifying oily

materials and removing debris from the dentin surface. Because they are surface-active agents containing both hydrophilic and hydrophobic groups, they can bind to components of the smear layer and maintain them in suspension within the irrigating fluid. The dynamic flow of the irrigation solution within the root canal system facilitates debris removal through this suspension mechanism.¹

Additionally, saponins act through emulsification and micelle formation mechanisms. Micelles, which are composed of surfactant molecules, are important for capturing and removing contaminants from canal walls. By attaching to debris and breaking it into smaller particles, the hydrophobic component of the saponin molecule facilitates its incorporation into water-based emulsions. Meanwhile, the hydrophilic component remains soluble in the aqueous medium and contributes to foam formation, which helps encapsulate and stabilize dispersed particles within the emulsion. These mechanisms may facilitate emulsification and solubilization, which are commonly used to describe how saponins solubilize organic materials in the smear layer and emulsify lipid-containing substances. Because of their detergent-like properties, saponins may help remove both organic and inorganic components of the smear layer. The emulsified debris can be effectively rinsed from the root canal system when active irrigation with dynamic back-and-forth fluid movement is applied.²¹

Oxalic acid is another component of green okra extract. Oxalic acid acts as a chelating agent that targets the inorganic component of the smear layer, particularly hydroxyapatite, by forming soluble calcium complexes through binding to calcium ions. As a result of this interaction, calcium is released from dentin through the formation of a calcium-chelate complex.⁴⁶ Chelating agents alter the mineral balance of dentin by interacting with hydroxyapatite crystals, thereby facilitating the removal of calcium from the root canal wall. This process decreases dentin microhardness, making the underlying surface softer and potentially easier to manipulate. As a result, irrigation solutions may more effectively dissolve and remove inorganic smear-layer components, such as calcium-based particles.⁸

Because of oxalic acid, green okra extract is acidic. Substances with a low pH can contribute to smear layer removal through demineralization, during which the mineral component of dentin, primarily hydroxyapatite, is dissolved. When an acidic solution interacts with the dentin surface, calcium and phosphate ions are released from the hydroxyapatite crystal structure, leading to dissolution of the mineralized framework. Higher concentrations of hydrogen (H^+) ions promote the replacement of hydroxyl (OH^-) ions in hydroxyapatite, resulting in the formation of water (H_2O). The depletion of hydroxyl ions facilitates the demineralization of inorganic phosphate, leading to the formation of hydrogen phosphate (HPO_4^{2-}). The formation of HPO_4^{2-} disrupts the structural stability of hydroxyapatite crystals, causing partial dissolution during irrigation. Therefore, acidic endodontic irrigants may contribute to the degradation of the mineralized smear layer and dentin demineralization by disrupting hydroxyapatite crystals, which may improve smear layer removal and promote exposure of the dentinal tubules.⁴⁷

SEM analysis showed that a small amount of smear layer remained after irrigation with 17% EDTA and 2.5% NaOCl. NaOCl removes organic tissue through saponification, amino acid neutralization, and chlorination, but it cannot eliminate inorganic components of the smear layer.⁴⁸ Therefore, it is commonly combined with 17% EDTA to achieve more complete smear layer removal. A 17% EDTA solution effectively removes the smear layer by chelating inorganic components, particularly calcium ions in dentin, resulting in decalcification and softening of the smear layer and underlying dentin.⁴⁹ Its demineralizing action enlarges dentinal tubules, reduces dentin hardness, and may affect collagen.⁵⁰ The formation of soluble calcium-EDTA complexes contributes to its cleaning effect,⁵¹ and when combined with 2.5% NaOCl, it provides more thorough smear layer removal because both inorganic and organic components are targeted.⁵²

Based on the results of this study, Group A (17% EDTA with 2.5% NaOCl) obtained a mode score of 3, whereas Group B (25% green okra extract) obtained a mode score of 4.

However, the two groups did not differ significantly. This finding suggests that 25% green okra extract may have a smear layer removal effect comparable to that of the combination of 17% EDTA and 2.5% NaOCl. Increasing the green okra extract concentration to 25% did not appear to yield significantly greater cleaning efficacy than the 17% EDTA and 2.5% NaOCl regimen.

Therefore, future studies using precisely standardized concentrations of green okra extract are recommended. The small sample size ($n = 5$ per group) may have reduced statistical power and limited the generalizability of the findings. One limitation of this study was that the irrigation solution formulated from pure green okra fruit extract had a limited shelf life and required refrigeration. Failure to use the solution promptly or to maintain appropriate storage conditions may affect the stability and activity of its bioactive compounds.

CONCLUSION:

The cleaning effect of 25% green okra extract on the root canal smear layer was comparable to that of a combination of 17% EDTA and 2.5% NaOCl. Further studies are needed to develop methods to preserve green okra fruit extract and extend its shelf life without compromising its chemical composition, and to evaluate its tissue-dissolving capacity, biocompatibility, and clinical performance before clinical application. These findings suggest that the smear layer removal efficacy of 25% green okra extract, together with its previously reported antimicrobial activity, may support its potential as a promising natural endodontic irrigant.

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