Comparison of the potential of green okra (*Abelmoschus esculentus*) extract and a combination of 17% EDTA and 2.5% NaOCl in removing the smear layer from dental root canal walls: experimental laboratory

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ABSTRACT

Introduction: The smear layer is a thin film of organic and inorganic substances that can inhibit intracanal medication penetration, necessitating removal with root canal irrigants. Commonly used irrigants include a combination of 17% EDTA and 2.5% NaOCl, but EDTA is ineffective against organic components, while NaOCl may cause periapical pain and instrument corrosion. Green okra extract contains active antibacterial compounds, showing potential as an alternative. This research aimed to compare the potential of green okra extract with that of 17% EDTA and 2,5% NaOCl in smear layer removal. **Methods:** This research is a laboratory experiment with a posttest only control group design. The treatment groups consisted of 12.5% green okra extract and a combination of 17% EDTA and 2,5% NaOCl root canal irrigation agents. Ten mandibular premolars were sectioned at the cemento-enamel junction then prepared using step-back technique. Five samples were irrigated with 0,1 ml of 12.5% green okra extract, while the other five received 0,1 ml 17% EDTA and 2.5% NaOCl. The cleanliness of the root canal walls was evaluated using a Scanning Electron Microscope (SEM) at 5000x magnification, and data were analyzed using the Mann-Whitney U test. **Result:** Both groups exhibited similar levels of root canal cleanliness (p=0.093), with a cleanliness score of 2, indicating mostly open dentin tubule orifices and minimal smear layer. **Conclusion:** Green okra extract (12.5) demonstrated comparable potential to a combination of 17% EDTA and 2.5% NaOCl in removing the smear layer from root canal walls.

Keywords

green okra extract, smear layer, root canal wall.

Perbandingan potensi ekstrak okra hijau (Abelmoscus esculentus) dengan kombinasi EDTA 17% dan NaOCl 2,5% dalam membersihkan smear layer dinding saluran akar gigi: eksperimental laboratoris

ABSTRAK

***Pendahuluan**: Smear layer adalah lapisan tipis yang berisi substansi organik dan anorganik. Smear layer dapat menghambat penetrasi medikamen intrakanal ke dalam tubulus dentin sehingga harus dibersihkan dengan bahan irigasi saluran akar. Bahan irigasi saluran akar yang biasa digunakan adalah kombinasi EDTA 17% dan NaOCl 2,5%. Namun, EDTA 17% tidak dapat membersihkan substansi organik dari smear layer, sedangkan NaOCl 2,5% dapat menimbulkan nyeri pada jaringan periapikal serta menimbulkan korosi instrumen endodontik. Ekstrak okra hijau mengandung senyawa antibakteri aktif, sehingga berpotensi sebagai alternatif bahan irigasi. Penelitian ini bertujuan untuk menganalisis perbandingan potensi ekstrak okra hijau dengan kombinasi EDTA 17% dan NaOCl 2,5% dalam membersihkan smear layer dinding saluran akar gigi. **Metode:** Jenis penelitian eksperimental laboratoris dengan rancangan posttest only control group design. Kelompok perlakuan konsentrasi ekstrak okra hijau 12,5% dan kombinasi EDTA 17% dan NaOCl 2,5%. Sepuluh premolar mandibula dipotong di cemento-enamel junction kemudian dipreparasi menggunakan teknik step back. Lima sampel di irigasi dengan ekstrak okra hijau 12,5% sebanyak 0,1 ml dan lima sampel di irigasi dengan EDTA 17% sebanyak 0,1 ml 17% dan NaOCl 2,5% sebanyak 0,1. Sepuluh sampel dinilai skor kebersihan dinding saluran akarnya menggunakan Scanning Electron Microscope (SEM) dengan perbesaran 5000x dan dilakukan uji beda menggunakan Mann Whitney U test. **Hasil:** Kebersihan dinding saluran akar pada dua kelompok sampel memiliki tingkat kebersihan yang sama. Analisis data menggunakan Mann Whitney U test. diperoleh nilai p=0,093 yang berarti bahwa tidak ada perbedaan yang signifikan antara dua kelompok. Kedua kelompok memiliki skor 2, artinya sebagian besar orifis tubuli dentin terbuka dan terdapat sedikit smear layer. **Simpulan:** Ekstrak okra hijau 12,5% mempunyai potensi yang sama dengan kombinasi larutan EDTA 17% dan irigasi NaOCl 2,5% dalam membersihkan smear layer dinding saluran akar.*

Kata kunci

ekstrak okra hijau, smear layer*, dinding saluran akar gigi.*

INTRODUCTION

Root canal treatment is the act of removing infected pulp tissue from the pulp chamber and root canal, then filling with root canal filling material to prevent further abnormalities or re-infection.[1] Biomechanical preparation in root canal treatment includes the use of endodontic instrumentation tools and the cleaning of infected pulp and dentin tissue. The friction between the endodontic tool and the root canal wall during preparation causes the formation of a layer of debris attached to the root canal wall called the smear layer.[2]

Dental smear layer is a thin layer containing organic and inorganic components, such as residual dentin, odontoblastic processes, pulp tissue and bacteria.[3] Smear layer consists of two parts, superficial layer which covers the surface of the dentin and smear plugs which block dentin tubules.[4] The presence of a smear layer on the root canal wall can lead to the growth of bacterial colonies, disrupt sealer adaptation and penetration of intracanal medicaments in the root canal. Therefore, an irrigation solution is needed that functions to clean the root canal from organic debris, microorganisms, residual pulp tissue, smear layer, and endotoxin as well as a lubricant.[5]

One of the irrigation materials widely used in root canal treatment is EDTA (Ethylene Diamine Tetraacetic Acid) with a concentration of 17%. EDTA is an irrigation material that can dissolve inorganic components of the smear layer, but is unable to dissolve organic tissue.[6] The use of EDTA must be combined with 2.5% NaOCl to remove organic material from the smear layer. The combination of the two irrigants complements root canal cleaning especially in areas that are difficult to access, such as dentinal tubules and lateral canals.[7] However, NaOCl has toxic effects on tissues that can cause allergic reactions, pain in periapical tissues, spontaneous swelling, is alkaline, and corrosive to metals that can damage the instruments used.[8] Thus, alternative natural materials are needed that can be used as irrigation materials.

One of the natural materials that can be used as an alternative irrigation material is green okra (*Abelmoschus esculentus*). Green okra contains oxalic acid compounds that can clean inorganic components of the smear layer.[9] Oxalic acid is a strong organic acid.[10] Oxalic acid has an ability to demineralizes inorganic smear layer components such as calcium hydroxyapatite so that it dissolves with the irrigation material.[11] In addition, green okra contains active compounds that are antibacterial in nature such as alkaloids, terpenoids, flavonoids, saponins and tannins.[12] The content of these active compounds is very necessary for one of the requirements of root canal irrigation materials, namely antibacterial. The saponin content in green okra acts as a natural surfactant. Surfactants are able to reduce surface tension which can remove organic and inorganic tissue, potentially removing the smear layer.[13]

The antibacterial properties of green okra have been supported by research which states that green okra extract with a concentration of 12.5% can inhibit *Enterococcus faecalis*.[14] Green okra extract 12.5% is able to inhibit *Staphylococcus aureus*.[15] Based on the earlier study described above, the concentration of green okra extract used was 12.5%. Based on the explanation above, there has been no research on the ability of green okra extract to remove a smear layer on the walls of dental root canals, making the author interested in studying the potential of 12.5% green okra extract in cleaning smear layer on the walls of dental root canals. The novelty of the research can be found in green okra extract that is used as an irrigation agent for root canal walls. The purpose of the research are Penelitian ini bertujuan untuk menganalisis perbandingan potensi ekstrak okra hijau dengan EDTA 17% dan kombinasi NaOCl 2,5% dalam membersihkan smear layer dinding saluran akar gigi.

METHODS

The type of research conducted is a type of laboratory experimental research with a Posttest Only Control Group Design. The research was conducted from December 2023 until January 2024 at Bioscience Laboratory Faculty of Dentistry University of Jember, Dental Conservation Clinic RSGM University of Jember, and Laboratory of the Institute of Life Sciences and Engineering (LIHTR) Airlangga University. This research used the Daniel (1991) formula for calculating the minimum amount of sample. In this study there are 5 samples of mandibular premolar irrigated with green okra extract 12,5% and 5 samples of mandibular premolar irrigated with combination of EDTA 17% and NaOCl 2,5%. The independent variable of this research is green okra extract 12,5%, meanwhile the dependent variable is the level of cleanliness of the smear layer on the root canal walls.

The tools and materials are used for make green okra extract are autoclave machine, knife, digital balance, Erlenmeyer flask, volumetric flask, blender, 40 mesh sieve, macerator, filter buncher, rotary vacuum evaporator, funnel glass, glass jar, mixing spatula, filter paper, aluminium foil, and gloves, green okra fruit and ethanol 96%. The tools and materials are used for root canal preparation and irrigation procedure are separating diamond disk, ruler, red paraffin block, paraffin knife, bunsen burner, type k file number 8-45, extirpation needle (Dentsply spiro colorinox barbed broach), disposable syringe, maxiprobe (Pro rinse), irrigation bottle, tweezers, clean stand (Dentsply maillefer), endo block (Dentsply maillefer), stainless steel endo, oven, insulated Petri dish, green okra extract 12,5%, EDTA 17%, and NaOCl 2,5%. The tools and materials used for observation of smear layer images are scanning electron microscope and transparent sheets.

The criteria of green okra fruit in this research were fresh, ripe, dark green, and the harvest time is 5-7 days after flowering. Firstly, green okra fruits were washed by water then cut into small pieces and dried at room temperature. After that, dried green okra were blended using a blender into powder then sifted using 40 mesh sieve until it became a fine powder. The fine powder of green okra was macerated by 96% ethanol solvent for 3 days. The macerated green okra extract were filtered using filter paper then evaporated with a rotary evaporator for 24 hours so it becomes green okra extract 100%. After that, green okra extract 100% was diluted by serial dilution method using distilled water to get green okra extract 12,5%.

The criteria of 10 first mandibular premolar in this research were root length at least 13 mm without root caries, fracture of the root, and the apex is completely closed. The crown of the mandibular first premolar tooth was cut using separating diamond disk in the mesio-distal direction at the level of the cemento-enamel junction, then the working length is measured with ruler starting from the CEJ to the apical then reduced by 1 mm, the first mandibular premolar roots are planted in a red paraffin block leaving ±2 mm CEJ area. After that, the root was preparated by k-file number 8-45 using step back technique. The root was irrigated using a disposable syringe in every change of k-file number. Root canal was irrigated by 0,1 ml green okra extract 12,5% for 30 seconds and 0,1 ml EDTA 17% and 0,1 ml NaOCl 2,5% for 30 seconds.

Samples were cut longitudinally in a mesio-distal direction into 2 parts, namely buccal and palatal, then samples were cut into 3 parts using separating diamond discs, so that the middle 1/3 of the tooth root is obtained. The parts observed by scanning Electron Microscope (SEM) is the middle 1/3 of the tooth root canal wall at 5000x magnification. The SEM results were observed by three observers by calculating the smear layer score of each box in each sample totaling 10 boxes and then calculating the score that appears most often (mode).
The images were analyzed independently by three blinded evaluators according to the following five score criteria for both the smear layer and debris removal evaluations. Score 1: no detectable smear layer, and clean root canal walls with very little to no debris; all dentinal tubules were clean and open, score 2: clean surfaces containing small agglomerations of debris and/or a thin homogeneous smear layer; most of the dentinal

tubules were open, score 3: many agglomerations of debris, and a homogenous smear layer covering <50% of the canal wall; only a few dentinal tubules were open, score 4: mostly contaminated surfaces with a heavy homogenous smear layer and a large amount of debris covering >50% of root canal walls; no dentinal tubules were open, score 5: contaminated root canal walls entirely covered by a heavy and inhomogeneous smear layer and debris.

The data obtained in this study was tabulated and then analyzed using the SPSS (Statistical Product and Service Solutions) program. The score of the smear layer is an ordinal-scale data then tested using the non-parametric Mann Whitney U test.[16] The research flow in this study is shown in the figure below:

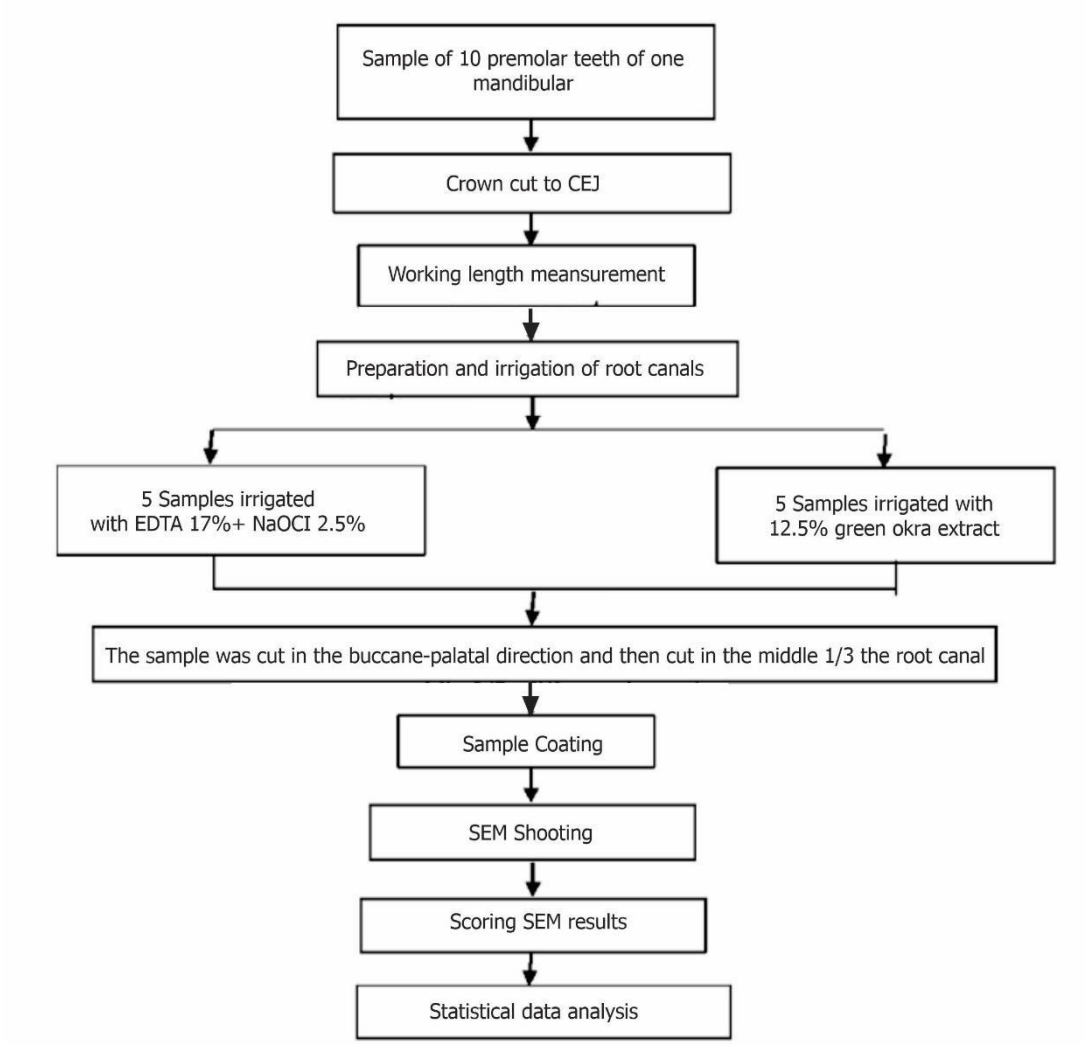

Figure 1. Research Flow

RESULTS

The cleanliness of the smear layer is indicated by the loss of the smear layer that covers the dentin tubules on the root canal wall as seen in SEM image. The smear layer of the root canal wall irrigated with a solution of 12.5% green okra extract and 17% EDTA solution and 2.5% NaOCl can be seen from the SEM photo with a 5000x magnification as shown in Figure 2

.

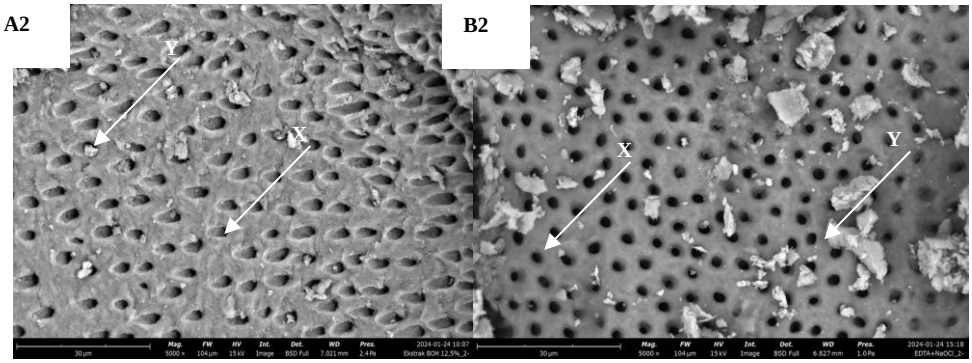

Figure 2. SEM Description The surface of the root canal wall after irrigation was 12.5% green okra fruit extract and 17% EDTA solution and 2.5% NaOCl with 5000x magnification (A2 and B2 samples). (X) = Dentin tubules that open on the surface of the root canal wall; (Y) = Smear layer that covers the dentin tubules on the surface of the root canal wall.

Figure 2 (A2) shows that on the surface of the root canal walls irrigated with 12.5% green okra extract, it appears that there is a slight smear layer with partially exposed dentinal tubules. Figure (B2) shows that on the surface of the root canal walls irrigated with 17% EDTA solution and 2.5% NaOCl, it appears that some dentinal tubules are open with a smear layer covering more dentinal tubules than the surface of the root canal irrigated with 12.5% okra extract. The most frequent score (mode) in each box is the smear layer frequency distribution of the sample shown in Table 1.

Table 1. Distribution of smear layer frequencies in each sample group

Green okra extract 12,5%					**EDTA 17% and NaOCl 2,5%**				
Sample	**Observer**			**Mode**	**Sample**	**Observer**			**Mode**
	O1	O2	O3			O1	O2	O3	
Sample 1	2	2	2	2	Sample 1	1	1	1	1
Sample 2	2	2	2	2	Sample 2	2	2	2	2
Sample 3	3	3	3	3	Sample 3	2	2	2	2
Sample 4	2	2	2	2	Sample 4	2	2	2	2
Sample 5	3	3	3	3	Sample 5	2	2	2	2
Total score			**12**		**Total score**			**9**	
Mode			**2**		**Mode**			**2**	

Table 1 shows that the cleanliness mode of the root canal wall smear layer in group A irrigated with 12.5% green okra extract is the same as that of group B irrigated with 17% EDTA solution and 2.5% NaOCl. Both groups received a layer 2 smear cleanliness score (there was a slight smear layer with some of the dentinal tubules exposed). The smaller the value of the smear layer, the cleaner the surface of the root canal wall. This shows that 12.5% green okra extract has the same ability as EDTA 17% and NaOCl 2.5% in cleaning the root canal wall smear layer. The difference in the cleanliness level of the root canal wall smear layer can be seen in Figure 3.

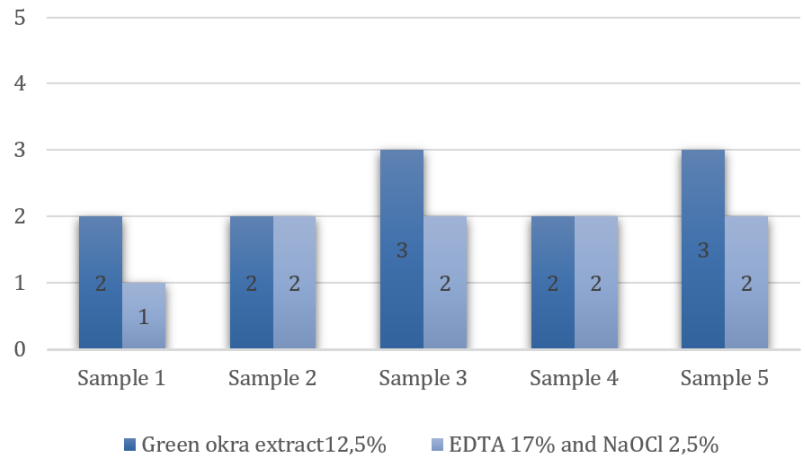

Figure 3. Frequency distribution (mode) of the cleanliness level of the root canal wall smear layer irrigated with green okra extract of 12.5%

Table 2. Statistical test result of Mann Whitney U Test

Rank				
	Group	**n**	**Mean rank**	**Sum of ranks**
Green okra extract - EDTA+NAOCL Test	Green okra extract	5	6.80	34.00
	EDTA+NAOCL	5	4.20	21.00
	Total	**10**		

Test statistics[a]	**Green okra extract -EDTA+NAOCL Test**
Mann-Whitney U	6.000
Wilcoxon W	21.000
Z	-1.68
Asymp.Sig.(2-tailed)	.093
Exact Sig.[2*(1-tailed Sig.)]	.222[b]

Note: a. Grouping variable: Group; b. Not corrected for ties

Table 2 shows that based on the results of the Mann Whitney U Test, a significance value of p=0.093 was obtained. This showed that there was no significant difference between the two groups, namely group A irrigated with green okra extract 12.5% and group B irrigated with EDTA solution 17% and NaOCl 2.5%.

DISCUSSION

The results showed that 12.5% green okra extract was able to clean the smear layer on the surface of the root canal wall. This is because green okra contains saponin compounds. Saponin is a surfactant active compound with the ability as a cleaning agent. Surfactant properties in saponins are caused by the presence of non-polar/hydrophobic groups and polar/hydrophilic groups.[17] The non-polar group on saponin will combine with fat to form a stable emulsion, can be carried away by water to remove the organic oil layer, while the polar group will clean the inorganic oil layer of hydroxyapatite Ca2+ polar groups bound to the root canal wall, so that surfactant molecules are more easily absorbed by water, resulting in a decrease in surface tension, and wetting of the root canal wall. Polar groups can form a hydrogen bond with water molecules so that saponin can dissolve in water so that the smear layer is dissolved in water. Polar groups clean inorganic smear layers, such as dentin, residual vital tissue or necrotic pulp, saliva, and bacteria, while non-polar groups clean organic smear layers, such as hydroxyapatite particles.[18]

Based on the results of the study, 12.5% green okra extract has a pH of 3.98 which is classified as acidic. This is due to the presence of acid content in 12.5% green okra extract such as oxalic acid. Oxalic acid / $H_2C_2O_4$ is an organic acid that is relatively stronger than

acetic acid.[10] Oxalic acid is able to demineralize the inorganic smear layer component calcium hydroxyapatite/ $Ca_{10}(PO_4)_6$. Acids can demineralize hydroxyapatite because ions (H^+) can affect hydroxyapatite crystal bonds. Hydroxyapatite is reactive to hydrogen ions. When H^+ ions increase, OH^- from hydroxyapatite crystals will be released because they are bound by H^+ ions from the acid and form water with the chemical reaction $H^+ + OH^- \leftrightarrow H_2O$. Then the release of OH^- from the hydroxyapatite crystal causes inorganic phosphate from the hydroxyapatite crystal to be demineralized which will form HPO_4^{2-}.[19] The change of phosphate ions in hydroxyapatite to HPO42- causes an imbalance in the hydroxyapatite bond so that some of the enamel hydroxyapatite crystals will dissolve along with the irrigation material.[11] Chemically, the ability of oxalic acid to dissolve inorganic smear layers is formulated as follows:

$$Ca_{10}(PO_4)_6\,(OH)^2 + 8H^+ \rightarrow 10Ca_2{}^+ + 6(HPO_4)^{2-} + 2H_2O$$

Oxalic acid also functions as a chelating agent. Chelating agents that react with hydroxyapatite can bind calcium ions so that calcium is released from the root canal wall and cause changes in the calcium ratio in the root canal wall. A decrease in the calcium ratio in the root canal wall has an impact on decreasing dentin hardness so that the root canal dentin becomes softer and makes the root canal easy to prepare so that calcium can dissolve along with the root canal irrigation material.[9]

Based on our study, it can be seen that there is no significant difference between the level of cleanliness of the smear layer on the root canal walls irrigated with 12.5% green okra extract and those irrigated with 17% EDTA and 2.5% NaOCl, which both have a score of 2 which indicates a slight smear layer with partially open dentinal tubules so that it can be seen that 12.5% green okra extract is able to clean the smear layer due to the content of saponin and oxalic acid. In addition, EDTA 17% and NaOCl 2.5% also have the ability to clean the smear layer. The two irrigation solutions have different ways of cleaning the smear layer on the root canal wall. EDTA 17% acts as a lubricant by taking calcium ions from dentin hydroxyapatite which causes dentin to become soft, so that instrumentation of the root canal wall becomes easier and faster, while NaOCl 2.5% acts as a solvent for necrotic and vital tissues and saponification reactions that can reduce surface tension. NaOCl 2.5% can be more effective in cleaning root canal walls that have been previously irrigated with 17% EDTA.[20]

Based on the results of the study, there are differences in the score of the cleanliness level of the smear layer in each sample which can be influenced by irrigation techniques. The length of the irrigation needle that can enter the root canal in the manual irrigation technique is limited and allows the formation of a vapor lock that inhibits the circulation of the irrigation solution so that the cleaning process of the root canal system does not run optimally.[21] In addition, other factors that affect smear layer cleaning such as the frequency and volume of irrigation material used.[22]

The amount of smear layer formed requires good cleaning. The thickness of the smear layer can be influenced by the abrasive instrument used. The larger the abrasive instrument particles used, the thicker the smear layer formed.[23]

The results showed that 12.5% green okra fruit extract has the ability to clean the smear layer on the root canal wall. The ability of green okra extract to clean the smear layer is the same as EDTA 17% and NaOCl 2.5% so it is expected to be an alternative root canal irrigation material in the field of dentistry. Furthermore, to obtain the ideal irrigation material, further tests are needed such as toxicity tests of 12.5% green okra extract and further research is needed on the preparation of green okra extract so that it has a longer shelf life.

However, further research is needed to evaluate its toxicity as a root canal irrigation material. The limitation of the research is it is necessary to have the same location of each sample in the middle third of the root of the tooth that is photographed using a scanning electron microscope to produce a homogeneous image of the field of view.

CONCLUSION

The 12,5% Green okra extract and EDTA 17% and NaOCl showed similar levels of root canal cleanliness, with a p-value of 0.093, indicating no difference between them. Both of the two groups have the ability to clean the smear layer on the root canal wall at score 2. The Ability of green okra extract 12.5% in cleaning the smear layer on the root canal wall is same with EDTA solution 17% and NaOCl 2.5%. It means that both of two groups can clean the smear layer with a small amount of the smear layer and part of the dentinal tubule is exposed. The implications of this study suggest that 12.5% green okra extract can remove the smear layer as effectively as a combination of 17% EDTA and 2.5% NaOCl solutions. This extract shows potential as an alternative irrigant.

Author Contribution: "Conceptualization, RAN, DWAF, and RN; methodology, RAN, DWAF, and RN; software, RAN; validation, DWAF and RN; formal analysis, RAN; investigation, RAN; resources, RAN; data curation, RAN; writing original draft, RAN; writing review and editing, DWAF and RN; visualization, RAN; supervision, DWAF, RN ; project administration, RAN; funding acquisition, RAN. All authors have read and approved the published version of the manuscript."

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