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Research Report

The differences in root canal smear layer removal between 6,25% pineapple *(Ananas comocus L. Merr.)* peel extract and 17% Ethylene diamine tetra-acetic acid

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ABSTRACT

Background: *The smear layer is attached to dentine and occludes the orifice and, consequently, must be removed in order to improve the success of inroot canal treatment. The ideal irrigation material removes both the organic and inorganic smear layers. Ethylene diamine tetra-acetic acid (EDTA) is one of the most commonly used root canal irrigation materials, but removes only inorganic smear layer. To overcome this problem, Pineapple (Ananas comosus L. Merr.) peel extract, which contains saponins, bromelain, polyphenol and flavonoid, is used during root canal irrigation.* ***Purpose:*** *The study aimed to analyze the difference in smear layer removal between the use of 6.25% pineapple peel extract and 17% EDTA.* ***Methods:*** *27 samples of mandibular premolar teeth with straight root canals were divided randomly into three groups (n = 9) and subsequently prepared using protaper. Irrigation was performed on the control group (aquadest), group I (17% EDTA) and group II (6.25% pineapple peel extract). The samples were dried, temporarily compressed and cut horizontally from the apical to the coronal. Samples were fixed with holder before the smear layer was observed through a scanning electron microscope (SEM). The resulting data was analyzed by means of an ANOVA test.* ***Results:*** *The highest score of root canal hygiene was recorded by group II, followed by group I and, finally, the control group. There were significant differences between the groups ($p < 0.000$).* ***Conclusion:*** *6.25% pineapple peel extract produces a higher smear layer removal effect than 17% EDTA on the apical 1/3 of the root canal.*

Keywords: *EDTA; pineapple peel extract; root canal irrigation; smear layer*

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INTRODUCTION

Root canal preparation can be performed using mechanical and irrigation instrumentation. Mechanical instrumentation is used to shape and enlarge the root canal in order to facilitate its irrigation and obturation.[1] This form of root canal instrumentation creates an organic and inorganic layer refered to as the "smear layer".[2] This is an amorphous and irregular thin layer composed of both organic materials (bacteria and bacterial products) and inorganic materials (calcium hydroxyapatite and tricalium phosphate). It can cover the prepared root canal walls and occludes the orifices of the dentinal tubules.[3] This layer will be firmly attached to the dentin and can potentially compromise the success of root canal treatment. The thickness, composition, and morphology of the smear layer depends on the instrumentation process and the location of the dentin from which it was formed.[4] The layer can act as a substrate for bacteria, thereby enabling them to survive and proliferate into the dentinal tubules. It may also interfere with the adaptation to and penetration of the filler into the dentinal tubules which will cause microleakage of 1/3 of the apical section of the root canal.[1,5] Smear layer removal requires what is termed irrigation material.

The ideal irrigation material should be able to remove the organic and inorganic smear layers without causing erosive effects on dentine, while also producing an antibacterial effect.[1,4] Ethylene diamine tetra-acetic acid

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(EDTA) is one of the most commonly used root canal irrigation materials. At a concentration of 17%, EDTA constitutes a chelating agent effective at removing the inorganic smear layer.[3,6] This material is non-toxic and, consequently, demonstrates an inability to remove organic smear layer, has a low antibacterial effect, and causes erosion of dentin.[1] EDTA proves effective in removing the smear layer in the coronal area and the 1/2 part of the root canal, but is ineffective in the 1/3 apical part of the root canal. Application of EDTA as the main irrigation material can lead to a significant reduction in dentin microhardness.[7] In order to overcome these disadvantages, pineapple *(Ananas comosus L. Merr)* peel extract is employed as a root canal irrigation material.

Pineapple peel extract contains active substances such as saponins, bromelain, polyphenol, and flavonoids. These active substances can lower the surface tension to remove organic and inorganic smear layer resulting in enhanced root canal hygiene. The minimum concentration capable of destroying Enterococcus faecalis (E. faecalis), thereby creating sterile conditions in the root canal is one of 6.25%.[1] Therefore, this study sought to analyze the difference in smear layer removal between the application of 6.25% pineapple peel extract and 17% EDTA.

MATERIALS AND METHODS

The material used in this research consisted of yellow-green pineapple peel extract from 1 kilogram of honey pineapple-skin The 60-day old pineapples whose peel had been dried for 24 hours had been derived from a plantation in Blitar, East Java. The pineapple peel was immersed in ethanol 96% in the agitator (The Original Resinator OG®, USA), agitated, covered with aluminum foil, allowed to stand for 24 hours and, finally, filtered to produce filtrate 1 and residue. The latter was subsequently added to ethanol solvent 96% and the resulting material was submerged in the agitator for two hours before being filtered to produce filtrate 2. At this point, Filtrate 1 and 2 were combined, covered with aluminum foil and allowed to stand for 24 hours before being filtered to produce 1000ml of filtrate. The filtrate was collected a second time and then concentrated through the use of an evaporator (Rufouz Hitek Engineers Pvt, India) at a temperature of 50-60^{o}C. Employing this process extracted, 500 ml of liquid-free ethanol.

A total of 27 permanent mandibular premolars satisfying the criteria of being caries-free and possessing closed apical foramen and straight root canals were immersed in 20 mm of saline solution. The samples were divided into three groups of nine randomly selected samples. Sample preparation was initiated by opening access through the use of a high speed drill (Dendia Dental, Germany). The working length of the entire sample was measured using a k-file no.10 (Dentsply Sirona, USA). Root canal preparation used k-files no.s 10 and 15 in sequence with a push-pull motion that inserted them up to 2/3 of their working length before extracting them in a straight motion. Root canal preparation was continued by means of a Protaper for Hand Use (Dentsply Sirona, USA) using a pressureless crown-down technique, up to 2/3 of the working length of an S1 file and and a k-file no.15 for recapitulation and irrigation. If the working length was appropriate, preparation was continued with files ranging from S1 to F3. Each tool replacement was irrigated with 3 ml aquadest depending on the treatment group.

The control group was irrigated with aquadest, group I was irrigated with 17% EDTA and group II with 6.25% pineapple peel extract. Irrigation was performed using an anoxygen tube at1 atm pressure (1.033 kg/cm^2). The root canal was dried with sterile paper points and cotton swabs and temporarily compressed in order to keep it dry. All the prepared and irrigated samples were contoured on the lingual, buccal, mesial, and distal surfaces using a diamond disc bur (Dendia Dental, Germany). As cutting guidance, the contour was traced as close as possible to the root canal to facilitate the cutting process. Samples were subsequently cut 6 mm horizontally from the apical to the coronal with the pieces being split into two using a chisel. The sample was fixed in the sample holder and the smear layer observed using a scanning electron microscope (Zeiss, Germany) at 1000x magnification. The root canal hygiene rating was determined based on the average hygiene score using the Schafer criteria as follows:[8] score 1 - no smear layer and dentinal tubules are unobstructed and clearly visible; score 2: < 25% of areas have smear layer and dentinal tubules are unobstructed; score 3: more than 50% of the area has an unevenly spread smear layer; score 4: the entire area is covered with a thin and homogenous smear layer; and score 5: the whole area is covered with a thick and non-homogeneous smear layer.

All data was statistically analyzed using both ANOVA and Tukey tests as comparative tests used to investigate the differences between all groups which showed a significant difference ($p < 0.05$).

RESULTS

In this study, the mean score result relating to root canal hygiene was obtained using the Schafer criterium in the control group, the group of teeth irrigated with 17% EDTA (group 1) and the group of teeth irrigated with pineapple peel extract (group II). The highest root canal hygiene score was obtained from the control group with a mean score of 4.097 followed by group I with a mean score of 2.483, while the lowest score was obtained from group II with a mean score of 1.484 (Figure 1).

The result of an ANOVA test showed that the p value = 0.0001 indicating that significant differences existed between the treatment groups ($p < 0.05$). Data analysis continued with the administering of a multiple comparison test in the form of a Tukey HSD to compare the two groups. The contents of Table 1 indicate that there were significant

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differences between the control groups and groups I and II, while a significant difference ($p < 0.05$) also existed between the latter two groups.

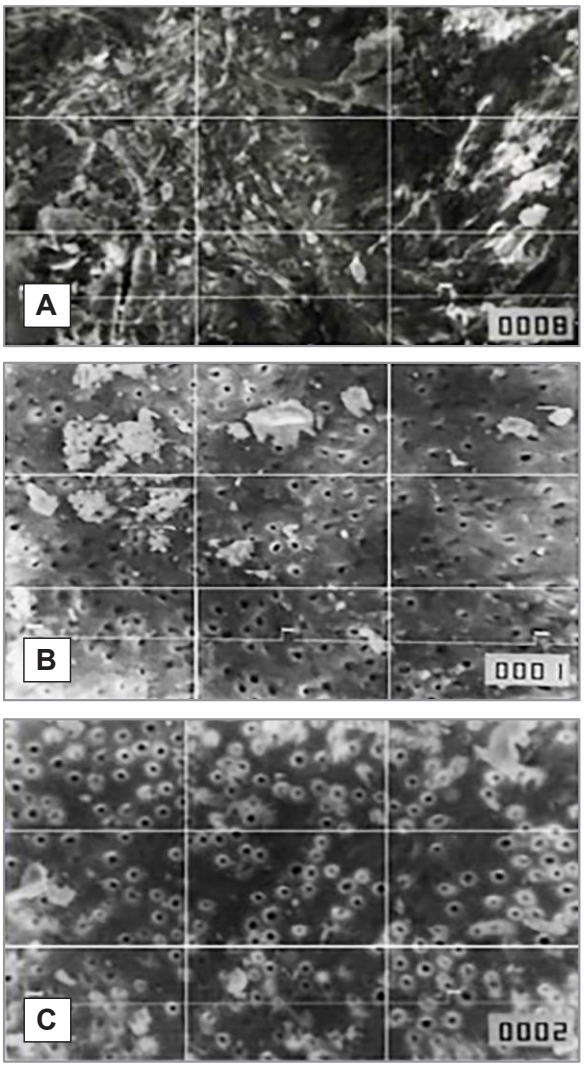

Figure 1. (A) Group control (Aquadest): many smear layers remain on 1/3 apical of the root canal. (B) Group I (17% EDTA): the smear layer appears slightly reduced. (C) Group II (6.25% pineapple peel extract) the smear layer has been removed.

Table 1. The results of different treatment groups using multiple comparisons Tukey HSD

Group	Group I (17% EDTA)	Group II (6.25% pineapple peel extract)
Control Group	0.001*	0.001*
Group I (17%EDTA)		0.001*

* indicated there was a significant difference ($p<0.05$)

DISCUSSION

In this study, root canal hygiene in the smear layer was assessed by comparing the smear layer removal effect between 6.25% pineapple peel extract and 17% EDTA as root canal irrigation material. Based on the results obtained, the lowest score was recorded by group I compared to the 17% EDTA group and the control group in terms of removal the smear layer on the 1/3 apical part of the root canal. This indicated that the irrigated samples group using pineapple peel extract experienced a smear layer removal effect higher than that of the other two groups, the 17% EDTA group and the control group (aquadest), on the 1/3 apical section of the root canal.

The high smear layer removal effect of 6.25% pineapple peel extract is influenced by potentially active substances such as saponins (2.48%), bromelain (1.44%), polyphenol (2.88%), and flavonoids (1.25%).[9] Saponins are substances with four hydrocarbon rings, divided into triterpenoids and steroid glycosides, that have properties as surfactants. Partial molecules of the surfactant constitute the active component of saponin in lowering the surface tension, whereas other surfactant molecules create a micelle ring. The low surface tension allows the irrigation material to reach 1/3 of the apical section of the root canal and increase the contact of the irrigation material with the dentine wall, thus enabling the smear layer to be removed and the dentinal tubule opened. Saponins also have a chemical structure consisting of glycosides (polar compounds) possessing hydrophilic properties and pentacyclic triterpenoids (non-polar compounds) that demonstrate hydrophobic properties. This non-polar saponin compound will dissolve the inorganic components of dentine which are predominantly composed of calcium hydroxyapatite and tricalcium phosphate. These hydrophilic and hydrophobic properties create a ring known as the micelle ring.[1]

Other elements contained within the pineapple peel include; bromelain, polyphenol and flavonoid which synergize with saponin to remove the smear layer by reducing the valence number of calcium ions that it contains. The decreasing valence number will create a metal attachment or an inorganic smear layer material on an unstable root canal which further renders this material loose and soluble. This loose inorganic smear layer will subsequently be surrounded by saponin molecules which create a micelle ring. At that point, the removal process initiated, namely; the absorption of the smear layer into the center of the micelle, thereby transforming it into a water soluble substance. Consequently, saponin content supported by the bromelain properties, polyphenols and flavonoids contained in the pineapple peel extract can remove the inorganic smear layer by releasing the bond and wrapping the smear layer. This results in enhanced root canal hygiene.[10,11]

A study conducted by Mancini *et al*. (2009) argued that, when employed as root canal irrigation material, EDTA proves ineffective at removing the smear layer on 1/3 of

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the apical section of the root canal.[12] 17% EDTA has a high surface tension of 0.0783 N/m. Consequently, it is difficult to gain access to the 1/3 apical section of the root canal. In addition, EDTA can also reduce the mineral components and noncollagenous components (NCPs) in dentine with the result that EDTA not only removes calcium ions, but also prevents calcium-bonding with NCPs.[13] Because of the NCP content reduction in 1/3 of the root, the ability of EDTA to decalcify that area is compromised. This finding corresponds with the theory proposed by Ozdemir *et al.* (2012) that EDTA proves ineffective at smear layer removal from the dentin of both young and old root canals.[14] Therefore, in this study, 17% EDTA proved less effective as an irrigation material compared to 6.25% pineapple *(Ananas comosus L. Merr)* peel extract during smear layer removal from 1/3 apical of the root canal. Based on the results of this study, it can be concluded that 6,25% pineapple peel extract proves more effective at higher smear layer removal compared to 17% EDTA on the 1/3 apical section of the root canal.

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