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Influence of *Sapindus mukorossi* extract in comparison to 17% EDTA as final root canal irrigant on the sealer penetration and microleakage of dentinal tubules

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Abstract. – OBJECTIVE: The study evaluated the effect of *Sapindus mukorossi* (SM) extract as a final root canal irrigant on sealer penetration (SP) in dentinal tubules and microleakage.

MATERIALS AND METHODS: Samples were selected based on inclusion and exclusion criteria. An access opening in all samples was performed and the working length was decided using pro taper for canal finishing along with constant irrigation. Specimens were randomly divided into 3 groups. Group 1 was irrigated with 3 ml of 17% EDTA; group 2 was irrigated with SM irrigant and group 3 samples were irrigated with 0.9% saline. After obturation, samples were vertically placed in 1% methylene blue dye cut in half longitudinally, and viewed under a stereomicroscope. Analysis of SP in the dentinal tubule was assessed using scanning electron microscopy (SEM). For microleakage assessment, mean and standard deviation were reported and One-Way ANOVA was applied. SP was compared using Kruskal-Wallis' test. For inspecting the interaction between SM/EDTA and NaOCl, Fisher's exact test was applied. No statistically significant difference between microleakage in any of the tested groups was observed. The control group showed minimum leakage as compared to EDTA and SM.

RESULTS: The results displayed that there was no significant difference, (p=0.67), between dentinal tubule SP at 2 mm. A significant difference between dentinal tubule SP among groups at 5 mm was observed (p<0.05).

CONCLUSIONS: SM ethanolic extract showed comparable outcomes of smear layer removal and sealer penetration to 17% EDTA, as a final irrigant in root canal cleaning. Therefore, SM has the potential to be used as an adjuvant final irrigant in conjunction with NaOCl.

Key Words:
Sealer penetration, Smear layer, Sapindus mukorossi, Canal irrigant, Microleakage.

Introduction

Preparation of the root canal is considered one of the most significant steps, which includes the exclusion of micro-organisms, necrotic and vital structures from the root canal space, as well as diseased root dentin[1]. The multifaceted morphology of the root canal system makes it difficult

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to prepare the root canal using instrumentation alone[2]. These anatomical variations provide a niche for bacteria by extruding through the apex and eliciting an inflammatory reaction in healthy periapical tissues[3]. In addition to mechanical debridement of the canal i.e., with the conventional hand files and nickel-titanium rotary instruments, endodontic irrigants are also used to appropriately disinfect the canal and achieve a sterile environment[4].

Irrigation is a critical aspect of root canal cleaning as it eliminates the bacteria, debris, and necrotic tissue that exist in the canal[5]. During and after instrumentation, the irrigants help in the removal of tissue residues, and dentin fragments from the root canal through flushing[6]. Sodium hypochlorite (NaOCl) is the most widely utilized irrigating solution. NaOCl interrupts numerous vital activities of the bacterial cell, causing cell death. In addition, it is cheap, easily available, and has a long shelf life[4,7]. NaOCl has a powerful antibacterial effect that kills the majority of bacteria in direct contact[8]. However, the limitations of hypochlorite are mainly its toxicity, damage to vital tissues, interaction with other irrigating solutions, and its inability to remove the smear layer (SL)[9,10]. Photon-induced photoacoustic streaming (PIPS) a novel laser agitation technique has been used to cleanse, disinfect, and shape the root canal system. It works on the principle of Er: YAG laser-activated flushing[11]. Recent research has demonstrated that 17% EDTA solution activated by the PIPS technique showed better canal disinfection and smear layer removal[12,13]

Smear layer removal is necessary for the sealer to penetrate in dentinal tubule and achieve a sterile environment. Penetration of sealer improves marginal adaptation, increases mechanical retention, entombs residual bacteria, and is antibacterial[14,15]. To remove the SL, adjuvant use of chelating agents like ethylene diamine tetra acetic acid (EDTA), citric acid, maleic acid, phytic acid, and chlorhexidine is mandatory along with NaOCl[16]. However, the use of EDTA causes erosion of the intra-radicular dentin with other undesirable effects[17].

Various herbal irrigants (green tea, neem extracts, and Triphala) have been tried to replace synthetic chemicals, to limit the adverse effects[18]. Among herbal irrigants, *Sapindus mukorossi* (SM) is a natural herbal extract found in plants with diverse therapeutic values. It comes from a tropical deciduous large tree with small flowers in large panicles. The fruits are smooth and yellow to orange. The plant is used for medicinal purposes and the seed is employed in fertilizers The major component of SM is saponins, extracted from the pericarp of the smooth yellow, orange fruit, which result in better surfactant properties[19]. SM has been evaluated for its influence on endodontic rotary instruments[17]. Although studies[20,21] have evaluated the SL removal ability of herbal agents in comparison to other chelating agents, the chelating influence of SM on root canal surface smear layer is not known. To our knowledge from indexed literature, limited evidence is available on the effect of SM as a final endodontic irrigant on sealer penetration, microleakage, and interaction with NaOCl. Therefore, this study aimed to evaluate the effect of SM extract on sealer penetration (SP) in dentinal tubule and adaptation with canal wall and its interaction with NaOCl.

Materials and Methods

Ethical Consideration and Review Board

The present *in vitro* study followed a checklist for reporting in-vitro studies (CRIS) guidelines. The study duration was seventeen months after the approval of the Institutional Review Board (IRB) IRB1478/DUHS/approval/2020/69. The IRB at Dow University of health sciences (DUHS) reviewed and approved the study.

Inclusion and Exclusion Criteria

Non-probability, the purposive sampling method was adopted for the collection of samples. The sample size was calculated through PASS version 11 for one-way ANOVA, taking a confidence interval of 95% and power of 90%, by using the means and standard deviations of the previous studies[22,23]. A minimum sample size of 8 samples per group was identified. Teeth were extracted at DUHS, in the Department of Oral Maxillofacial Surgery. Each tooth was radiographed to confirm the presence of a single canal. After extraction, teeth were cleaned in tap water to remove any visible debris and stored in 0.1% thymol at 4°C in the refrigerator, till further use. Samples were selected based on the following inclusion criteria, single-rooted permanent human teeth with mature apices; patients aged between 18 to 40 years. The exclusion criteria consisted of intracanal calcification. (assessed from pre-operative radiograph before extraction); root dilacerations; root caries, fractured or cracked tooth; root re-

sorption and previously treated or initiated root canal treatment. Expired irrigants were excluded and turbid or flaccid or granular irrigants were discarded.

Preparation of Ethanolic Extract of S. mukorossi

The desiccated fruit of SM was used for the formulation of an experimental root canal irrigant. The dry pericarps of SM (≈ 1.0 kg) present around the seed nut, were separated with a sharp blade. These pericarps were blended to produce fine particles in a sterilized home blender. The resultant 10-gram powder was soaked in 100ml of absolute ethanol (99%) for 24 hours at normal room temperature. The solvent was filtered and removed with help of a rotary evaporator, and the extract was kept in cleaned screw-capped vials (Premium Vials B4702-12 Glass with Screw Cap) at -20º C until use. To form an operational concentration of 5 mg/ml, the extract was re-dissolved in distilled water (Figure 1).

Sample Preparation

Sixty teeth were included in the present study, with 10 samples in each group. An access opening in all samples was performed with a # 4 diamond cutting round bur, in a high-speed handpiece under the air-water spray. A #10 K-type file (Maillefer/Dentsply) was introduced into each root canal till it could be seen outside the apical foramen. The working length was decided by decreasing this length by 1 mm. A single operator used K-type files (Maillefer/Dentsply) initially, then with the ProTaper Universal System (Dentsply-Maillefer) preparation of root canal was performed up to F2 file size. Irrigation was performed at each change of file with 2.5mL of 3% NaOCl. Specimens were randomly divided into 3 groups through the lottery system.

Group 1 (n=20): The sample received final irrigation with 3 ml of 17% EDTA which was left in the canal for 1 minute.

Group 2 (n=20): The sample received final irrigation with 3ml of SM irrigant (1 minute).

Control group (n=20): The sample received final irrigation with 0.9% saline using standard irrigation protocol (1 min).

Specimens were dried with F2 sterile absorbent paper points and an F2 gutta-percha (GP) cone was selected as the master cone. Sealapex (Sybron-Endo, Glendora, CA, USA) sealer was mixed according to the manufacturer's instructions and placed in the canal. Mastercone was inserted with the sealer up to the working length and tug back was assessed. The GP was inserted up to the working length after the apical 2mm of the master cone was evenly coated with the sealer. Excess GP was seared off using a hot condenser, and coronal GP was vertically compacted to produce a good coronal seal. The access cavity was sealed with GIC restorative type. All samples were stored in an incubator (binder GmbH, Germany) at 37°C under 100% humidity for 1 week.

Stereomicroscopic Analysis of Dye Penetration

After complete drying, ten samples in each group (for dye penetration) were vertically placed in 1% methylene blue dye (WellcoSol, TM) for 72 hours. All specimens were cut in half longitudinally, parallel to their long axis using a rotation diamond disk. The samples were viewed under a stereomicroscope (Olympus VM-ILA-2) at 30X

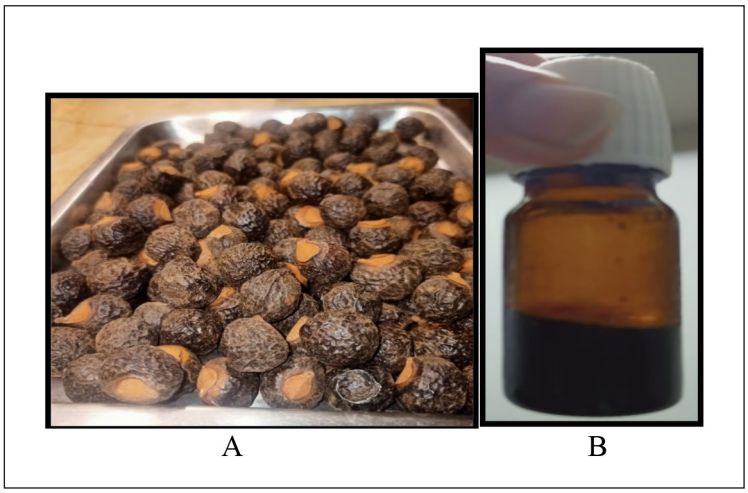

Figure 1. A, *Sapindus mukorossi* fruit. **B,** Ethanolic extract of *Sapindus mukorossi*.

magnification to evaluate apical microleakage. Independent reviewers examined the samples. Apical microleakage was measured by the linear depth of dye penetration, which is defined as the maximum dye penetration distance from the root apex to the coronal extent. All procedures were measured in millimeters (mm).

Sealer Penetration Using Scanning Electron Microscopy (SEM)

Ten sectioned teeth for each group, were marked at 2 mm and 5 mm. Specimens were mounted on metallic stubs and titanium-sputtered in Auto Fine Coater: JEC-3000FC, on 20 mA for 30 seconds. Under an electron microscope, (JEOL, Tokyo, Japan Model No. JSM-IT 100), images were taken. An independent (blinded) observer examined samples for dentinal tubule penetration of sealers at two levels (2 mm and 5 mm) from the root apex. The depth of penetration was estimated by measuring the distance between the points and the sealer-dentin contact.

Interaction of Experimental Irrigant with Sodium Hypochlorite

For the assessment of precipitate formation, solutions of 17% EDTA, SM, and 3% NaOCl were utilized. Two polystyrenes round-bottom tubes were used (Falcon; Thermo Fischer Scientific, Waltham, MA, USA). One was filled with 1 ml extract of SM (labeled A), and the other was filled with 17% EDTA (labeled B). Similarly, the other two polystyrene tubes were filed with SM and 3% NaOCl (Labeled C) and 17% EDTA and 3% NaOCl (labeled D), respectively. All four tubes were assessed at 15 mins, 2 hours, 24 hours, and 1 week by a single observer[24].

Statistical Analysis

SPSS version 23 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. For microleakage assessment, mean and standard deviation were reported and one-way ANOVA was applied. Group-wise comparison of microleakage was determined through the Games-Howell test. The data for sealer penetration was assessed with Kruskal-Wallis' test. Group-wise comparison of sealer penetration was computed by the Mann-Whitney test. A comparison of sealer penetration at different tooth levels was computed by Wilcoxon signed-rank test. The correlation between microleakage and sealer penetration was assessed by Spearman's correlation. For evaluating the interaction between investigated irrigants and NaOCl, Fisher's exact test was applied. A p-value of < 0.05 was suggested to be statistically significant.

Results

The outcomes of microleakage among different investigated groups are given in Table I. The results showed that there was no statistically significant difference (p=0.84) between microleakage in any of the tested groups. The control group showed lower leakage as compared to EDTA and SM (Figure 2). The results displayed that there was no significant difference (p=0.67) between sealer penetration in the dentinal tubule at 2 mm. A statistically significant difference among groups for sealer penetration in the dentine at 5 mm was observed (p<0.05). Sealer penetration at 5mm showed a significant difference between the control group and EDTA ($p\leq0.001$); and the control group to SM ($p\leq0.001$). Whereas, EDTA to SM comparison revealed no significant difference (p=0.806) (Table II).

The relationship between microleakage and sealer penetration showed a weak negative association that was not significant between microleakage and dentin penetration at 2 mm in the control group and the EDTA group (rs=-0.08, p=0.66)(rs=-0.15, p=0.43). Whereas, at the 5 mm level, results indicated that there was a negative association (not significant) between microleakage and sealer penetration,

Table I. Mean Comparison of Microleakage & Sealer Penetration among groups.

Parameters (unit)	Control	EDTA	SM	*p*-value
Microleakage (mm)	1.4 (.855)	1.6 (.755)	1.7 (.91)	0.84[β]
Penetration at 2 mm[b] (µm)	0.00 (0.00-6.5)	0.00 (0.00-13.6)	0.00 (0.00-14.4)	0.668[^]
Penetration at 5 mm[b] (µm)	8.4 (5.4-9.5)	14.7 (9.9-21.5)	14.2 (12.5-17.2)	< 0.001[^]

[a]Value is represented as the mean (Standard deviation). [b]Values are represented as median (Interquartile range). [β]One Way ANOVA; ^Kruskal Wallis.

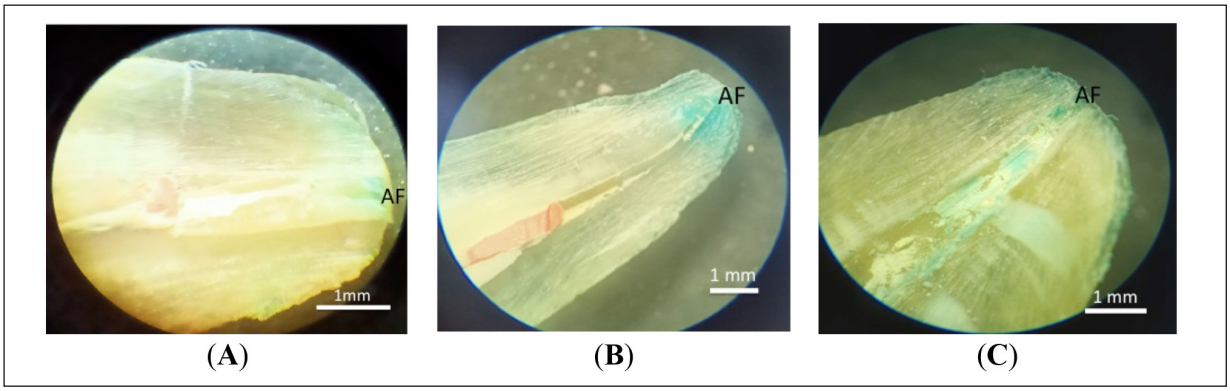

Figure 2. Assessment of microleakage under a stereomicroscope at 30× magnification. AF: Apical foramen. (**A**) Control, (**B**) EDTA group and (**C**) S*apindus mukorossi.*

Table II. Group-wise comparison of microleakage & sealer penetration.

Parameters (unit)	Control *vs.* EDTA	Control *vs.* SM	EDTA *vs.* SM
Microleakage (mm)$^{\Omega}$	-.234 (0.50)	-.28 (0.43)	-.05 (0.97)
Penetration at 5 mm (µm)$^{\#}$	< 0.001	< 0.001	0.806

$^{\Omega}$Values are represented as mean difference (*p*-value) computed using the Games-Howell test. $^{\#}$Values are represented as p-value computed using the Mann-Whitney test.

in the control group and SM group (Table III and Figure 3). (rs =-0.04, *p*=0.8)(rs=-0.18, *p*=0.34). In the SM group, there was a positive association between microleakage and sealer penetration at 2 mm (rs=0.05, *p*=0.76). On the contrary, a weak negative association was observed at the 5 mm level (rs=-0.18, *p*=0.344) (Table IV).

Throughout the observation period (15 mins, 2 hrs, 24 hrs, and 1 week), tube 1 (SM) remained transparent. Tube 2 (EDTA) appeared as a curdy white solution that does not precipitate. Tube 3 (SM+ NaOCl) revealed a visible soap bubble production on the surface that gradually developed over time (after 24 hours), with a brown tint but no precipitate formation at any point throughout the observation. Tube 4 (EDTA+NaOCl) became milky white after mixing, and a suspended precipitate was visible after 15 minutes, with part of it gradually settling down after standing and remaining unaltered for 12 hours. After 24 hours, a white clear liquid on top remained unaffected for one week (Table V).

Discussion

Discussing the results of the present study, the null hypothesis was accepted which asserts that there is no significant difference between EDTA and ethanolic extract of SM as a final irrigant for microleakage and SP. To avoid the hazardous effects of EDTA, SM irrigant can be used as a smear layer removal agent. Further studies are still required to deduce the findings of the present study and to make easy commercial availability of SM as an irrigant.

Table III. Comparison of sealer penetration among different levels of the tooth among all investigated groups.

Group	Penetration at 2 mm	Penetration at 5 mm	*p*-value
Control group	0.00 (0.00-6.5)	8.4 (5.4-9.5)	0.002$^{\infty}$
EDTA group	0.00 (0.00-13.6)	14.7 (9.9-21.5)	< 0.001$^{\infty}$
The experimental group (SM)	0.00 (0.00-14.4)	14.2 (12.5-17.2)	0.001$^{\infty}$

Values are reported as (Median ± Q1-Q2 µm). $^{\infty}$2-mm *vs*. 5-mm level: Wilcoxon signed-rank test, $p < 0.05$.

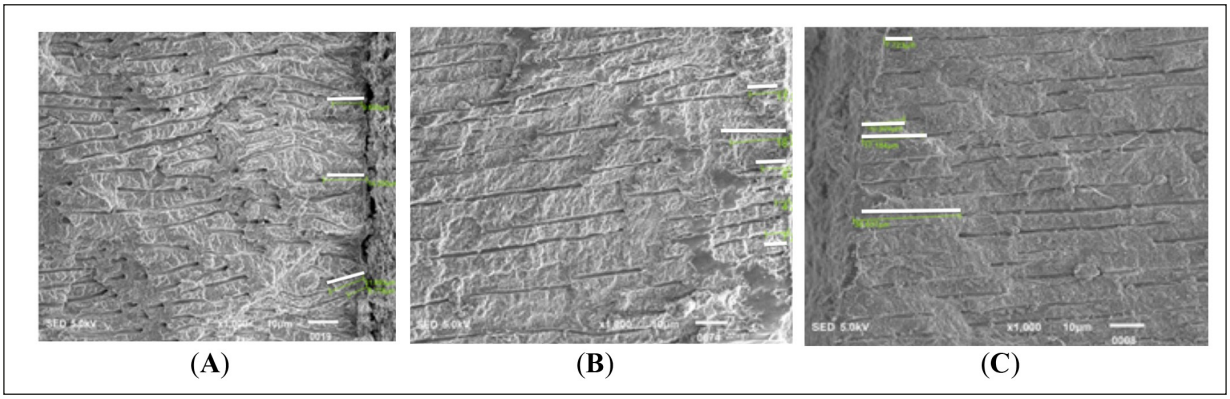

Figure 3. Assessment of sealer penetration into dentinal tubule using SEM images at 1000×. (**A**) Control group, (**B**) EDTA group (**C**) *Sapindus mukoross*i. The length of the white lines indicates the dentine penetration in the specimens.

Table IV. Correlation between Microleakage & Sealer Penetration.

Parameters	Control	EDTA	SM
Microleakage & Penetration at 2 mm	-0.08 (0.668)	-0.15 (0.430)	0.05 (0.766)
Microleakage & Penetration at 5 mm	-0.04 (0.835)	0.119 (0.530)	-0.18 (0.344)

Values are represented as Spearman's Correlation (*p*-value).

The sealing of the root canal apically by the sealer is important to anticipate the communication of root canal substance with the periapical tissue[25,26]. The ideal outcome of root canal treatment is the sealer penetration in dentinal tubules, which prevents bacterial recolonization and halts bacterial activity within the tubules. In this study, a seal-apex Calcium hydroxide Ca $(OH)_2$ based sealer was used[27]. Calcium hydroxide-based sealers have been in use for two prime reasons. Firstly, for stimulation of the periapical tissues, which aids in healing, and secondly because of antimicrobial properties. Additionally, the seal apex not only possesses sufficient biological but also physical and chemical characteristics, such as sealability[28,29].

Results of the present study revealed that there is no significant difference between the microleakage of any investigated group (*p*-value=0.84). But the

Table V. Interaction of group with formation of precipitate at the different time duration.

Formation	EDTA+ NaOCl	SM+ NaOCl	*p*-value[#]
15 minutes			
Present	(100%)	(0%)	0.10
Absent	0 (0%)	(100%)	
2 hours			
Present	(100%)	(0%)	0.10
Absent	(0%)	(100%)	
24 hours			
Present	(100%)	(0%)	0.10
Absent	0 (0%)	03 (100%)	
1 week			
Present	(100%)	(0%)	0.10
Absent	(0%)	(100%)	

[#]Fisher exact test for association.

control group showed minimum leakage with a mean of 1.4 mm, and EDTA samples mean of 1.6 mm and 1.7 mm in SM specimens. The combination of EDTA with NaOCl resulted in decreased microleakage, according to the findings of the current investigation in comparison with SM. Similar results have been obtained in a study by Agarwal et al[30].

Sealer penetration among different final irrigation methods revealed that at the apical 2 mm level there was no statistically significant difference among examined groups. Evidence[31] suggests the outcomes are poised for the following reasons, difficulty in irrigating the apical area and removing the smear layer, which directly correlates to sealer penetration. In addition, even with cautious sample selection, it was not possible to assure a homogeneous distribution of sclerotic dentin, which may impact dentin penetration[32]. The results of the present study are in harmony with previously reported work[33,34]. Sealer penetration at the 5 mm level possesses significant differences between the inspected groups. EDTA when used as the final irrigant showed the highest sealer penetration followed by SM ethanolic extract used as an irrigant. The main elements of the SM fruit pericarp include saponins (11.5%) and sugars (10%)[33]. The high contents of saponins allow for a surfactant action on any surface. When the SM extract is added to an irrigant, it reduces its surface tension due to its detergent-like quality, leading to the dissolution of water-insoluble substances and a reduction of surface tension[34]. SM ethanolic irrigant can, therefore, eliminate debris, pulp tissues, and microbes, all part of the smear layer. The results of the present study followed outcomes of previously reported work by Moon et al[35], who investigated the effects of final irrigation in curved root canals comparing EDTA and NaOCl, they showed that at 5 mm depth, dentinal penetration by the sealer was observed after using EDTA under laser confocal microscopy. The overall results of this study are in agreement with the earlier studies[36,37] which showed smear layer removal can result in better sealer penetration.

The outcomes of the present study revealed that sealer penetration at different levels showed a snowballing trend from the apical to the coronal level. The conclusions can be based on the following reasoning, in the coronal or middle third greater diameter and a larger number of dentinal tubules facilitate the action of irrigation further effectively, therefore increasing the penetration into the tubules as compared to the apical third[38-40]. The outcomes of the existing study are in line with recently reported work by Generali et al[41] who investigated the sealer penetration at different levels after using variable irrigating systems.

In the present study, a significant correlation of microleakage with sealer penetration is not reported, although an inverse relationship existed. This result is in agreement with the study by Sen et al[42]. However, Richard et al[43] noted a significant link between sealer penetration and a decrease in leakage. More studies are required to generalize the verdicts of the present study. Numerous variables may impact the rate and depth of sealer infiltration. The sufficiency of the smear layer removal, the obturation procedure, the physical and chemical features of the sealer, and the architecture of the root canal framework are all aspects to consider[44,45].

The SM use as a final irrigant showed encouraging outcomes in the removal of the smear layer and sealer penetration in the present study. It is pertinent to mention that the biocompatibility of SM is critically important for its clinical use in dental applications. Du et al assessed the oral and dermal toxicity of saponins in rats. They suggested that the saponins extraction from SM Gaerth was non-oral-toxic and nondermal irritant[46].

Limitations

The present study is imperiled by some inherent limitations. The evaluation of apical microleakage was assessed using a two-dimensional method of dye penetration, however, contemporary 3-dimensional techniques may show different outcomes. The dye penetration method does not provide any information about the volume or trace that penetrated along the root canal and provides measurement in two dimensions only. In addition, the present study included only single-rooted teeth using an *in vitro* study design. Therefore, further studies comparing the physical, mechanical, and biological influence of SM on root dentin in a clinical trial employing contemporary techniques are recommended.

Conclusions

Sapindus mukorossi ethanolic extract showed comparable outcomes of smear layer removal and sealer penetration to 17% EDTA, as a final irrigant in root canal cleaning. Therefore, *Sapindus mukorossi* has the potential to be used as an adjuvant final irrigant in conjunction with NaOCl.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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Authors' Contribution
Conceptualization: AT, FRQ, UM, ZC, MAA, KAA, TA, FV; methodology: AT, FRQ, UM, ZC, MA, SM, AAM, KA, MFA, software: AT, MFA, MAA, KAA, TA, FV; validation, formal analysis: AT, FRQ, UM, ZC, SM, AAM, MAA, KAA, TA, FV; resources: FRQ, UM, ZC, MA, SM, KAA, KA, TA, FV; data curation: AT, FRQ, UM, KA, ZC, MAA, KAA, TA, FV; writing-original draft preparation: AT, FRQ, MFA, MAA, KAA, TA, FV; writing-review and editing: UM, ZC, MA, SM, KA, AAM, AA. Funding: TA. The authors hsve read and agreed to the published version of the manuscript.

Ethics Approval
The Institutional Review Board (IRB) at Dow University of Health Sciences (DUHS) reviewed and approved the study IRB1478/DUHS/approval/2020/69.

Informed Consent
nformed consent was obtained for the removal of teeth and use of extracted teeth.

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