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Comparative Evaluation of Microhardness, Smear Layer Removal Efficacy and Depth of Penetration Using Punica granatum, Emblica officinalis and Sodium Hypochlorite As Endodontic Irrigants: An In Vitro Study

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Abstract

Introduction

Root canal morphology tends to be complicated by nature and dealing with this intricacy can be challenging because it makes it difficult to completely disinfect the root canal space. The success of root canal therapy is also determined by the biomechanical preparation of the canal with the application of instruments and irrigating solutions. Due to the fact that the root dentin surface continues to interact with the irrigating solution during preparation, it's critical to evaluate the mechanical characteristics and smear layer removal. Though sodium hypochlorite (NaOCl) is the most commonly used irrigant due to its tissue-dissolving abilities, it has certain drawbacks which include the inability to remove the smear layer and also affects the mechanical properties of root dentin. To overcome these limitations, a variety of herbal substitutes like *Punica granatum* and *Emblica officinalis* which possess anti-bacterial and anti-fungal properties can be used as endodontic irrigants. Several studies on the anti-bacterial properties of natural irrigants of pomegranate and amla were reported. However, the mechanical properties and smear layer removal of *Punica granatum* and *Emblica officinalis* have not been explored in the field of endodontics.

Aim

The main aim of this in vitro study is to compare and evaluate microhardness, smear layer removal efficacy and depth of penetration of herbal and conventional irrigants.

Materials and methods

Thirty-six palatal roots of maxillary molars were decoronated and instrumented up to F3. These roots were sectioned longitudinally and divided into three test groups: Group 1: 12.5% *Punica granatum*; Group 2: 6.25% *Emblica officinalis*; control: Group 3: 2.5% NaOCl. All specimens were irrigated with 5ml of each irrigant for 5 minutes. Microhardness of root dentin was measured using a Vickers diamond intender, smear layer removal using a scanning electron microscope (SEM) and depth of penetration using a stereomicroscope. The data was analyzed using one-way ANOVA and the inter-group comparison using Tukey's post hoc test.

Results

Statistical analysis was done using one-way analysis of variance (ANOVA) and Tukey's post hoc test using SPSS software version 17.0 (SPSS Inc., Chicago). The highest microhardness was seen in Group 1 (cervical: 53.8375 ± 1.35956, middle: 53.9875 ± 1.01761, apical: 53.6875 ± 1.63133) while Group 2 (cervical: 43.2750 ± 1.73596, middle: 43.3125 ± 1.17648, apical: 43.8000 ± 1.32665) and Group 3 (cervical: 42.7250 ± 2.93391, middle: 41.9625 ± 1.66985, apical: 42.0250 ± 2.21085) showed significant reduction in root dentin hardness. Regarding smear layer removal Group 1 (1.3750 ± 0.51755), and Group 2 (1.2500 ± 0.46291) reveals greater smear removal in the middle third of the root dentin compared with Group 3 (2.3750 ± 0.51755), which showed the least smear layer removal. A greater depth of penetration was seen in Group 1 (0.5488 ± 0.05222) and Group 2 (0.5263 ± 0.05181) than in Group 3 (0.3087 ± 0.05743).

Conclusion

The present study reveals that the least reduction of microhardness was seen in *Punica granatum* followed by *Emblica officinalis* and NaOCl. The smear layer removal efficacy and depth of penetration were greater in *Punica granatum* and *Emblica officinalis* than in NaOCl. It was concluded that as these herbal irrigants are biocompatible agents, they can be considered for future use in root canal treatment.

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Introduction

The primary factor of endodontic failure is the retention of pathogens because of the complex structure of root canals [1]. An effective endodontic treatment requires the entire removal and disinfection of microorganisms and subsequent derivatives from the infected root canal, which is achieved in part by canal shaping but primarily through antimicrobial irrigants [2]. Root canals are prepared using manual and rotary instruments under copious irrigation. Regardless of the canal preparation procedure, 35% or more of root canal walls (along with the isthmus) were found to remain untouched in a study using micro-CT images taken before and after root canal shaping [3]. Thus, irrigation is an important component of endodontic treatment because it facilitates thorough disinfection beyond instrumentation.

Sodium hypochlorite (NaOCl) is a commonly used endodontic irrigant because of its antimicrobial and tissue-dissolving abilities [4]. Instrument corrosion, terrible taste, high toxicity [5], inability to remove the smear layer, loss of elastic modulus, and reduced flexural strength of dentin are some of the drawbacks of employing NaOCl [6]. As a result, many herbal alternatives have been explored as endodontic irrigants. The advantages of using herbal extracts in endodontics are that they have minimal cost, easy accessibility, extended shelf life, low toxic effects, lack of microbial resistance, are better tolerated by patients, and are eco-friendly [7].

Punica granatum (Pomegranate) exhibits antibacterial properties because of the presence of phytocompounds such as hydrolysable tannins, polyphenolics, and flavonoids [8,9]. The fruit of *Emblica officinalis* (Amla) contains antioxidants such as emblicanin A and B which are two hydrolyzable tannins that might explain Amla's antibacterial properties [8-10]. Flavonoids and tannins cause increased antibacterial action against gram-positive anaerobes because of their ability to decrease a variety of microbial virulence processes, including suppression of biofilm formation, reduction of host ligand adherence, and neutralization of bacterial toxins [8,11,12]. The elimination of the smear layer from the root canal walls and dentinal tubules, as well as the reduction of harmful effects on dentin, is necessary for effective endodontic therapy [13,14].

As root dentin interacts with the irrigants during irrigation, it is important to assess the influence of irrigants on microhardness, smear layer removal efficacy and depth of penetration into dentin. This study investigates the effects of Punica granatum, Emblica officinalis, and NaOCl in dentinal root wall microhardness, smear layer removal efficacy, and depth of penetration. The null hypothesis was that there is no significant difference in microhardness, smear layer removal efficacy and depth of penetration of root dentin among the various irrigants.

Materials And Methods

The study received approval from the institutional human ethics committee (CARE IHEC-I/0363/21). Thirty-six permanent human maxillary first molars, freshly extracted for periodontal reasons, were used in this study. Maxillary molar teeth with straight roots extracted from patients between the age group of 25-50 years were selected. Carious teeth and teeth with developmental anomalies were excluded.

The palatal roots were decoronated at the cementoenamel junction using a diamond disc with a straight handpiece under constant water cooling. The root canal length was standardized to 14mm. A stainless steel K-type hand file #15 (Mani, Japan, 2022) was used to establish patency. Using the crown-down approach, the canals were prepared using Protaper Gold rotary NiTi files up to F3 (Dentsply Sirona, USA). During instrumentation, irrigation was performed using saline.

Preparation of herbal extracts

Punica granatum (Pomegranate) peels and *Emblica officinalis* (Amla) fruits were sun-dried and ground into fine powder. The powders underwent cold maceration, which involved intermittent stirring with a sterile glass rod and being left undisturbed for 48 hours before being filtered through a sterile muslin cloth and heated in a water bath to 110°C to produce crude extracts. A total of 1.25g of dried amla crude extract is mixed with 20 ml of distilled water to obtain 6.25% of *Emblica officinalis* extract. And 2.5g of dried pomegranate crude extract is mixed with 20ml of distilled water to obtain 12.5% of *Punica granatum* extract (Figure *1*) [8].

FIGURE 1: Prepared extract of Punica granatum and Emblica officinalis

Sample distribution

The sample size was estimated using G* power software (version 3.1.9.2) (Erdfelder, Faul, & Buchner, 1996) and other input parameters:

1. Effect size - 0.55 (Payal et al., 2019)

2. Level of significance - 5%

3. Power (1-β) - 80%

The prepared specimens were divided randomly into three groups (n=12) based on the irrigant used: G1: 12.5% *Punica granatum,* G2: 6.25% *Emblica officinalis and* G3: 2.5% NaOCl.

Evaluation of microhardness

Pre-treatment Microhardness

Twelve prepared teeth were taken and longitudinal grooves were made on the buccal and lingual sides using a diamond disc without piercing the root canal space. Splitting of the teeth was done using a chisel into two halves. The specimens were stored in normal saline till use. Root segments were mounted using auto-polymerizing acrylic resin and further smoothened using fine emery papers. Microhardness values (M1) of all samples were measured with Vickers diamond intender at three different locations (cervical, middle and apical parts) using a 200g load and at 20s dwell time. The indentations were positioned 0.5 mm from the root canal surface at a depth of 100 µm from the pulp-dentin interface, with no overlap between them.

Post-treatment Microhardness

All these specimens were immersed in their respective irrigants for 5 minutes. Finally, they were rinsed with distilled water to avoid continuous exposure to the irrigants. Post-treatment microhardness values (M2) were recorded for each section after irrigation with corresponding irrigants. Microhardness values of all samples were measured similarly to the pretreatment microhardness. The decreased microhardness for each specimen was calculated.

Evaluation of smear layer removal

Twelve prepared teeth were irrigated with 5ml of respective irrigants for 5 minutes. Finally, rinsed with 1ml of distilled water for 1 minute. Splitting of teeth was done. Later, specimens were gold-platinum sputtered and then viewed under a scanning electron microscope (SEM). The middle third of the root canal was examined under 5000x magnification. The smear layer removal was measured by three blinded observers according to the criteria (Table *1*) given by Torabinejad et al. [15].

Scores	Evaluation Criteria
SCORE 1	No smear layer (no smear layer on the surface of the root canals; all tubules were clean and open)
SCORE 2	Moderate smear layer (no smear layer on the surface of root canal, but tubules contained debris)
SCORE 3	Heavy smear layer (the smear layer covered the root canal surface and tubules)

TABLE 1: Evaluation criteria for smear layer removal

Evaluation of depth of penetration

The crystal violet dye was made to flow from the apical end of each prepared tooth (12 teeth) for about five seconds. The teeth were then soaked in the dye for two days and washed for 10 minutes under tap water. The teeth were then irrigated with 5ml of respective irrigants for 5 minutes. Splitting of the teeth was done as mentioned previously. Each sample was then evaluated in the middle third of the canal using a Stereomicroscope (Leica Microsystems, Germany) under 20x magnification. ImageJ software (National Institutes of Health, USA) was used to calculate the penetration depth. The depth of the bleaching zone was calculated in millimetres.

Statistical analysis

The results were collected and tabulated. Mean and standard deviation were calculated for all the groups. Statistical analysis was done using analysis of variance (one-way ANOVA) and Tuckey's post hoc test. Statistical significance was considered at a level of $P<0.05$. Data were analyzed using Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, IBM Corp., Version 17.0, Chicago).

Results

Microhardness

The variations between the M1 and M2 scores at all three levels of root dentin were examined using one-way ANOVA. All the irrigating solutions used resulted in reduced microhardness compared with baseline values. They showed a statistically significant difference ($p<0.05$) between the initial and post-treatment microhardness values. In Table 2 Group 2 (cervical: 43.2750 ± 1.73596, middle: 43.3125 ± 1.17648, apical: 43.8000 ± 1.32665) and Group 3 (cervical: 42.7250 ± 2.93391, middle: 41.9625 ± 1.66985, apical: 42.0250 ± 2.21085) showed reduced microhardness compared with Group 1 (cervical: 53.8375 ± 1.35956, middle: 53.9875 ± 1.01761, apical: 53.6875 ± 1.63133). The post-treatment microhardness values were most decreased NaOCl and *Emblica officinalis* whereas, *Punica granatum* showed the least changes.

Groups			Mean	Standard Deviation	Std. Error Mean	P-value
Group 1	Cervical	Pre	56.6500	0.77275	0.27321	0.001
		Post	53.8375	1.35956	0.48068	
	Middle	Pre	57.4125	1.31740	0.46577	0.001
		Post	53.9875	1.01761	0.35978	
	Apical	Pre	57.1750	0.89562	0.31665	0.001
		Post	53.6875	1.63133	0.57676	
Group 2	Cervical	Pre	55.4250	1.54712	0.54699	0.001
		Post	43.2750	1.73596	0.61376	
	Middle	Pre	56.2875	1.70749	0.60369	0.001
		Post	43.3125	1.17648	0.41595	
	Apical	Pre	55.2375	1.67156	0.59099	0.001
		Post	43.8000	1.32665	0.46904	
Group 3	Cervical	Pre	53.0250	2.52516	0.89278	0.001
		Post	42.7250	2.93391	1.03730	
	Middle	Pre	52.9500	0.85021	0.30059	0.001
		Post	41.9625	1.66985	0.59038	
	Apical	Pre	52.9375	0.97825	0.34586	0.001
		Post	42.0250	2.21085	0.78165	

TABLE 2: Inter-group comparison for pre- and post-microhardness using ANOVA

Statistically significant p<0.05; SD: standard deviation, ANOVA: analysis of variance

Smear layer removal

The mean value for the smear layer removal of all three groups was analyzed at 5000x magnification. Table 3 depicts the mean values of Group 1 (1.3750 ± 0.51755), and Group 2 (1.2500 ± 0.46291) revealing greater smear removal in the middle third of the root dentin compared with Group 3 (2.3750 ± 0.51755), which showed the least smear layer removal. Group 1 (Figure 2) and Group 2 (Figure 3) showed that the orifices of the dentinal tubules were patent and no smear layer was present (score of 1 with no smear layer present). The NaOCl group (Figure 4) depicts a variable distribution of smear particles over the dentinal tubules (score of 3 with up to 50% of root canal area). However, using one-way ANOVA and Tukey's post hoc test, Groups 1 and 2 showed better smear layer removal, followed by Group 3, which showed the least removal.

Groups	Mean	Standard Deviation	95% Confidence Interval for Mean		F	P-value
			Lower Bound	Upper Bound		
Group 1	1.3750	0.51755	0.9423	1.8077	12.16	0.001
Group 2	1.2500	0.46291	0.8630	1.6370		
Group 3	2.3750	0.51755	1.9423	2.8077		

TABLE 3: Inter-group comparison for smear layer removal

Statistically significant p<0.05; F: ANOVA (analysis of variance) value

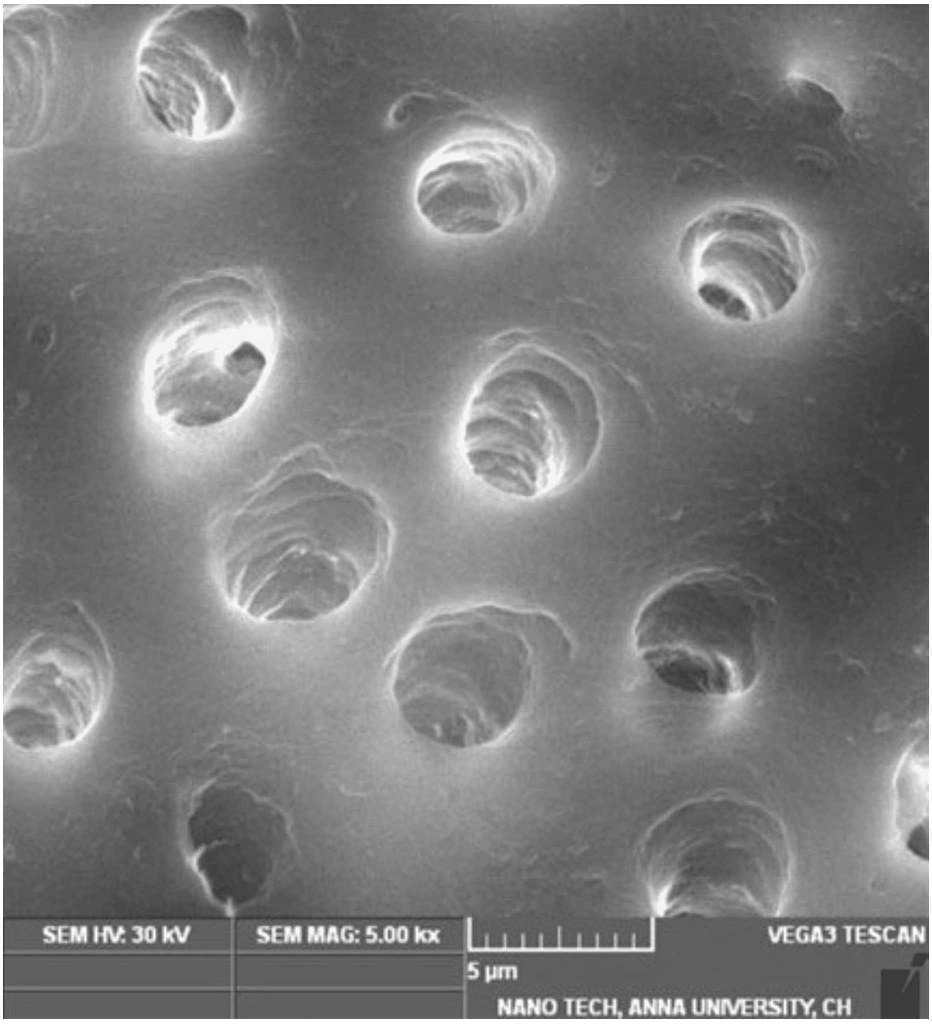

FIGURE 2: SEM photomicrograph example of root canal wall treated with 12.5% Punica granatum in 5000x with score 1 showing patent orifices of dentinal tubules and no smear layer particles in the examined field.

SEM: scanning electron microscope

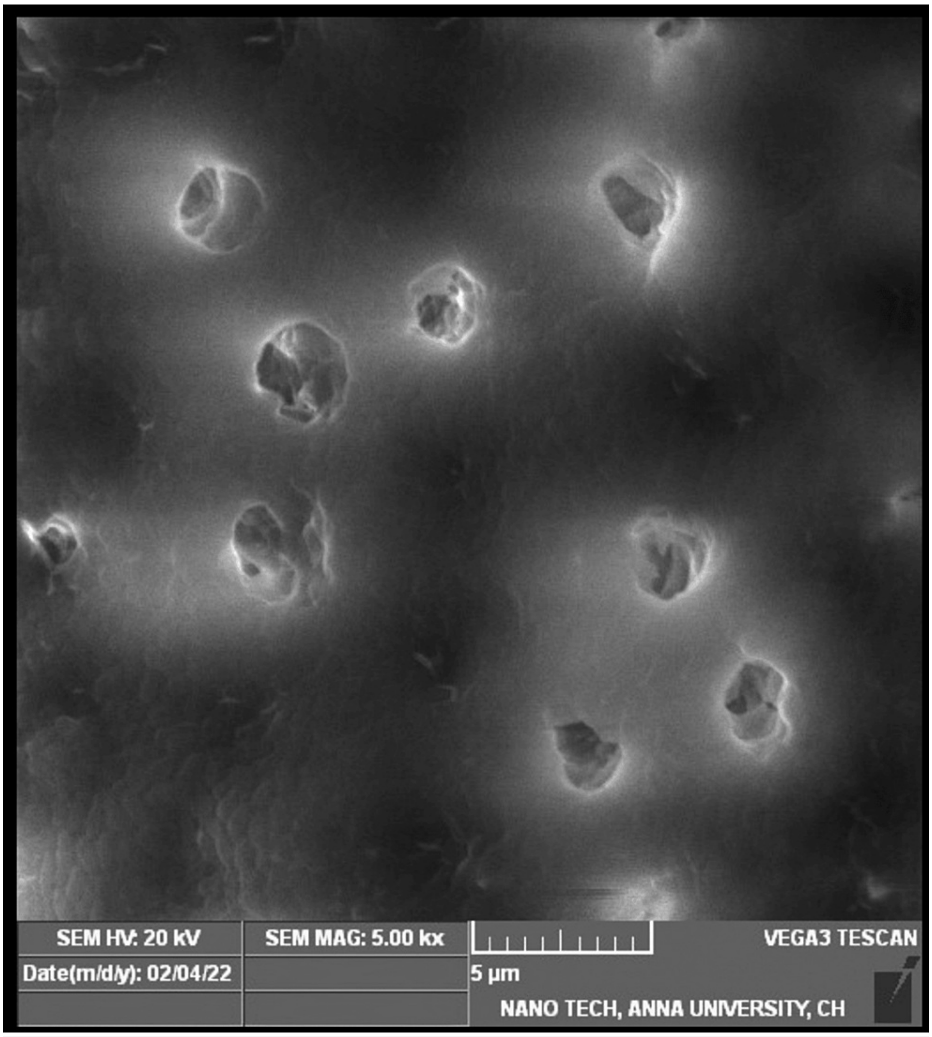

FIGURE 3: SEM photomicrograph of an example of root canal wall treated with 6.25% of Emblica officinalis in 5000x with score 1 showing patent orifices of dentinal tubules and no smear particles in the examination field.

SEM: scanning electron microscope

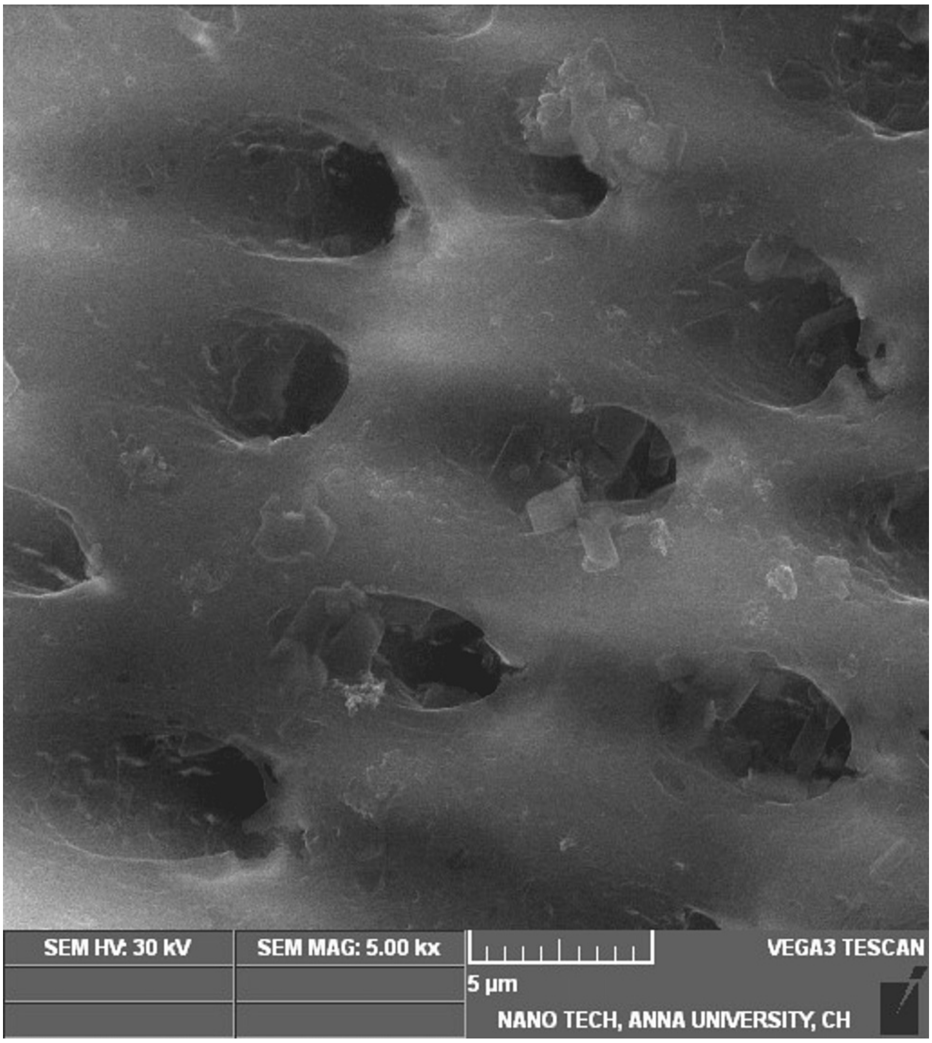

FIGURE 4: SEM photomicrograph example of root canal wall treated with NaOCl in 5000x with score 3 showing patchy distribution of smear layer up to 50% of root canal area.

SEM: scanning electron microscope, NaOCL: sodium hypochlorite

Depth of penetration

Data for depth of penetration were calculated in millimetres (Figure *5*). ImageJ software was used to calculate the depth of penetration at the middle third of the canal. The values were statistically analyzed using ANOVA and tabulated in Table *4*. The mean value of Group 1 (0.5488 ± 0.05222) and Group 2 (0.5263 ± 0.05181) showed greater depth of penetration than Group 3 (0.3087 ± 0.05743), which was statistically significant ($P<0.05$). There was no difference between Groups 1 and 2.

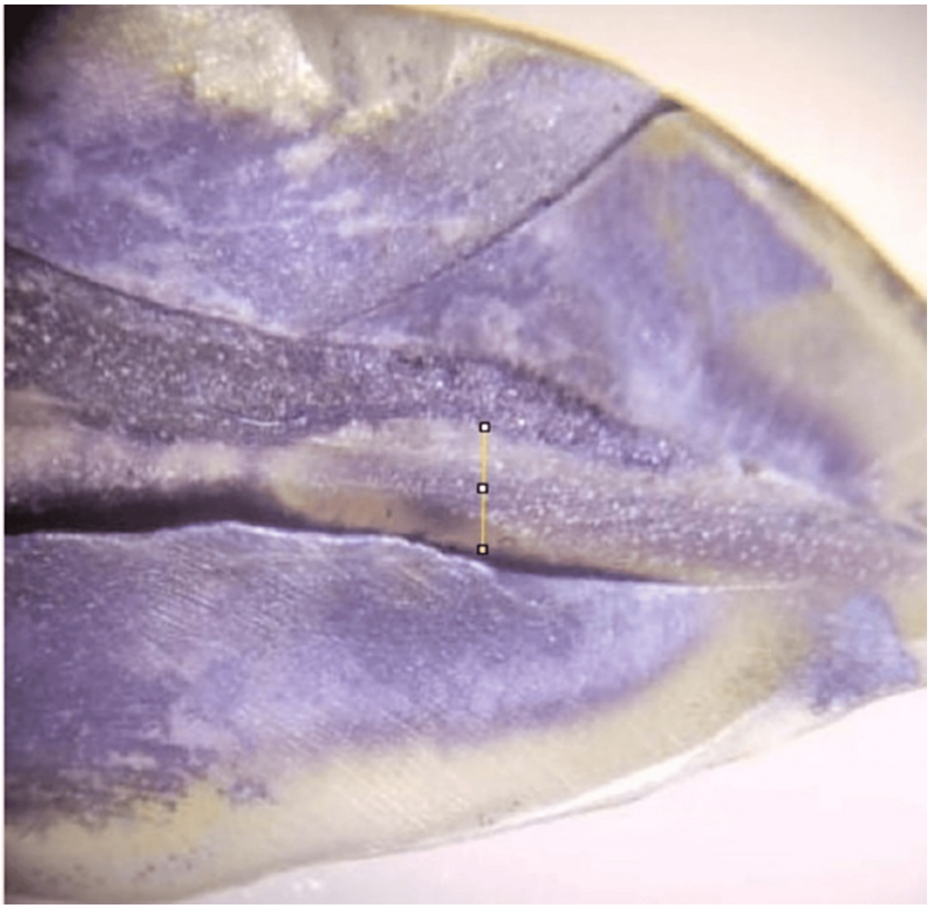

FIGURE 5: Example of depth of penetration determined under Stereomicroscope

Groups	Mean	SD	95% Confidence Interval for Mean		F	P-value
			Lower Bound	Upper Bound		
Group 1	0.5488	0.05222	0.5051	0.5924		
Group 2	0.5263	0.05181	0.4829	0.5696	48.41	0.0001
Group 3	0.3087	0.05743	0.2607	0.3568		

TABLE 4: Inter-group comparison for smear layer removal

Statistically significant p<0.05; SD: standard deviation; F: ANOVA (analysis of variance) value

Discussion

The complex root canal system must be cleaned and shaped properly for effective endodontic therapy. Root canal bacteria play a key role in the development of dental pulp inflammation and necrosis, resulting in periapical lesions [15,16]. *Enterococcus faecalis* is one of the most resistant strains in the canal and causes re-infection. [17]. Eradication of microorganisms during endodontic treatment is essential and depends on biomechanical preparation, irrigation, and intra-canal medications [18]. An ideal irrigant should have antibacterial properties, and the potential to remove the leftover necrotic pulpal tissue and cause only mild irritation to the periapical tissues. Existing formulations mostly comprise antibiotics, antibacterial agents, surfactants, and alcohol, which have been shown to be cytotoxic and only partially effective in eliminating oral infections. Herbal medicines are increasingly becoming the treatment of choice as endodontic irrigants [19].

In vitro experiments have shown tannins containing herbal extracts are substances that have a variety of antibacterial and antifungal properties. *Punica granatum* and *Emblica officinalis* both contain a significant amount of tannins and other components [7,8]. Payal et al. [8] compared the antibacterial efficacy of 6.25% *Emblica officinalis*, 12.5% *Punica granatum*, and 1% NaOCl against *E. faecalis* and found that these aqueous extracts have good antimicrobial properties as endodontic irrigants.

NaOCl is the most commonly used irrigant because of its greater tissue-dissolving ability [4]. However, it has a number of drawbacks, including the ability to irritate the tissue and produce dentin collagen denaturation, significant cell toxicity, and disintegration of the stem cells at the periapex, causing an unpleasant taste and perhaps causing allergies and leading to hypochlorite mishaps [20]. Currently, herbal compounds are preferred as endodontic irrigants. Hence, the present study evaluated the effect of various endodontic irrigating solutions such as 12.5% *Punica granatum*, 6.25% *Emblica officinalis*, and 2.5% NaOCl on microhardness, smear layer removal, and depth of penetration of irrigants. In addition to its advantages, irrigants may have negative effects on root dentin [21].

Irrigating solutions can affect the microhardness of root dentin, which in turn impacts the clinical performance of teeth that have had endodontic treatment, according to Saha SG et al. [22]. Even though reduced microhardness makes instrumentation easier, it might weaken the root structure [22]. Dentin is made up of 22% organic material, primarily collagen type I. Its depletion produces morphologic disarray and mechanical property changes [23,24]. According to Pashley et al. [25], tubular density impacts microhardness, as the density of the tubules increases, dentin microhardness decreases, likely because of a reduction in intertubular dentin and an increase in individual tubular diameter [25]. NaOCl causes a reduction in the organic content of root dentin. It also has the propensity to degrade the long peptide chains and chlorinate the protein terminal groups, which results in further breakdown of N-chloramines into other species. According to Dayal C et al. [26], instrumentation and irrigation with NaOCl alter the dentin’s biomechanical properties. In our study, NaOCl lowered the microhardness values. *Emblica officinalis* showed a decrease in microhardness which can be related to its pH value of 2.5 (acidic pH) [10]. This results in alterations in the ratio of the organic to the inorganic component of radicular dentin, which in turn affects the microhardness. *Punica granatum* showed a slightly acidic pH (3.25-4.14), which may be the reason for a decreased tendency to reduce microhardness and can be considered an effective endodontic irrigant [27].

The removal of the smear layer from the root canal walls and dentinal tubules is another crucial part of effective endodontic treatment. Because it prevents a hermetic closure between the guttapercha and the sealer, the smear layer must be removed [28]. The results of the present study showed a greater smear layer in *Emblica officinalis* and *Punica granatum* than in NaOCl. The pH of Amla is acidic because of the presence of gallic acid. The availability of calcium ions from hydroxyapatite for chelation decreases as the pH increases. As a result, a higher dissociation of the acidic irrigant results in greater attraction of calcium ions [7,10]. *Punica granatum* also exhibits greater smear layer removal because of its slightly acidic pH. The presence of ascorbic acid and flavonoids in these herbal irrigants may be the reason for their efficient removal of the smear layer [28].

An increased penetration depth of irrigants into dentinal tubules may increase the success rate of endodontic therapy. The shaping procedure showed inadequate debridement and regions unaffected by the manual K-files or rotary instruments, elimination of bacteria is mostly influenced by the depth to which irrigants penetrate to scavenge microorganisms that are firmly embedded inside the dentinal tubules [29]. According to Bystrom and Sundqvist [30], the diffusion of anti-microbial agents such as intracanal irrigants and sealers in the dentinal tubules can be initiated because of the presence of the smear layer. The dentinal tubules were stained with crystal violet dye because it allows for greater stereomicroscopic visualization [30]. The herbal irrigants showed better depth of penetration into the dentinal tubules than NaOCl. Greater penetration of irrigants into the dentinal tubules may be because of the efficient removal of the smear layer. The results of this study show that *Punica granatum* and *Emblica officinalis* can be used as alternative irrigants. The main benefits of utilizing herbal substitutes are their accessibility, affordability, longer shelf life, low toxicity, and absence of known microbial resistance.

Limitations

This study was in vitro the outcomes in actual patients may differ. Hence, lengthy in vivo investigations must be conducted to assess their effectiveness.

Another drawback of the study is that the irrigants were used individually rather than in combination. Hence, further investigations must be conducted to examine the interactions between these natural extracts and various other irrigants.

To assess their toxicity on live cells, toxicological studies must be conducted on living organisms.

Conclusions

Herbal irrigants like *Punica granatum* and *Emblica officinalis* have proved to be advantageous as endodontic irrigants. The present study reveals that the least reduction of microhardness was seen in *Punica granatum* followed by *Emblica officinalis* and NaOCl. The smear layer removal efficacy and depth of penetration were greater in *Punica granatum* and *Emblica officinalis* than in NaOCl. It was concluded that these herbal irrigants are biocompatible agents, it can be considered for future use in root canal treatment.

Additional Information

Disclosures

Human subjects: Consent was obtained or waived by all participants in this study. Institutional Human Ethics Committee issued approval CARE IHEC-I/0363/21. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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