Original Article

Comparative Evaluation of Pulp Dissolution and Smear Layer Removal Properties of Various Herbal Extracts: An *in vitro* Study

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Abstract

Introduction: Root canal treatment involves eliminating microorganisms and necrotic tissue through biomechanical debridement and root canal filling to prevent reinfection. Disinfection is enhanced by removing the smear layer, composed of organic and inorganic debris, using irritants like sodium hypochlorite (NaOCl) for organic dissolution and ethylenediaminetetraacetic acid (EDTA) for chelation. Despite their efficacy, these agents have limitations, such as cytotoxicity and dentinal erosion. Research into biocompatible alternatives, including herbal extracts and essential oils, shows promise for safer and more effective root canal disinfection. **Materials and Methods:** Eighty extracted single-rooted premolars were studied for pulp dissolution and smear layer removal. Neem leaf (Group A), orange peel (Group B) and grape seed (Group C) extracts were tested against 3% NaOCl (Group D) for pulp dissolution and 17% EDTA (Group E) for smear layer removal. Pulp dissolution was assessed by weighing specimens before and after immersion in solutions, while smear layer removal was evaluated using scanning electron microscopy. Results were statistically analysed. **Results:** On statistical analysis, NaOCl showed significantly higher pulp dissolution compared to experimental herbal extract at all-time intervals, i.e. 30, 60 and 90 min. On intergroup comparison of smear layer removal scores, 17% EDTA produced significantly higher smear layer removal with a mean score of 0.4 compared to other herbal extracts. **Conclusion:** Herbal extracts exhibited no significant pulp dissolution at 30, 60 and 90 min compared to NaOCl. Similarly, none of the herbal extracts were comparable to 17% EDTA in smear layer removal efficacy. However, amongst herbal extracts, grape seed showed significantly better smear layer removal than Orange peel extract.

Keywords: Grape seed extract, herbal irrigants, neem extract, orange peel extract, pulp dissolution, smear layer

Introduction

Root canal treatment is a critical procedure in endodontics aimed at addressing pulpal pathosis.[1] The primary goal of this treatment is to eliminate microorganisms and necrotic pulp tissue through biomechanical debridement, followed by the application of an appropriate root filling to seal the canals and prevent reinfection.[2] Effective disinfection of the root canal system is a key aspect of this process and is accomplished during bio- or chemo-mechanical preparation.[3] This involves several steps, including the extirpation and/or dissolution of necrotic pulp tissue, shaping the root canal system and cleaning out debris and smear layer using suitable irrigating solutions.[4]

The smear layer is an amorphous layer composed of remnants of organic pulp tissue, inorganic dentinal debris, bacteria and bacterial byproducts.[5] Its removal from canal walls is of significant importance in root canal therapy as it exposes the dentinal tubules, facilitating the penetration of irrigants.[6] This deeper penetration enhances the disinfection of the root canal system and contributes to the success of the treatment.[7] Research has highlighted the importance of thoroughly removing the smear layer to improve the outcome of root canal treatment and minimise the risk of reinfection.[8]

An ideal irrigating solution should possess the ability to dissolve organic tissues, such as pulp tissue, collagen and biofilm, and effectively remove the smear layer formed during biomechanical preparation.[9] Sodium hypochlorite (NaOCl)

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is amongst the most widely used root canal irrigants due to its potent tissue-dissolving and antibacterial properties.[10] NaOCl can break down organic material within the root canal system, thereby aiding in the decontamination process.[11] On the other hand, ethylenediaminetetraacetic acid (EDTA) is the most commonly used agent for smear layer removal in dentistry.[12-14] EDTA works by chelating calcium ions within the smear layer, thereby breaking down its structure and facilitating its removal.[15]

The combined use of NaOCl and EDTA has been widely documented in the literature.[16] NaOCl targets the organic component of the smear layer and canal contents,[17] while EDTA focuses on the inorganic portion.[18] This synergistic action is crucial for achieving comprehensive cleaning and disinfection of the root canal system. Studies have shown that the sequential use of these agents significantly improves the removal of the smear layer and enhances the penetration of subsequent root canal sealers.[19]

Despite their effectiveness, both NaOCl and EDTA have notable disadvantages. NaOCl is known for its cytotoxicity, which can lead to irritation or hypersensitivity reactions if extruded beyond the root apex.[20] In addition, prolonged exposure to NaOCl can negatively affect the mechanical properties of dentin, such as elasticity and microhardness.[21] Similarly, EDTA, while effective in smear layer removal, can contribute to dentinal erosion and weaken the structural integrity of the tooth.[22] Moreover, both agents raise concerns regarding biocompatibility, which has driven researchers to explore alternatives to minimise these undesirable effects.

Recently, a growing interest has been in identifying more biocompatible and sustainable alternatives to conventional irrigants. Amongst these, herbal extracts have garnered significant attention due to their natural origin and minimal side effects. Herbal extracts are being investigated for their antimicrobial, anti-inflammatory, and tissue-dissolving properties, which can potentially provide a safer and more effective alternative for root canal disinfection.

Several studies have evaluated the efficacy of various herbal extracts, such as neem (*Azadirachta indica*), tulsi (*Ocimum sanctum*), turmeric (*Curcuma longa*), and green tea (*Camellia sinensis*), as potential endodontic irrigants.[23] Neem extract has demonstrated antibacterial and antifungal properties, making it a promising candidate for reducing microbial load in the root canal.[24] Similarly, tulsi and turmeric possess antimicrobial and anti-inflammatory characteristics that can aid in disinfection and promote healing.[25] Green tea extract, rich in polyphenols, has shown potential in disrupting biofilms and providing antioxidant benefits that support tissue repair.[26]

In addition, propolis, a resin-like material produced by bees, has gained prominence as a natural endodontic irrigant.[27] Propolis exhibits broad-spectrum antimicrobial activity, biocompatibility, and anti-inflammatory properties.[28] Studies comparing propolis to conventional irrigants have found it effective in microbial reduction and smear layer removal while being less cytotoxic.[29]

Another notable advancement is the exploration of essential oils, such as tea tree oil and eucalyptus oil, which possess potent antimicrobial properties.[30] When appropriately formulated, these oils can be used as adjuncts or alternatives to traditional irrigants.[4] Moreover, their natural composition ensures better biocompatibility and reduced adverse effects.

While the use of herbal extracts and other natural agents shows promise, further research is required to standardise their formulation, concentration and application protocols.[31] Clinical trials are essential to establish their efficacy and safety compared to conventional agents such as NaOCl and EDTA. Moreover, advancements in nanotechnology are being integrated with natural products to enhance their efficacy. For example, nanoparticles derived from herbal extracts can improve penetration into dentinal tubules and increase antimicrobial activity.[32]

Root canal treatment relies heavily on effective cleaning and disinfection of the root canal system to ensure long-term success. While conventional irrigants such as NaOCl and EDTA remain the standard, their limitations highlight the need for safer alternatives. Exploring herbal extracts and other natural agents represents a promising direction in endodontic research. As scientific advancements continue, these biocompatible alternatives may be pivotal in improving patient outcomes while reducing the potential risks associated with conventional treatments.

Aim

The aim of the present study was to evaluate the pulp dissolution property and smear layer removal efficacy of three different herbal extracts such as neem leaf, orange peel and grape seed extract as root canal irrigant.

Materials and Methods

This clinical study was conducted in the conservative dentistry department of our hospital. This clinical study evaluated the pulp dissolution property and smear layer removal efficacy of three herbal extracts, such as neem leaf, orange peel and grape seed extract, as root canal irrigants. The institution's ethical committee reviewed and approved this study with Reference No.: SEDC/2022/43, Dated 10 June 2021. This study was planned and conducted under the declaration of Helsinki (version 2008) and carried out from November 2022 to April 2023.

Following institutional research ethical approval, 80 single-rooted mandibular 1st premolar teeth with a single root canal (confirmed radiographically), extracted due to orthodontic purpose, and with fully developed apices were selected for this study and further divided into two study groups containing 40 sample teeth each.

Preparation of herbal extracts

A total of 30 g of each experimental herbal product was added to 300 ml of distilled water in a glass beaker. After obtaining a homogeneous mixture, the solutions were boiled till 30 ml of the herbal extract solutions were left in the beaker. The herbal

extracts were then filtered to obtain the irrigating solutions and stored in opaque glass bottles.

The irrigating solutions thus prepared were as follows:

- Group A – Neem leaves (HerbLand, Rajasthan, India)
- Group B – Orange peel (HerbLand, Rajasthan, India)
- Group C – Grape seed (Herbal engine, Punjab, India)
- Group D – 3% NaOCl (Parcan, Septodont Healthcare India Pvt. Ltd) (Control group for assessing pulp dissolution property)
- Group E – 17% EDTA (Waldent Innovations Pvt. Ltd, India) (Control group for evaluation of smear layer removal property).

Evaluation of pulp dissolution property

A total of 40 teeth samples were randomly distributed and equally divided into four different groups (Groups A, B, C and D). Each tooth was sectioned using a diamond disc by placing longitudinal mesial and distal grooves, avoiding damage to the root canal. Then, the tooth was divided into two halves using a chisel and mallet. The pulp specimen was removed from the separated roots using a straight probe and tissue-holding forceps.

Each of the pulp specimens was washed with distilled water, blotted on a filter paper and weighed using Shimadzu AY220 (Kyoto, Japan) weighing balance. The weight of the pulp specimens was recorded for all four groups at a base level (t0), and then, they were immersed into the respective solutions. The quantity of total pulp dissolution was observed at 30 min (t1), 60 min (t2) and 90 min (t3), as described in Figure 1.

Evaluation of smear layer removal property

40 sample teeth were randomly distributed and divided into four different groups (Groups A, B, C and E) containing

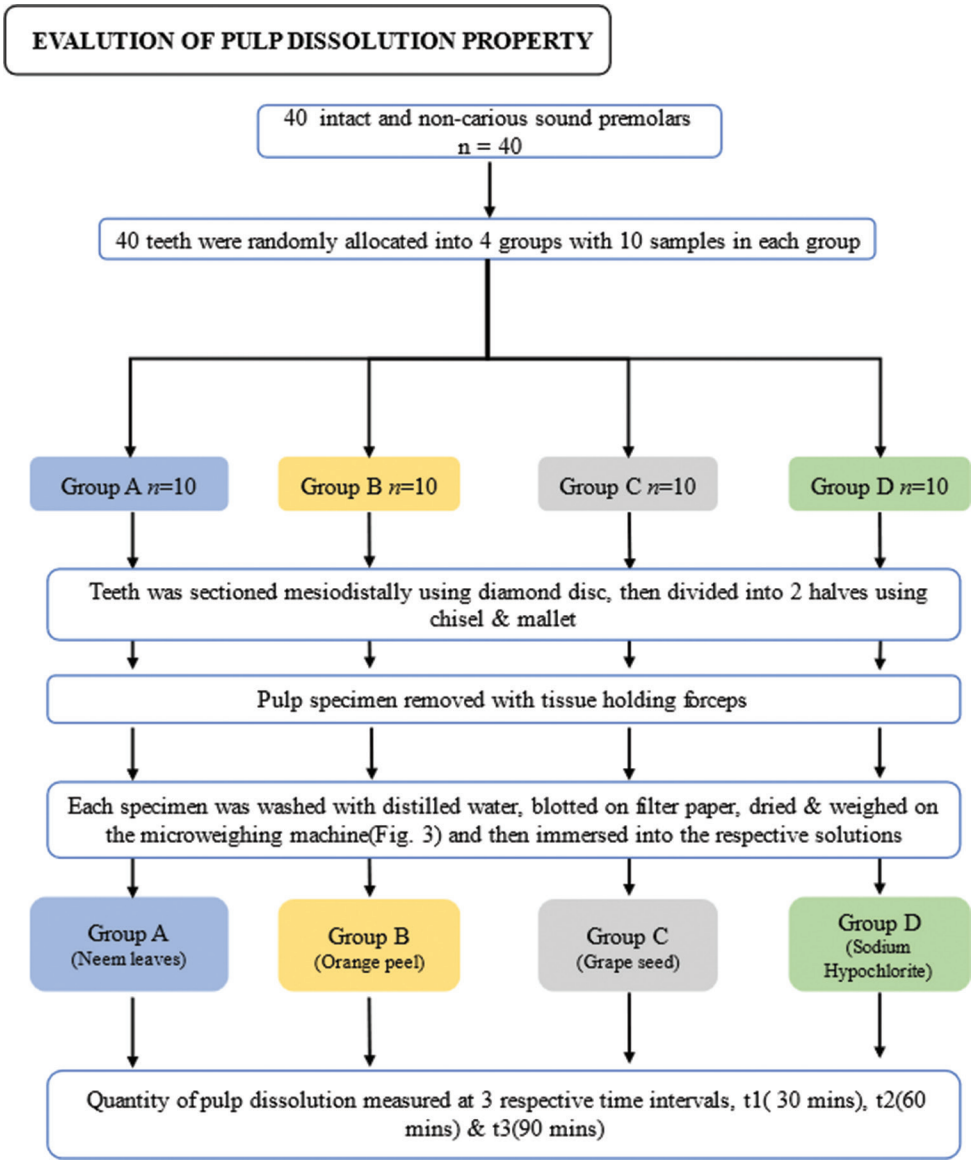

Figure 1: Evaluation of pulp dissolution

10 samples each. Following this, the apical foramen of each root was coated with cyanoacrylate glue. The teeth were then embedded into a polyvinyl-siloxane impression material of dimension 1 cm × 1 cm to obtain the closed system. An ideal access cavity was prepared on each tooth, followed by pulp extirpation with a Size #15 barbed broach. Working length was determined radiographically using a #15 K file. The root canals were then prepared up to apical size 25, 0.06 taper using rotary nickel–titanium files (Woodpecker) at specific speed and torque by manufacturer's instructions.

After each instrumentation, canals were irrigated with 0.5–0.7 ml of the experimental herbal solutions in Groups A, B and C and with 17% EDTA solution in Group E. Sterile distilled water was used as a final rinse for all teeth. Canals were then dried using sterile absorbent paper points. Thereafter, each of the sample teeth was removed from its embedded block of polyvinyl-siloxane impression material, and the teeth were decorated at the cementoenamel junction using a diamond disc to obtain a standard root length of 10 mm. Mesial and distal longitudinal grooves were placed on the tooth using a diamond disc so that the root canal was not perforated or damaged during the procedure. After the desired depth of groove was obtained, the roots were split into two halves (buccal and lingual) using a chisel and mallet, and then the samples were sent for SEM study, as shown in Figure 2.

Scanning electron microscopic evaluation

The specimens were coded and then mounted onto metallic stubs with carbon strip backing with the entire root canal visible and facing upward and then subsequently viewed under the scanning electron microscope. The photomicrographs were obtained at ×3000 using digital image analysis software and were stored appropriately for subsequent analysis [Figures 3-6]. The results were then scored following Rome *et al*. criteria, as shown in Table 1.

Statistical analysis

Statistical analysis was done using the Statistical Package of the Social Science (SPSS Version 22.0; Chicago Inc., USA).

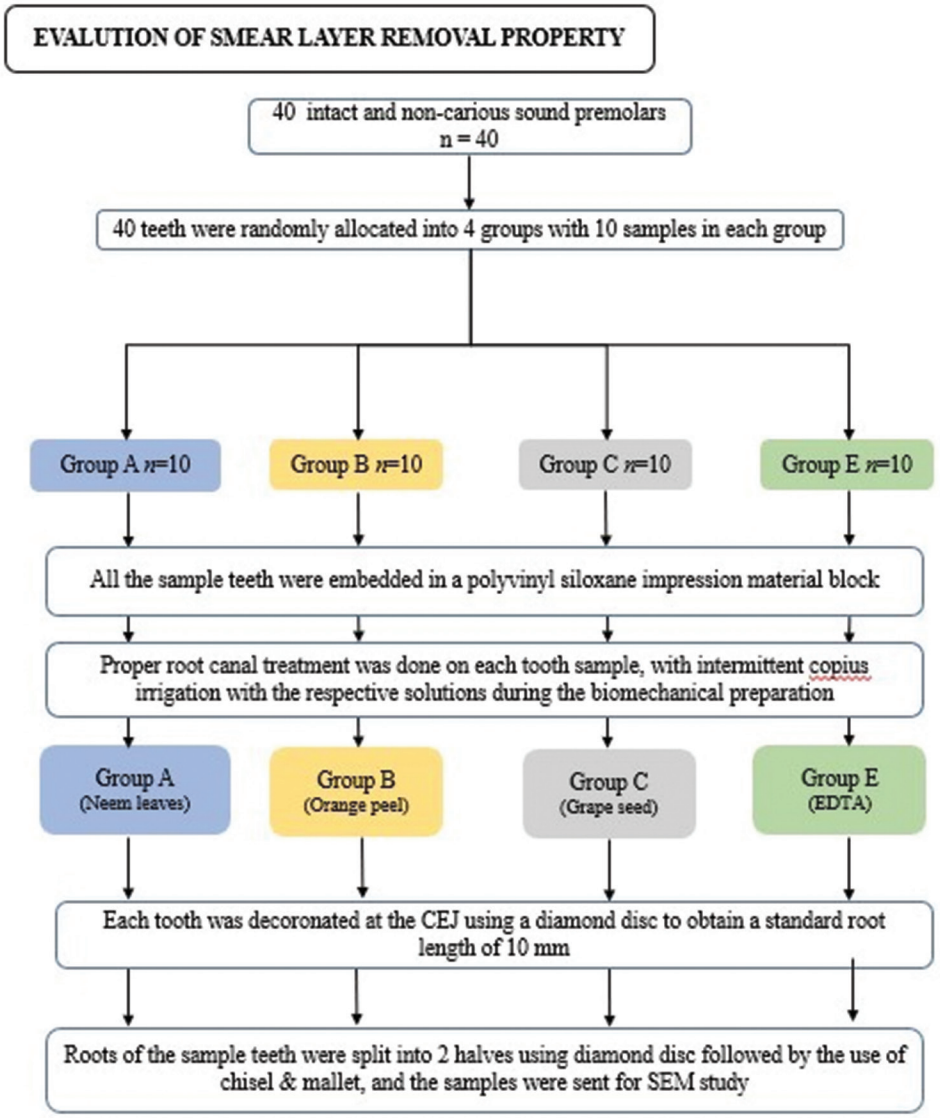

Figure 2: Evaluation of smear layer removal

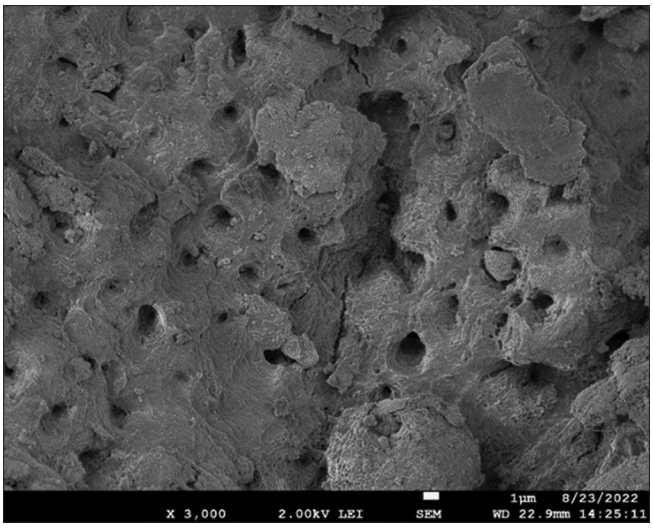

Figure 3: SEM image of canal wall after the application of Neem extract

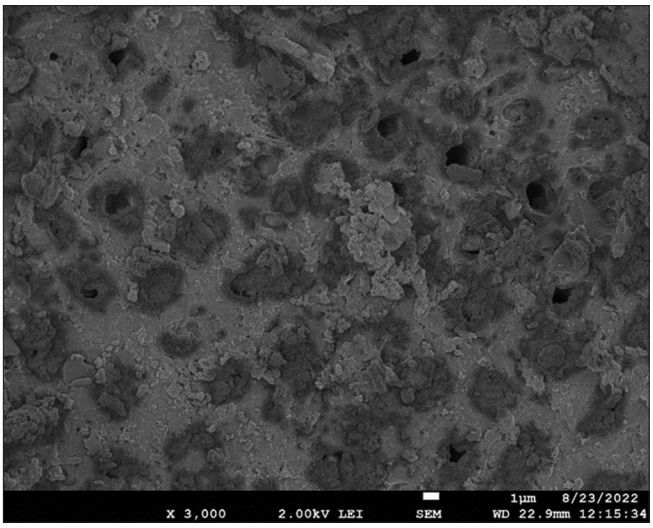

Figure 4: SEM image of canal wall after the application of Orange peel extract

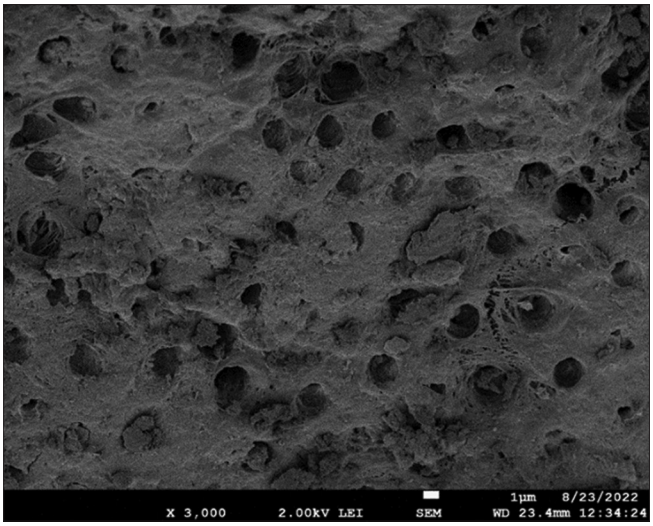

Figure 5: SEM image of canal wall after the application of Grape seed extract

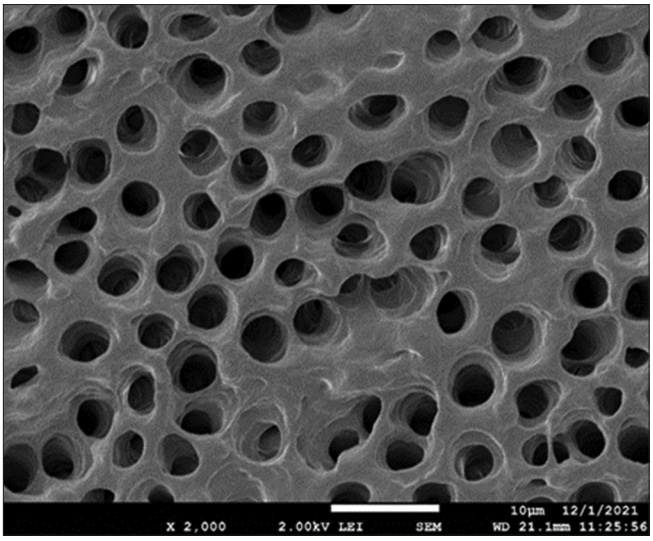

Figure 6: SEM image of canal wall after the application of 17% EDTA

Table 1: Smear layer removal scores

Score	Interpretation
0	No smear layer
1	Smear layer present only in aperture of dentinal tubules
2	The smear layer covering the root canal surfaces and dentinal tubules apertures
3	Heavy smear layer masking dentinal tubule apertures

Rome *et al*., 1985[33]

Data comparison was made by applying specific statistical tests to find out the statistical significance of the comparisons, which included one-way ANOVA to compare the means of the groups and the *post hoc* Tukey HSD test for intergroup mean comparison. The significance level was fixed at $P < 0.05$.

Results

On statistical analysis, NaOCl showed significantly higher pulp dissolution followed by grape seed extract, orange peel extract and neem extract at 30, 60 and 90 min, as seen shown in Table 2. After 30 min, the fibrous pulp tissue in NaOCl was almost completely dissolved leaving only fine particulate sediments on the filter paper. Herbal extracts did not exhibit significant pulp dissolution at 30, 60 or even after 90 min, and no significant difference in pulp dissolution was found amongst herbal extracts.

On intergroup comparison of smear layer removal scores, results concluded that 17% EDTA produced significantly higher smear layer removal with a mean smear layer score of 0.4, followed by grape seed extract (1.4), neem extract (2.1) and orange peel extract (2.9) as shown in Table 3. Thus, the lowest smear layer mean score (0.4) was obtained with 17% EDTA, while the highest score (2.9) was found with orange peel extract, indicating that orange peel extract was least efficacious in removing the smear layer.

Table 2: Pulp dissolution efficacy (weight reduction in mg)

Group	30 min, mean±SD	60 min, mean±SD	90 min, mean±SD	Statistical test (ANOVA)	*P*
Neem extract	0.05±0.01	0.09±0.02	0.12±0.02	*F*=96.34	<0.05*
Orange peel extract	0.04±0.01	0.08±0.02	0.10±0.03		
Grape seed extract	0.06±0.02	0.11±0.02	0.15±0.03		
3% NaOCl	0.40±0.05	0.78±0.08	1.20±0.10		

*Significant at $P<0.05$, NaOCl showed significantly higher pulp dissolution than all herbal extracts at all-time intervals. NaOCl: Sodium hypochlorite, SD: Standard deviation

Table 3: Smear layer removal scores (mean±standard deviation)

Group	Smear layer score, mean±SD	Statistical test (ANOVA)	*P*
Neem extract	2.1±0.4	*F*=134.25	<0.05*
Orange peel extract	2.9±0.3		
Grape seed extract	1.4±0.3		
17% EDTA	0.4±0.2		

*Significant at $P<0.05$, EDTA showed significantly higher smear layer removal than all herbal extracts. EDTA: Ethylenediaminetetraacetic acid, SD: Standard deviation

Discussion

NaOCl has been considered one of the most commonly used irrigants for several decades owing to its excellent tissue dissolution and antimicrobial properties. However, NaOCl with an alkaline pH of approximately 11–12 shows toxic effects on organic tissues, ranging from skin ulceration to rupture or destruction of red blood cells, leading to hemolysis, eventually leading to cell death or necrosis.[33] Whereas EDTA is the most commonly used agent in the removal of smear layer from the root canal, which acts by chelation action with the inorganic portion of the canal debris and has been reported to decalcify dentine to a depth of 20–30 μm in 5 min. However, in case of extrusion of EDTA beyond the apical foramen into the periapical tissues, it can inhibit macrophage function, alter inflammatory response in periapical infections, cause irreversible decalcification of periapical bone and also have an effect on neuroimmunological regulatory mechanisms.[18]

This fact motivated us for the present study, which aimed to evaluate the pulp dissolution and smear layer removal efficacy of various herbal extracts and compare it with NaOCl and EDTA and thus an attempt to find alternative irrigants in order to overcome the risk in clinical practice. Apart from this, herbal products are also easily available and cost-effective.

Any irrigant's ability to dissolve organic tissue is influenced by its concentration, its type, how long it stays in contact with the tissues, and the area and mass of the organic tissue. As a result, in the current study, these variables were strictly standardised using the same volume of solutions and the similar shape and weight of human pulp tissue. The weight of the pulp tissue was determined in the current investigation using a precision scale. The pulp tissue is weighed before and after contacting the irrigating solutions. This method is more accurate because precise tissue dissolution can be estimated by deducting the weight of residual pulp from the weight of the pulp before contact with the irrigating solution. The weight of the pulp was measured first at the baseline before subjecting the samples to dissolution solutions. Then, the time intervals at which the weight of the pulp was measured were 30, 60 and 90 min after exposing the sample to the solutions.[34,35]

Pulp dissolution

Statistical analysis showed that NaOCl showed significantly higher pulp dissolution than herbal counterparts at 30, 60, and 90 min. While herbal extracts did not exhibit significant pulp dissolution at 30, 60, or even 90 min, no significant difference in pulp dissolution was found amongst herbal extracts.

The observations in our study are in accordance with various other studies that have reported NaOCl as an ideal irrigant and has a high level of efficacy as a pulp tissue dissolving agent. The tissue dissolution property of NaOCl is due to the free chlorine in the solution.

Several studies have been carried out on grape seed extract regarding its anti-microbial properties against root canal pathogens, especially *Enterococcus faecalis*,[36,37] in cleaning smear layer from the root canal wall,[38] its influence on microhardness of the root canal dentin,[39] and its effect on tensile bond strength of resin-dentin bond[40] due to its property of serving as a dentin collagen cross-linking agent. However, the pulp dissolution property of Grape seed extract has not been investigated to date. In the present study, although marginally higher pulp dissolution was seen with Grape seed extract than the rest of the herbal extract, it was significantly less than NaOCl. This finding of the present study cannot be compared with another study due to the lack of literature.

Similar to our findings, Bhavsar *et al*.[41] reported that the pulp dissolution was significantly higher with NaOCl compared to neem leaf extract. Sajjan *et al*.[42] also conducted a study on the pulp dissolution property of NaOCl on bovine pulp tissue and reported that 3% NaOCl showed the highest tissue dissolution, followed by neem. The pulp dissolution property of Neem, although very less, can be attributed to the fact that it has certain active phytochemical constituents such as nimbidin, nimbin, Azadirachtin, gallic acid, etc., which might play a role in pulp tissue dissolution.[43]

Orange peel extract acts as an anti-microbial, anti-inflammatory and anti-plaque agent. It punctures the

cell membrane as it has a strong affinity for lipid components of the cell membrane.[44] However, there is a lack of literature regarding its pulp dissolution ability. In the present study, orange peel extract showed significantly less pulp dissolution than NaOCl.

SMEAR LAYER REMOVAL

The smear layer is composed of organic and inorganic phases, and its removal from the canal walls is crucial because this permits the irrigants to enter the dentinal tubules. Eliminating the smear layer increases the effectiveness of intracanal medications and irrigants and decreases the time required for canal disinfection. In addition, it makes it easier for root canal filling to adapt to canal walls and reduce apical leakage.[45,46]

The efficacy of the smear layer removal was evaluated after root canal preparation using experimental herbal extracts as root canal irrigants and compared with EDTA. The smear layer was assessed using the scoring criteria given by Rome *et al.*[47] shown in Table 1.

The results of the present study concluded that 17% EDTA produced significantly higher smear layer removal with a smear layer mean score of 0.4 when compared with other herbal extracts. Amongst herbal irrigants, grape seed extract (1.4) performed significantly better in comparison to orange peel extract (2.9), but there was no significant difference between grape seed and neem extract (2.1). Thus, the lowest smear layer mean score (0.4) was obtained with 17% EDTA, while the highest score (2.9) was found with orange peel extract, indicating that orange peel extract was least efficacious in removing the smear layer.

Studies have reported 17% EDTA to be the most effective in terms of smear layer removal as it dissolves the inorganic portion of the debris by chelation and detaches biofilms adhering to root canal walls, thus removing the calcifications hindering the biomechanical preparation of the root canal.[48]

In a few studies, grape seed extract has been found to be effective in removing the smear layer.[36,38] This can be attributed to the fact that there is the presence of polyphenol content,[49] which consists of 74-78% proanthocyanidin, serving as a natural cross-linking collagen, thus increasing the potential of Grape seed extract to clean the smear layer when used as an irrigating solution.[50] In the present study, it was concluded that Grape seed extract was effective in removing the smear layer compared to the other herbal extracts. However, it was significantly less than EDTA. This result is in agreement with the study done by Margono *et al*. (2017)[50] in which they investigated the ability of grape seed extract in different concentrations to clean the smear layer from the root canal and concluded that, at all the concentrations, grape seed extract was effective in smear layer removal. Still, they were less effective than 17% EDTA.

Neem has various biological properties such as anti-microbial action, anti-fungal, anti-inflammatory effects, etc. Various studies have suggested that neem extract can be effective in removing the smear layer, owing to the fact that it consists of active phytoconstituents such as acid metabolites, flavonoids, isoprenoids, alkaloids, glycosides, steroids, tannins, nimbin, nimbidin and nimbidol.[51] In the present study, Neem produced significantly less smear layer removal than EDTA. Similarly, in their SEM study, Kumar *et al.*[52] found that 17% EDTA showed significantly higher smear layer removal than neem leaf extract.

In line with our results, Sebatni *et al*.[45] did an *in vitro* SEM study to compare the efficacy of neem and orange peel extracts on the smear layer removal and found no significant difference between them. However, Ranjitha *et al.*[51] found significantly higher smear layer removal with neem than with orange peel extract.

Orange peel extract consists of certain ingredients, d-limonene, flavonoids, acid metabolites and citric acid, which acts as a chelating agent and may aid in removing the smear layer. However, in the present study, orange peel extract was found to be the least effective amongst all herbal extracts and the control EDTA. In agreement with our study, Bolhari *et al.* (2011)[53] observed that citrus aurantifolia (orange) extract could not effectively remove the smear layer from the root canal.

One of the limitations of the present study is the preparation of herbal extract, which is a time-consuming and complex process. It is also prudent to compare these herbal formulations at various concentrations. Further research with more herbs at different concentrations is necessary before considering herbal irrigants as an alternative to conventional irrigants.

Conclusion

Based on the findings of this *in vitro* study, the following conclusions can be drawn:

The pulp dissolution capability of 3% NaOCl was significantly greater than that of the herbal extracts tested at all-time intervals.

Herbal extracts showed minimal pulp dissolution even after 30, 60, or 90 min, with no significant differences, indicating they are unreliable alternatives to conventional irrigants like NaOCl.

The study found that 17% EDTA was significantly more effective in removing the smear layer than the herbal extracts.

Amongst the herbal irrigants, grape seed extract outperformed orange peel extract in smear layer removal, though no significant difference was observed between grape seed and neem extracts.

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Conflicts of interest

There are no conflicts of interest.

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