

# Comparative Evaluation of Smear Layer Removal Efficacy of Neem Leaf Extract, Propolis, and Orange Oil when used as Endodontic Irrigants: An *in vitro* Scanning Electron Microscopic Study

## Abstract

**Introduction:** In root canal treatment, chemical debridement of the root canal with the help of irrigants is important due to the complex internal anatomy of the teeth. Biomechanical root canal preparation produces a smear layer. It covers the dentinal tubules, which may interfere with the penetration of various irrigants and intracanal medicaments, so the smear layer has to be removed. The potential side effects and certain limitations of chemical irrigants for smear layer removal have led to a shift toward herbal alternatives. **Objective:** The aim of the present study was to evaluate and compare the smear layer removal efficacy of Neem leaf extract, Propolis, and Orange oil as endodontic irrigants using the scanning electron microscope. **Materials and Methods:** Ninety samples were taken and then randomly (simple random sampling method) divided into three experimental groups ( $n = 30$ ) depending upon different endodontic irrigants used: Group 1-Neem leaf extract, Group 2-Orange oil and Group 3-Propolis and smear layer removal evaluation was done under the scanning electron microscope Scanning Electron Microscope (SEM) at all the levels, i.e., coronal, middle, and apical. **Results:** The collected data were analyzed using the Chi-square test. Group 1 (Neem leaf extract) showed the highest smear layer removal efficacy, which was followed by Group 2 (Orange oil) and the least efficacy of smear layer removal was shown by Group 3 (Propolis) at coronal, middle, and apical levels. **Conclusion:** Group 1 (Neem leaf extract) performed significantly better in removing the smear layer at coronal, middle, and apical levels as compared to the other two groups.

**Keywords:** Herbal, irrigation, neem leaf extract, orange oil, propolis, SEM, smear layer

## Introduction

Root canal anatomy is complicated, and it is characterized by accessory features such as isthmus, fins, and cul de sacs, which makes root canal treatment challenging. A clinician's main target is to completely disinfect the root canal system more effectively and efficiently. The success rate of root canal treatment relies on cleaning, shaping, disinfecting, and obturating the root canal.<sup>[1]</sup> Cleaning and disinfecting the root canal system can be achieved through irrigation and instrumentation, which is one of the foremost goals of biomechanical preparation.<sup>[2]</sup> Biomechanical root canal preparation forms an irregular layer of debris and microorganisms known as "Smear layer."<sup>[3]</sup>

The smear layer should be removed from the root canal as it is infected, and harbor microorganisms and their toxins within

the dentinal tubules. It may interfere with the penetration of various antimicrobial irrigating solutions, intracanal medicaments, and sealers into dentinal tubules. To decrease the risk of micro-leakage, the smear layer should be removed; otherwise, it can lead to endodontic failure.<sup>[4]</sup> In this aspect, endodontic irrigating solutions play a key role in the removal of the smear layer.

Various endodontic irrigants are available, which can be categorized into Chemical agents (Normal saline, Sodium hypochlorite [NaOCl], Ethylene diamine tetra acetic acid [EDTA], Chlorhexidine, Maleic acid, Chitosan, and Polyacrylic acid) and Natural agents (Triphala, Green tea, Neem, Aloe vera, Propolis, Tea tree oil, Turmeric, Miswak, Orange oil).

NaOCl is mostly used in dentistry as irrigating solutions in root canal treatment as it has good organic and necrotic pulpal

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Ria Setia,  
Nitika Bajaj,  
Meenu Bhola,  
Gurlal Singh Brar

Department of Pedodontics and Preventive Dentistry, Dasmesh Institute of Research and Dental Sciences, Faridkot, Punjab, India

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## Address for correspondence:

Dr. Ria Setia,  
Department of Pedodontics and Preventive Dentistry, Dasmesh Institute of Research and Dental Sciences, Faridkot, Punjab, India.  
E-mail: setiaria92@gmail.com

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tissue dissolving capacity and bactericidal properties. However, it has limited effect on the dissolution of dentin debris and inorganic component of the smear layer.<sup>[5]</sup> It also has an unpleasant smell, and taste is toxic to periradicular tissues, has allergic potential, and its excessive periapical extrusion can cause hypochlorite accidents. It reacts with the protein present in the dentin matrix and decreases the flexural strength of dentin.<sup>[6]</sup>

The use of certain acids such as EDTA, citric acid, and maleic acid is effective for smear layer removal. However, there are some side effects as EDTA and citric acid reacts with calcium ions in dentin, produces chelation, and reduces dentinal microhardness.<sup>[7]</sup> These chemical endodontic irrigants, such as NaOCl, EDTA, maleic acid, and chlorhexidine, with their cytotoxic effects, have led several researchers to discover natural substitutes as endodontic irrigant.

Herbal and natural products are gaining their attention as endodontic irrigants mainly to overcome the drawbacks of chemical endodontic irrigants. Natural products such as Triphala, Green tea, Neem, Aloe vera, Propolis, Tea tree oil, Turmeric, Miswak, and Orange oil are well known for their biocompatibility. These products have natural therapeutic properties like antioxidant, antibacterial and anti-inflammatory properties which have favored their use in dentistry for canal disinfection. The main advantages of using these herbal and natural products are due to its natural origin, easy availability, cost-effectiveness, increased shelf life, less toxicity, safety, fewer side effects, and lack of microbial resistance.

*Azadirachta indica* also known as Indian Neem or Margosa tree, is a common medicinal and traditional plant of India. Each part of the tree has some medicinal property, and extract from the leaf is found to have antibacterial efficacy.<sup>[8]</sup> Neem has antibacterial, antifungal, anti-inflammatory, and immunomodulatory properties due to its active constituents, such as alkaloids, glycosides, terpenoids, steroids, and tannins.<sup>[9]</sup> Neem constitutes a number of compounds, such as nimbin, nimbidin, and nimbolide, which has anti-adherence activity by altering the bacterial adhesion and ability of the microorganism to colonize.<sup>[10]</sup> *In vitro* study by Babaji *et al.* evaluated and found an inhibitory zone against *Enterococcus faecalis* by *Morinda citrifolia*, *A. indica*, and *Aloe vera* due to their antibacterial effect and concluded that these could be used as root canal irrigants.<sup>[11]</sup>

Propolis (Bee Glue) is a natural antibiotic resinous substance extracted by bees from plants such as poplars, conifers and from flowers of Genric clusia. It is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% various other substances. The active ingredients are flavonoids, cinnamic acid, and caffeic acid phenethyl ester. It is well known for antimicrobial, anti-inflammatory, antioxidant,

cytotoxic, anesthetic, and cariostatic properties.<sup>[12]</sup> It has drawn its attention in endodontics as a pulp capping agent, intracanal medicament, cariostatic agent, mouth rinse, storage media for avulsed teeth, and endodontic irrigant.<sup>[12]</sup> Verma *et al.*, in an *in vivo* study, demonstrated the antibacterial efficacy of propolis extract and advised its use as a root canal irrigant in endodontic treatment.<sup>[13]</sup>

Orange oil is also known as *Citrus sinensis*. *C. sinensis* is the most widely grown fruit crop which belongs to the family *Rutaceae*. It mainly consists of 94% d-limonene and 3% of myrcene and also has long-chain aliphatic hydrocarbon alcohol and aldehydes.<sup>[12]</sup> Orange oil is rich in antioxidants as it contains Vitamin C. Phytochemical analysis of *C. sinensis* done by Oikeh *et al.*, revealed the presence of Tannins, flavonoids, saponins, phenolic compounds, and essential oils, which are responsible for its antibacterial, antioxidant, and anti-inflammatory properties.<sup>[14]</sup> Due to these benefits, it was decided to use orange oil as one of the endodontic irrigants in this study.

While research is on the rise in this field but there has been little literature available to evaluate the efficacy of these products as endodontic irrigants on smear layer removal. Hence, the purpose of this study was to assess and compare the smear layer removal efficacy of three different herbal endodontic irrigants such as Neem leaf extract, Propolis, and Orange oil using a scanning electron microscope.

## Materials and Methods

The study was conducted in the Department of Pediatric and Preventive Dentistry. Ninety single-rooted premolars with fully developed apices extracted for orthodontic purposes were used for the study

### Preparation of extracts

Neem leaf extract – Neem leaf powder was taken and weighed (50 g) mixed with distilled water (500 ml), and then boiled at 100°C so as to get 50 ml of neem leaf extract.

Orange oil – Orange peel powder was taken and weighed (50 g) mixed with distilled water (500 ml) and then boiled at 100°C so as to get 50 ml of orange oil.

Both of the prepared extracts were filtered using filter paper and then obtained extracts were stored in opaque bottles and used as irrigant.<sup>[15]</sup>

Propolis extract – 5 ml of Propolis was diluted in 95 ml of distilled water to make a 5% solution and then it was used as irrigant.

### Methodology

Ninety orthodontically extracted straight and single-rooted premolars were taken for the study. Using ultrasonic scaler debris was cleaned from teeth and then stored in distilled water until use. Crowns were removed at the level of the

cement-enamel junction, and only the roots were taken for the study. The working length determination was done using 15 K-file. The working length was kept 1 mm short of the apical foramen. The apical foramen of each root was sealed using sticky wax in order to restrict the flow of irrigants through it and also to simulate the clinical situation. The selected teeth were divided randomly (Simple random sampling method) into three experimental groups depending on the different endodontic irrigants used.

- Group 1– Neem leaf extract as an irrigant ( $n = 30$ )
- Group 2 – Orange oil as an irrigant ( $n = 30$ )
- Group 3 – Propolis as an irrigant ( $n = 30$ ).

Root canal preparations were done till size # 25 using hand K-files which was followed by Protaper Gold rotary instrumentation up to size F3 with crown down preparation technique. During the instrumentation, the root canals were irrigated using 2 ml of the prepared irrigating solution of the respective group for 10 s after the use of each file. For irrigation, a 2.5 ml syringe (0.55 mm × 25 mm, 24G) was used to deliver the irrigating solutions into the canal and it was kept 2 mm short from the working length. Finally, the root canals were flushed with sterile distilled water and then dried using sterile absorbent paper points.

### Scanning electron microscopic evaluation

Using a diamond disc, longitudinal grooves were placed on the buccal and lingual surfaces of the root portion of all the samples without penetrating into the root canal. Using

chisel, the root portion was split into two halves. One-half of the root portions were prepared for scanning electron microscopic (SEM) analysis. All the samples were mounted on an aluminum stub and gold sputter coating was done. The samples were viewed under JSM-6510 LV Scanning electron microscope to evaluate the smear layer removal efficacy. For each sample, photomicrographs were taken at the coronal, middle, and apical sites of the root canal at ×1500 [Figures 1-3].

Scoring of the smear layer removal at coronal, middle, and apical sites of the root canal was evaluated using a five-point scale described by Al-Kilani *et al.* in 2003 as follows:<sup>[16]</sup>

- Score (1) Clean root canal wall, only a few small debris particles
- Score (2) Light coverage of debris, <25% tubules covered
- Score (3) Moderate coverage of debris, >25% but < 50% tubules covered
- Score (4) Heavy coverage of debris, >50% but <75% tubule covered
- Score (5) Complete or nearly complete root canal wall covered by debris.

### Statistical analysis

Data were tabulated and statistically analyzed for each section (coronal, middle, and apical) and of all three

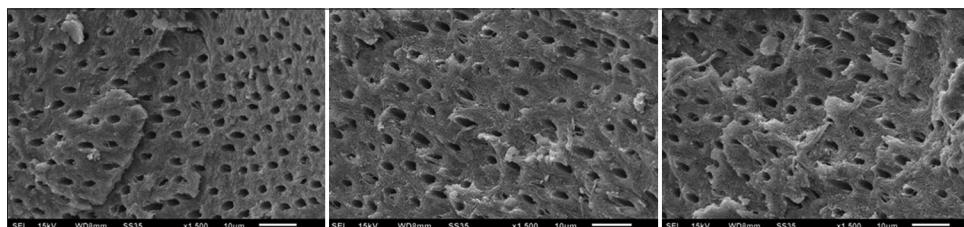


Figure 1: SEM image showing smear layer removal by Group 1 (Neem) at coronal, middle and apical site respectively

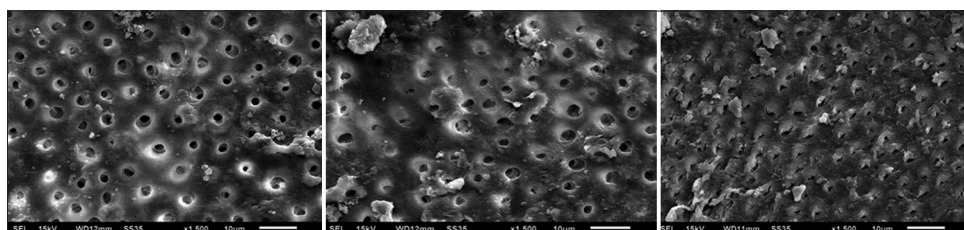


Figure 2: SEM image showing smear layer removal by Group 2 (Orange) at coronal, middle, and apical site, respectively

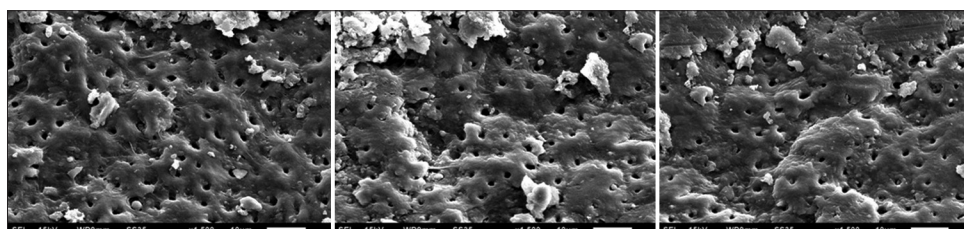


Figure 3: SEM image showing smear layer removal by Group 3 (Propolis) at coronal, middle, and apical site respectively



experimental groups, and overall comparison of smear layer removal scores by different experimental groups using the Statistical Package for the Social Sciences (SPSS) 19.0 version, IBM Corp. Armonk, Newyork statistical software 19.0 version. The intergroup comparison of frequency and percentages between the groups was made using the Chi-Square test.

### Results

Table 1 shows the comparison of smear layer removal scores of different experimental groups at the coronal, middle, and apical levels. The highest smear layer removal efficacy was observed in Group 1 (Neem leaf extract), followed by Group 2 (Orange oil) and Group 3 (Propolis), found to be the least efficacious.

Table 2 and Figure 4 show the overall comparison of the smear layer removal score of different experimental groups. Group 1 (Neem leaf extract) showed better smear layer removal efficacy as compared to the other two groups i.e., Group 2 (Orange oil) and Group 3 (Propolis).

The difference between the groups was statistically significant when analyzed using the Chi-square test at  $P \leq 0.05$ .

### Discussion

The success of the root canal treatment depends on a variety of factors such as proper diagnosis, cleaning, shaping, disinfection of root canals with the help of various irrigating solutions, and creating a three-dimensional seal of the root canal system. Successful root canal treatment aims at the complete eradication of microorganisms and

debridement of the root canal system, which can be achieved through irrigation and instrumentation of the root canal system. Mechanical root canal instrumentation produces an irregular amorphous layer of debris and microorganisms known as the smear layer.

Scanning electron microscope studies have shown that cleaning and shaping produces a smear layer on instrumented walls of the root canal, which contains organic and inorganic substances, remnants of odontoblastic processes, bacteria, and necrotic pulpal tissue.<sup>[17]</sup> These components of the smear layer can get penetrated into the dentinal tubules at varying distances. It could be due to the capillary action produced by the adhesive forces between the dentinal tubules and the smear layer material.<sup>[18]</sup>

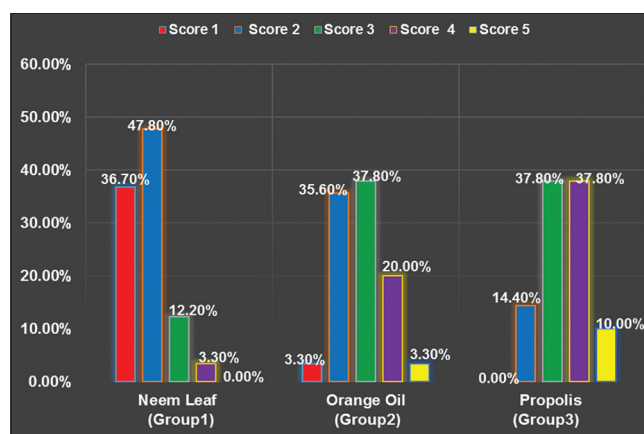


Figure 4: Comparison of overall score of smear layer removal by different experimental Groups (Group 1 [Neem Leaf Extract], Group 2 [Orange Oil], and Group 3 [Propolis])

**Table 1: Comparison of smear layer removal score of different experimental groups (Group 1-neem leaf extract, Group 2-orange oil, and Group 3-propolis) at coronal, middle, and apical sites**

Group	Score 1, n (%)	Score 2, n (%)	Score 3, n (%)	Score 4, n (%)	Score 5, n (%)	$\chi^2$	P
Neem leaf (Group 1)							
Coronal	11 (36.7)	15 (50.0)	4 (13.3)	0	0	41.341	0.001 (significant)
Middle	15 (50.0)	11 (36.7)	3 (10.0)	1 (3.3)	0		
Apical	7 (23.3)	17 (56.7)	4 (13.3)	2 (6.7)	0		
Orange oil (Group 2)						43.076	
Coronal	2 (6.7)	13 (43.3)	11 (36.7)	4 (13.3)	0		
Middle	1 (3.3)	11 (36.7)	11 (36.7)	7 (23.3)	0		
Apical	0	8 (26.7)	12 (40.0)	7 (23.3)	3 (10.0)		
Propolis (Group 3)						48.474	
Coronal	0	4 (13.3)	15 (50.0)	10 (33.3)	1 (3.3)		
Middle	0	7 (23.3)	13 (43.3)	8 (26.7)	2 (6.7)		
Apical	0	2 (6.7)	6 (20.0)	16 (53.3)	6 (20.0)		

**Table 2: Comparison of overall score of smear layer removal by different experimental groups (Group 1-neem leaf extract, Group 2-orange oil, and Group 3-propolis) using the Chi-square test**

Group	Score 1	Score 2	Score 3	Score 4	Score 5	$\chi^2$	P
Neem leaf (Group 1)	33 (36.70)	43 (47.80)	11 (12.20)	3 (3.30)	0	121.54	0.001 (significant)
Orange oil (Group 2)	3 (3.30)	32 (35.60)	34 (37.80)	18 (20.00)	3 (3.30)		
Propolis (Group 3)	0	13 (14.40)	34 (37.80)	34 (37.80)	9 (10.00)		

The smear layer being a loosely adherent structure, can harbor bacteria and provide an avenue for leakage and bacterial contamination. Hence, the smear layer removal can improve the diffusion and action of irrigants and intracanal medications, promotes dentin permeability, facilitates the penetration of sealer into the dentinal tubules and lateral canals at greater depth, and enhances the fluid-tight seal of the root canal system.<sup>[19]</sup>

In the root canal treatment, irrigants play an important role as it helps in the lubrication of the root canal, elimination of microorganisms, dissolution of the organic matter in the canal, and to flushing away the loose and necrotic pulpal tissue remnants. In addition, irrigants increase dentin permeability by removing the smear layer and further disinfecting the root canal system. With the help of various irrigants, chemical debridement of the root canal system is of utmost importance due to complex internal anatomies such as fins, isthmus, lateral canals, and those areas which might be missed by mechanical instrumentation.

Various chemical irrigants (Normal saline, NaOCl, EDTA, Chlorhexidine, Maleic acid, Chitosan, and Polyacrylic acid) are available in the market, but increased resistance to the antimicrobial action of chemical irrigants, potential side effects, safety concerns and their certain limitations had led to shift from chemical irrigants to herbal alternatives.

The present study assessed and compared the smear layer removal efficacy of three different natural endodontic irrigants such as Neem leaf extract, Orange oil and Propolis using scanning electron microscope (SEM).

Scanning electron microscope is a powerful magnification tool that can detect accurate surface characteristics such as texture, shape and size of the particles, composition and crystallographic information of the object. It was opted for as it provides detailed three-dimensional and topographical images of the prepared root canal.

Table 1 shows the statistical evaluation of smear layer removal score by different experimental groups at coronal, middle, and apical sites. Group 1 (Neem leaf extract) performed best, which was followed by Group 2 (Orange oil), whereas Group 3 (Propolis) was least efficacious. This could be attributed to the anti-adherence activity of Neem leaf extract, which alters the bacterial adhesion and ability of the microorganism to colonize. A similar study was conducted by Ranjitha *et al.* who evaluated and compared the smear layer removal efficacy of neem leaf extract, orange peel extract, and tulsi extract at the apical third and concluded that neem leaf extract had the highest efficacy in removing the smear layer from the apical third.<sup>[20]</sup>

On overall intergroup comparison [Table 2], the smear layer removal efficacy of Group 1 (Neem leaf extract) was found to be best among all the three groups. The highest smear layer removal efficacy of Group 1 (Neem leaf extract) can be due to the presence of biologically active compounds

such as acid metabolites, flavonoids isoprenoids, alkaloids, glycosides, steroids, and tannins.<sup>[15,20-22]</sup> The findings of the present study were in accordance with the previous studies conducted by Bhargava *et al.*<sup>[23]</sup> and Kumar *et al.*<sup>[7]</sup> who compared neem leaf extracts with other herbal irrigants such as triphala, amla, and tulsi and concluded neem leaf extract to be most efficacious in removing the smear layer.

Group 2 (Orange oil) exhibited less amount of smear layer removal efficacy as compared to Neem leaf extract. The lesser efficacy of orange oil could be due to the lack of the required amount of acid metabolites needed for the removal of the smear layer. Furthermore, the reduced efficacy might be attributed to the high surface tension of oil-based orange peel extract, which could limit the penetration of orange oil into the root canal.<sup>[20]</sup> An *in vitro* study done by Sebatni and Kumar,<sup>[15]</sup> compared and evaluated the smear layer removal efficacy of herbal extracts, i.e., Green tea extract, Orange oil, and Neem leaf extract as endodontic irrigant and found that neem leaf extract was highly efficacious in removing the smear layer, followed by orange oil, NaOCl and green tea extract.

Propolis (Group 3) showed the least efficacy in removing the smear layer when compared to the other two groups, i.e., neem leaf extract (Group 1) and orange oil (Group 2). This could be due to the intrinsic difficulty of introducing propolis and removing it from the root canal, as it is highly viscous in nature because it contains resins, essential oils, and waxes. This further leads to high surface tension by lowering the wet ability of the root canal surface. The results of our study were in agreement with the findings of Sharaf *et al.*,<sup>[24]</sup> who assessed the smear layer removal efficacy of MTAD and propolis extract as irrigant on the root canal walls and found that MTAD is more efficacious than propolis at the coronal, middle, and apical third.

Propolis has the capability to inhibit bacterial cell division and break down the bacterial cell wall and cytoplasm. Hence, it is a good antimicrobial agent. The study done by Awawdeh, Jamleh, and Beitawi 2018, evaluated the antifungal effect of Propolis with MTAD, Chlorhexidine, and NaOCl in the presence and absence of smear layer. They suggested that propolis is a promising endodontic irrigant that works even in the presence of smear layer.<sup>[25]</sup>

The present study was performed under *in vitro* conditions and the results of the herbal irrigants used in the study appeared promising as they all had the ability to remove the smear layer from the root canal. Group 1 (Neem leaf extract) was significantly better in removing the smear layer at all coronal, middle, and apical levels as compared to the other two groups, i.e., Group 2 (Orange oil) and Group 3 (Propolis).

#### Limitation of the study

In the present study, the sample size was less hence larger sample size is required for a more detailed evaluation of

these herbal endodontic irrigants. The present study was performed under *in vitro* conditions, although the best simulation of intraoral conditions was tried with the help of available resources, but the oral cavity being a complex structure, exact simulation could not have been reproduced, which might have affected the result.

## Conclusion

As the root canal is a complex structure, it is important to use suitable irrigating solutions while cleaning and shaping the root canal. Endodontic irrigating solutions should help to remove the smear layer, dissolve the organic matter, and disinfect and lubricate the root canal wall. Within the limitations of the study, the following conclusions have been drawn:

- All three herbal endodontic irrigants removed the smear layer from coronal, middle, and apical sites
- Group 1 (Neem leaf extract) effectively removed the smear layer from coronal, middle, and apical sites as compared to the other two groups
- Group 3 (Propolis) showed the least smear layer removal from coronal, middle, and apical sites among all three experimental groups.

Results of the herbal extracts have appeared to be favorable as they all had the ability to remove the smear layer from the root canal. In the modern era, the inclination toward natural treatment has been increased. Hence, the use of herbal extracts in pediatric patients might be advantageous as they are more bio-friendly, have plant origin, better biocompatibility, have greater therapeutic activity, and are more affordable. Further *in vivo* research is needed to evaluate the potential use of these herbal endodontic irrigants and the effect of these herbal irrigants on the long-term prognosis of root canal treatment.

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## Conflicts of interest

There are no conflicts of interest.

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