



# A Comparative Evaluation of Smear Layer Removal Efficacy by Herbal Extracts Used as Endodontic Irrigants – An In-Vitro Study

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## KEYWORDS

Herbal extract, Endodontic, Irrigant, neem leaf extract, lemongrass, triphala, sodium hypochlorite, EDTA, herbal irrigants, root canal irrigation, SEM, smear layer.

## ABSTRACT:

**Introduction:** Smear layer formation during root canal instrumentation hinders irrigant penetration, reduces dentinal permeability, and compromises the sealing ability of obturation. Although NaOCl and EDTA remain the gold standard combination for organic and inorganic smear removal, their cytotoxicity and dentin-altering effects have driven interest in herbal irrigants. Neem, Triphala, and Lemongrass contain bioactive phytochemicals with antimicrobial and chelating properties that may offer safer alternatives.

**Objectives:** To evaluate and compare the smear layer removal efficacy of three herbal irrigants—Neem extract, Triphala extract, and Lemongrass extract—with conventional NaOCl + EDTA and saline, using scanning electron microscopy.

**Methods:** Seventy-five extracted single-rooted human teeth were collected and instrumented to size 25/0.06 taper using Protaper NiTi files. Samples were randomly allocated into five groups (n = 15): Group 1 – Saline, Group 2 – 3% NaOCl + 17% EDTA, Group 3 – Neem extract, Group 4 – Triphala extract, and Group 5 – Lemongrass extract. Each canal was irrigated with 5 ml of the respective solution during preparation. Following instrumentation, roots were decoronated, longitudinally sectioned, and examined under SEM at ×1500 magnification. Smear layer was scored using the Rodig et al. scoring system. Data were analyzed using ANOVA and Tukey HSD post hoc tests.

**Results:** Significant differences were observed among the groups (p < 0.001). NaOCl + EDTA exhibited the lowest mean SEM score (0.87 ± 0.64), indicating maximum smear layer removal. Neem extract showed the highest efficacy among the herbal groups (1.13 ± 0.35), with no significant difference compared to NaOCl + EDTA (p = 0.584). Lemongrass (1.93 ± 0.59) and Triphala (2.07 ± 0.26) demonstrated moderate smear removal. Saline showed the poorest performance (3.00 ± 0.00). Overall efficacy followed the order: NaOCl + EDTA > Neem > Lemongrass > Triphala > Saline.

**Conclusions:** Neem extract showed smear layer removal efficacy closest to the conventional NaOCl + EDTA regimen and may serve as a potential herbal alternative. Lemongrass and Triphala displayed moderate effectiveness but inferior chelating action compared to Neem. Further in-vivo and long-term studies are recommended to validate the clinical applicability of these natural irrigants.

## 1. Introduction

Lorem Root canal instrumentation produces a smear layer<sup>[1]</sup> which is composed of a superficial layer (1-2 μm) on the root canal walls and a deep layer (40 μm) packed into dentinal tubules<sup>[2]</sup> American Association of Endodontists defines smear layer as “a surface film of

debris retained on dentin or other tooth surfaces, such as enamel or cementum, after instrumentation with either rotary instruments or endodontic files”.<sup>[3]</sup> It consists of organic and inorganic particles of dentin, including coagulated proteins, odontoblastic processes, saliva,



blood cells, vital or necrotic remnants of the pulp tissue, and microorganisms.

Smear layer blocks the dentinal tubule orifices hindering irrigants, intracanal medicaments, and sealer penetration into dentinal tubule hampering fluid-tight seal of root canal treatment [3]. For smear layer removal, instrumentation must be supplemented with irrigation [2].

Various synthetic chemical substances like sodium hypochlorite and EDTA are being used as endodontic irrigants for their effectiveness in removal of the smear layer and disinfection. Sodium hypochlorite [NaOCl] is a synthetic chemical used due to its exceptional antibacterial activity and distinct capacity to disintegrate pulp tissue, this chemical is preferred over other irrigants. NaOCl has several drawbacks, such as a nauseating smell, toxicity, and the inability to dissolve inorganic material, which prevents it from removing the smear layer [4].

For this reason, Ethylenediaminetetraacetic acid (EDTA) was used, which effectively demineralizes the smear layer but has to be used along with NaOCl to remove the organic part and also reduces the dentin microhardness.

To overcome the side effects of the above agents and to meet the requirements of an ideal irrigant, there is an increase in the exploration and use of natural phytochemicals to be a part of dental treatment procedures due to ease of availability, cost-effectiveness, and better biocompatibility [4,5]. The method of using herbs to treat a variety of disorders is referred to as "Phytotherapy" or "Phytomedicine" or "Ethnopharmacology".

Herbal extracts such as neem leaf, triphala, propolis, aloe vera, tulsi, orange oil etc, are gaining significant prominence as endodontic irrigants as they contain active components with antioxidant, antimicrobial, anti-inflammatory, and other favorable properties [6].

Triphala is an ayurvedic herbal formulation of dried, powdered fruits of three medicinal plants viz; *Terminalia bellerica*, *Terminalia chebula*, and *Embllica officinalis*. The principal constituent is tannic acid. It is used in treatment of headaches, constipation, and hepatic disorders.[24] Various studies have proven the bacteriostatic or bactericidal effect of tannic acid on gram-positive and gram-negative pathogens. It is safe

and is composed of compounds with anti-oxidative and anti-inflammatory properties.

Neem, also known as *Azadirachta Indica*, is a species of tree in the mahogany family, which is a common medicinal plant, having multiple properties and a wide spectrum of biological activity. It contains the active constituents like sodium nimbin, salannin, gedunin, nimbin, azadirachtin, nimbidol, quercetin, and nimbidin. Various studies have shown that neem extract can be used as an endodontic irrigant due to its antimicrobial and therapeutic effects against root canal microorganisms. Also, studies have found its ability to remove the smear layer [2].

*Cymbopogon flexuosus* (lemongrass) belongs to the family Poaceae and is a medicinal grass which is used as a food, in perfumery, and pharmaceutical industries. Previous studies showed the antimicrobial effect of lemongrass due to its component monoterpenes such as citral, which is an effective antibacterial against *E. faecalis*. However, very few studies have been done on its ability to remove the smear layer [23].

## 2. Objectives

Comparing the efficacy of these herbal agents has not been reported. Therefore, the present study aims to compare to evaluate the smear layer removal efficacy of lemongrass extract and to compare and evaluate smear layer removal efficacy of lemongrass, neem extract and triphala using scanning electron microscope.

## 3. Methods

A total of 75 non-carious single-rooted single canal human permanent teeth extracted for orthodontic or periodontal purposes will be collected for study. Teeth with complete root formation and single patent root canal were included in this study, and teeth with open apices, calcified canals, multiple canals, any anomaly, internal resorption, previous restoration, or endodontic treatment were excluded.

### PREPARATION OF HERBAL EXTRACT:

For the preparation of neem leaf and triphala extract, neem leaf and triphala powder will be weighed at 75g each, and separately mixed with 750ml of distilled water and will be boiled at 100°C to get 75ml of each extract. The prepared solution will be filtered using



Whatman filter paper, and the final irrigating solutions will be obtained and stored in opaque bottles.

For lemongrass extract, fresh lemongrass leaves will be placed in distilled boiling water for 5 minutes, at a solid: liquid ratio of 1:1. Later, this will be cooled for 5 minutes. The prepared solution will be filtered using cheesecloth and will be stored in opaque bottles at 4°C.

### SPECIMEN PREPARATION:

A total of 75 intact single-rooted, single-canal human permanent teeth that fulfill the inclusion and exclusion criteria will be collected. The teeth will be cleaned using an ultrasonic scaler, followed by sterilization in autoclave. The teeth will be stored in distilled water until use.

The working length will be measured radiographically to be 1mm short of the apical foramen. The Glide path will be established using a size 15 K-file. All samples will be randomly divided into 5 groups, with each group having 15 samples (2 control groups and 3 experimental groups).

**GROUP 1:** Normal saline

**GROUP 2:** 3% NaOCl & 17% EDTA

**GROUP 3:** Neem leaf extract solution

**GROUP 4:** Triphala extract solution

**GROUP 5:** Lemongrass extract solution

Root canals will be prepared up to apical size 25, 0.06 taper using Protaper NiTi files at specific speed and torque according to the manufacturer's instructions using crown down preparation method. During instrumentation, canals will be irrigated using 5ml of prepared solution corresponding to the respective group. In all the groups, study specimens will be finally rinsed with sterile distilled water and dried using sterile absorbent paper points. Samples will be stored at humidity.

Then, teeth will be decoronated at cemento-enamel junction using a diamond disc to obtain a standardized root length of 10mm. One longitudinal groove will be placed on buccal and lingual surfaces of each root using diamond disc, avoiding penetration into the canal. The roots will then be split into 2 halves using a chisel and mallet.

### SCANNING ELECTRON MICROSCOPIC EVALUATION:

Specimens will be mounted onto metallic stubs with carbon strip backing and will be viewed under Scanning Electron Microscope. Scanning Electron Microscope photomicrographs will be taken at a magnification of x1500 to evaluate smear layer removal and Rodig et al scoring system will be used for evaluation.

Score Description

- 1 - No smear layer, dentinal tubules open.
- 2 - Small amount of smear layer, some dentinal tubules open.
- 3 - Homogenous smear layer covering the root canal wall, only a few dentinal tubules open.
- 4 - Complete root canal wall covered by a homogenous smear layer, no open dentinal tubules.
- 5 - Heavy inhomogeneous smear layer covering the complete root canal wall.

### 4. Results

The mean SEM scores of smear layer removal among different irrigant groups demonstrated significant variations ( $p < 0.001$ ). The saline group (control) exhibited the highest mean score of  $3.00 \pm 0.00$ , indicating the presence of a heavy smear layer with no dentinal tubule openings. The NaOCl + EDTA group recorded the lowest mean score ( $0.87 \pm 0.64$ ), showing maximum smear layer removal and effective dentinal tubule exposure. Among the herbal irrigants, Neem extract showed a comparatively low mean score ( $1.13 \pm 0.35$ ), suggesting better smear layer removal efficacy than Triphala and Lemongrass. Triphala displayed a mean score of  $2.07 \pm 0.26$ , while Lemongrass showed  $1.93 \pm 0.59$ , both demonstrating moderate smear layer removal. Overall, NaOCl + EDTA exhibited significantly superior performance compared to all experimental groups ( $p < 0.05$ ), whereas Neem extract showed promising results approaching the conventional irrigant, as shown in Table 1.

Post hoc Tukey HSD analysis revealed statistically significant differences among the irrigant groups ( $p < 0.05$ ). The NaOCl + EDTA group exhibited a significantly lower mean SEM score when compared

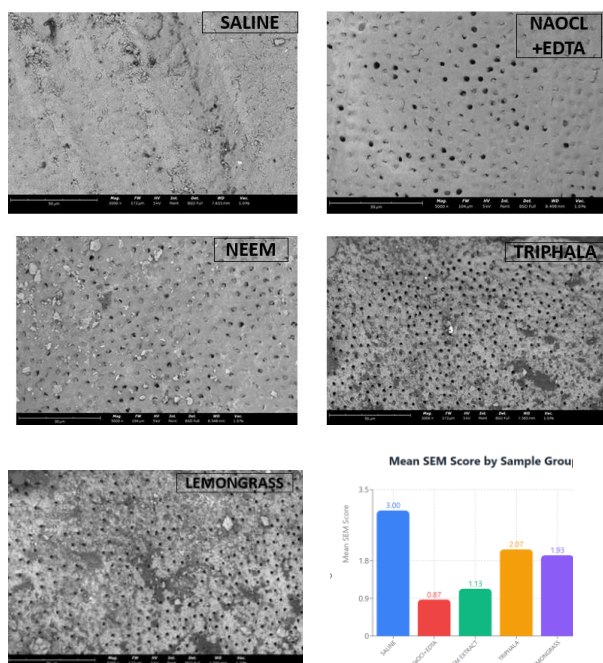


with Saline (mean difference = -2.133,  $p = 0.001$ ), Triphala (mean difference = -1.200,  $p = 0.001$ ), and Lemongrass (mean difference = -1.067,  $p = 0.001$ ) indicating superior smear layer removal efficacy. However, the difference between NaOCl + EDTA and Neem extract was not statistically significant ( $p = 0.584$ ), suggesting that Neem extract demonstrated smear layer removal potential comparable to the conventional irrigant.

When compared with the Saline group, all experimental irrigants (Neem, Triphala, Lemongrass) showed statistically significant reductions in smear layer scores ( $p = 0.001$ ), confirming their cleaning efficacy over the control. Among herbal agents, Neem extract performed significantly better than Triphala ( $p = 0.001$ ) and Lemongrass ( $p = 0.001$ ).

Thus, based on intergroup comparisons, the overall smear layer removal efficacy followed the descending order:

NaOCl + EDTA > Neem extract > Lemongrass > Triphala > Saline.



## 5. Discussion

The present study evaluated and compared the smear layer removal efficacy of three herbal irrigants—Neem, Triphala, and Lemongrass extracts—with the conventional irrigation regimen of NaOCl and EDTA.

The findings revealed significant differences among the groups, with NaOCl + EDTA demonstrating the highest smear layer removal efficacy, followed closely by Neem extract. Lemongrass and Triphala extracts showed moderate activity, while saline performed the poorest.

The superior performance of NaOCl + EDTA aligns with previous studies reporting the strong ability of sodium hypochlorite to dissolve organic matter and the chelating action of EDTA on the inorganic portion of the smear layer<sup>16</sup>. The combination is widely recognized as the gold standard for root canal irrigation due to its synergistic cleaning and antimicrobial properties<sup>10</sup>. The low mean SEM scores in the NaOCl + EDTA group observed in this study further support their established effectiveness in smear layer removal and dentinal tubule exposure.

Among herbal irrigants, Neem extract demonstrated the most promising results, showing smear layer removal efficacy statistically comparable to NaOCl + EDTA. This corroborates earlier research that highlighted Neem's antimicrobial and cleansing capabilities<sup>2,4</sup>. Neem contains bioactive components such as nimbidin, quercetin, and azadirachtin, which possess antibacterial, anti-inflammatory, and mild chelating properties<sup>11,12</sup>. The chelating ability of neem is attributed to its rich content of tannins, flavonoids, nimbidin, azadirachtin, and gallic acid, which form stable complexes with calcium ions, facilitating inorganic smear dissolution<sup>12</sup>. These phytochemicals disrupt the smear layer matrix while simultaneously exhibiting strong antibacterial effects, particularly against *E. faecalis*, as documented in multiple studies<sup>11-14</sup>. This indicates its potential dual action as both a disinfectant and smear layer remover<sup>13,14</sup>. In the present study, the significantly lower SEM scores in the Neem group compared to Triphala and Lemongrass reinforce its superior dentin-cleaning potential.

Triphala displayed moderate smear layer removal efficacy. Its constituents—*Terminalia chebula*, *Terminalia bellerica*, and *Embolica officinalis*—are rich in tannins and polyphenols that have reported antimicrobial and antioxidant effects<sup>5,9</sup>. Its chelation mechanism is primarily due to citric acid and ascorbic acid, organic acids known for binding calcium ions, in addition to polyphenols such as chebulinic acid and tannins that enhance smear dissolution<sup>9</sup>. These agents weaken the inorganic phase of the smear layer and increase dentinal



permeability, which explains its effective performance. However, its ability to decalcify the inorganic smear layer appears weaker compared to Neem and chemical chelators. Similar moderate results for Triphala have been reported in previous SEM-based studies evaluating its capacity as a final rinse solution<sup>5</sup>.

Lemongrass extract also showed intermediate smear layer removal ability. Citral, its primary active component, has been shown to exhibit broad antimicrobial properties, particularly against *E. faecalis*<sup>23</sup>. Lemongrass (*Cymbopogon citratus*) removes the smear layer mainly due to its high **citral** content, which binds to calcium ions and causes mild decalcification. Its flavonoids and phenolic acids (like caffeic and chlorogenic acid) also act as natural chelators, forming complexes with Ca<sup>2+</sup> and weakening the inorganic smear layer. Essential oil components such as **geraniol and limonene** provide mild solvent action, enhancing smear dissolution. However, limited literature exists on its smear layer removal capacity, and most studies have focused on its antibacterial effects. The moderate scores recorded in the current study suggest that while Lemongrass possesses some cleaning capability, it lacks strong chelating action needed for complete smear layer elimination.

As expected, saline showed no smear layer removal, confirming that mechanical instrumentation alone results in a compacted debris layer, as widely reported in literature<sup>8</sup>. This highlights the necessity of using a chemically active irrigant during endodontic treatment.

The positive performance of all herbal extracts compared to saline reflects a growing body of evidence favoring plant-derived irrigants for their biocompatibility, availability, and reduced toxicity<sup>1,3,6</sup>. These findings are consistent with studies advocating the potential of herbal solutions as alternatives to conventional irrigants, especially in patients sensitive to synthetic chemicals.

However, several limitations should be acknowledged. This in-vitro study does not entirely replicate clinical conditions such as the influence of anatomical variations, biofilm complexity, and irrigant dynamics. Additionally, only single concentrations and preparation methods were evaluated. Future studies should examine different concentrations, activation techniques, and long-term effects on dentin structure and bonding.

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**Table 1 – Mean smear layer removal comparison among various test groups**

Descriptives									
SEM SCORE									
	N	Mean SEM score	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	P-VALUE <sup>A</sup>
					Lower Bound	Upper Bound			
<b>SALINE</b>	15	3.00	.000	.000	3.00	3.00	3	3	<b>&lt;0.001**</b>
<b>NAOCL+EDTA</b>	15	0.87	.640	.165	.51	1.22	0	2	
<b>NEEM EXTRACT</b>	15	1.13	.352	.091	.94	1.33	1	2	
<b>TRIPHALA</b>	15	2.07	.258	.067	1.92	2.21	2	3	
<b>LEMONGRASS</b>	15	1.93	.594	.153	1.60	2.26	1	3	