Original Article

# Evaluation of smear layer removal and antimicrobial efficacy of intracanal herbal irrigants

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## ABSTRACT

**Aim:** To evaluate and compare the efficiency of *Triphala, Neem,* the combination of *Triphala, Neem* and 3% sodium hypochlorite (NaOCl) in the removal of smear layer (SL) evaluated using scanning electron microscope (SEM) and antimicrobial efficacy against standard culture strains of *Enterococcus faecalis.*

**Methods:** Seventy-five extracted human permanent teeth were divided into Group I control and Group II experimental, which was further subdivided into Group IIA, IIB, IIC, IID with 3% NaOCl, 5% *Triphala* extract, 7.5% *Neem* extract, and alternate use of *Triphala* and *Neem* and extracts as irrigants, respectively. The microbial sample was streaked on the agar plates to check colony-forming units/ml (CFU's) after inoculation and incubation at pre- and postirrigation. Teeth that were instrumented, and irrigated were split longitudinally, and examined using SEM under ×400, ×1000 to determine the debris and SL.

**Results:** Statistically significant reduction of CFU's was noted at postirrigation in Sub Group IIA, IIB, IIC, and IID with a mean rank of 31.77, 46.7, 34.53, and 9, respectively. SL removal was significant ($P = 0.001$), with Group IID exhibiting a lower mean rank, followed by B, C, A, and Group I.

**Conclusion:** The antimicrobial effect and SL removal efficacy were maximum for Group IID, which can be considered an effective herbal alternative in endodontic therapies.

**Keywords:** Antimicrobial efficacy, *Enterococcus faecalis*, Neem, smear layer, Triphala

## INTRODUCTION

Tooth tends to become nonvital either by physical, chemical, and mechanical or pathological insult. These necrotic teeth are invaded by obligatory anaerobic bacteria, which establish an infectious process, leading to pulp and periapical pathosis. The pathogenesis of these pulpal diseases through bacteria was first reported by Miller in 1894.[1] To retain an infected tooth, endodontic therapy is advocated, which involves thorough instrumentation and disinfection. However, facultative bacteria such as *Enterococci, Non-Mutans Streptococci,* and *Lactobacilli,* once established, are more likely to survive instrumentation, either within the dentinal tubules or bound within the apical dentin plug. This leads to endodontic failure and reinfection. In 2001, Hancock et al. showed that the most prevalent bacterial strain detected in teeth with endodontic treatment failure is *Enterococcus faecalis*,[2] with a prevalence range of 24%–77%.[3] Other drawbacks of instrumentation are formation of smear layer (SL), which is made up of a superficial layer (1–2 um) on the root canal walls

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and a deep layer (40 um) packed into the dentinal tubules.[3-4] It penetrates into dentinal tubules by capillary action and inhibits the passage of irrigants or medication.[5] American Association of Endodontists defined the SL 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.[6] This SL consists of organic, inorganic particles of dentin, including coagulated proteins, odontoblastic processes, saliva, blood cells, vital or necrotic remnants of the pulp tissue, and microorganisms.[5]

To increase the efficacy of mechanical preparation, microorganisms, and removal of SL, instrumentation must be supplemented with irrigation (chemo-mechanical preparation).[7] Various chemical irrigants have been used for this purpose, among which scientific evidence suggests that sodium hypochlorite (NaOCl) is currently the irrigant of choice,[8] which has excellent properties of tissue dissolution and antimicrobial activity.[3] However, on the other side, it has several limitations.[9] Since then, many studies have focused on biological medicaments derived from plants to be used as an irrigant. They might be advantageous over chemical agents.[10] The rationale of this study was to find alternatives to currently available chemical irrigants. Herbal extracts such as *Triphala* and *Neem* have been studied and found to be antibacterial with the ability to remove SL. Comparing the efficacy of these herbal agents and 3% NaOCl was not reported. Therefore, the present study aimed to compare the antimicrobial and SL removal properties of *Triphala*, *Neem*, their combination, and 3% NaOCl.

## MATERIALS AND METHODS

The present *in vitro* study was conducted in the Department of Paediatric and Preventive Dentistry, Panineeya Mahavidhyalaya Institute of Dental Sciences and Research Centre, Dilsukhnagar, Hyderabad, after obtaining Institutional Ethics Committee approval (Reference No. PMVIDS and RCIEC/PEDO/DN/0253-18).

### Methodology

One hundred and twenty human permanent incisors which were extracted due to periodontal reasons, were collected. They were thoroughly cleaned of debris, and soft-tissue remnants and stored in deionized water (AMF Enterprises) at room temperature in a moisture-free plastic container with a secure lid until the procedure began (approximately 2 months) to prevent dehydration. To confirm the presence of a patent canal, each sample was radiographed.

### Selection criteria

Permanent incisors with single relatively straight roots, and mature root apex having patent canals without any anatomic variations were included. Teeth with calcified canals, fillings or posts, root resorption or perforations, caries, or fracture were excluded.

### Sample size calculation

The sample size was calculated using G*power software version 3.1.9.4 with an error probability of 0.05, power of 0.80, and effect size of 0.42. The total sample was estimated to be 75, with 15/group.

### Preparation of the herbal extracts

Commercially available sealed packs of *Neem* leaf and *Triphala* fruit powder (IMC distributor-Natural herbal product store, Hyderabad, Telangana, India) were weighed at 37.5 g and 25 g, respectively, using a precision balance. They were mixed with distilled water (1000 ml), and boiled at 100°C to get 500 ml of each extract. The prepared solutions were filtered using Whatman filter paper, and the final irrigating solutions were obtained with 7.5% and 5% concentrations of *Neem* and *Triphala* crude aqueous extract, respectively.[11,12] Before usage, irrigating solutions were placed in Ultraviolet Chamber.

### Preparation of sample (tooth)

One hundred and twenty teeth were collected, among which 75 were considered satisfying selection criteria. Ultrasonic Scaling (woodpecker (UDS-J)), followed by decoronation below the cemento-enamel junction (standardized to 13 mm of root length) with the aid of diamond disc was done,[11] and sterilized in an autoclave. Instrumentation in step back technique was done up to 40 no. K-file (25 mm), (Mani Inc., Japan) till the apical foramen to form SL and the working length was set 0.5 mm short of the apex. Apical foramen was sealed with wax to prevent leakage of bacterial broth and irrigating solutions. Out of 75, 15 samples were irrigated with saline and were grouped as control for scanning electron microscope (SEM) (ZEISS EVO 18) analysis (Group I) and 60 samples were inoculated with *E. faecalis* (Group II).

### Microbial sample preparation

$1 \times 10$ cells/ml concentration of *E. faecalis* (ATCC 29212 strain, obtained from American type Culture Collection, Pune, India) was grown in Brain heart infusion broth (HIMedia Laboratory Private Limited, Mumbai, India) for 24 h. Each sample was inoculated with $20 \pm 1$ µl of *E. faecalis* suspension under laminar airflow chamber. K file was used to uniformly distribute bacterial suspension in the entire root canal length.[3] Contaminated teeth were kept moist (placed in saline) to prevent evaporation of bacterial suspension. Sixty inoculated samples were placed in Eppendorf tubes and incubated at 37°C for 48 h. Each tooth was assigned a serial number. The infection of the sample was confirmed by sampling the culture on Mueller Hinton agar (HIMedia

Laboratory Private Limited, Mumbai). Forty size paper point was placed in the canal to the working length for 1 min (to provide a "pooling effect of bacteria).[13] This was streaked onto the agar and incubated for 2 days at 37°C. After incubation, the colony forming units (CFU) counts for all the teeth were recorded (preirrigant culture) using colony counter.[8] Inoculated teeth with assigned serial numbers were randomly allocated to four subgroups based on the irrigant used with 15 samples each, using block randomization.

- Group IIA – 3% NaOCI
- Group IIB – 5% *Triphala* extract
- Group IIC – 7.5% *Neem* leaf extract
- Group IID – Alternate use of *Triphala* and *Neem* extracts.

### Irrigation protocol

Group II A, B, and C samples were passively irrigated with 20 mL of the corresponding irrigant, and in Group IID, initial irrigation was done with 10 mL of *Triphala* extract, followed by 10 mL of *Neem* extract. They were irrigated for over a 5-min period[8] using a sterile 5 mL disposable 24 G conventional syringe. Final irrigation of all the samples was done with distilled water to prevent the carryover of irrigants.

The microbial samples were taken in a manner identical to preirrigant culture methods. This constituted the "postirrigant culture." The CFU counts for all teeth were recorded.

### Scanning electron microscope sample preparation

Five samples from each group randomly selected for the SEM study were split longitudinally in a buccolingual direction. To prevent insertion of dentinal particles during root notching by diamond disc, adhesive tape was used to seal the canal orifice. Diamond disc was used to place one longitudinal groove on the buccal and lingual/palatal surfaces of each root without penetrating into the canal. The roots were split gently into two halves with the aid of a chisel and mallet and the optimum half of each specimen was used for the SEM examination with other discarded.[6] One-half of each section was demarcated at 4 mm (apical), 8 mm (middle), and 12 mm (coronal) from the apex. These sections were prepared for analysis with SEM under ×400 and ×1000 to evaluate debris and SL, respectively, at the center of each third. SL and debris removal were evaluated using photomicrographs [Figure 1], following the five-point scoring reported by Hulsmann et al[14] [Figure 2].

The assigned sample was given to the investigator to do the procedure. Microbiologist who did the counting of CFU's and a person who was trained in the evaluation of SL scores from SEM photomicrographs were blinded of irrigant.

The recorded values were analyzed statistically using SPSS software version 20 (SPSS, IBM, Armonk, NY, U.S.A.) $P < 0.05$ was considered statistically significant. Kruskal–Wallis test was used to explain if there was a significant difference between three or more than three groups containing nonparametric data. Other tests used were Mann–Whitney *U*-test, and Wilcoxon Sign–Rank test.

## RESULTS

Table 1 shows no statistically significant difference at preirrigation ($P = 0.959$) and significant difference was observed at postirrigation among groups ($P = 0.001$) [Table 1].

Mann–Whitney test compared microbial reduction in postirrigation among groups and significant difference was observed between Groups showing reduced CFU's/ml as follows: IIA < IIB ($P = 0.002$), IIA > IID ($P = 0.001$), IIB > IIC ($P = 0.012$), IIB > IID ($P = 0.001$), IIC > IID ($P = 0.001$). No statistically significant difference was observed between Group IIA and IIC, relatively IIA showed a better reduction in CFU's/ml than IIC (IIA < IIC) [Table 1].

Table 2 shows comparison of the debris and SL in the coronal, middle, and apical third of the root by Kruskal–Wallis test, there was a significant difference between groups. Group IID exhibited a lower mean rank in SL score, followed by IIB, IIC, IIA, and Group I [Table 2].

Statistically significant difference was observed among groups as follows: Group IIA showed a significant reduction in the amount of debris or SL compared to IIB under ×400 ($P = 0.002$), ×1000 ($P = 0.003$) in the coronal

**Table 1: Intra- and intergroup comparison of colony-forming units/ml**

| Intra group | | | | | |
|---|---|---|---|---|---|
| Preirrigation | | | Postirrigation | | |
| Groups | Mean rank | *P* | Groups | Mean rank | *P* |
| IIA | 30.43 | 0.959 | IIA | 31.77 | 0.001* |
| IIB | 30.43 | | IIB | 46.7 | |
| IIC | 32.13 | | IIC | 34.53 | |
| IID | 29 | | IID | 9 | |
| **Inter group** | | | | | |
| Groups | | | | *P* | |
| IIA | | | | | |
| IIB | | | | 0.002* | |
| IIC | | | | 0.555 | |
| IID | | | | 0.001* | |
| IIB | | | | | |
| IIC | | | | 0.012* | |
| IID | | | | 0.001* | |
| IIC | | | | | |
| ID | | | | 0.001* | |

*Statistical significance set at 0.05

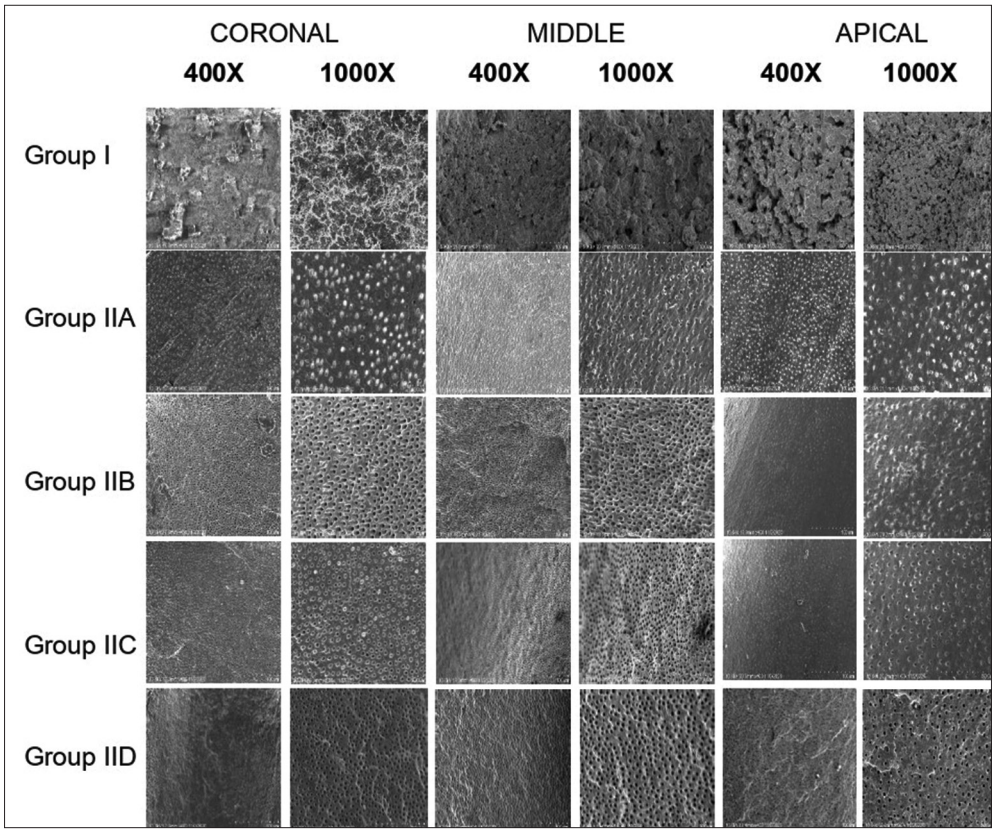


**Figure 1: SEM photomicrographs under ×400 (Debris), and ×1000 (Smear layer), of all the groups at different levels. Group I (Saline), Group IIA (NaoCl), Group IIB (*Triphala*), Group IIC (*Neem*), and Group IID (Alternate use of *Triphala* and *Neem*). SEM: Scanning electron microscope**

portion, and ×1000 ($P = 0.043$) in apical portion. Group IIA showed higher amount of debris or SL when compared to IID at coronal ($P = 0.001$) and middle third ($P = 0.03$) under ×400, ×1000, and apical third under ×1000 ($P = 0.002$). Group IIB showed significant reduction in amount of debris or SL when compared to IIC under ×400 ($P = 0.012$), ×1000 ($P = 0.004$) in coronal; ×400 ($P = 0.019$), ×1000 ($P = 0.007$) in middle; and ×1000 ($P = 0.012$) in apical portion. Group IIC showed significantly higher amount of debris or SL when compared to IID at coronal, middle, and apical third under ×400 and ×1000.

Group IIA versus (vs.) IIC showed no significant difference in the amount of debris or layer under × 400 and ×1000 in the coronal (IIA > IIC), middle (IIA = IIC) and apical region (IIA < IIC). Group IIB versus IIC under ×400 at apical third showed no statistically significant difference. Group IIB versus IID showed no statistically significant difference under × 400 and ×1000 in coronal, middle, and apical regions, with IID showing a reduced amount of debris and SL compared to IIB [Table 2].

## DISCUSSION

As there is limited literature comparing herbal agents with NaOCl in evaluating antimicrobial efficacy and SL removal in the same specimen, the present study was undertaken using *Neem, Triphala*, and their combination as root canal irrigants to evaluate their efficacy. Most of endodontic therapies required retreatment due to failure in reduction and elimination of infection. *E. faecalis* is one among the major causative factor for unsuccessful endodontic treatment,[13] whose biofilm has a dynamic structure of bacterial populations enclosed in a polymeric polysaccharide matrix.[15] This anaerobic bacterium has several virulence factors and can even tolerate high alkaline pH (such as calcium hydroxide), dry climate when compared to other species. It may explain its survival in root canal infections, where nutrients are scarce, and it has a proved resistance to a wide range of antimicrobial agents.[13] Although endodontic infections are polymicrobial, considering the nature of this organism as described, the single-species culture of *E. faecalis* was an imperative choice for this study.

Mechanical instrumentation is considered the core method for bacterial reduction in the infected root canal. According to Ghorbanzadeh *et al*.,[16] mechanical instrumentation only eliminates 50% of bacteria from the root canal. Moreover, it leaves more than 35% of root canal walls untouched and inadvertently forms an amorphous layer termed as "SL" over prepared dentinal walls. The removal of this layer aids in the opening of the dentinal tubules, allowing penetration of

| SMEAR LAYER SCORE | INTERPRETATION |
|---|---|
| 1 | No smear layer and all dentinal tubules open |
| 2 | A small amount of smear layer and some dentinal tubules open |
| 3 | Homogenous smear layer covering the root canal wall and only a few dentinal tubules open |
| 4 | Complete root canal wall covered by a homogeneous smear layer and no open dentinal tubules |
| 5 | Heavy homogeneous smear layer covering the complete root canal wall |
| **DEBRIS SCORE** | **INTERPRETATION** |
| 1 | Clean root canals, only few small debris particles |
| 2 | Few small agglomerations of debris |
| 3 | Many agglomerations of debris covering <50% of the root canal wall |
| 4 | More than 50% of the root canal wall covered by debris |
| 5 | Complete or nearly complete root canal wall covered by debris. |

**Figure 2: Hulsmann Criteria of debris and smear layer removal**

irrigants and intracanal medicaments.[11] Shahravan *et al*.[17] found that there was a reduction of microbial leakage after SL removal. For the removal of infected pulp and debris from the root canal, it is necessary to use irrigation solution simultaneously with instrumentation.[18] The irrigation solution must have both chelating and proteolytic actions for the removal of inorganic and organic parts of the SL.[19] Various chemical agents during instrumentation have been used, among which NaOCI is one of the most widely used endodontic irrigants because of its broad antibacterial spectrum, oxidizing, hydrolyzing properties, and strong proteolytic effect.[20] However, it has detrimental effects such as tissue toxicity, allergic potential, disagreeable taste, reduction of flexural strength and elastic modulus of dentin and inability to remove the SL.[10]

Torabinejad and Walton outlined the ideal properties of an endodontic irrigating solution: organic and inorganic tissue solvent, antimicrobial, nontoxic, low surface tension, and lubricant action.[21] To meet the requirements of an ideal irrigant, the search for more biocompatible and dentin-friendly irrigants is on the rise. An ancient idea of plants having healing potential has gained renewed interest and importance in recent times due to their high antimicrobial, anti-inflammatory, anti-oxidant properties, biocompatibility, easy availability, cost-effectiveness, increased shelf life, low toxicity, and lack of microbial resistance reported so far.[20] Therefore, herbal irrigating solutions with strong antibacterial activity and SL removal efficacy are the current focus of interest.

*Triphala* is an Indian herbal formulation containing dried and powdered fruits of *Amalaki*-Emblica officinalis,

**Table 2: Intra- and intergroup comparison of debris and smear layer under different magnifications**

| Intragroup comparison of debris and smear layer | | | | | | |
|---|---|---|---|---|---|---|
| **Groups** | **Coronal** | | **Middle** | | **Apex** | |
| | **Mean rank values** | | | | | |
| | **400X** | **1000X** | **400X** | **1000X** | **400X** | **1000X** |
| I | 67.87 | 67.23 | 67.73 | 67.47 | 66.5 | 66 |
| IIA | 42.5 | 41.83 | 36.63 | 35.63 | 32.6 | 39.8 |
| IIB | 23.67 | 23.17 | 25.47 | 25.07 | 29.2 | 25.6 |
| IIC | 37.2 | 39.5 | 38.4 | 40.4 | 42.1 | 40.63 |
| IID | 18.77 | 18.27 | 21.77 | 21.43 | 19.6 | 17.97 |
| **Intergroup comparison of debris and smear layer** | | | | | | |
| **Groups** | ***P*** | | | | | |
| IIA | | | | | | |
| I | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* |
| IIB | 0.002* | 0.003* | 0.084 | 0.099 | 0.665 | 0.043* |
| IIC | 0.255 | 0.063 | 1 | 0.572 | 0.147 | 0.948 |
| IID | 0.001* | 0.001 | 0.033* | 0.038* | 0.059 | 0.002 |
| IIB | | | | | | |
| I | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* |
| IIC | 0.012* | 0.004* | 0.019* | 0.007* | 0.054 | 0.012* |
| IID | 0.203 | 0.203 | 0.443 | 0.443 | 0.12 | 0.164 |
| IIC | | | | | | |
| I | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* |
| IID | 0.001* | 0.001* | 0.005* | 0.002* | 0.001* | 0.001* |
| IID | | | | | | |
| I | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* | 0.001* |

*Statistical significance set at 0.05

*Vibhitaki*-Terminalia bellirica, and *Haritaki*-Terminalia chebula. It is reported to scavenge the free radicals generated by the bacteria due to their phenolic nature or inhibits cell division or damage the cell walls of the bacterium[12] or deactivate microbial adhesins, cell envelope transport proteins, and enzymes.[22] As it is rich in citric acid, it acts as a good chelating agent and removes SL.[23]

*Neem* is other Indian herbal formulation, where each part of it has some medicinal properties.[24] The leaf extract has tetranortriterpenes which possess antibacterial property that is by inhibition of cell membrane synthesis.[25] Due to the above-mentioned properties, among the herbal irrigants *Neem* and *Triphala* ready-made powder extracts were chosen as experimental irrigating solution in comparison with NaOCl, which is considered as a gold standard.[19]

In a study conducted by Bag et al.,[26] hot aqueous extract of *Triphala* was found to be more potent against *Escherichia coli* strains. Rajshekharan et al.,[27] and Nayak *et al*.[28] stated that aqueous *Neem* leaf extracts exhibited significant anti-bacterial activity with no much difference from the alcoholic extract. Therefore, hot aqueous extract of *Neem* and *Triphala* was used as irrigants in this study. Nayak *et al*.[28] study showed minimum inhibitory concentration (MIC) of *Neem* at 7.5%, and Satti *et al*.[12] study showed MIC of *Triphala* at 50 mg/ml; both were considered for preparation of aqueous herbal extract. The antimicrobial efficacy of NaOCl was shown at concentrations of 3% or even lower,[29] and the same was chosen for comparison.

The present study considered the 5 min as irrigation time following a study conducted by Divia *et al*.[8] A conventional 5 ml syringe was used for irrigation which was considered to be the most widely used technique according to Buldur and Kapdan.[30] and Hata et al.[31] Grossman et al.[32] used paper points for obtaining the root canal samples. Chandwani *et al*.[18] collected microbial samples from the apical third of the canal, which is the most difficult area to be cleaned. Microbiological research techniques often rely on the accurate determination of CFUs.[29]

SEM, which can detect accurate surface characteristics, was used to assess the effectiveness of irrigants in the debris and SL removal[33] by SEM Photomicrographs and was first reported by Eick et al.[34] Debris which was defined as dentin chips, pulp remnants, and particles loosely attached to the root canal wall,[14] can easily be observed at low magnification. Higher magnification is, however, required for the identification of dentinal tubules and observation of the remnants of the SL. Among the various evaluating criteria of SL removal under SEM, Hulsmann criteria were followed as it quantifies the differences in debris and SL removal under ×400 and ×1000, respectively.[35] In the present study, there is not much difference noted between the debris and SL scores among the groups.

### Antimicrobial efficacy of irrigants

On intergroup comparison, alternate use of *Triphala* and *Neem* irrigants had shown higher antimicrobial efficacy. NaOCl group, when compared to *Triphala* group, has shown a significant reduction in CFU's/ml, which was in accordance with a study conducted by Divia *et al*.[8] Prabhakar *et al*.[20] stated that *Triphala* achieved 100% killing of *E. faecalis* at 6 min, which was in contrast with the present study where the 100% killing was not noted. This could be due to variations in extract preparation and microbial procedure involved in evaluating antimicrobial efficacy. When the NaOCl group and *Neem* group were compared, there was no statistically significant difference observed at postirrigation CFU's/ml, but NaOCl has a better mean rank, which was in accordance with Dubey *et al*.[36] This was in contrast to a study conducted by Rosaline et al.,[37] and Ghonmode *et al*.[38] where *Neem* has shown high effectiveness than 5.2% and 3% NaOCl, respectively. This could be due to ethanolic extract of *Neem*.

In the present study, NaOCl is equally effective to *Neem* and more effective than *Triphala* in reducing bacterial count, which could be due to the release of hypochlorous acid (HOCl) disrupting the metabolism of the microorganisms.[39] According to a study conducted by Saxena *et al*.,[40] mean zone of bacterial inhibition in descending order was found as NaOCl > Propolis > *Azadirachta Indica* > *Triphala* > *Curcuma longa* = *Morinda Citrifolia* > ethanol, which was in accordance to the findings in the present study.

### Smear layer removal property of irrigants

Another parameter evaluated was SL removal, where the results have shown statistically significant differences in debris or SL removal scores among five different intracanal irrigants ($P = 0.001$).

*Triphala* group has shown a significantly lower mean rank in the amount of debris or SL removal when compared to *Neem* group and NaOCl group. There was no significant difference observed between NaOCl and *Neem* group, but relatively, *Neem* group had better SL removal property with a lower mean rank, and this might be because NaOCl dissolves the organic component and leaves the SL of inorganic tissue. These results were in accordance with a study conducted by Charlie KM *et al*.[4]

Among all the groups, a statistically significant difference was observed between the alternate use of *Triphala* and *Neem* group and other groups, showing better antimicrobial efficacy and SL removal property. This could be due to the synergistic effect of two herbal agents used.

There are few limitations to be considered, such as need of fresh herbal extract preparation, technique sensitivity due to *E. faecalis* contamination, and limited samples were subjected to SEM examination. These results cannot be applied to multirooted teeth as they have anatomic complexity, which poses a challenge for root canal disinfection.

## CONCLUSION

Within the limitations, it can be stated that alternate use of *Triphala* and *Neem* aqueous extract can serve as an alternative available natural extract for irrigation. The observations of herbal products appear promising. The results can be further justified by a larger sample size, and clinical trials before *Neem* and *Triphala* use can be recommended conclusively as an intracanal irrigating solution.

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### Conflicts of interest

There are no conflicts of interest.

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