

Comparative Evaluation Of Neem Extract, Green Tea Extract, And Sodium Hypochlorite On Smear Layer Removal And Dentinal Tubule Penetration: An In Vitro Scanning Electron Microscopic And Stereomicroscopic Study

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ABSTRACT

Background: Persistence of microorganisms within the smear layer and dentinal tubules is a principal cause of endodontic treatment failure. Sodium hypochlorite (NaOCl) remains the irrigant of choice, but its inability to remove the inorganic component of the smear layer, its cytotoxicity, and its adverse effect on dentin have prompted interest in biocompatible herbal alternatives such as neem (*Azadirachta indica*) and green tea (*Camellia sinensis*).

Aim: To comparatively evaluate the smear layer removal efficacy and dentinal tubule penetration depth of neem extract, green tea extract, and 3% NaOCl.

Materials and Methods: Sixty extracted single-rooted human mandibular premolars were decoronated to a standardized root length of 14 mm, instrumented with ProTaper Gold rotary files to size F3, and divided into three groups of twenty (n = 10 for smear layer evaluation and n = 10 for dentinal tubule penetration per group): Group 1, 3% NaOCl; Group 2, neem extract; Group 3, green tea extract. Smear layer was scored at the middle third under field emission scanning electron microscopy (FESEM, 5000×) using the Torabinejad criteria. Dentinal tubule penetration depth was assessed at the middle third with crystal violet dye under a stereomicroscope (20×). Data were analyzed with the independent samples t-test ($\alpha = 0.05$).

Results: Mean smear layer scores were 2.2 ± 0.4 (NaOCl), 1.3 ± 0.5 (neem), and 2.9 ± 0.3 (green tea) ($p < 0.001$). Neem showed significantly lower (better) scores than both NaOCl ($p = 0.045$) and green tea ($p < 0.001$); NaOCl and green tea were statistically similar ($p = 0.086$). Mean dentinal tubule penetration depths were 0.51 ± 0.02 mm (NaOCl), 0.59 ± 0.04 mm (neem), and 0.36 ± 0.02 mm (green tea) ($p < 0.001$), with all pairwise differences significant (NaOCl–neem, $p = 0.044$; neem–green tea, $p < 0.001$; green tea–NaOCl, $p = 0.029$).

Conclusion: Within the limitations of this in vitro study, neem extract demonstrated superior smear layer removal and dentinal tubule penetration compared with 3% NaOCl and green tea extract, indicating its potential as an alternative or adjunctive endodontic irrigant. NaOCl retained moderate, reliable performance, while green tea extract was the least effective irrigant for these two parameters.

Keywords: Smear layer; dentinal tubule penetration; sodium hypochlorite; neem extract; green tea extract; herbal irrigant; scanning electron microscopy.

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INTRODUCTION

Endodontic disease results primarily from microbial invasion of the root canal system, and the persistence of microorganisms within its complex anatomy remains the principal cause of endodontic treatment failure¹. The objective of root canal therapy is therefore thorough disinfection of the canal system while preserving dentin structure and periapical biocompatibility².

Biomechanical preparation combines mechanical instrumentation with chemical irrigation to reduce

microbial load and shape the canal, but anatomical complexities—lateral canals, fins, isthmuses, and the dentinal tubules that permeate the root dentin—mean that instrumentation alone cannot achieve complete debridement³. These niches allow bacteria such as *Enterococcus faecalis* to penetrate deep into dentin and evade conventional cleaning, making effective irrigation essential.

During biomechanical preparation an amorphous smear layer forms on the canal walls, composed of dentinal

debris, pulp remnants, and microorganisms with their by-products⁴. It exists as a superficial layer loosely attached to the canal wall and a deeper layer that penetrates the tubules as smear plugs. Its management depends on three mechanisms: dissolution of organic material, for which NaOCl is well known⁵; removal of the inorganic component by chelating agents such as EDTA⁶; and modification of the layer without complete removal⁴. Clinically, the smear layer occludes dentinal tubules, reduces permeability, limits penetration of irrigants and medicaments⁴, harbors microorganisms¹, and can compromise the seal of obturating materials².

Dentinal tubule penetration is an equally critical determinant of disinfection success, since resistant species such as *E. faecalis* can survive deep within the tubules and cause reinfection¹. Penetration depth depends on the physicochemical properties of the irrigant such as surface tension and viscosity⁷, its method of delivery and activation³, and prior removal or modification of the smear layer, which opens the tubule orifices to deeper irrigant contact⁴.

Sodium hypochlorite is widely regarded as the gold-standard endodontic irrigant because of its potent antimicrobial activity and its capacity to dissolve organic tissue through oxidation and chloramination reactions⁵. However, it cannot remove the inorganic component of the smear layer and is therefore typically combined with chelating agents⁶; it is also cytotoxic when extruded beyond the apex and can reduce dentin microhardness⁸. These drawbacks have driven interest in herbal irrigants that combine antimicrobial activity with biocompatibility, antioxidant properties, and minimal adverse effects on dentin, owing to bioactive constituents such as flavonoids, tannins, alkaloids, and polyphenols⁹. Neem (*Azadirachta indica*) is among the most extensively studied herbal agents in endodontics, with antimicrobial, anti-inflammatory, antifungal, and antioxidant properties attributed to constituents such as nimbidin and azadirachtin, and demonstrated efficacy against endodontic pathogens including *E. faecalis*¹⁰. Green tea (*Camellia sinensis*), rich in catechins such as epigallocatechin gallate (EGCG), likewise shows well-documented antimicrobial and antioxidant activity and has shown promise in smear layer removal and dentinal tubule penetration in earlier reports¹¹. Despite this, herbal irrigant efficacy varies with concentration, preparation, and application protocol¹², and comprehensive studies that simultaneously evaluate smear layer removal and dentinal tubule penetration of herbal irrigants against NaOCl under standardized conditions remain scarce; most prior work has examined these parameters, or antimicrobial activity, in isolation rather than in combination¹³.

The present study was therefore designed to comparatively evaluate the efficacy of neem extract, green tea extract, and sodium hypochlorite with respect to smear layer removal and dentinal tubule penetration, in order to determine their potential role as alternative or adjunctive irrigants in modern endodontic practice.

Aim

To evaluate the effect of different herbal irrigants and sodium hypochlorite on removal of the smear layer and depth of dentinal tubule penetration.

MATERIALS AND METHODS

This in vitro study was conducted in the Department of Conservative Dentistry and Endodontics, Regional Dental College, Guwahati, Assam. Herbal extracts were prepared at the Government Ayurvedic College, Guwahati; root sample analysis was performed at the Material Science Laboratory, Department of Mechanical Engineering, and FESEM analysis at the Central Instrument Facility, Indian Institute of Technology, Guwahati. Ethical clearance was obtained from the Institutional Ethical Committee Board, Regional Dental College, Guwahati, prior to the study.

Sample size and selection

Sample size was calculated using $n = \{(Z\alpha/2 \times s) / E\}^2$ ($Z\alpha/2 = 1.96$ at 95% confidence). A total of 60 extracted single-rooted human mandibular premolars, obtained from the Department of Oral and Maxillofacial Surgery for orthodontic reasons, were included and divided into three groups of 20 teeth each. Inclusion criteria were non-carious single-rooted mandibular premolars with a single canal (radiographically verified), a mature apex, and a root length of at least 14 mm. Teeth with root caries, dilacerations, morphological deformities, cervical abrasions, calcifications, cracks, more than one canal, immature roots, prior restoration or endodontic treatment, or resorption were excluded.

Sample preparation

Teeth were cleaned of soft-tissue tags, bony fragments, stain, and calculus, then decoronated with a diamond disc to a standardized root length of 14 mm, verified with a digital vernier caliper. Working length was established with an ISO size #10 K-file taken to the apical foramen, minus 1 mm. Canals were instrumented with the ProTaper Gold rotary system to size F3 under saline irrigation.

Preparation of herbal extracts

Fresh neem leaves and processed green tea leaves were washed, shade- and sun-dried to constant weight, and ground into fine powder. The powders were cold-macerated in distilled water for 48 hours, filtered through sterile muslin cloth, and the filtrates concentrated by heating in a water bath at 110°C to obtain crude extracts. Working 12.5% w/v aqueous solutions were prepared by dissolving 2.5 g of each crude extract in 20 mL distilled water.

Experimental groups

Prepared roots were irrigated with 5 mL of the respective irrigant for 5 minutes: Group 1 ($n = 10$ per outcome) – 3% NaOCl; Group 2 ($n = 10$ per outcome) – neem extract solution; Group 3 ($n = 10$ per outcome) – green tea extract solution.

Evaluation of smear layer removal

After irrigation and a final 1 mL distilled-water rinse for 1 minute, teeth were split longitudinally with a chisel and the middle third of the canal wall examined under

FESEM at 5000× magnification. Smear layer was scored using the criteria of Torabinejad et al.¹⁴: Score 1, no smear layer with all tubules clean and open; Score 2, moderate smear layer (clean canal surface but tubules containing debris); Score 3, heavy smear layer covering both surface and tubules.

Evaluation of dentinal tubule penetration

Crystal violet dye was flowed from the apical end of each prepared tooth for 5 seconds, and the teeth were soaked in the dye for 2 days before a 10-minute tap-water wash. Samples were then irrigated with 5 mL of the respective irrigant for 5 minutes, split longitudinally, and the middle third examined under a stereomicroscope at 20× magnification to measure dye penetration depth (mm).

Statistical analysis

Data were entered and analyzed in IBM SPSS Statistics version 20.0. Descriptive statistics (mean, SD, minimum, maximum) summarized smear layer scores and penetration depths for each group. Intergroup comparisons (neem vs. NaOCl, neem vs. green tea, and Table 1. Smear layer removal scores by irrigant group (Torabinejad criteria, FESEM, 5000×).

Group	Mean	SD	Minimum	Maximum
3% NaOCl	2.2	0.4	2	3

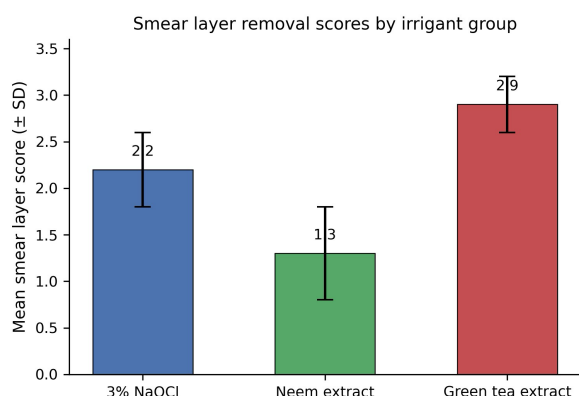


Figure 1. Mean smear layer removal scores (± SD) for 3% NaOCl, neem extract, and green tea extract. Lower scores indicate more effective smear layer removal ($p < 0.001$ across groups).

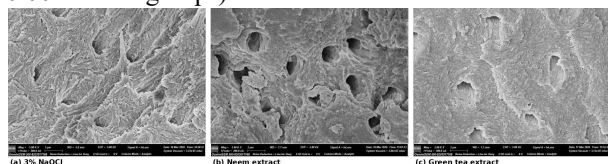


Table 2. Dentinal tubule penetration depth (mm) by irrigant group (crystal violet dye, stereomicroscope, 20×).

Group	Mean (mm)	SD	Minimum	Maximum
3% NaOCl	0.51	0.02	0.48	0.55

green tea vs. NaOCl) were performed with the independent samples t-test; $p < 0.05$ was considered statistically significant at a 95% confidence interval.

RESULTS

Sixty extracted mandibular premolars were successfully prepared, irrigated, and analyzed with no sample attrition. Results for smear layer removal and dentinal tubule penetration are summarized below.

Smear layer removal

Mean FESEM smear layer scores (lower score = more effective removal) were 2.2 ± 0.4 for 3% NaOCl (range 2–3), 1.3 ± 0.5 for neem extract (range 1–2), and 2.9 ± 0.3 for green tea extract (range 2–3). The difference among the three groups was statistically significant ($p < 0.001$). On pairwise comparison, neem extract produced significantly lower scores than both NaOCl ($p = 0.045$) and green tea extract ($p < 0.001$), whereas NaOCl and green tea extract were not significantly different from one another ($p = 0.086$), indicating that neem achieved the cleanest canal walls of the three irrigants tested.

Neem extract	1.3	0.5	1	2
Green tea extract	2.9	0.3	2	3

Figure 2. Representative FESEM photomicrographs (5000× magnification) of the root canal middle third after irrigation with (a) 3% NaOCl, showing a moderate, partially occluding smear layer; (b) neem extract, showing open, clearly visible dentinal tubules with minimal residual smear layer; and (c) green tea extract, showing a heavier smear layer covering the canal surface and tubule orifices.

Dentinal tubule penetration

Mean dye penetration depth at the canal middle third was 0.51 ± 0.02 mm for NaOCl (range 0.48–0.55 mm), 0.59 ± 0.04 mm for neem extract (range 0.54–0.66 mm), and 0.36 ± 0.02 mm for green tea extract (range 0.32–0.39 mm). The overall difference among groups was statistically significant ($p < 0.001$), and all pairwise comparisons reached significance: NaOCl vs. neem, $p = 0.044$; neem vs. green tea, $p < 0.001$; green tea vs. NaOCl, $p = 0.029$. Neem extract achieved the greatest penetration depth, followed by NaOCl, with green tea extract showing the shallowest penetration of the three irrigants.

Neem extract	0.59	0.04	0.54	0.66
Green tea extract	0.36	0.02	0.32	0.39

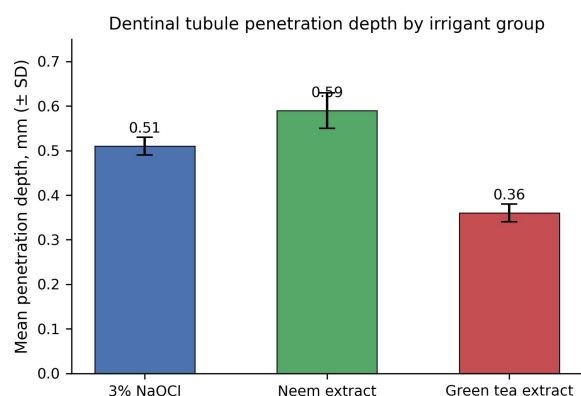


Figure 3. Mean dentinal tubule penetration depth ($\pm$ SD, mm) for 3% NaOCl, neem extract, and green tea extract Table 3. Intergroup pairwise comparisons (independent samples t-test, $\alpha = 0.05$).

Comparison	Outcome	p value	Significance
NaOCl vs. Neem	Smear layer score	0.045	Significant
Neem vs. Green tea	Smear layer score	< 0.001	Significant

DISCUSSION

Successful endodontic therapy depends fundamentally on thorough debridement and disinfection of the root canal system. Because mechanical instrumentation alone cannot eliminate microorganisms from fins, isthmuses, lateral canals, and dentinal tubules, chemical irrigation plays a pivotal role in canal cleanliness and microbial eradication³. The smear layer formed during instrumentation is a well-recognized barrier that occludes dentinal tubules and limits penetration of irrigants and intracanal medicaments, so its removal is considered essential for effective disinfection and optimal sealing of obturating materials⁴.

The present study found statistically significant differences among all three irrigants for both smear layer removal and dentinal tubule penetration ($p < 0.001$), confirming that irrigant selection substantially influences both outcomes. Neem extract produced the most favorable results in both parameters, followed by sodium hypochlorite, while green tea extract was least effective – highlighting the potential of herbal irrigants, and neem in particular, as alternatives or adjuncts to conventional agents.

The superior smear layer removal observed with neem extract may relate to its phytochemical composition. Neem contains nimbidin, azadirachtin, flavonoids, alkaloids, and tannins, a complex profile that has been associated with a wide range of pharmacological activity and may facilitate disruption of both organic and inorganic components of the smear layer; tannins in particular are able to precipitate proteins and interact with dentinal components, aiding smear layer removal¹⁰. This finding is consistent with earlier reports that herbal

($p < 0.001$ across groups; all pairwise comparisons significant).

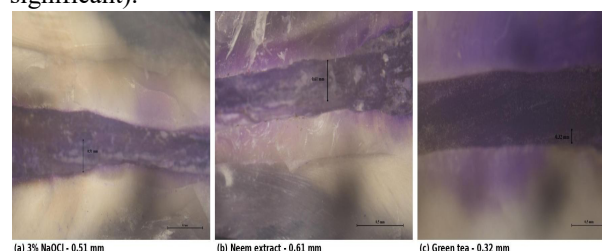


Figure 4. Representative stereomicroscopic images (20 $\times$ magnification) of crystal-violet dye penetration at the canal middle third for (a) 3% NaOCl (0.51 mm), (b) neem extract (0.61 mm), and (c) green tea extract (0.32 mm).

Green tea vs. NaOCl	Smear layer score	0.086	Not significant
NaOCl vs. Neem	Penetration depth	0.044	Significant
Neem vs. Green tea	Penetration depth	< 0.001	Significant
Green tea vs. NaOCl	Penetration depth	0.029	Significant

irrigants, including neem, achieve significant smear layer removal under scanning electron microscopy¹⁵, that herbal irrigants are particularly effective in the apical third where conventional irrigants often underperform¹⁶, and that herbal agents can achieve smear layer removal comparable to, or better than, conventional irrigants owing to their natural chemical constituents¹⁷.

Sodium hypochlorite, despite being the most widely used endodontic irrigant, showed only moderate smear layer removal in this study. This is consistent with its known mechanism of action, which dissolves organic tissue but does not remove the inorganic component of the smear layer when used alone⁵; the literature has consistently emphasized that chelating agents such as EDTA are required for complete smear layer removal¹⁸, and that NaOCl is ideally combined with such agents to achieve optimal canal cleanliness³. The present findings are in agreement with this established understanding.

Green tea extract demonstrated the least effective smear layer removal of the three irrigants. Although its polyphenolic catechins confer well-documented antioxidant and antimicrobial properties, these effects appear unrelated to tissue dissolution or chelation, which may explain its limited ability to interact chemically with mineralized dentin and remove the smear layer¹¹. This pattern mirrors earlier reports of green tea polyphenols being less effective at smear layer removal compared with other irrigants¹¹, and of herbal extracts that lack chelating properties showing similarly limited smear layer removal¹⁹.

Dentinal tubule penetration is equally important for disinfection, since bacteria that persist within the tubules are a recognized contributor to periapical disease and

treatment failure¹. In the present study, neem extract achieved the deepest penetration, followed by NaOCl, with green tea extract showing the shallowest penetration; all differences were statistically significant. The superior penetration seen with neem may relate to lower surface tension and the presence of natural surfactant-like constituents that enhance wettability and diffusion into dentinal tubules²⁰, consistent with the view that the physicochemical properties of an irrigant – including surface tension, viscosity, and irrigation dynamics – govern both penetration and antimicrobial effectiveness²¹.

Sodium hypochlorite's moderate penetration in this study is consistent with its relatively higher surface tension compared with the herbal extracts tested, and with the broader observation that irrigant penetration is governed by fluid dynamics within the canal system²² and is reduced by the presence of an intact smear layer⁷. Green tea extract's shallow penetration may relate to its larger molecular size and lack of surfactant character, limiting diffusion into the tubules and potentially compromising its ability to reach and eliminate intratubular bacteria²⁰.

A notable observation of the present study is the concordance between smear layer removal and dentinal tubule penetration: neem extract, which achieved the best smear layer scores, also achieved the deepest penetration. This is consistent with the concept that the smear layer acts as a physical barrier limiting irrigant penetration⁷, and that its removal improves fluid distribution and interaction between irrigant and dentinal walls within the canal system²².

The growing interest in herbal irrigants reflects their biocompatibility, availability, cost-effectiveness, and reduced toxicity relative to conventional chemical agents⁹. Neem in particular has been repeatedly shown to exert significant antimicrobial activity against root canal pathogens, including biofilms of *E. faecalis*, with efficacy in some reports comparable to sodium hypochlorite¹⁰. Green tea extract, while antimicrobial and antioxidant, may not provide the same degree of mechanical cleaning or smear layer removal as conventional irrigants²³, limiting its suitability as a stand-alone irrigant even though it retains value for its other biological properties. Combining herbal extracts with adjunctive measures such as sonic agitation, or comparing them against benchmarks such as *Punica granatum* and *Emblica officinalis*, has similarly been shown to improve smear layer removal and penetration outcomes^{24,25}, supporting the broader principle that irrigant performance depends on both chemical composition and delivery.

Taken together, these findings support sodium hypochlorite's continued role as a dependable, moderately effective conventional irrigant²⁶, while suggesting that neem extract, and to a lesser extent green tea extract, merit further consideration as biocompatible adjuncts or alternatives – particularly given neem's combination of superior smear layer removal and deeper dentinal tubule penetration observed here²⁷.

LIMITATIONS

This study is limited by its in vitro design, which cannot fully replicate clinical conditions such as complex canal anatomy, the presence of vital pulp tissue, and variation in irrigation technique and activation. Antimicrobial efficacy, cytotoxicity, and long-term clinical performance of the tested irrigants were not evaluated. Future work should include in vivo evaluation and clinical trials to validate these findings and to establish standardized protocols for the use of herbal irrigants in endodontic practice.

CONCLUSION

Within the limitations of the present in vitro study, the following conclusions can be drawn:

- Neem extract demonstrated the highest efficacy in smear layer removal, followed by 3% NaOCl, while green tea extract showed the least effectiveness; the differences among groups were statistically significant.
- Neem extract also exhibited the greatest dentinal tubule penetration depth, followed by NaOCl, with green tea extract showing the lowest penetration; differences between all groups were statistically significant.
- Neem extract outperformed both NaOCl and green tea extract in both smear layer removal and dentinal tubule penetration, suggesting superior efficacy as an irrigating solution.
- NaOCl showed moderate effectiveness in both parameters, reaffirming its role as a reliable conventional irrigant.
- Green tea extract demonstrated the least efficacy in both smear layer removal and dentinal tubule penetration among the tested groups.
- Neem extract may serve as a promising natural alternative to conventional irrigants, offering enhanced cleaning ability together with deeper penetration into dentinal tubules.

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