https://doi.org/10.33472/AFJBS.6.6.2024.5978-5987

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

A Comparative Evaluation between Neem, Triphala and Ethylenediaminetetraacetic Acid to Determine Smear Layer Removal Efficacy Using Scanning Electron Microscope –An in Vitro Study

Dr. Nitin Lokhande[1*], Dr. Aditya Idhate[2], Dr. Sathish Abraham[3], Dr. Pradnya Nagmode[4], Dr. Treesa Mathew[5], Dr. Priyani Jaiswal[6]

[1]Associate Professor, Department of Conservative Dentistry and Endodontics, SMBT Dental College, Sangamner
[2]Post Graduate Student, Department of Conservative Dentistry and Endodontics, SMBT Dental College, Sangamner
[3]Professor and Head, Department of Conservative Dentistry and Endodontics, SMBT Dental College, Sangamner
[4]Professor, Department of Conservative Dentistry and Endodontics, SMBT Dental College, Sangamner
[5]Post Graduate Student, Department of Conservative Dentistry and Endodontics, SMBT Dental College, Sangamner
[6]Post Graduate Student, Department of Conservative Dentistry and Endodontics, SMBT Dental College, Sangamner

***Corresponding author:**
Dr. Nitin Lokhande
Email: drnlokhande@gmail.com
ORCID ID: Dr. Nitin Lokhande- https://orcid.org/0000-0001-6802-7793

Article Info

Volume 6, Issue 6, June 2024

Received: 18 April 2024

Accepted: 27 May 2024

Published: 21 June 2024

doi: 10.33472/AFJBS.6.6.2024.5978-5987

ABSTRACT:

Background: Chemo-mechanical preparation is a crucial component of an effective endodontic treatment plan. It is accomplished by the tools and efficient irrigation products. Irrigating solutions are used to dissolve organic and necrotic tissue, avoid dentin chip packing in the apical region, and eliminate smear layers and debris. Healing potential of plants is an age-old idea that has recently attained renewed interest. Considering the ineffectiveness, potentially harmful effects, and safety concerns of commonly used synthetic irrigants, the herbal alternatives for endodontic usage might prove to be advantageous. The aim of this study was to compare of smear layer removal efficacy of Neem, Triphala and 17 % EDTA as root canal irrigants.

Materials and Methodology: thirty human mandibular first premolars were decoronated to a root length of 17mm. They were cleaned and shaped upto 40k size file using saline as an irrigant throughout the instrumentation. The specimens were equally divided into 3 groups according to final irrigant used for smear layer evaluation; group 1- Neem solution, group 2 -Triphala solution, group3-17% EDTA. Samples were split longitudinally and examined under microscope for smear layer evaluation at coronal, middle and apical levels.

Results: Triphala demonstrated the maximum efficiency in eliminating the smear layer than EDTA and Neem solution.

Conclusion: Neem and Triphala both showed the potential to eliminate the smear layer. Triphala demonstrated the maximum efficiency in eliminating the smear layer.

Keywords: EDTA, Triphala, Neem, smear layer, SEM

1. INTRODUCTION

A successful endodontic treatment strategy must include the removal of smear layers and microbial biofilms from the root canal walls.[1,2] Chemical irrigants work to eliminate the smear layer that embedded dirt and germs during the mechanical preparation.[3]. However, the disadvantages of chemical irrigants prompted the researchers to discover more dentin-friendly and biocompatible irrigants.[4,5] Natural substances including as herbal extracts are gaining popularity as endodontic irrigants because of the antioxidant, anti-inflammatory, immune-

modulatory, antibacterial, antifungal, antiviral, antioxidant, anti-carcinogenic, and radical scavenging characteristics of the active components.

Although the concept of plants having the ability to heal is not new, it has recently attracted more attention. Herbal remedies are safe and contain active ingredients that, in addition to their medicinal properties, have positive physiological effects. Triphala is a traditional Indian herbal remedy made from the powdered and dried fruits of three healing herbs. It has tannic acid as its principal constituent. It has been used in Indian traditional medicine for treatment of headaches, constipation, and hepatic disorders. Initial studies have shown bacteriostatic or bactericidal effect of tannic acid on gram-positive and gram-negative pathogens. The advantages of Triphala include easy availability, low cost, long-term substantivity, less toxicity, and absence of microbial resistance.[1]

In root canal therapy, ethylene-diaminetetra-aceticacid (EDTA) is used and it works incredibly well to remove smear layers[6]. It generally has no effect on Gram-positive organisms and is not a very effective bactericide. Azadirachta indica, sometimes referred to as "Indian lilac," "Margosa tree," or "Indian neem," is one of the most efficient medicinal plants with a broad range of biological activity[7].

Neem (Azadirachta indica) is one of the ancient medicinal plants used most frequently in India. Every branch of the tree has been studied in phytotherapy. About 140 physiologically active compounds that possess anti-inflammatory, antifungal, antibacterial, antiviral, antioxidant, antimutagenic, and anticarcinogenic properties have been discovered from this plant. The plant's antibacterial properties are due to a variety of compounds, including nimbidin and cyclic trisulfide, alkaloids, tetranortriterpenoid, glycosides, saponins, flavonoids, steroids, anthraquinone, and tannic acid. The isoprenoid group of the neem plant, which is made up of nimbin, nimbinin, nimbidinin, nimbolide, and nimbidic acid, has a number of therapeutic and antibacterial qualities that suggest the plant may be used as an endodontic irrigant. Neem has great biocompatibility and remarkable antioxidant qualities thus tissue toxicity is not an issue. Neem extract is regarded as an effective irrigant for the apical section of the root canal that removes smear layers [8] Using scanning electron microscopy (SEM), McComb & Smith (1975) were the first to describe the smear layer in instrumented root canals. There are both organic and inorganic materials in the smear layer.[9] Although root canal shaping can be executed with precision and efficiency using instruments, thoroughly cleaning the entire root canal system with an appropriate irrigant is still a difficult task[10].Thus, using scanning electron microscopy (SEM) image analysis, this study compares the effectiveness of triphala, and Azadirachta indica (neem) for smear layer eradication with EDTA.

2. MATERIALS AND METHODS

The present cross-sectional comparative study included 30 freshly extracted human permanent maxillary and mandibular single-rooted teeth collected from the Oral Surgery Department of SMBT Dental College and hospital, sangamner (fig.1). The collected teeth were properly washed with running tap water to remove any blood and debris from the surface. The teeth were decoronated to obtain a uniform working length of 17 mm for all samples using a diamond disk (Fig.2,3). The samples were stored in a saline solution. The samples were divided randomly into three groups with 10 teeth each. They were organized according to the irrigants used: Group A: Neem, Group B: Triphala, and Group C: 17 % EDTA.[11,12,13] The root canals were accessed, and the biomechanical preparation were done using a standardized crown down technique. The initial coronal preparation was done with Gates-Glidden drills up to number 3 size. Hand instrumentation were done in step down method using K files in sequentially smaller sizes up to ISO size 40. According to the manufacturer's specifications, the root canals were prepared in a crown down manner using ProTaper Gold rotary system (Dentsply Sirona, United

States) at 300 rpm speed and 5.10 N cm torque (Fig.4). The canals were irrigated using 5 mL of the prepared solutions corresponding to the respective group during the instrumentation (fig.5 a,b,c,) The study samples were subsequently rinsed with sterile distilled water in each group and dried with sterile absorbent paper sheets. Diamond discs were used to cut deep grooves on the buccal and lingual surfaces of the roots, without perforating the root canals. The roots were then split with a chisel and mallet. One half of each tooth is selected and prepared for SEM examination. The specimens were dehydrated by ethyl alcohol: 30% for 10 min, 50% for 20 min, 90% for 30 min, and 100% for 30 min. The coronal, middle, and apical one-third of root dentin was observed with SEM under 1000X.[11] (fig.6 a,b,c)

Smear layer evaluation criteria Score

The scoring system described by Prado et al. in 2011 was used to evaluate the degree of smear layer removal

- · Score 1: no smear layer and all tubules are clean and open
- · Score 2: a few areas covered by smear layer, with most tubules cleaned and opened
- · Score 3: smear layer covering almost all the surface, with a few tubules, opened.
- · Score 4: smear layer covering all the surfaces.

Statistical Methodology

The data was obtained and entered in Microsoft Excel version 13. The data subjected to Statistical analysis using IBM Statistical Package for Social Science version 21. For the Categorical Scores in Each group Frequency and Percentage was obtained. The Mean Smear Layer Score removal was obtained. For intergroup comparison Kruskal Wallis H was applied for Categorical variables and the same evaluation of the variables on continuous scale was evaluated using ANOVA with Post Hoc Tukey's. All the statistical tests were applied keeping confidence interval at 95% and ($p<0.05$) was considered to be statistically significant.

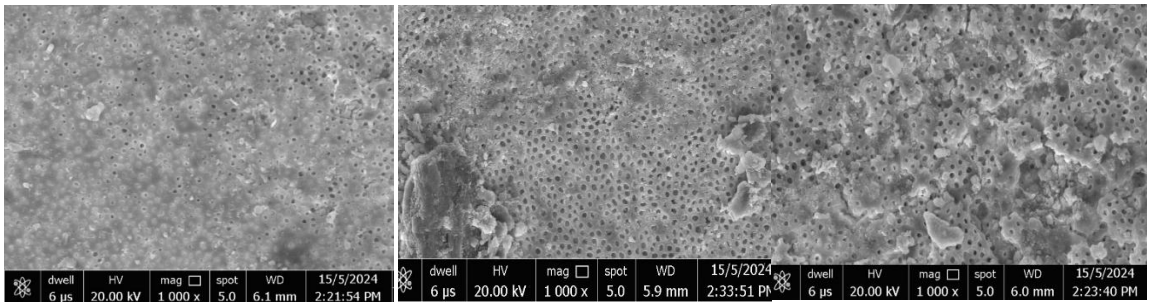
(fig.1) single rooted premolars

(fig.2) Decoronation of specimen

(fig.3) Decoronated specimens at the level of CEJ

(Fig.4) Root canals were prepared using ProTaper Gold rotary system

(fig.5) Canals were irrigated using 5 ml (a) neem leaf extract (Group I), (b) Triphala (Group II), (c) 17% liquid EDTA as irrigants of the prepared solution corresponding to the respective group.



(fig.6) Scanning electron microscope images (×1000) depicting smear layer removal using (a) neem leaf extract (Group I), (b) Triphala (Group II), (c) 17% liquid EDTA as irrigants

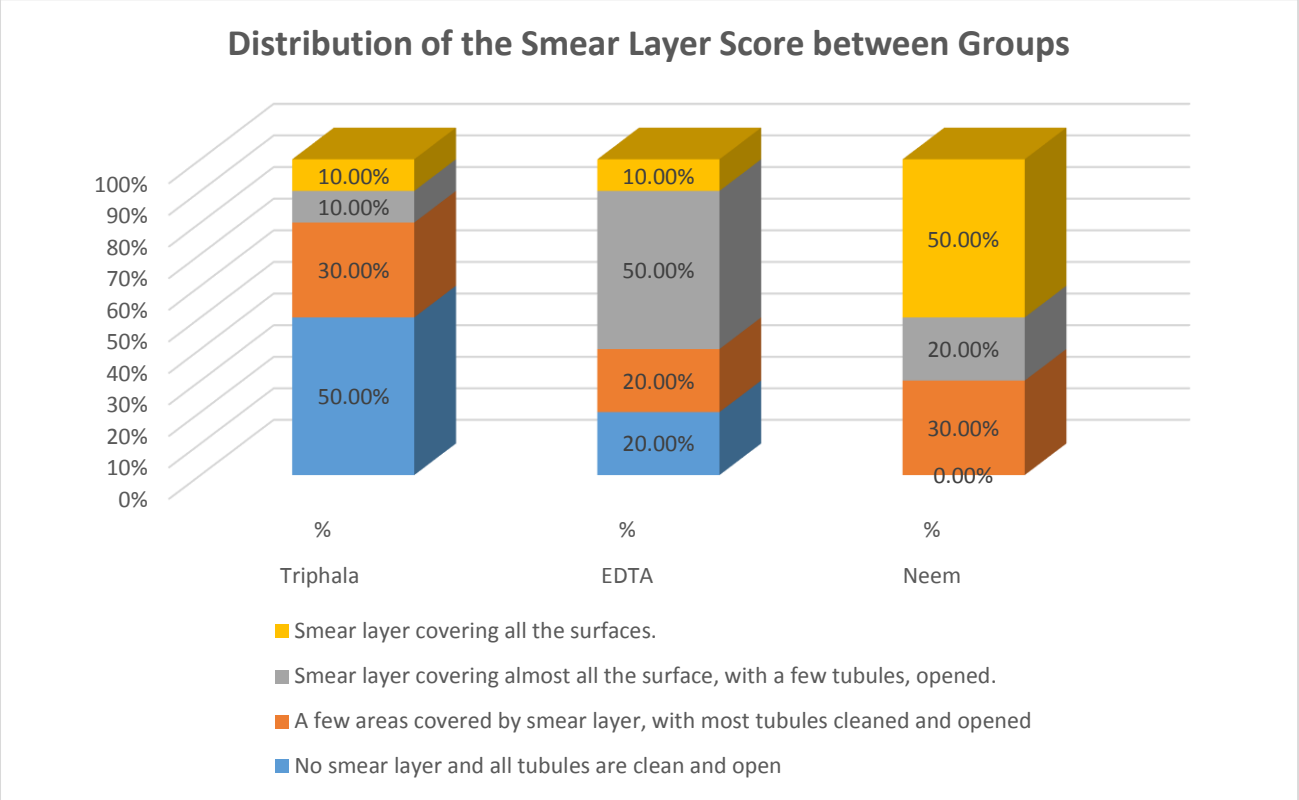
3. RESULT

Distribution of the Smear Layer Score between Groups

			Smear Layer Removal Score				Total	P Value	P Value between Groups
			No smear layer and all tubules are clean and open	A few areas covered by smear layer, with most tubules cleaned and opened	Smear layer covering almost all the surface, with a few tubules, opened.	Smear layer covering all the surfaces.			
Groups	Triphala	n	5	3	1	1	10	0.21	0.004*
		%	50.0%	30.0%	10.0%	10.0%	100.0%		
	EDTA	n	2	2	5	1	10	0.308	
		%	20.0%	20.0%	50.0%	10.0%	100.0%		
	Neem	n	0	3	2	5	10		

		%	0.0%	30.0 %	20.0%	50.0%	100.0 %	0.49 7	
Total		n	7	8	8	7	30		
		%	23.3 %	26.7 %	26.7%	23.3%	100.0 %		

When the Distribution of the Smear Layer Score between Groups was evaluated, it was observed that of the 10 Teeth Irrigated with Triphala Solution 5 (50%) had no smear layer, EDTA had 2 (20%), Neem had 0 (0%) teeth which had no smear layer with all tubules are clean and open. It was observed that Triphala Solution was more efficacious followed by EDTA followed by Neem Solution.



Mean Distribution of the Smear Layer Score

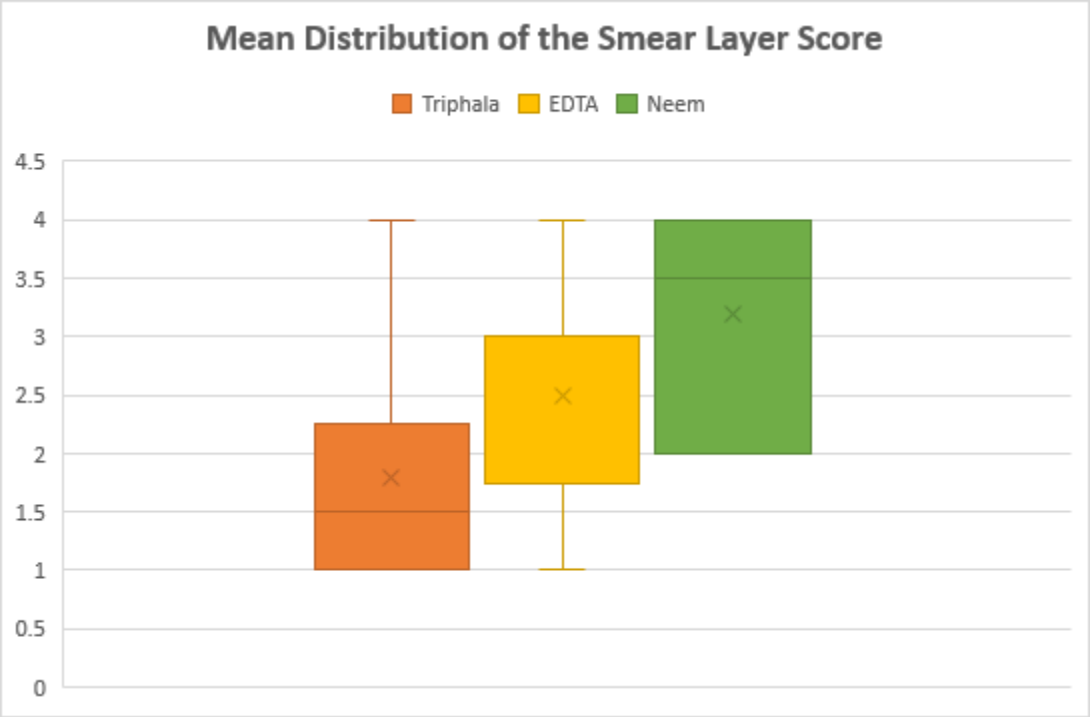
	N	Minimum	Maximum	Mean	Std. Deviation	Std. Error	P Value
Triphala	10	1.00	4.00	1.8000	1.03280	.32660	0.013
EDTA	10	1.00	4.00	2.5000	.97183	.30732	
Neem	10	2.00	4.00	3.2000	.91894	.29059	

The Mean Distribution of the Smear Layer Score was 1.80 ± 1.03, 2.50 ± 0.97 and 3.20 ± 0.91. The score was lowest for Triphala indicating a better removal of Smear Layer followed by EDTA followed by Neem and this difference in Mean was statistically significant ($p<0.05$).

Pairwise Comparison of Smear Layer Removal Score

Multiple Comparisons						
Dependent Variable: Smear Layer Removal Score						
Tukey HSD						
(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	**P Value**	95% Confidence Interval	
					Lower Bound	Upper Bound
Triphala	EDTA	-.70000	.43631	.261	-1.7818	.3818
	Neem	-1.40000	.43631	.009	-2.4818	-.3182
EDTA	Neem	-.70000	.43631	.261	-1.7818	.3818

When Pairwise comparison was done between Triphala – EDTA, Triphala Neem and EDTA and Neem it was observed that the Triphala Solution was more effective followed by EDTA followed by Neem Solution. The difference between Scores was found to be statistically significant between Triphala and Neem. ($p<0.05$)



4. DISCUSSION

A strong correlation has been found and supported in literature[14] between the clinical science of effective endodontic treatment and the fundamental science of microbiology. Root canal therapy depends on the removal of bacterial and diseased pulpal tissues and their poisons to create a sterile environment and a hermetic seal. Endodontic therapy seeks to eradicate microbial colonization and establish a three-dimensional obturation of the root canal system.[15] In chemo-mechanical preparation comprises the usage of a chemical solution in conjunction with mechanical instruments to clean the root canal system.[16] The elimination of pulp remains, microbial toxins and germs performed by chemo mechanical debridement, is required for the effectiveness of root canal therapy[17].

Good root canal cleaning is accomplished using irrigation and instrumentation.[18] Massive dentin debris and microbial toxins that adhere to the root canal wall produce a smear layer

during the instrumentation procedure when viable and necrotic pulp tissue persists. First to document the development of a smear layer on instrumented root canals was McComb and Smith in 1975. This layer is composed, the researchers say, of microorganisms, necrotic pulp tissues, odontoblastic processes, and bits of dentin.[19] A major contributing factor to many treatment failures is the ongoing infections in these canal areas. To this aim, irrigation solutions in conjunction with biomechanical preparation have been successfully applied. Since then, a lot of study has been done on how the smear layer forms and how to manage it during root canal therapy. Almost always, during root canal instrumentation, a smear layer forms.[15]

Researchers are attempting to revive their quest for herbal substitutes in view of the safety issue, antibiotic-resistant microorganisms, and absence or very little possible adverse effects.[20] The antioxidant, anti-inflammatory, immunomodulatory, antibacterial, antifungal, antiviral, antioxidant, anticarcinogenic, and radical scavenging properties of the active components make natural substances like herbal extracts more important as endodontic irrigants.[21] Because natural herbal remedies are readily available, reasonably priced, and biocompatible, they are being studied and used more and more as part of dental treatment regimens.[1] Because antibiotic-resistant bacteria are becoming more common, synthetic drugs are causing negative effects, and irrigants like sodium hypochlorite carry hazards, researchers have been searching for safer and more patient-friendly herbal alternatives.[20] Researchers have developed substitutes for synthetic irrigants using a variety of herbal extracts including Citrus Aurantifolia, Apple Vinegar, Castor Detergent+Papain Enzyme, Orange Oil, Green Tea Polyphenol, Salvadora Persica, Tea Tree Oil, Turmeric, Chitosan, Morinda citrifolia, Terminalia chebula, Triphala, Neem Leaf, German Chamomile, Tulsi, Passion Fruit Juice, Nutmeg, and Phytic Acid.[1]

In the present work, the effectiveness of herbal irrigants in relation to EDTA was investigated using triphala and neem extract. Triphala shown promising effectiveness in the trial, as 50% of the prepared teeth had no smear layer in the canal. Similar findings were reported in a second investigation on the effectiveness of triphala as a last rinse solution in curved canals by Susan A et al.[24] In this work, premixed triphala activated by ultrasonics removed smears almost as well as 17% EDTA at the coronal and apical thirds of the curved canals.

This is because of the main component of triphala, tannic acid, which has been shown in earlier research to have bacteriostatic and bactericidal effects against some gram-positive and gram-negative bacteria. They have been linked to physiological human processes including host-mediated tumour, phagocytic cell activation, and anti-infective effects.[1] By nonspecific forces of hydrophobicity, covalent bond formation, and hydrogen bonding, they bind with proteins. They function by deactivating enzymes, cell envelope transport proteins, and microbial adhesins.[22] Triphala is one of the natural alternatives to synthetic chemicals that is useful as a root canal irrigant. Strong inhibitory action of triphala against matrix metalloproteinases and collagenases, which can lead to periodontal damage. The purpose of inhibition of these enzymes is to stop this deterioration. It has been successful to use doxycycline to suppress collagenase. Herbal derivatives help to prevent synthetic medicine side effects.[23,24]

Neem extract was a further herbal substitute used in the current investigation. Neem leaf extract is a possible root canal irrigation agent since it has been demonstrated to be efficient against Enterococcus faecalis and Candida albicans. 67% Because it contained flavonoids and acid metabolites, it removed smear layers with the highest efficacy of all the groups in the study by Sebatni et al.[3] Though 50% of the prepared teeth had a smear film covering all surfaces, neem extract was shown to be ineffective in completely removing the smear layer.

But in just a handful of the prepared teeth—mostly cleaned and opened tubules—only a few places were covered. It therefore performed worse than EDTA, which in 20% of all prepared teeth revealed no smear layer. In another work, orange oil, propolis, and neem leaf extract were evaluated as endodontic irrigants by Setia et al.[7] The smear layer was removed at the coronal,

intermediate, and apical levels by neem leaf extract substantially more effectively than by the other two groups, the authors reported.
In an vitro experiment by Bhargava et al. [25] compared the effectiveness of EDTA with that of amla, neem, and triphala. According to the authors, EDTA was the most effective in removing the smear layer, however amla was more effective than triphala and neem. Although synthetic chemical compounds designed as solutions for use as irrigants in endodontics aid in the cleaning and disinfection of the canal system, they also have drawbacks including toxicity, allergic potential, bad taste, and cost. As the native herbal remedies have less toxicity and are more affordable, there is knowledge of and a significant shift in trend towards their use with the appropriate pharmacological properties.[3,7]

5. CONCLUSION

Neem and Triphala both showed the potential to eliminate the smear layer. Triphala demonstrated the maximum efficiency in eliminating the smear layer. Since the smear layer removal abilities of Triphala were shown to be superior than EDTA, and it is a biocompatible agent, it can be evaluated for usage in the root canal yet further investigations are need to prove its efficiency as an endodontic irrigant.

6. REFERENCES

1. Susan AC, Bharathraj AR, Praveen M, Karunakaran JV. Intraradicular smear removal efficacy of Triphala as a final rinse solution in curved canals: A scanning electron microscope study. Journal of Pharmacy and Bioallied Sciences. 2019 May 1;11(Suppl 2):S420-8.
2. Chhabra N, Gyanani H, Kamatagi L. Smear layer removal efficacy of combination of herbal extracts in two different ratios either alone or supplemented with sonic agitation: An: in vitro: scanning electron microscope study. Journal of Conservative Dentistry and Endodontics. 2015 Sep 1;18(5):374-8.
3. Sebatni MA, Kumar AA. Smear layer removal efficacy of herbal extracts used as endodontic irrigants: An in vitro study. Endodontology 2017;29:35-8.
4. Sowjanyaa J, Thomas T, Chandana CS. Comparative evaluation of the efficacy of smear layer removal by ethylenediaminetetraacetic acid, Triphala, and German chamomile as irrigants – A scanning electron microscopy study. J Adv Pharm Edu Res 2017;7:261-71.
5. Kumar A, Sarthaj AS, Antony SM. Comparative evaluation of antibacterial and smear layer removal efficacy of two different herbal irrigants: An in vitro study. Int Healthc Res J 2018;1:350-4.
6. Rome WJ, Doran JE, Walker III WA. The effectiveness of Gly-Oxide and sodium hypochlorite in preventing smear layer formation. Journal of Endodontics. 1985 Jul 1;11(7):281-8.
7. Setia R, Bajaj N, Bhola M, Brar GS. 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. Contemporary Clinical Dentistry. 2023 Apr 1;14(2):128-34.
8. Sudhakar S, Gupta N, Ghambir N, Singh R, Singh D. Comparative Evaluation of Intracanal Smear Layer Removal by Different Root Canal Irrigants: A Scanning Electron Microscope Study. International Journal of Clinical Pediatric Dentistry. 2023 Jul;16(4):633.
9. Anusha Y. *Comparative evaluation of antimicrobial efficacy of bitter guard (Momordica Charantia) and GARLIC (Allium Sativum) as endodontic Irrigants against E. Faecalis:*

An In Vitro Study (Doctoral dissertation, Adhiparasakthi Dental College and Hospital, Melmaruvathur).

10. Ali SA, Hussain M, Shah H, Khan H. Smear layer removal efficacy of conventional endodontic irrigants v/s phytochemical extracts-an in vitro study. Pakistan Oral & Dental Journal. 2018 Sep 10;38(2):254-8.
11. Mukherjee M, Kalita T, Barua P, Barman A, Thonai S, Mahanta Sr P, Medhi H. Efficacy of smear layer removal of human teeth root canals using herbal and chemical irrigants: an in vitro study. Cureus. 2023 Jun 15;15(6).
12. Prabhakar J, Mensudar R, Geethapriya N, Prabhakar J. Cleaning efficacy of Triphala (an Indian herbal medicine) and green tea polyphenol used as irrigants on removal of smear layer: a sem study. Biomed. Pharmacol. J. 2015;8:303.
13. Rao SA, Sowjanya KI, Sunitha L. Comparison of smear layer removal ability and the effect on root dentin strength of garlic extract and EDTA used as final irrigants–An in vitro study. Indian J Conserv Endod. 2016 Oct;1:81-5.
14. Paul J, Prakash V, Mensudar R, Sukumaran VG, Subbiya A, Mitthra S. Evaluation Of Three Root Canal Irrigants In Their Ability To Remove Smear Layer With Significantly Least Erosio–Ansem Analysis. Int. J. of Aquatic Science. 2021 Jun 1;12(3):2045-54.
15. Violich DR, Chandler NP. The smear layer in endodontics–a review. International endodontic journal. 2010 Jan;43(1):2-15.
16. Alharbi A, Aliuddin S, Alharbi TA, Alharbi S, Alanzi AO, Alharbi AA. Herbal endodontic irrigants. Int J Prev Clin Dent Res. 2017;4(4):311-14. Doi: https://doi.org/10.5005/jp-journals-10052-0133. Irrigants HE. Herbal Endodontic Irrigants. Int J Prev Clin Dent Res. 2017;4(4):311-4.
17. Abdelghany ME, Kamel WH, Bastawy HA. Efficacy of apple vinegar as final irrigating solution in removing smear layer using XP-Endo Finisher file. Al-Azhar Dental Journal for Girls. 2020 Jan 1;7(1):95-103.
18. Karteek BS, Reddy KS, Prasad SD, Reddy MS, Jain A, Lakshmi C. Evaluation of efficacy of three herbal extracts on smear layer removal during root canal preparation using scanning electron microscope: An in vitro study. Int J Dent Sci Innov Res. 2020,3(4):240-53
19. Alamoudi RA. The smear layer in endodontic: To keep or remove–an updated overview. Saudi Endodontic Journal. 2019 May 1;9(2):71-81.
20. Manasa G, Kumar MG, Nallanchakrava S, Bala GN, Rao KN. Evaluation of smear layer removal and antimicrobial efficacy of intracanal herbal irrigants. Endodontology. 2023 Jan 1;35(1):35-42.
21. Ranjitha GR, Mutha SR, Anithakumari R, Vikram R, Kumar N, Shivekshith AK. Comparative evaluation of smear layer removal efficacy of three herbal irrigants in the apical third of the root canal: An: in vitro: scanning electron microscopic study. SRM Journal of Research in Dental Sciences. 2020 Apr 1;11(2):61-5.
22. Jagadish L, Kumar VA, Kaviyarasan V. Effect of Triphala on dental bio-film. Indian Journal of Science and Technology. 2009 Jan 1:30-3.
23. Agrawal V, Kapoor S, Agrawal I. Critical review on eliminating endodontic dental infections using herbal products. Journal of dietary supplements. 2017 Mar 4;14(2):229-40.
24. Pujar M, Patil C, Kadam A. Comparison of antimicrobial efficacy of Triphala,(GTP) Green tea polyphenols and 3% of sodium hypochlorite on Enterococcus faecalis biofilms formed on tooth substrate: in vitro. Journal of International Oral Health. 2011 Apr 1;3(2).
25. Bhargava KY, Aggarwal S, Kumar TA, Bhargava SH. Comparative evaluation of the efficacy of three anti-oxidants vs NaOCl and EDTA: Used for root canal irrigation in smear layer removal-SEM study. Int J Pharm Pharm Sci. 2015;7:366-71.