"COMPARATIVE EVALUATION OF SMEAR LAYER REMOVAL OF CUMINUM CYMINUM, 17% EDTA AND CUMINUM CYMINUM AND 17% EDTA AS A FINAL RINSE AT THE APICAL THIRD OF THE ROOT CANAL: AN IN VITRO CONFOCAL LASER SCANNING MICROSCOPY STUDY."

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LIST OF ABBREVIATIONS

SR.NO	ABBREVIATIONS	FULL FORM
1	**EDTA**	Ethylene diamine tetra acetic acid
2	**CC**	Cuminum cyminum
3	**CLSM**	Confocal Laser Scanning Microscope
4	**ANOVA**	Analysis of variance
5	***µm***	Micrometre
6	**SD**	Standard Deviation
7	**et al**	Additional persons involved in the same study
8	**NaOCl**	Sodium Hypochlorite
9	**mL**	Milliliter
10	**mg**	Milligram
11	**° C**	Degrees Celsius
12	**mm**	Millimeter
13	**hrs**	Hours
14	**min**	Minutes
15	**n**	Number of specimens
16	**p-value**	Probability value
17	**i.e.**	That is

18	**nm**	Nanometer
19	**<**	Less than
20	**>**	Greater than
21	**SE**	Standard error
22	**PM**	Premolar
23	**PUI**	Passive ultrasonic irrigation
24	**WL**	Working Length
25	**MAF**	Master Apical File
26	**NS**	Not significant
27	**Conc.**	Concentration
28	**PP**	Paper Point
29	**GP**	Gutta Percha
30	**MIC**	Minimum Inhibitory Concentration
31	**MBC**	Minimum Bactericidal Concentration
32	**HEDP**	Hydroxyethylene Diphosphonic Acid
33	**CFU**	Colony Forming Units
34	**CHX**	Chlorhexidine

ABSTRACT

Aim- To evaluate and compare the efficacy of smear layer removal of Cuminum cyminum, 17% EDTA and Cuminum cyminum, and 17% EDTA as a final rinse, at the apical third of the root canal.

Methodology: Cumin seeds were hydrodistilled to form an oil extract which was then diluted with DMSO and distilled water to the calculated MIC and MBC of the extract. 60 extracted human mandibular premolar teeth with single root and single canal were selected. They were disinfected in 0.1% thymol solution, cleaned of calculus and soft tissues and stored in 0.1% thymol solution till use. All teeth were radiographed and selected as per the inclusion and exclusion criteria. The teeth were decoronated using a diamond disk under copious water spray to acquire a standardized root length of 14 mm. Working length was established by inserting a size 10 K file into each root canal until it is visible at the apical foramen and by subtracting 1mm from the recorded length. Instrumentation of the root canal was done till master apical file size of F2/F3 using ProTaper universal, rotary instruments. The canals were irrigated with 2 mL of 3% sodium hypochlorite between successive files. Teeth were randomly divided in 4 groups (n= 15) according to the intervention. Passive ultrasonic irrigation was used to activate the irrigants. Final irrigation was performed with saline. The teeth were obturated using AH plus sealer coated with rhodamine B dye. Depth of sealer penetration into dentinal tubules was assessed using Confocal Laser Scanning Microscope

Results – Highly significant difference was seen between the groups with EDTA and Cuminum cyminum, with EDTA demonstrating the highest penetration of sealer. The

least penetration was seen with the negative control group that was irrigated with saline followed by the group irrigated with Cuminum cyminum irrigant.

Conclusion - It can be concluded that while EDTA continues to remain the gold standard irrigant for smear layer removal, further research is required to find an irrigant which is as efficient as EDTA however, has a less aggressive effect on the dentinal tissues.

Keywords: Cuminum cyminum, EDTA, AH plus, Dentinal tubule penetration, Rhodamine dye, Confocal Laser Scanning Microscope, Herbal

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INTRODUCTION

Successful endodontic therapy relies heavily on the standard of biomechanical preparation followed by the water tight seal of the root canal.[1] Canal instrumentation by either hand or machine-run methods results in a "smear-layer" which is made up of organic and mineralised debris from dental tissues. The smear-layer possesses constituents such as pulpal debris, dentinal tissue cells, microbiota and haematic cells in the radicular tubules.[2]

This smear-layer is known to form spaces between the root canal walls and the sealers used for obturation, thus inhibiting a total lock and attachment of obturating materials to radicular dentinal tubules.[3] It consists of microorganisms, their by-products and therefore should be cleaned entirely from the root canal system.[4] To add to this, cleaning the smear-layer facilitates efficient entry of dressings and sealants into tubules leading to better disinfection and obturation.[5]

Irrigants help in mechanical debridement of the canal by washing out detritus formed from dissolved cells and disinfecting the canal. Among various endodontic irrigants available for smear-layer removal, Ethylene-diamine-tetra-acetic acid (EDTA) as well as Sodium-hypochlorite, (NaOCl) are used universally.[2,6]

Baumgartner and Madely in their study, observed that when EDTA and NaOCl come in contact, it causes a progressive dissolution of dentin thereby dissolving and weakening the peritubular and intertubular regions.[7] Furthermore, EDTA has proven to have a weakening effect on the tooth structure and erodes the radicular tubular dentin if it is exposed to the root dentin for increased time periods.[8]

These and many other harmful side effects of synthetic irrigants have encouraged research in the field of herbal medicine. Herbal alternatives employed in

endodontics have numerous pros such as economics, biocompatibility, easy use, as well as prolonged shelf life.[9]

Sodium hypochlorite (NaOCl) is a universal choice for endodontic therapy due to the unparalleled organic tissue dissolving properties. This irrigant, however, has little to no power on mineralised component of the smear-layer.[10] Its oxidizing properties interfere with the resin cement's free radical polymerization at its interface with dentin.[11]

Research has shown that some natural antioxidants can fight the deteriorating effect of NaOCl on the bond formed between dentin and resin, eg. Citric acid, green tea extract[12] and can also diffuse the adverse effect of laboratory made antimicrobials, thereby supporting their use in endodontic irrigation and inter-appointment dressings.[13]

A variety of herbals such as Neem, Propolis, Curcumin etc. have been used as irrigants in endodontics over the years and have yielded good results especially as potent antimicrobials.[14]

Cuminum cyminum (cumin) is an important and popular spice locally known as 'Jeera' that is used around the world for numerous purposes. It belongs to the plant family known as *Apiaceae*.[15] The spice is rich in vitamins, especially B complex, and minerals like Cu, Fe, K, Zn, and Ca. Anti-oxidant vitamins like vitamin A, C and E are also known constituents.[16] The seeds have proven to be of great use in traditional medicine for the treatment of jaundice, diarrhoea, toothaches etc.[17]

Research has proven that cumin is a strong antioxidant, antibacterial and antifungal against a variety of microorganism such as *E. faecalis* and *Candida albicans*.[18] Amalia et. al in 2019 reported that Cuminum cyminum in the strength of

1.0 mg/ml has superior anti-bacterial properties when compared to 2% chlorhexidine against *E. faecalis* strains which has also proven to be comparable to anti-microbial effect of 5.25% NaOCl.[19]

High antioxidant activity shown by *C. cyminum* is attributed to the existence of polyphenolic groups such as flavonoids, linalool, anethole, monoterpene alcohols, and carvacrol.[20] These compounds have been known to increase shelf life and scavenge free radicals which in turn delays the process of lipid peroxidation of materials.[21]

The use of C. cyminum in irrigation has not been researched in depth. Since there is a lack of literature on the effects of Cuminum cyminum and its combination with EDTA, on the clearance of smear-layer during biomechanical preparation, the goal of this study is to evaluate and compare the smear-layer clearing capacity of Cuminum cyminum, a combination of 17% EDTA and Cuminum cyminum, and 17% EDTA at terminal third of root canal.

AIMS AND OBJECTIVES

AIM OF STUDY

To evaluate and compare the efficacy of smear-layer removal of Cuminum cyminum, 17% EDTA and Cuminum cyminum, and 17% EDTA as a final rinse, at the apical third of the root canal.

OBJECTIVES

- To assess the effect of Cuminum cyminum on smear-layer removal at the apical third of the root canal.
- To assess the effect of combination of 17% EDTA and Cuminum cyminum on smear-layer removal at the apical third of the root canal.
- To assess the effect of 17% EDTA on smear-layer removal at the apical third of the root canal.
- To compare the effect of Cuminum cyminum, combination of 17% EDTA and Cuminum cyminum, and 17% EDTA on the smear-layer removal at the apical third of root canal.

HYPOTHESIS

NULL HYPOTHESIS:-

There will be no difference in the effect of Cuminum cyminum, combination of 17% EDTA and Cuminum cyminum and 17% EDTA on the smear-layer removal at the apical third of the root canal.

ALTERNATE HYPOTHESIS:-

There will be a difference in the effect of Cuminum cyminum, combination of 17% EDTA and Cuminum cyminum and 17% EDTA on the smear-layer removal at the apical third of the root canal.

REVIEW OF LITERATURE

1. A research study by Ahmed Abo El-Hamd Ali and co-workers aimed to assess the disinfectant efficacy and cytotoxic effects of Myrtus communis (M. communis) essential oil, chlorohexidine (CHX) 2%, and sodium hypochlorite (NaOCl) on mature single-canaled maxillary central incisors infected with Enterococcus faecalis. Forty-seven teeth were segregated in four different groups (control, Myrtus communis, CHX, NaOCl), along with which antibacterial activity was analyzed using confocal laser microscopy. Cytotoxicity was analyzed using the MTT colorimetric assay. Results indicated that NaOCl had the highest antibacterial effect, while M. communis essential oil showed the greatest cell viability. CHX demonstrated moderate antibacterial efficacy and cytotoxicity compared to NaOCl. These findings highlight M. communis as a promising alternative with lower cytotoxicity for root canal therapy.[22]
2. The observations by Asikali Idayadullah et al. when they evaluated the impact of two free radical inhibitors on debris removal and microhardness of radicular dentin using an individuals unanchored teeth showed scanning electron microscopy (SEM) revealed both antioxidants effeciently eliminated the debris film, with one performing more effectively in the terminal one third of radicular canal. Microhardness, assessed using a Vickers hardness test, showed both antioxidants influenced dentin hardness, but one caused a lesser reduction, preserving structural integrity. The study emphasized the importance of selecting an antioxidant that balances effective smear-layer removal with minimal impact on dentin hardness. These findings offer valuable insights for optimizing endodontic treatments.[23]

3. The study by Rasha Sameh and co-workers investigates the comparative effectiveness of sodium hypochlorite (NaOCl) and strawberry extract as endodontic irrigation solutions. Utilizing single-rooted premolars infected with Enterococcus faecalis biofilm, the research examines bacterial reduction through ultrasonic, sonic, and non-activation methods. CLSM was employed to analyze the samples, with data processed using ANOVA and Tukey's tests. Outcomes revealed that ultrasonic activation of NaOCl and strawberry extract produced the highest bacterial reduction rates, with strawberry extract demonstrating potential as an alternative irrigation solution in endodontic therapy. The statistical analysis supported the significance of the findings.[24]
4. A research by Vinuta Mukundreddy Bhandi et al focuses on the in vitro antibacterial activity of Cuminium cyminium (C. cyminium) ethanol extract compared to calcium hydroxide ($Ca[OH]_2$) as intracanal medicaments. Utilizing a direct contact test (DCT), bacterial growth kinetics were monitored at intervals of 1 hour, 24 hrs, 48 hrs, and 72 hrs. OD values were calculated for test, positive, and negative control groups. Statistical analyses, including paired t-tests and post hoc procedures, confirmed a significant reduction in bacterial viability with C. cyminium extract over time. The study highlights C. cyminium as a promising natural alternative to traditional endodontic medicaments for combating pathogens in root canal infections.[25]
5. A study by Regina Gascón and co-workers attempted to assess the impact of antioxidant treatment on instant bond strength of fixatives in dentistry to endodontically therapised dentine. Data from studies published in English between 2001 and 2022 were collected through five databases using specific descriptors. A total of 18 studies were analyzed by independent reviewers to ensure methodological quality. Findings suggest that substrate preparation and

irrigation procedures during endodontic therapy influence bond strength. Antioxidants have been shown to enhance bond strength and adherence, especially after using common irrigants in endodontic treatments. Consequently, antioxidant treatments are effective techniques to improve bond strength in endodontically treated teeth.[26]

6. Shahzad Sharif Mughal's study highlights the vital role of spices in cooking, enhancing dishes with aroma, texture, and color while providing preservation and health benefits. Cumin (Cuminum cyminum), or 'zeera,' a versatile spice from the Apiaceae family, is highly valued for its antioxidant properties and extensive use in traditional medicine. Its bioactive compounds, including terpenes, phenols, and flavonoids, contribute to its therapeutic effects. Research has explored cumin's seed oil, essential oil, and thymoquinone (TQ), revealing its potential in managing diabetes, dyslipidemia, hypertension, and inflammatory diseases. Furthermore, it shows promise in treating respiratory conditions, allergic rhinitis, digestive disorders, and cancer, reinforcing its role as a complementary therapy alongside conventional treatments. This versatile spice remains significant in both culinary and medicinal contexts.[17]
7. Nabavizadeh and colleagues performed a study to estimate the adhesive bond energy of resin-based sealer to dentine treated by herbal irrigating solutions. Forty mandibular first premolars were divided into four groups after smear film removal: Group 1 - Cinnamomum zeylanicum (CZ), Group 2 - 2.5% NaOCl, Group 3 – NaOCl at its minimum inhibitory concentration (M.I.C.), and Group 4 - Cuminum cyminum (CC) at MIC. The canals were irrigated for one minute, followed by rinsing with distilled water. After obturation, 1-mm root slices underwent push-out testing using a UTM and failures were analyzed under a stereomicroscope (30X). Statistics using ANOVA and

Tukey's-post-hoc test revealed CZ exhibited significantly less bond strength compared to NaOCl. No significant differences were noted amongst CC and NaOCl groups, with most failures classified as mixed. The study concluded that Cuminum cyminum does not compromise AH Plus bond strength and may serve as a promising alternative for final irrigation.[13]

8. An experimental study by Ramin Ghaseminejad and coworkers evaluated the penetration depth of methylene blue and curcumin into dentinal tubules, with and without smear films. Thirty-two human incisors were divided into four groups, and penetration was measured across apical, middle, and coronal sections using buccal, mesial, distal, and palatal cross-sections. Results revealed that curcumin had significantly greater penetration depth than methylene blue in the presence of smear films across all sections. Smear elimination enhanced penetration in the apical and middle 1/3rd but did not significantly impact coronal third. The study concludes that curcumin is more effective than methylene blue at penetrating dentine tubular channels in the presence of smear coatings.[27]
9. A research by Taiane Correa Furtado et al investigated the impact of fluorophores on intratubular penetration of four endodontic sealers using CLSM 80 cow teeth were treated, filled by cold lateral compaction, and analyzed after seven days. Results demonstrated that "Sealer-Plus-BC/Rhodamine and Endosequence BC/Rhodamine" achieved significantly greater tubular penetratin compared to AH Plus/Fluo-3 and Sealer Plus/Fluo-3. The penetration was consistent across canal thirds except in the terminal third for "AH Plus/Fluo-3 and Sealer Plus BC/Fluo-3" groups. The study concludes that fluorophore choice affects calcium-silicate-sealers' penetration but not

epoxy-resin-based ones, recommending against using Rhodamine with BC sealers for CLSM evaluation.[28]

10. This SR and meta-analysis by Kasidid Ruksakiet et al. assessed the anti-microbial strength of CHX and NaOCl, two commonly used irrigants in endodontic therapy. A search across electronic databases identified eight eligible randomized controlled trials. Results showed no significant differences between CHX and NaOCl in bacterial growth reduction or mean bacterial number changes, as analyzed by relative risk (RR) and standardized mean difference (SMD). Subgroup analyses revealed similar performance in both culture and molecular bacterial detection methods. Heterogeneity was minimal in RR analysis but substantial in SMD analysis. Despite comparable efficacy, the molecular mechanisms of CHX and NaOCl differ, supporting their use as primary antibacterial irrigants. However, the findings are limited by inconsistencies and the lack of clinically relevant outcomes, highlighting the need for further research.[29]
11. The study conducted by Ahmed Mostafa Farghaly and Mohamed Ahmed Wakwak examines the antibiofilm efficacy of various disinfecting agents, including nano Punica granatum herbal extract, calcium hydroxide, photodynamic therapy using Rose Bengal Dye, and sodium hypochlorite (NaOCl), on multispecies biofilms in dentin section blocks. The study involved preparing and sterilizing 100 dentin sections, cultivating Enterococcus faecalis and Staphylococcus epidermidis strains to form biofilms, and dividing the sections into five groups for testing. Confocal Laser Scanning Electron Microscopy (CLSEM) was used for analysis. Results showed nano Punica granatum extract had the highest antibacterial efficacy, followed by photodynamic therapy and sodium hypochlorite. Calcium

hydroxide showed lesser efficacy, while the control group exhibited the lowest bacterial reduction. These findings highlight the potential of herbal and photodynamic therapies as effective root canal disinfection strategies.[30]

12. A study by Anis Dien Hartini and collaborators explores the antifungal properties of Cuminum cyminum (cumin) extract against Candida albicans ATCC 10231, focusing on both planktonic and biofilm forms. The extract, obtained via steam distillation in various concentrations (0.1 to 1.6 µL/mL), demonstrated promising antifungal effects when assessed through ELISA-based turbidity measurements. The planktonic form showed maximum susceptibility at 0.4 µL/mL concentration, while the biofilm form was less responsive, with 0.1 µL/mL being the most effective among biofilm groups. These findings suggest cumin extract as a viable antifungal agent, with stronger efficacy against planktonic forms, offering potential applications in endodontic infection treatments.[31]
13. Amalia, R. and collaborators conducted a study comparing the anti-bacterial strength of C. cyminum essence with 2% CHX against Enterococcus faecalis isolated from non-vital teeth with chronic apical abscesses. E. faecalis was cultured in Chrom-Agar and DNA was extracted and standardized via PCR. Cumin extract, obtained through steam distillation, was diluted to concentrations of 0.2, 0.5, 0.7, 1.0, and 1.2 milligram/milliLitre. Anti-bacterial activity was measured through turbidity reduction in bacterial biofilms using an ELISA reader. The study found that cumin extract at 0.7 and 1.0 milligram/milliLitre demonstrated significantly greater antibacterial effects compared to lower concentrations, with 1.0 milligram/milliLitre being better than two percent CHX ($p < 0.05$). DNA analysis ensured consistency across samples and biofilm turbidity reduction served as a marker of antibacterial

activity. The findings highlight cumin extract as a promising natural antibacterial agent.[19]

14. "Emre Erik C, Onur Orhan E, and Maden M" conducted a laboratory study to analyse the efficiency of varied etidronate (HEDP) volumes for smear film removal. 78 instrumented roots were segregated in six different categories (n=13): sterile saline (control), 17% EDTA, 9% HEDP, 18% HEDP, 1% NaOCl + 9% HEDP and 2% NaOCl + 18% HEDP. Every radicular canal was washed with 5 ml solution for three minutes, and the smear film was evaluated under SEM. Results showed that the 2% NaOCl + 18% HEDP group achieved substantially there was superior debris elimination in comparison to other categories ($p < .05$), particularly in the root end. Insignificant variations were found in the cervical and mid - regions among treatments. The investigation inferred that 18% HEDP, when combined with NaOCl, is the more efficient option for clinical debris elimination.[10]
15. According to Arasappan Rajakumaran and colleagues, spices performed a vital part in improving the flavor, aroma, and color of food, while also providing health benefits and acting as natural preservatives. Cumin (Cuminum cyminum), generally called as 'Zeera,' is a widely used herb from the Apiaceae family, known for its potent antioxidant properties. Beyond its culinary applications, cumin is extensively utilized in traditional medicine for managing various health conditions. Its therapeutic potential is related to bioavailable compounds like terpenes, phenols, and flavonoids. Research has shown that cumin is effective in addressing diabetes, hypertension, dyslipidemia, and inflammatory diseases. Additionally, it has been found beneficial for respiratory ailments, allergic rhinitis, and dyspepsia, with promise as a complementary therapy for cancer. Scientific studies further

support the use of cumin's seed oil, essential oil, and thymoquinone (TQ). In summary, cumin holds immense potential in both the culinary and medicinal realms.[32]

16. A study by Tariq S. Abuhaimed and Ensanya A. AbouNeel highlights the importance of effective shaping and cleaning for successful endodontic treatment, as the complex anatomy of root canals often prevents complete bacteria removal through instrumentation alone. Sodium hypochlorite (NaOCl) is widely utilized during irrigation for its antimicrobial, enzymatic, and cleaning aspects. However, sodium hypo-chlorite can modify the constituents of dentine, potentially impacting the binding force of sticky resins. The review examines how sodium hypo-chlorite affects adhesive forces, detailing refinement mechanisms, and explores strategies to mitigate its negative effects on dentin. Integrating NaOCl with restorative techniques has the potential to enhance treatment outcomes.[11]
17. This study by Jason Gagliardi et al assessed the ability of Pro-Taper Gold (PTG) system versus Pro-Taper Next (PTN) and Pro-Taper Universal (PTU) using micro–computed tomography. Twenty-four mandibular first molars with anatomically matched mesial canals were prepared and analyzed for canal transportation, centering ability, untouched canal walls, and remaining dentin thickness. Results indicated that PTN produced significantly fewer transportation errors and maintained more static voxels compared to PTG and PTU. PTG and PTN preserved more dentin thickness than PTU, highlighting their effectiveness in minimally invasive instrumentation. Despite PTN having less canal wall contact, all systems performed adequately for moderately curved canals without significant clinical errors.[33]

18. Abbaszadegan A and collaborators conducted a study to analyze the chemical composition, antimicrobial efficacy, and cytotoxicity of Cuminum cyminum (cumin) extract. The oil was tested against aerobic and anaerobic bacteria, including Enterococcus faecalis isolated from teeth with persistent apical periodontitis. Antimicrobial performance was evaluated using methods like zone of inhibition (ZOI), MIC, MBC, MBIC, and time-kill assays. Cytotoxicity was tested on L929 fibroblasts. The oil's main components were cumin aldehyde and γ-terpinene. While co-trimoxazole had the highest Zone of inhibition, zeera demonstrated effective antimicrobial activity, particularly against E. faecalis, with a lower MBC. CHX exhibited the highest cytotoxicity, whereas cumin and co-trimoxazole had similar, lower cytotoxicity levels. The study concluded that cumin oil possesses notable antimicrobial properties and is biocompatible with fibroblasts, making it a promising alternative in endodontic treatments.[18]
19. A study by Dayane Oliveira et al evaluated the impacts of a fluorescing liquid (rhodamine B) on material and structural attributes of 3 luting cements: RelyX ARC (conventional adhesive), RelyX U-200 (self-adhesive), and SeT PP (self-etching and self-adhesive). Bar-shaped specimens containing 0.03 wt% rhodamine B were light-cured and analyzed for Knoop hardness number (KHN), flexural strength (FS), and Young's modulus (YM) after 24 hours. Statistical analyses, including two-way ANOVA and Tukey's test, revealed negligible discrepancies in FS or YM among groups, while the incorporation of Rhodamine B enhanced durability in all tested cements. These findings indicate that the fluorescent agent does not adversely impact the polymerization. [34]

20. Shenoy A and colleagues executed a laboratory study to know the effect of final irrigants on debris elimination and radicular canal sealer penetration. Thirty decoronated teeth were standardized to 15 mm and prepared using telescopic method and step down method with 3% NaOCl as the irrigant during instrumentation. The teeth were segregated in 3 groups (n=10): section 1- 17% Ethylenediaminetetraacetic acid, section 2 - Tubulicid Plus, and section 3- Biopure MTAD®. Acroseal sealer mixed with 0.1% rhodamine B dye was used for obturation. Following 2,880 minutes, roots were segmented and examined with CLSM. Quantitative research using two-way ANOVA and Newman-Keuls post-hoc test revealed notable differences in depth of penetration of a sealer. Tubulicid Plus displayed maximum penetration, followed by MTAD and EDTA. The study concluded that Tubulicid Plus is highly efficient irrigant for debris elimination and improving ingress of the filling material.[35]
21. Dineshkumar MK and colleagues conducted a laboratory study for examining the effects of various irrigation solutions on radicular dentine microhardness using the Vickers test. Eighty specimens, obtained from forty single-rooted human teeth, were segregated in 4 categories (n=20). 1st group (control) was processed with demineralized water, while 2nd – 4th groups were exposed to 1.3% Sodium hypo-chlorite for 20 minutes trailed by 17% ethylenediaminetetraacetic acid, mixture of tetracycline isomer, acid and detergent or 18% HEDP, sequentially. Post-treatment, microhardness was measured using a Vickers hardness tester. Outcomes revealed that every tested irrigating solution decreased dentine micro-hardness relative to the control group, but 18% HEDP caused the least reduction. The study concluded that

HEDP is a promising final rinse due to its minimal impact on dentin mineral composition.[2]

22. An in vitro study by Candeiro and his colleagues analysed debris elimination in radicular canals utilizing varied irrigants. 40 human tooth roots were equipped and segregated in 4 categories (n=10): apple vinegar (A), apple vinegar + 17% EDTA (B), 1% NaOCl + 17% EDTA (C), and saline (D, control). After preparation, roots were split and analyzed via SEM (×1,000) in the mid and root end. Two calibrated examiners scored smear film removal (1=poor, 2=good, 3=excellent). Details were evaluated utilizing the Kruskal-Wallis, Dunn's, and Wilcoxon tests (α=0.05). All categories revealed elevated debris elimination in the middle 3rd . Group B (apple vinegar + EDTA) had the best results in the apical 3rd (p=0.0373). A notable dissimilarity was noted within categories in the mid- third (p=0.0402). The study concluded that apple vinegar, with or without ethylenediaminetetraacetic acid, is efficient for debris elimination in endodontics.[36]
23. Iness Bettaieb et al conducted an analysis of Cuminum cyminum L. (cumin), examining its essential oil yield, polyphenolic compounds, and antioxidant properties across various structures of plants , comprising rootlets , stalks, leaves, and blooms . Their findings revealed that flowers had the highest essential oil yield (1.7%), with γ-terpinene as the primary component, constituting 51%. The total phenolic content varied from 11.8 to 19.2 mg GAE/g DW, with quercetin being highly prominent in rootlets, rosmarinic acid dominating in stems and leaves, and vanillic acid accounting for 51% in flowers. Acetone extracts from flowers demonstrated strong antioxidant properties, including DPPH scavenging and inhibition of lipid peroxidation. Stalks and leaves demonstrated maximum chelation, while essential oils

demonstrated average antioxidant activity. These findings emphasize the substantial bioactive potential of cumin.[37]

24. The experiment performed by Young-Mi Moon et al examined the efficiency of different final irrigating regimens on obturating material ingress in arched radicular canals. The researchers aimed to determine how various irrigation protocols influenced the depth and distribution of sealer within the dentinal tubules. The experiment involved the use of various irrigants, including EDTA and sodium hypochlorite, to assess their impact on sealer penetration. The results indicated that the choice of final irrigation solution played a significant role in enhancing or limiting sealer penetration. Subsequently it was descerned that EDTA effectively removed the debris, allowing for deeper ingress of the sealer. In contrast, sodium hypochlorite alone was less effective in promoting sealer penetration. The study concluded that an appropriate final irrigation regimen was crucial for optimizing the binding capability of pulp therapy materials in curved radicular canals.[38]
25. Semra Çalt and Ahmet Serper conducted an experiment to analyse the duration-based outcomes of Ethylenediaminetetraacetic acid on dentin structures. The researchers investigated how prolonged exposure to EDTA influenced the composition and integrity of dentin. Their findings indicated that EDTA effectively eliminated the debris from dentin film, enhancing the exposure of dentinal tubules. However, extended exposure resulted in excessive demineralization and erosion of the dentin matrix. The experiment unveiled that a short application interval of EDTA was adequate for smear film removal without causing significant structural damage. Conversely, prolonged use led to detrimental effects, weakening the dentin and increasing its susceptibility to degradation. The authors concluded that while

Ethylenediaminetetraacetic acid played a crucial part in endodontic treatment by facilitating the cleaning process, its application time needed to be carefully controlled to prevent unwanted deterioration of dentin structures.[7]

26. A study by "Michael J. Jeansonne and Robert R. White" explored the disinfectant efficacy of 2.0% chlorhexidine-guconate (CHX) compared to 5.25% sodium hypochlorite (NaOCl) as endodontic irrigants. Newly removed dentition with pulp diseases were treated with CHX, sodium hypo-chlorite, or isotonic solution, and pathological specimens were analyzed at various intervals post-irrigation. Both CHX and NaOCl showed significant reductions in after - irrigant positive cultures and colony-forming components corelated to isotonic water -treated dentition. While CHX exhibited slightly lower bacterial counts than NaOCl, the discrepancies were analytically insignificant. This experiment highlights the potential of CHX as a safer alternative to NaOCl due to its comparable antimicrobial activity with fewer associated issues like toxicity and discoloration.[39]

MATERIALS AND METHODS

STUDY DESIGN:-

In Vitro Study

SOURCE OF DATA/LABORATORY DETAILS:-

The study was conducted in the Department of Conservative Dentistry and Endodontics, KLE Academy of Higher Education & Research, KLE VK Institute of Dental Sciences, Belagavi and the laboratory procedures were carried out in Dr. Prabhakar Kore's Basic Science Research Laboratory, KLE University, Belagavi.

Seeds of Cuminum cyminum were procured and authenticated by KLE's Ayurveda Pharmacy.

Extracted human mandibular premolar teeth, indicated for orthodontic extraction were obtained from Department of Oral and Maxillofacial Surgery, KLE Academy of Higher Education & Research, KLE VK Institute of Dental Sciences, Belagavi..

Specimens were evaluated by Confocal Laser Scanning Microscope at Birla Institute of Technology and Science, Pilani, K.K Birla Goa Campus.

INCLUSION CRITERIA

- Extracted human mandibular teeth having single patent canals
- Teeth with apical diameter of #20 K-file or less

EXCLUSION CRITERIA

- Teeth with apical diameter greater than #20 K-file size
- Teeth with calcified canals
- Teeth with root caries
- Teeth with fracture/crack or a restoration
- Teeth with internal and external resorption
- Teeth with anatomic variations
- Teeth with more than 5^0 curvature

PERMISSIONS TAKEN

- Ethical committee approval
- Dr. Prabhakar Kore's Basic Science Research Centre (BSRC), KLE University, Belagavi
- Specimens were evaluated under the Confocal Laser Scanning Microscope at Birla Institute of Technology and Science, Pilani, K.K Birla Goa Campus

SAMPLE SIZE ESTIMATION:

Sample size at 95 % confidence interval and 95% power

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \ (SD_1{}^2 + SD_2{}^2)}{(\bar{x}_1 - \bar{x}_2)^2}$$

n = 15 per group

Where,

$\bar{x}1 = 656.33$

$\bar{x}2 = 425.00$

SD1 = 206.305

SD2 = 104.136

Z1-a=Alpha error at 5%

Z1-B=Beta power at 95 %

MATERIALS:

- 60 human mandibular premolar teeth
- 0.1% thymol (S D FINE-CHEMICALS LIMITED, MUMBAI)
- 3% Sodium Hypochlorite (VISHAL DENTOCARE, AHEMDABAD)
- 17% Ethylenediamine-tetraacetic-Acid (EDTA) (CANALARGE)
- 0.9% Saline (JEDUX PARENTERAL PRIVATE LIMITED)
- Cuminum Cyminum seeds
- Paper-points (DIADENT GROUP INTERNATIONAL)
- Distilled-water (NICE LIFE CARE, NEW DELHI)
- Gutta-percha-points (DIADENT GROUP INTERNATIONAL)
- Rhodamine B dye
- AH Plus sealer (DENTSPLY, GERMANY)

ARMAMENTARIUM:

- K Files (#10-40)
- ProTaper Universal Nickel-Titanium files
- Airotor
- Endomotor
- Lentulospiral
- Micromotor
- 5 ml, 25-gauge syringe
- Diamond disk
- Confocal Laser Scanning Microscope

Preparation of the Cuminum Cyminum irrigant :

To provide the requisite form of cumin, a grinder was used to grind the seeds to create a fine powder. Powder was then hydrodistillated in a Clevenger-apparatus. The condensed vapours were then collected and the top layer of the oil was parted to obtain the oil extract.

Primary concentration of the irrigant was calculated using G.C.M.S. (Gas Chromatography-Mass Spectrometry) and was estimated to be $4.4 \times 10^5 \mu g/ml$. It was then diluted by distilled water and dimethyl sulfoxide to obtain the M.I.Cs (minimum inhibitory concentration)

M.I.C. and M.B.C. of the Cumin Extract:

Zone of Inhibition test for Cumin oil extract

Brain Heart Infusion (BHI) agar plates were prepared, and wells of 6 mm in diameter were punched into the medium. A 100 µL aliquot of Enterococcus faecalis microbial sample was spread plated onto the agar surface. Different concentrations of the test sample (10 µL, 20 µL, 40 µL, and 50 µL) were added to the wells, and the plates were left to incubate at 37°C for 24 hrs.

Fig 1: Antimicrobial efficacy of the Cumin oil at various conc. (10, 20, 40, and 50 µL) against E. faecalis.

Minimum Inhibitory Concentration (M.I.C.) & Minimum Bactericidal Concentration (MBC)

Ten milliliters of Brain Heart Infusion (BHI) broth in 15 screw-cap test tubes were inoculated with 100 µL of *Enterococcus faecalis* culture and various concentrations of the test cumin aldehyde concetration [50-100µg/mL], then left to incubate at 37°C for 24 hours. After incubation, the Optical Density of the samples was estimated at 600 nm. A 100 µL aliquot from the samples with the lowest Optical Density was plated onto BHI agar plates and incubated for another 24 hrs at 37°C. Following this incubation, plate that showed no bacterial colony growth was recorded as the Minimum Bactericidal Concentration (M.B.C).

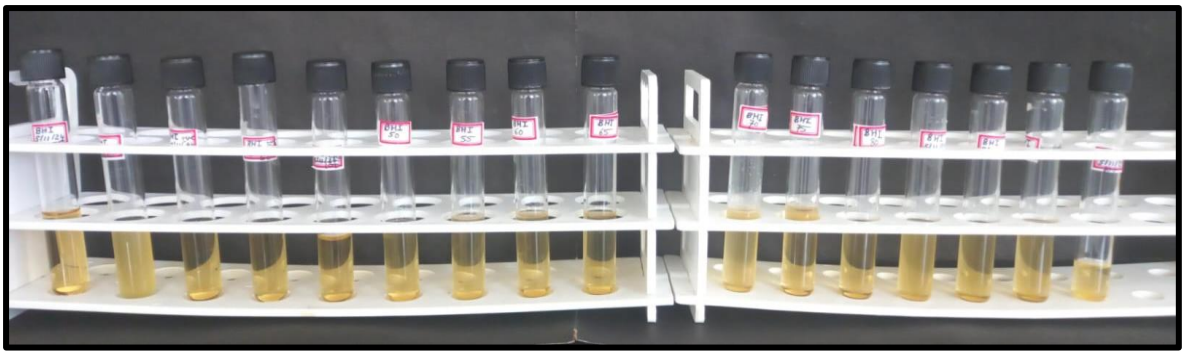


Fig 2: M.B.C. by tube dilution method- Culture broth + CA in varying concentrations after 24 hrs of incubation.

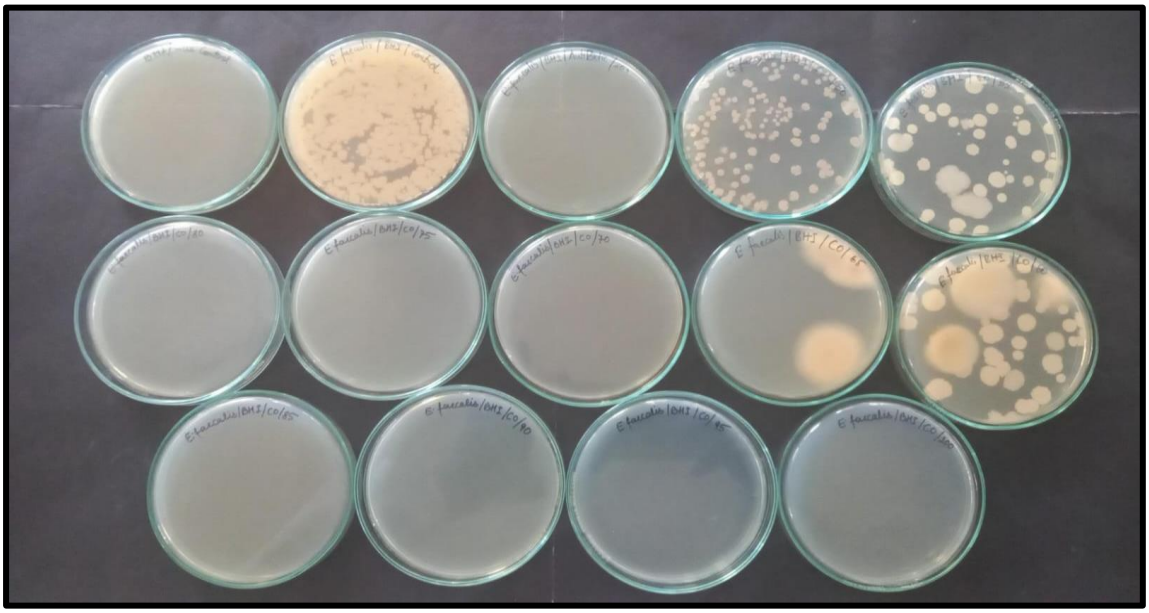


Fig 3: CFU analysis for MBC

Result

Cumin aldehyde µg/mL	OD@600nm	% Reduction	CFU/mL
50	0.31	74.29519071	46
55	0.322	73.30016584	31
60	0.331	72.55389718	24
65	0.258	78.60696517	0
70	0.218	81.92371476	0
75	0.117	90.29850746	0
80	0.1	91.70812604	0
85	0.078	93.53233831	0
90	0.049	95.93698176	0
95	0.021	98.25870647	0
100	0.019	98.42454395	0
Control +Ab (30 µg/mL)	0.109	90.96185738	0
+ve Control *E. faecalis*	1.206		160

*Where, control/ +ve control was the test organism, sample was cumin aldehyde at different concentrations, and ‘Ab’ stands for antibiotics [Metronidazole]

Table: Minimum Bactericidal Concentration

Interpretation

CFU suggested that the concentration (65 µl onwards) used is effective at killing the test organism *E.faecalis*. Therefore, cumin oil effectively inhibits and kills *E.faecalis* at the tested concentrations.

M.I.C. and M.B.C. of the irrigant against a strain of *E. Faecalis* was estimated to be 65µg/ml through laboratory testing.

Sixty lower premolar teeth with single roots and single canals were chosen following its preservation in 0.1% thymol suspension. After RVG assessment and meeting inclusion criteria, specimens were decoronated at 14 mm from apex for standardisation. WL was recorded by using #10 K file in each root canal till it was seen at the anatomic apex and by cutting 1 mm from the length measured. Canal instrumentation was then done with ProTaper universal rotary files up to F2/F3 MAF size depending on apical gauging. Chemomechanical preparation with 3% sodium hypochlorite was done after each successive file. Subject teeth were assigned to one of four intervention subgroups.

- GROUP 1: Cuminum cyminum irrigating solution
- GROUP 2: 17% EDTA
- GROUP 3: Combination of 17% EDTA and Cuminum cyminum irrigating solution
- GROUP 4: Saline

All experimental irrigating solutions were administered utilizing 5 ml syringe with 25-gauge-needle inserted 1 mm short of the WL. Each specimen received a 5 ml flush of experimental irrigating solution for 1 min Following this, root canal walls were thoroughly irrigated with 3 ml saline for 1 min and dried with paper points.

Agitation of the irrigating solution

Ultrasonic agitation of the irrigants was done with PUI utilizing no.25 ultrasonic tip for a 30-second cycle which enhances root canal cleaning. This method involves irrigating canals with 5 mL of respective solution, thereby removing debris and smear-layer efficiently.

Preparation of the specimens for observation under Confocal Laser Scanning Microscope

To enhance fluorescence for visualization under confocal laser scanning microscopy, Rhodamine B isothiocyanate dye was mixed with AH Plus sealer at a conc. of approximately 0.1% by weight during manipulation. AH Plus was applied to canal wall with a no. 30 lentulospiral, with GP cones coated in the labelled epoxy resin-based sealer placed till the working length. Excess GP was sheared and cavity was double sealed with GIC. Specimens were preserved at temperature 37°C with 100% humidity for one week for sealer polymerisation. After complete setting, specimens were horizontally sliced at apical third with diamond disc, followed by polishing. Sections were then placed on glass slides and assessed using Confocal Laser Scanning Microscope (CLSM).

METHODOLOGY WITH FLOWCHART

60 extracted human mandibular premolar teeth were cleaned of calculus and soft tissue and was handled according to OSHA guidelines and stored in 0.1% thymol solution till use

↓

All teeth were radiographed and selected as per the inclusion and exclusion criteria

↓

The teeth were decoronated using a diamond disk under copious water spray to acquire a standardized root length of 14 mm

↓

WL was established by inserting a size 10 K file into each root canal until it was visible at the apical foramen and by subtracting 1mm from the recorded length

↓

The root canal was prepared using ProTaper rotary instruments up to F2/F3 based on apical gauging

↓

Irrigation was done with 2 ml of 3% sodium hypochlorite between successive files

Teeth were randomly divided in 4 groups (n= 15) according to the intervention

GROUP 1	GROUP 2	GROUP 3	GROUP 4
Cuminum cyminum irrigating solution	17% EDTA	Combination of 17% EDTA and Cuminum cyminum irrigating solution	Saline

Canals were irrigated with 3mL of saline to terminate the action of irrigating solutions

The root canals were dried with paper points and obturated with gutta percha and AH Plus sealer and prepared for CLSM

To promote fluorescence under confocal laser scanning microscopy, Rhodamine B isothiocyanate dye was added, 0.1% by weight, to AH Plus sealer during manipulation

The endodontic sealer was applied in the canal 1 mm shorter than the working length using no. 30 lentulospiral and single cone obturation was done with ProTaper Universal F3 gutta-percha cone (Dentsply Maillefer)

Excess gutta-percha was removed using a heated plugger and the access cavity was sealed with GIC

↓

The teeth were stored in an incubator at 37°C and 100% humidity for 7 days to allow the sealer to set

↓

At a distance of 2 mm from apex, a horizontal section of 1 mm was taken and the surfaces of the sections were polished with a sandpaper

↓

All specimens were mounted on glass slides and examined under a Confocal Laser Scanning Microscope

Fig 4: Seeds of Cuminum cyminum

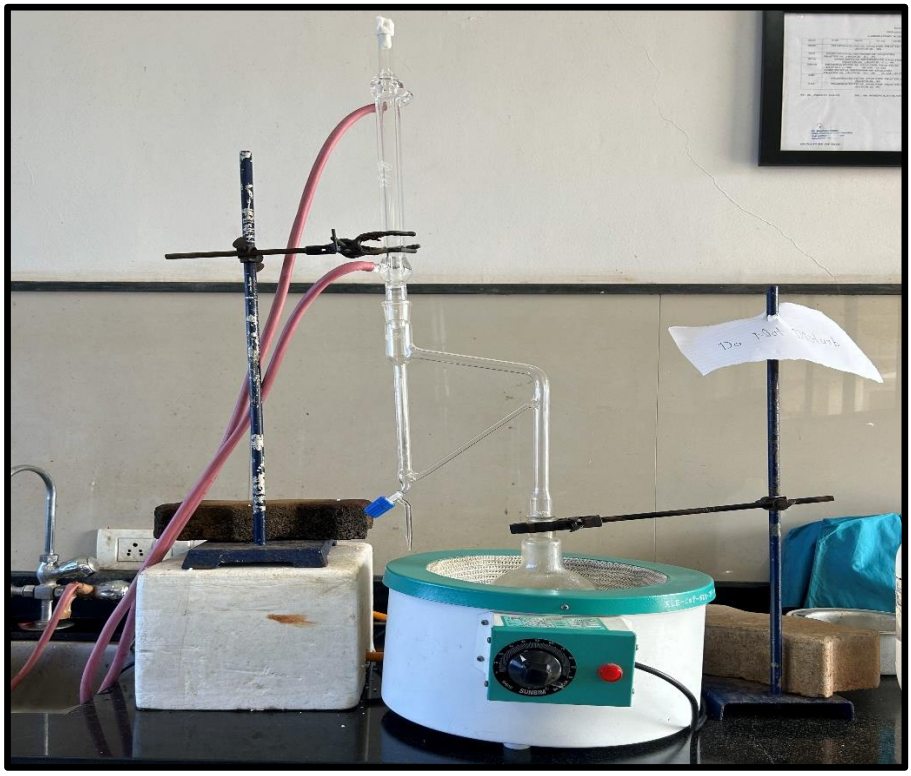


Fig 5: Clevenger Apparatus

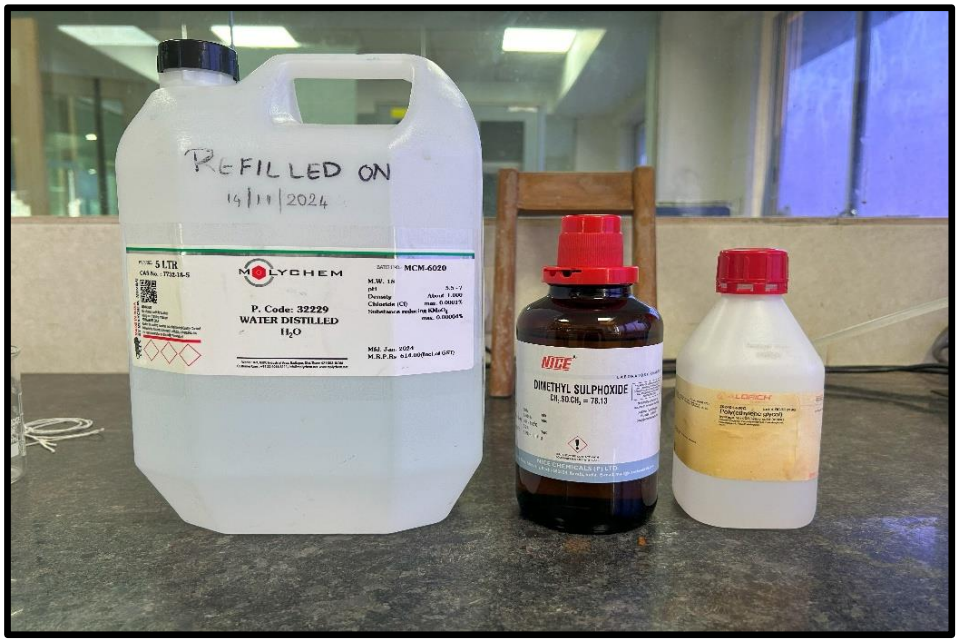


Fig 6: Materials For Irrigant Preparation (Distilled Water, DMSO4, Polyethylene Glycol)

Fig 7: Preparation of Irrigant

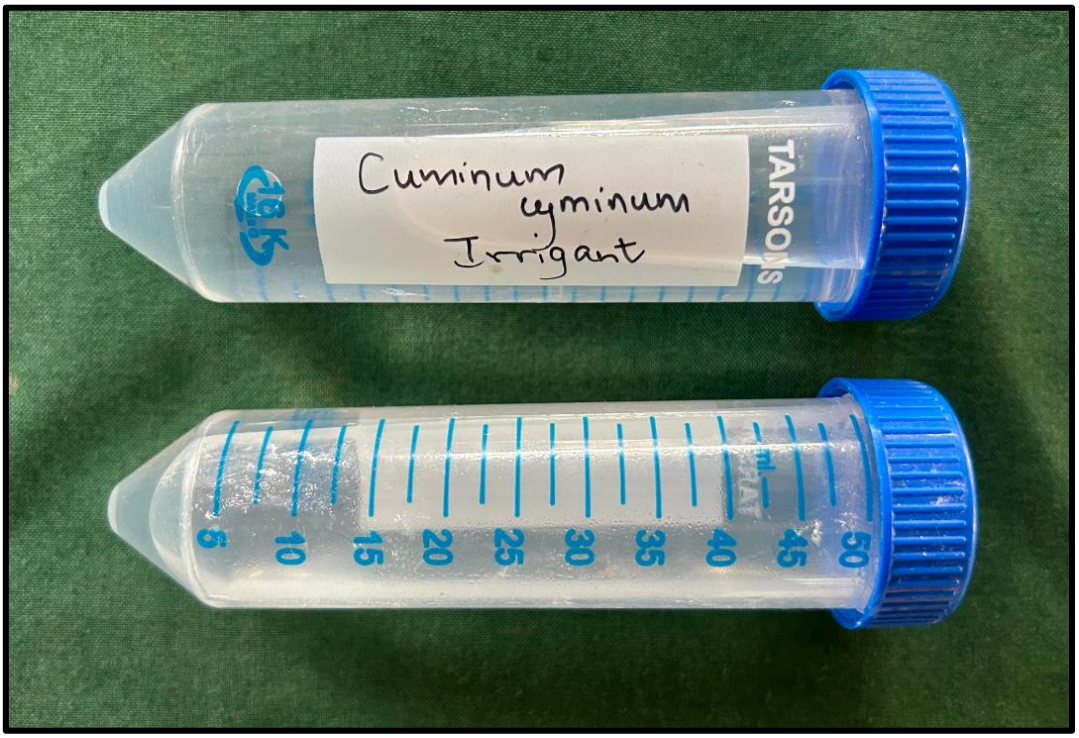


Fig 8: Prepared Irrigant Of Cuminum Cyminum

Fig 9: Armamentarium

Fig 10: Materials

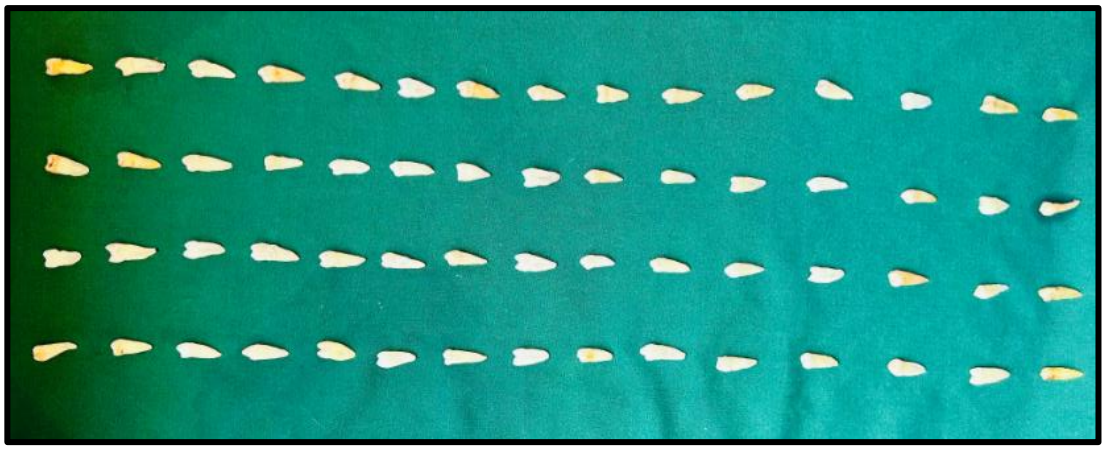


Fig 11: Sixty Mandibular Premolars

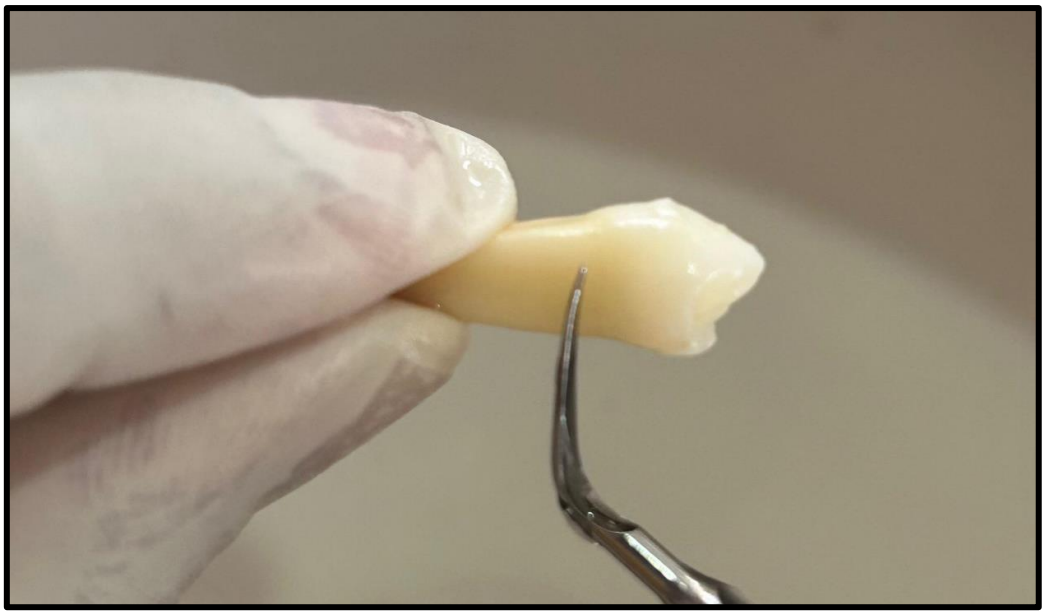


Fig 12: Debris Removal

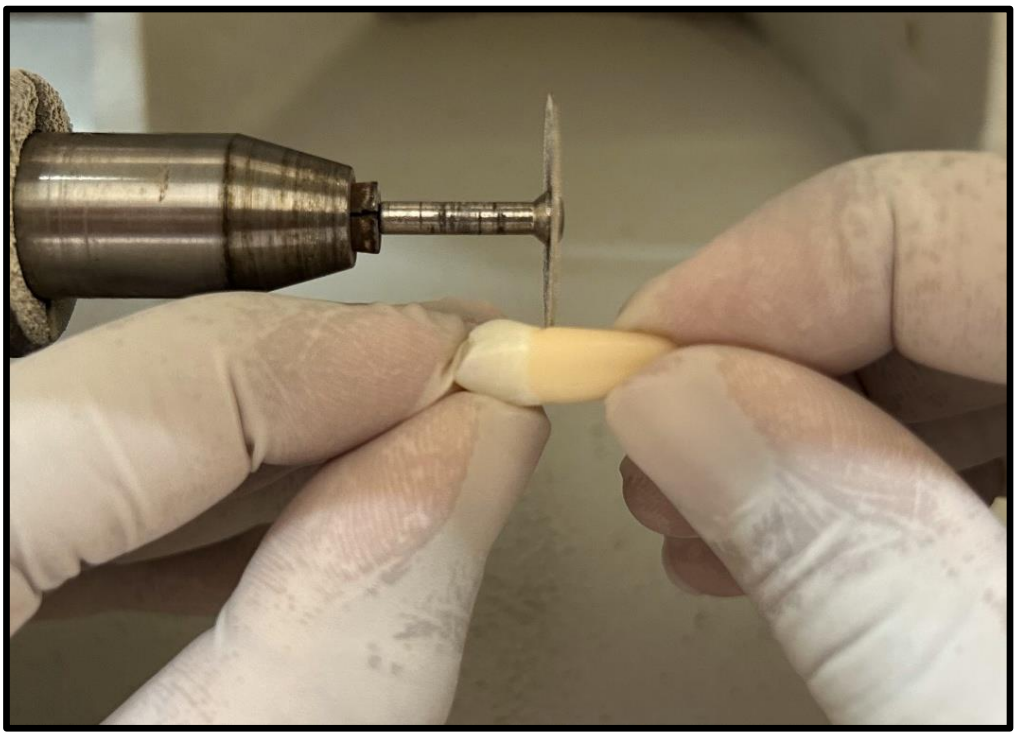


Fig 13: Decoronation

Fig 14: Working Length Determination

Fig 15: Biomechanical Preparation

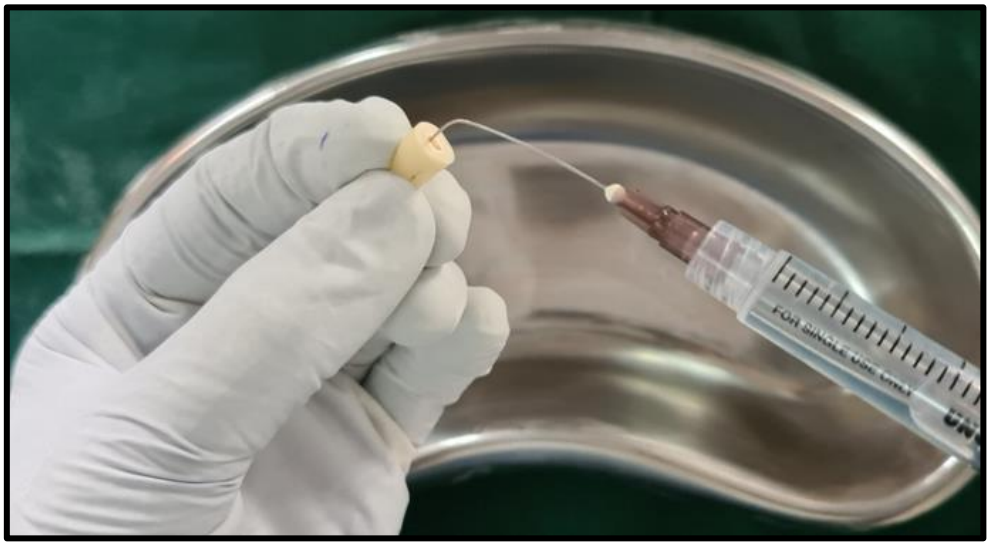


Fig 16: Irrigation

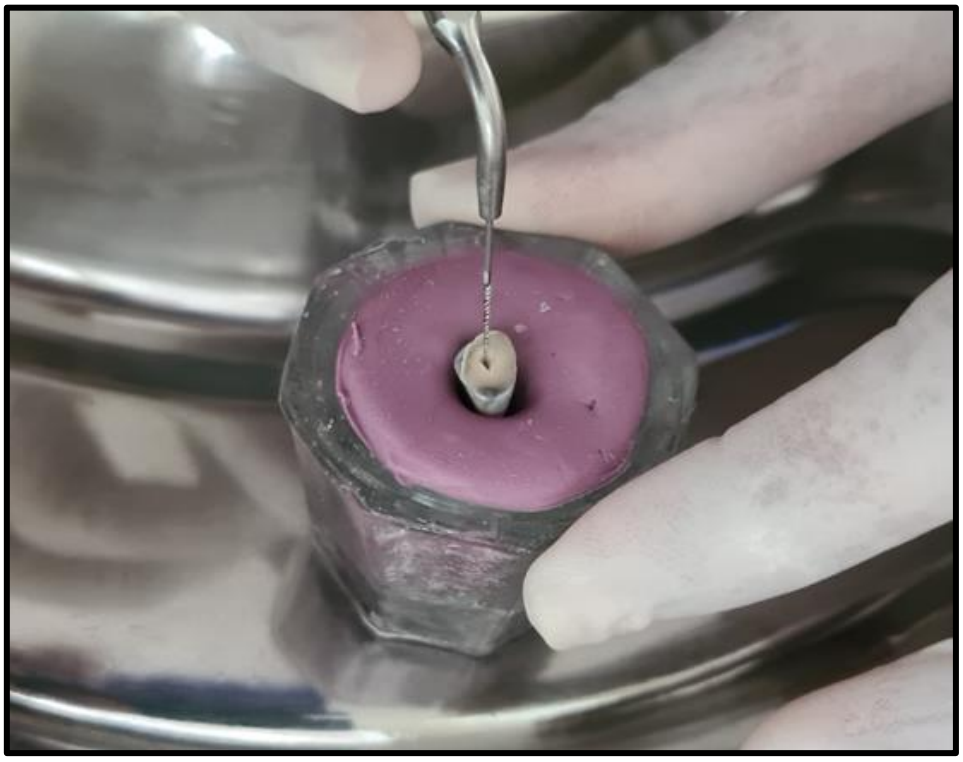


Fig 17: Activation of the Irrigant with PUI

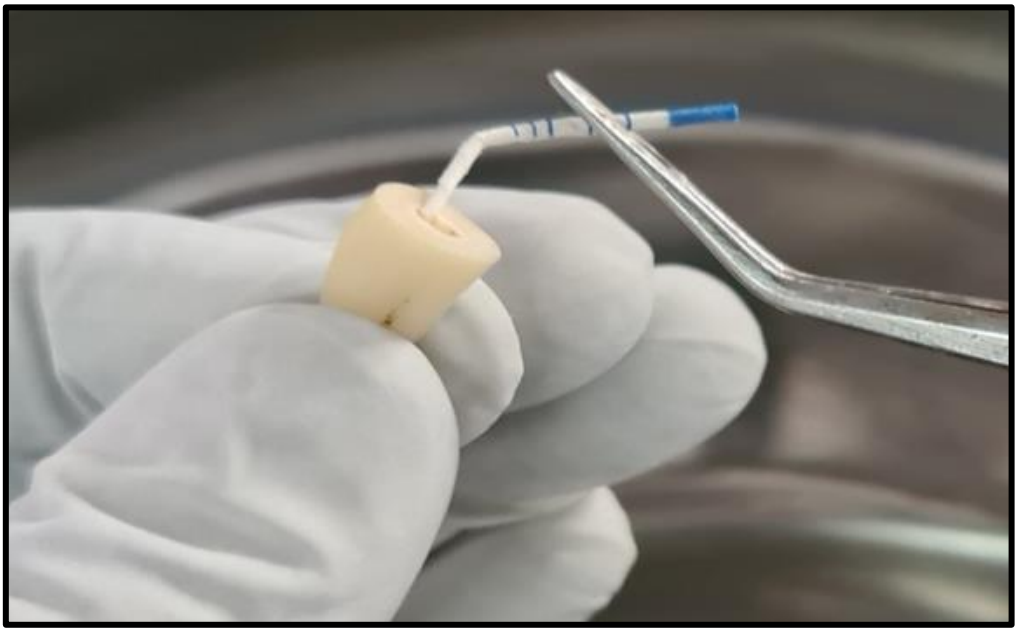


Fig 18: Drying the Canals with Paper Points

Fig 19: Dye Incorporation with Sealer

Fig 20: Application of Sealer with Lentulospiral

Fig 21: Obturation

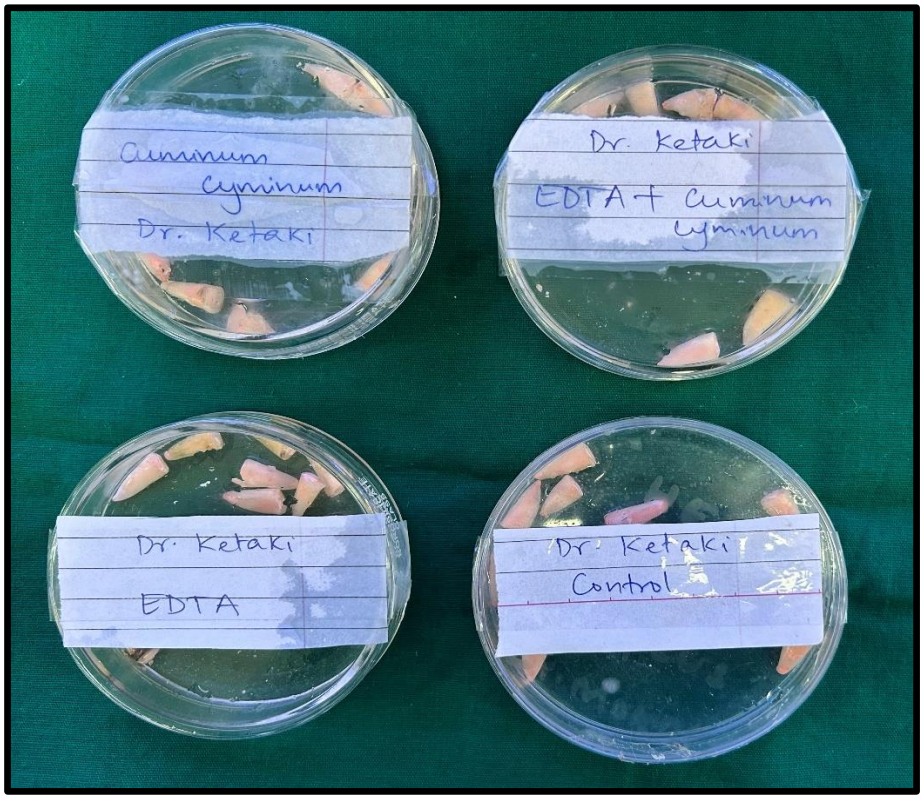


Fig 22: Samples According To Interventional Group

Fig 23: Incubation

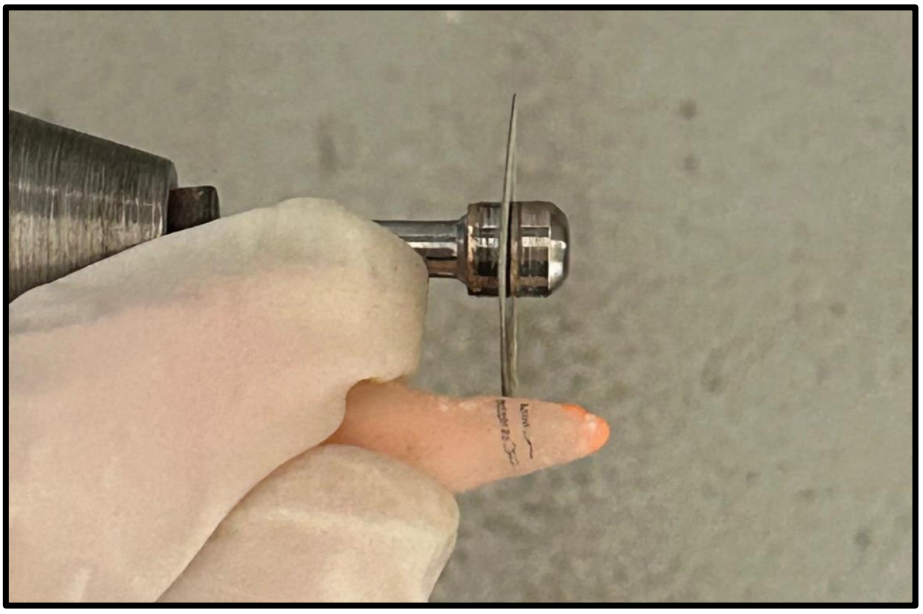


Fig 24: Sectioning at the Apical Third

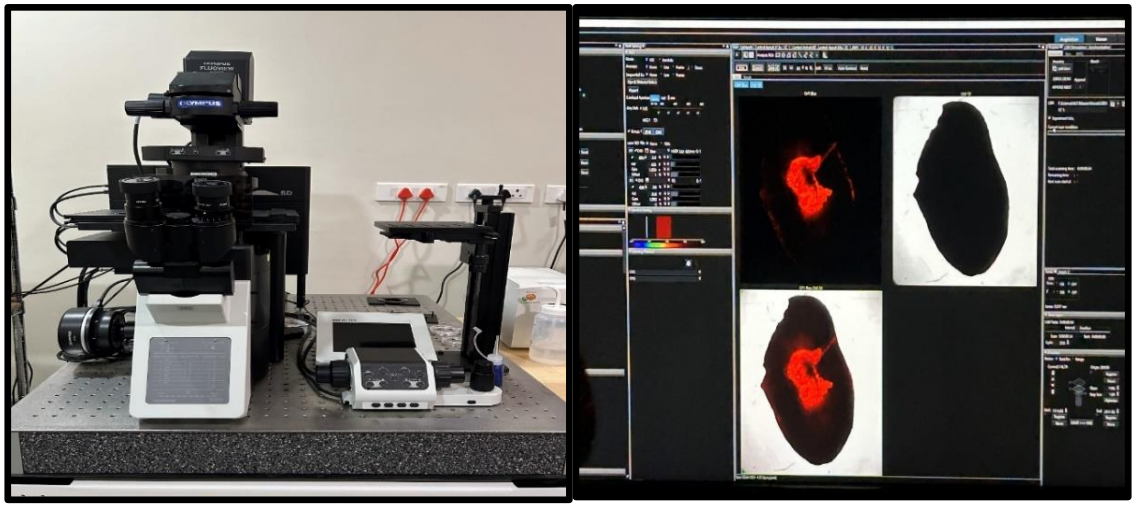


Fig 25: Confocal Laser Scanning Microscope(CLSM)

RESULTS

Overall, 15 specimens per group were analysed for radicular tubule penetration of sealer in terminal thirds.

Table 1 depicts, mean depth value of penetration, Standard Deviation and Standard Error of the categories, constituting 15 per group by One way ANOVA. Highest mean depth of penetration in terminal third was observed with 17% EDTA, depth being 1047.65 µm and results were statistically significantly (P=0.0001). Teeth irrigated with saline showed lowest penetration, i.e., 556.15 µm.

Tukey's-multiple-post-hoc test was done for comparing of the 2 groups in the terminal third and highly significant difference was seen between CC (Group 1) and 17% EDTA (Group 2). Statistically significant difference was seen amongst these 2 groups.

When 17% EDTA, CC and 17% EDTA + CC were compared, 17% EDTA showed better penetration.

Table 1: Summary of depth of penetration in four groups

Groups	Mean	Std.Dev.	Std.Err.	95% CI for mean	
				Lower	Upper
CC	744.07	183.60	47.41	642.39	845.74
17% EDTA	1047.65	227.83	58.83	921.48	1173.82
CC+17% EDTA	899.16	218.15	56.33	778.36	1019.97
Control	556.15	129.54	33.45	484.42	627.89

- 17% EDTA appears to be the most effective in enhancing sealer penetration, likely due to its strong chelating properties.
- Combination of CC and 17% EDTA is also effective, but slightly less than 17% EDTA alone.
- CC showed lower values of sealer penetration when measured against other experimental groups.
- Control group has least penetration, acting as a baseline.

Table 2: Comparison of four groups with depth of penetration by one way ANOVA

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	p-value
Between groups	3	1998014.54	666004.85	17.7617	0.0001*
Within groups	56	2099813.06	37496.66		
Total	59	4097827.60			

*p<0.05

- Significant difference could be seen amongst four categories with depth of penetration (F=17.7617, p=0.0001). It means that, the mean penetration depth is different in four categories and is found to be statistically different.

Table 3: Pair wise comparison of four groups with depth of penetration by Tukeys multiple posthoc procedures

Groups	CC	17% EDTA	CC+17% EDTA	Control
Mean	744.07	1047.65	899.16	556.15
Std.Dev.	183.60	227.83	218.15	129.54
CC	-	p=0.0005*	p=0.1377	p=0.0489*
17% EDTA	p=0.0005*	-	p=0.1658	p=0.0002*
CC+17% EDTA	p=0.1377	p=0.1658	-	p=0.0002*
Control	p=0.0489*	p=0.0002*	p=0.0002*	-

*p<0.05

- Significant difference was seen between CC with control, 17% EDTA with control, CC+17% EDTA with control group with mean depth of penetration. It means that, the mean depth of penetration is significantly higher in CC, 17% EDTA and CC+17% EDTA as compared to Control group
- 17% EDTA outperforms CC and Control significantly but is not significantly different from CC+17% EDTA, even though its mean is highest.
- CC+17% EDTA also significantly outperforms Control, but is not significantly different from CC or 17% EDTA.
- CC vs CC+17% EDTA is not significant, suggesting combining CC with 17% EDTA may not produce a statistically better result than CC alone

Fig A: Comparison of all groups, penetration depth

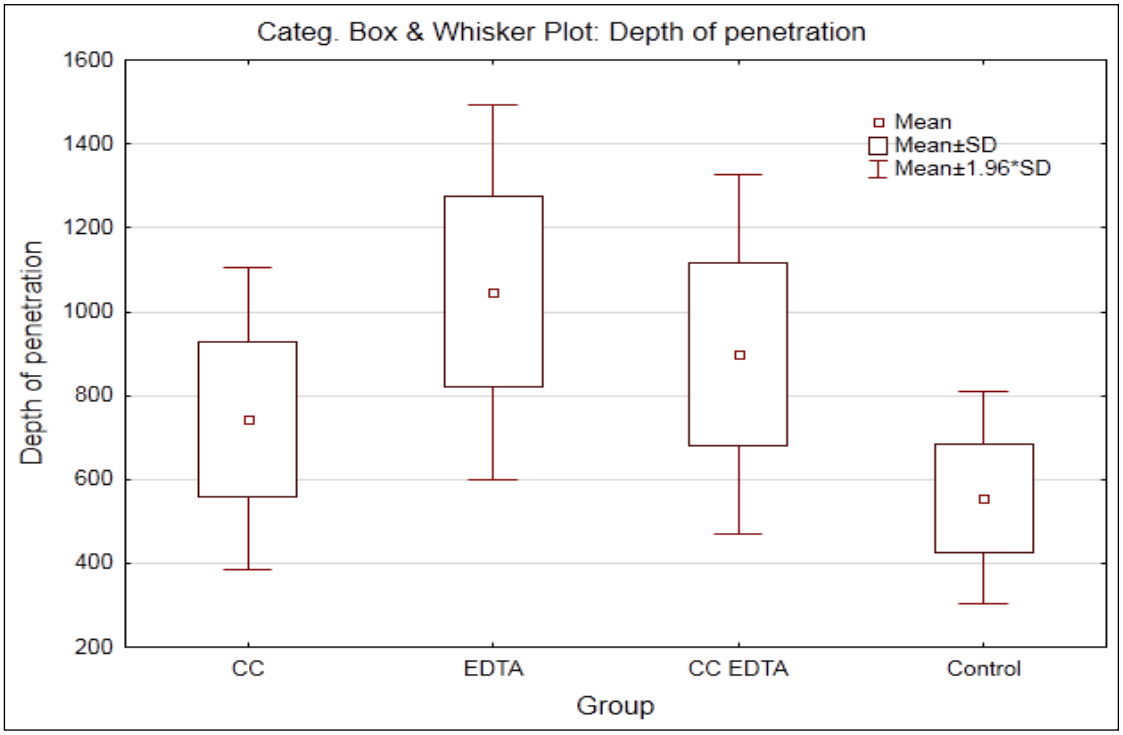
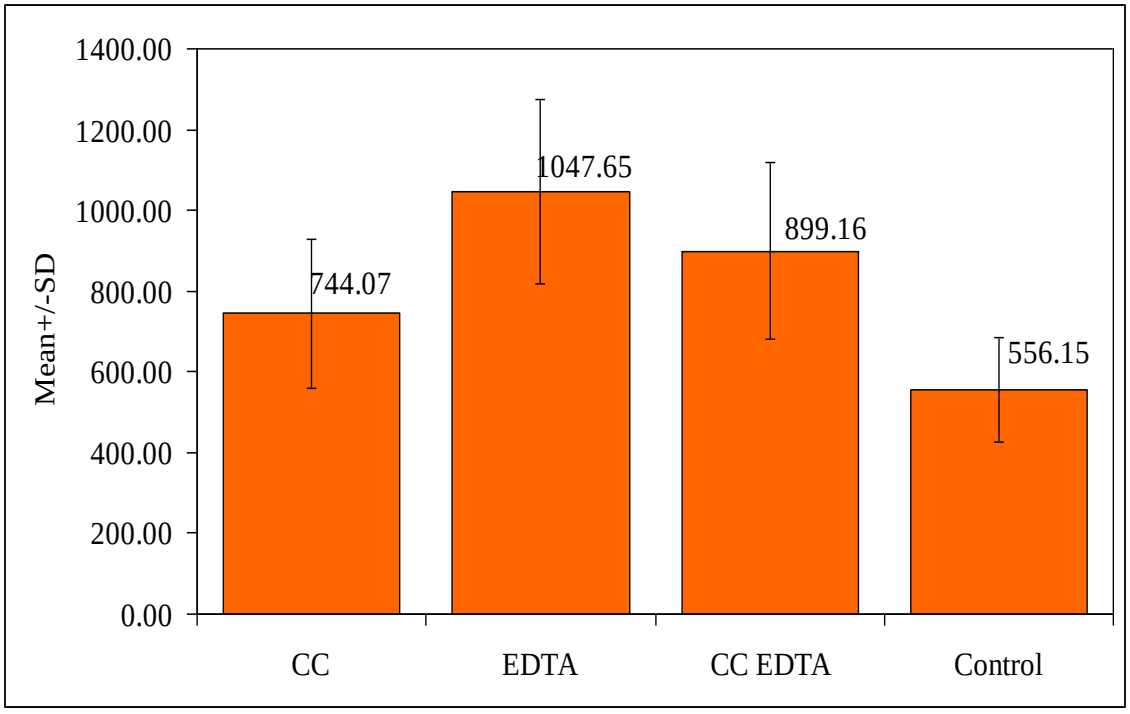


Fig B: Comparison of all groups, penetration depth



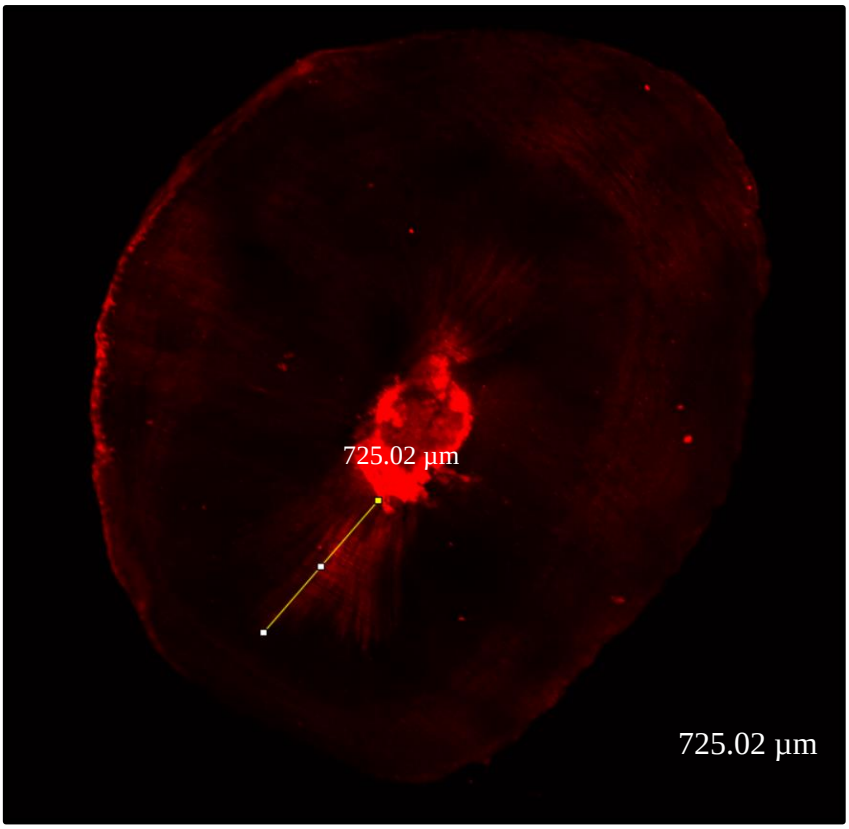


Fig 26: CLSM Image of CC Group (Group 1)

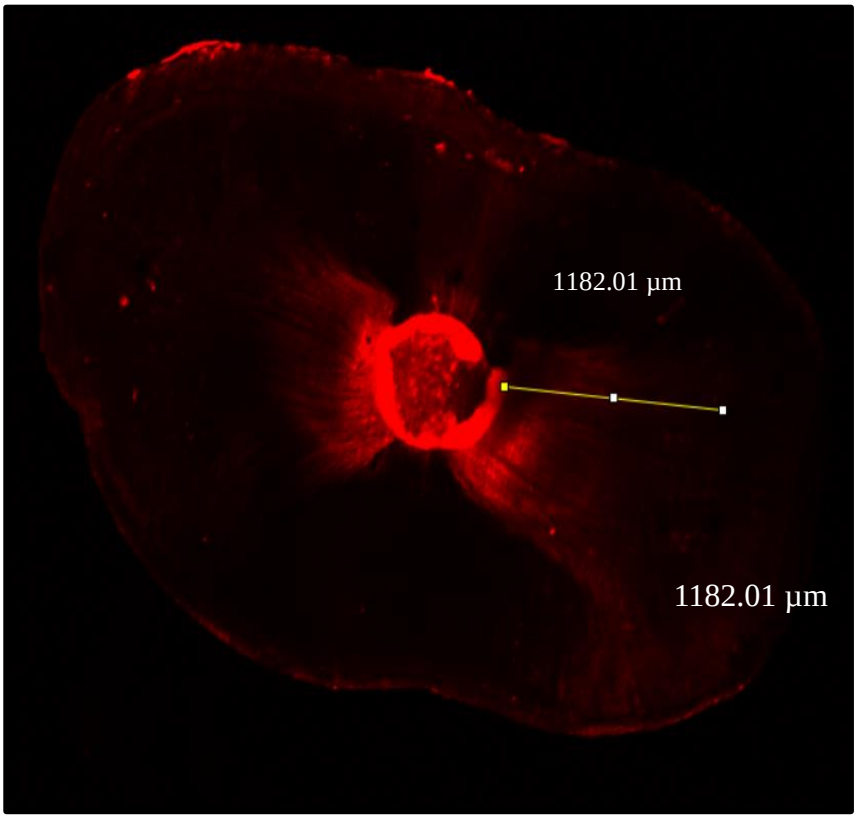


Fig 27: CLSM Image of 17% EDTA Group (Group 2)

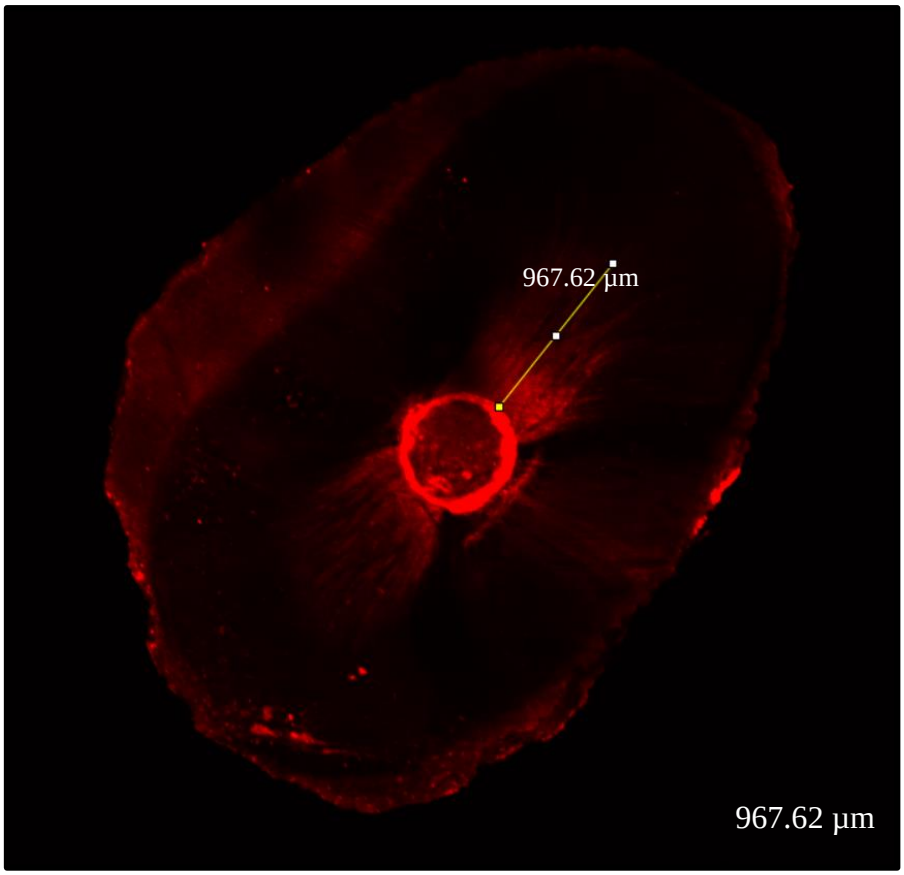


Fig 28: CLSM Image of CC + 17% EDTA Group (Group3)

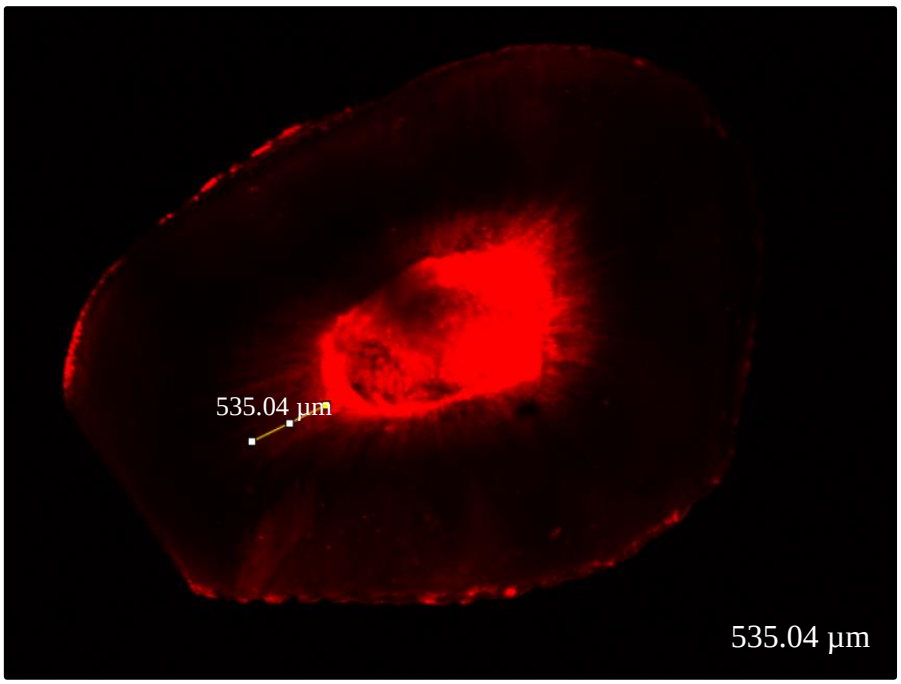


Fig 29: CLSM Image of Negative Control, Saline Group (Group 4)

DISCUSSION

Antibacterial activity of sealers and intracanal medicaments are effective in killing and entombing microorganism.[40] This is enhanced by the deep spread of these sealers and medicaments in the root dentin tubules.[41] To add to this, the mechanical interlocking of the plugs formed by these sealers in the root dentine is believed to increase adherence of the product.[42] This phase which is formed between sealer and dentinal tissue is improved by sealer entry in tubules, thereby increasing the sealing ability.[38,43]

During biomechanical preparation, a layer of debris is formed called the smear-layer. This coating impedes the penetration of irrigants, inter-appointment dressings as well as sealers during obturation into root dentine tubules.[44] Materials are employed to eliminate this smear-layer but it results in adverse effects on the radicular dentin and transforms its physical properties and chemical constitution. Such as a reduction in microhardness of the radicular dentine, which facilitates instrumentation through the length of the canal but also weakens the tooth structure.[45]

The universally used chelating solution is Ethylenediaminetetraacetic Acid (EDTA).[46] Ayce Eldinez has reported that this chelating effect has a profound demineralizing effect on dentin which results in largening of tubules, demineralising of calcified tissues, and breaking up of collagen.[32,47]

"C¸ alt and Serper" observed that exposure of EDTA up to 1 minute was effective to clean the smear-layer. They also observed, 10-minute long exposures to dentinal tissues resulted in excess erosion. Thus, they concluded exposure of EDTA must not be longer than 1 minute.[7] Saleh and Ettman studied H2O2/NaOCl and EDTA as endodontic irrigants on the microhardness of radicular dentine and reported

that the solutions studied resulted in decreased microhardness of dentin however EDTA had highest reduction.[48] This was also in accordance with the study conducted by Taner and coworkers where they studied the effect of EDTA and other irrigants such as EDTAC, EGTA etc. on the microhardness of dentin with and without irrigation with NaOCl and concluded that EDTA had the highest reduction in microhardness of dentinal tissues.[49]

The need for a milder chelator that was as efficient and safer than EDTA has led to great research on numerous materials.[50,51,52,53] Most materials have not shown as much promise in smear-layer clearance, especially in terminal-third of the root.[32]

NaOCl is a widely used irrigant in endodontics. It also causes alterations in chemical makeup and mechanical characteristics of dentinal tissues resulting in decreased bond-strength and reduced micro-hardness of dentin.[54] The ill-effect of sodium hypochlorite on dentine is proven to be concentration-dependent.[11] Baumgartner and Madely observed the reason for this to be the action of NaOCl, on the organic part of the dentine, and EDTA, on the mineralised part.[7]

A depletion in Cl^- ions is seen in research when NaOCl and EDTA interact, with increased loss of Cl^- ions in the first five mins. Over time the loss becomes gradual. This explains the reduction in antibacterial force of NaOCl when it is mixed with a chelating agent.[55]

As an oxidizer, sodium hypochlorite inhibits the resin and free radical interaction at the resin-tooth junction. The presence of remnants of hypochlorite is believed to be a reason for deterioration in bond strength.[56]

It has been proven that antioxidants enhance resin strength to hypochlorite treated dentin and can enhance the resin-dentin phase because of their antioxidant

properties. They can eliminate remnants of NaOCl by redox reactions.[57] Some of nature's antioxidants, like vitamin C, green tea extracts, and proanthocyanidin, can combat the cons of hypochlorite on dentine bond strength.[23]

Recently, there has been an increased inclination towards herbal irrigants with medicinal characteristics. Because of toxic effect on tissues of most synthetic irrigants, there is an increased tendency to use plant derived biologic medication in endodontic therapy.[13]

Bansal et. al in 2014 was successfully able to conclude that high antioxidant activity is demonstrated by the oil of *C. cyminum* (CC) because of polyphenolic like flavonoids, linalool, anethole, monoterpene alcohols, and carvacrol. [17, 20]

Along with this, CC has proven antimicrobial activity. Abbas Abbaszadegan et al observed the anti-microbial efficacy of CC against E faecalis with chlorhexidine (CHX) (control) as an intracanal medicament. The study concluded that cumin oil-based extract was a stronger disinfecting agent when compared with chlorhexidine against the study's micro-organisms. This potent anti-microbial action can be a result of the of cumin aldehyde present in the essential oil. Another advantage of using herbal medicines is the increased biocompatibility and reduced cytotoxicity when compared to the synthetic irrigants in the market.[18]

Having such an overpowering disinfecting action even on the toughest of bacteria that reside in the dentinal tubules prompted its use in this study.

Once the cumin seeds were procured and authenticated, they were pulverised to a smooth powder and steam-distilled with a Clevenger-apparatus to obtain the oil extract. The oil extract was volatile in nature[58] therefore in order to get an accurate initial concentration of the cumin aldehyde content present in this extract, Gas

Chromatography Mass Spectroscopy was done.[59] The initial concentration was estimated to be 4.4 x 10^5µg/ml.

The M.I.C.and M.B.C. against a strain of E. Faecalis were calculated of the extracts and they were estimated to be 65µg/ml. In accordance with the Clinical and Laboratory Standards Institute, M.I.C. is the least concentration of an extract that blocks the growth of bacteria post incubation. It indicates the extract's ability to inhibit bacterial growth, but not necessarily kill it.[60] M.B.C.is the least concentration of a plant extract that kills 99.9% of the bacteria after incubation. It indicates the extract's ability to kill the bacteria, not just stop their growth.[61]

In this study, the irrigants were used at a concentration of MIC against E. faecalis because this strain of bacteria is not only a normal occupant of the mouth but also is known to cause a variety of periradicular pathoses such as endodontic and persistent infections.[62] The incidence of E. faecalis in non-healing periradicular pathoses is much higher. Furthermore, failed endodontic cases are 9 folds more likely to have E. faecalis as oppose to primary endodontic disease.[63] E. faecalis survives in the canal system by many ways. It can demonstrate various genetic polymorphisms.[64] Therefore, the irrigant also aims at eradicating all kinds of pathogens that may pose a threat to the long lasting success of treatment.

Sixty human lower premolars were chosen because of their common availability due to extraction for orthodontic treatment as well as their unpredictable anatomic variations. Braga et. al studied the antifungal properties of thymol against common oral fungi species and concluded its high efficacy at inhibiting the growth of Candida albicans.[65] Jarahi et. al researched the effect of thymol to store dentinal tissues for various periods of time and concluded that storing teeth up to six months showed no significant effect on the dentine-composite bond strength.[66] Due to this,

0.1% thymol solution stored the teeth until use. To get standard root length to 14 ± 1 mm with reproducible reference point, samples were decoronated with a diamond disk. This was done by flattening the coronal surface to counter the variations in access preparations. ProTaper Universal system was used for biomechanical instrumentation because they have better cutting ability and less torsion stress.[67]

Canal cleansing regimen was 3% sodium hypochlorite between each instrument due to its ability to dissolve tissue and antimicrobial characteristics, succeeded by a final wash of 17% EDTA for smear-layer cleansing. In the second group, last rinse was irrigated by equal parts mixture of 17% EDTA and CC and in the third group final flush was done with CC alone. The fourth group was the negative control group which received a final flush of 0.9% saline.

Passive Ultrasonic Irrigation activated the irrigants. Ultrasonic energy has been utilized in endodontics for a significant period to debride canals and aid in disinfection. When combined with an irrigant, ultrasonics provide higher cleaning efficacy of canal system versus syringe irrigation.[68]

Better performance of PUI is attributed to activating the irrigant through acoustic microstreaming, generated by oscillating a smooth wire at an ultrasonic frequency of 25-40 kHz.[69] Due to this, a high speed vortex-like motion of the liquid and cavitation lead to the formation of spontaneous cavities in the liquid, which increased penetration of irrigants into the dentine channels.[70] Last irrigation used 0.9% saline to terminate the action of irrigants on the polymerization of sealers.[71]

The specimens were then obturated with AH Plus sealer. Takashi et. al in a review article studied the various properties of AH Plus and reported ideal adherence to dentine, biocompatibility and superior physical characteristics, hence deeming it

the gold standard.[72,73] The sealer was coated with Rhodamine B dye before obturating the specimens. A single cone was used to obturate the specimens. Single cone obturation technique is the most popular obturating method due to its advantages such as faster treatment, less apical stresses and less root dentin stress, less sealer and GP extrusion.[74]

To inhibit micro-leakage, Glass Ionomer cement was used over GP as temporisation. To allow sealer's set and to mimic the intra-oral conditions, all teeth were kept at 37°C in 100% humidity for one week.

The premolars were then sectioned 3 mm from the root terminus into 1 mm thin sections to be studied under the Confocal Laser Scanning Microscope. Due to its complex anatomy comprising lateral canals, apical deltas, accessory canals etc., the persistence of microorganisms in these irregularities is much higher. This in turn has effects on the long-term prognosis of endodontically therapized teeth, which is why apical sections of the teeth were chosen for this study.[75,76]

To assess penetration depth into the radicular dentine channels, in this study, CLSM was the microscope chosen over SEM, due to its potential to form a 3-Dimensional image, visualize samples at various points and make calculation more accurate.[77] CLSM has no artifacts because analysis is done to a depth of 20-30 microns. Miniscule quantities (0.1% w/w) of Rhodamine B dye were added to generate fluorescence hence sealer's characteristics did not alter.[78]

In this study, 17% EDTA (positive control) showed the highest sealer penetration into the dentine tubular channels. This was followed by its combination with Cuminum cyminum. The group that had the final flush of CC irrigant showed lowest penetration when calculated against other experimental groups however the

negative control group which received saline as its final flush showed least values of penetration of the sealer over all.

Godeau et al. studied the structure of cumin aldehyde and suggested that the carbonyl groups in CC gives a varitey of structural modifications for interfaces, thereby increasing the wettability, thereby increasing the interaction of irrigant and dentine resulting in superior penetration of sealer into dentinal channels when compared to other herbal irrigants.[79]

Iness Bettaieb et. al. researched chelating property of C. cyminum in comparison to 17% EDTA and concluded that Cuminum cyminum was a weak chelator with an affinity for Fe^{3+} ions.[37] EDTA forms stable complexes with Ca^{2+} ions in the smear-layer and removes it.[80] This could explain the deeper penetration of the resin-based sealer of the pure 17% EDTA group when compared to CC and the combination group.

As there is no established synergistic relationship between the two chemicals, The combination group was believed to have an intermediate performance between that of pure 17% EDTA and pure CC likely due to the lower concentration of 17% EDTA in the 1:1 mixture. More studies are warranted to explore the relationship between EDTA and CC.

Therefore, it can be concluded from this study that while Cumin cyminum wasn't as good in cleaning the smear-layer, its strong antimicrobial properties can make it a vital irrigant in endodontics. However, due to the aggressive nature of EDTA on the dentine, more research to find another irrigant to clean the smear-layer without resulting in excessive harm to dentin is required.

LIMITATIONS

As this study is in vitro, it is unable to accurately simulate the in vivo conditions. There is insufficient literature on Cuminum cyminum as an endodontic irrigant therefore the evidence to support the findings in this study require more in depth research. The oily nature of the cumin extract can pose challenges during removal from the root canal due to its high surface tension and immiscibility with water based irrigants that flush out debris. However, with the limitations of this study, it is clear that Cuminum cyminum with its anti-microbial strengths can be used as an adjunct to commonly used endodontic irrigants.

CONCLUSION

The power of the chelating agent used during endodontic therapy and the type of ions that the chelator has an affinity to plays a vital role in the elimination of smear-layer. This study demonstrated that irrigation with 17% EDTA had maximum penetration depth of sealer while Cuminum cyminum showed the least. It is confirmed that while EDTA continues to remain the gold standard for the removal of smear-layer, more studies are needed to find an irrigant which is as efficient as EDTA but, has a less aggressive effect on the dentinal tissues.

SUMMARY

The guaranteed prognosis of Endodontic treatment relies upon the efficient preparation of the canal system prior to a compact, water tight and three dimensional obturation. Various materials have been researched to achieve a near complete sterile environment within the canal, resulting in successful outcomes. The introduction of herbals into endodontics has proven to better the efficiency of irrigants within the canal ecosystem as well in complex morphologies present.

In this study, dentinal penetration after using Cuminum cyminum, 17% EDTA and a combination of the two has been assessed and compared as it is established that the smear-layer hinders sealer penetrability into the tubules. EDTA has shown to have an aggressive effect on the tooth structures therefore a milder chelator with good antimicrobial and antioxidant activity has been researched in this study.

Cumin seeds once procured were subjected to hydrodistillation to obtain the oil extract. The initial concentration of this extract was then diluted down to M.I.C. concentration and converted into an irrigant.

Standardized root length of 14±1mm with the help of a diamond disk was achieved. WL was calculated using a #10 K-file and the teeth were divided into 4 categories (n = 20), CC, 17% EDTA, CC+17% EDTA and Control group. Biomechanical preparation was completed with ProTaper Universal till F2/F3.

GROUP 1: Cuminum cyminum irrigant

GROUP 2: 17% EDTA

GROUP 3: CC + 17% EDTA

GROUP 4: Saline

Obturation was done utilizing a sealer with 0.1% Rhodamine B dye and teeth were incubated for a week. Sectioning was done for CLSM evaluation and images obtained were analyzed using Image J software.

Null hypothesis of no difference between the sealer penetration depth after irrigating the specimens with the 17% EDTA group (positive control), Cuminum cyminum group and their combination was rejected.

Superior results were attained with 17% EDTA group and CC group demonstrated least penetration depth of the sealer.

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ANNEXURE I – ETHICAL CLEARANCE CERTIFICATE

Research and Ethics Committee
KLE VK INSTITUTE OF DENTAL SCIENCES
A Constituent Unit of KLE Academy of Higher Education & Research
Accredited 'A' Grade by NAAC | Placed in Category 'A' by MHRD (GoI)
Nehru Nagar, Belagavi - 590 010, Karnataka State
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Sl. No. : 1637

CERTIFICATE

This is to Certify that the synopsis titled

Comparative evaluation of smear layer removal of cuminum cyminum Combination of 17% EDTA and cuminum cyminum and 17% EDTA as a final rinse at the apical third of the root canal. a scanning electron Microscope study.

Submitted by Dr. **REG. NO. IE0222003** *P. G. Student / Staff, Guided by* _____ *from Department of* Conservative Dentistry & Endodontics *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 8/4/25

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

ANNEXURE II – BIOSTATISTICS CLEARANCE CERTIFICATE

K L E
VISHWANATH KATTI
INSTITUTE OF DENTAL SCIENCES,
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Department of Conservative Dentistry and Endodontics

Biostatistics Clearance Certificate

This is to certify that the Biostatistics aspect of this Dissertation/ Thesis work of **REG. NO. IE0222003** ı, post-graduate student, under the guidance of ɔr, **Department of Conservative Dentistry and Endodontics**, entitled **"COMPARATIVE EVALUATION OF SMEAR LAYER REMOVAL OF CUMINUM CYMINUM, COMBINATION OF 17% EDTA AND CUMINUM CYMINUM AND 17% EDTA AS A FINAL RINSE AT THE APICAL THIRD OF THE ROOT CANAL: A SCANNING ELECTRON MICROSCOPE STUDY"** has been done under my guidance and completed satisfactorily.

Date : 16/4/2025
Place: Belagavi, KA

Name and Signature of Biostatistician

Dr. S. B. Javali. Ph.D.
Professor In Statistics
Department of Community Medicine
USM KLE International Medical Programme
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ANNEXURE III – PLAGIARISM CHECK REPORT

Scientific Correspondence and Review Committee

KLE VK Institute of Dental Sciences

A Constituent Unit of KLE Academy of Higher Education and Research
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Nehru Nagar, Belagavi - 590 010, Karnataka State

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Date : 16/04/2025 Serial No. : 416

PLAGIARISM CHECK REPORT

Name of the Applicant : **REG. NO. IE0222003**
UG / PG / Ph.D / Staff : PG
Batch & Year : 2022-2025
Department : Department of Conservative Dentistry and Endodontics

The soft copy of Research Work / Manuscript by **REG. NO. IE0222003** .. entitled "Comparative evaluation of smear layer removal efficacy of cumium cymium, combination of 17% EDTA and cumium cymium and 17% EDTA as a final rinse at apical 1/3rd of root canal : An invitro confocal laser scanning microscope study."
under the guidance of .....has been submitted for Anti-Plagiarism check to the Scientific Correspondence & Review Committee of KLE VK Institute of Dental Sciences using "Turn-it-in" software.

The scan has been carried out and the scanned output reveals a Similarity Index of .....4.....%, which is **within / not within** the acceptable limits of 10% as per the UGC guidelines.

16/04/2025

Member Secretary
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER-Belagavi

Chairman
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
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