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Dentistry Section

# Efficacy of Chelating Agents on Calcium Hydroxide Removal from the Root Canals and their Influence on the Penetration of a Bioceramic Sealer: An In-vitro Evaluation

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## ABSTRACT

**Introduction:** Eliminating Calcium Hydroxide {$Ca(OH)_2$} remnants from root canal walls presents a significant challenge. Nevertheless, its complete removal enhances the deeper penetration of a sealer, which improves the three-dimensional (3D) seal against the canal walls.

**Aim:** To analyse the intracanal $Ca(OH)_2$ removal efficacy of different chelators and evaluate their influence on the depth of penetration of a bioceramic sealer.

**Materials and Methods:** The present in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics at GITAM Dental College and Hospital, Visakhapatnam, Andhra Pradesh, India, from October 2024 to April 2025. In this study, 120 extracted mandibular premolars were selected. After biomechanical preparation, $Ca(OH)_2$ was placed in all root canals, except those assigned to the control group, and the teeth were incubated for 7 days. Intracanal medicament removal was carried out using various chelating agents: 17% Ethylenediaminetetraacetic acid (EDTA), 9% etidronic acid, 7% maleic acid, or 0.7% fumaric acid (n=24 each), irrigation in combination with ultrasonic activation. A total of 60 teeth were sectioned longitudinally to calculate the number of open dentinal tubules and assess canal cleanliness with Scanning Electron Microscopy (SEM). In the remaining 60 teeth, following $Ca(OH)_2$ removal, the canals were obturated with gutta-percha using CeraSeal. Sealer penetration depth was evaluated utilising Confocal Laser Scanning Microscopy (CLSM). The data obtained were subjected to statistical analysis using one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test, with a significance threshold of p-value <0.05.

**Results:** The use of 7% maleic and 0.7% fumaric acids resulted in significantly cleaner canals and enhanced sealer penetration compared to 17% EDTA and 9% etidronic acid (p-value <0.05). The coronal third of the canal demonstrated the greatest efficiency in both medicament removal and sealer penetration, whereas the apical third showed the lowest effectiveness (p-value=0.0001).

**Conclusion:** Compared to 17% EDTA and 9% etidronic acid, increased dentinal tubule patency and superior penetration of sealer were observed with 7% maleic and 0.7% fumaric acids.

**Keywords:** Confocal, Electron, Etidronic acid, Fumaric acid, Maleic acid, Microscopy, Root canal irrigants, Scanning, Smear layer

## INTRODUCTION

Different antimicrobial agents applied in the form of endodontic irrigants and intracanal dressings ensure the maximum possible root canal disinfection [1]. Calcium hydroxide, a well-known and regularly applied medicament in infected root canals, is on account of its biocompatibility, strong antibacterial and antiresorptive activities [1]. However, intricate root canal system anatomy and low solubility of $Ca(OH)_2$ in conventional irrigants make it challenging to remove the medicament completely from canals before obturation [2].

Residual $Ca(OH)_2$ gradually dissociates into calcium and hydroxyl ions, which can negatively influence the endodontic sealers' setting time, flow characteristics, and ability to penetrate dentinal tubules [3]. These remnants may contribute to void formation and microleakage, and can also disrupt the interaction between sealers and dentinal collagen, potentially compromising the success of endodontic therapy [4]. Although various irrigating solutions and approaches have been suggested to improve the removal of $Ca(OH)_2$, there is still no universally accepted method or solution considered the most effective [5].

Earlier studies have demonstrated that EDTA, maleic acid, and etidronic acid vary in their effectiveness for removing $Ca(OH)_2$ and the smear layer, while limited information is available on the role of fumaric acid and on how these chelators influence the penetration of modern bioceramic sealers [6,7].

The present investigation stands out as it directly compares four chelating agents (EDTA, etidronic acid, maleic acid, and fumaric acid) for their $Ca(OH)_2$ removal capacity using SEM and simultaneously evaluates their effect on CeraSeal bioceramic sealer penetration into root dentin using CLSM, thus addressing a relevant gap in irrigation-obturation protocols.

Accordingly, the objectives were: (i) to assess and compare the $Ca(OH)_2$ removal potential of 17% EDTA, 9% etidronic acid, 7% maleic acid, and 0.7% fumaric acid using SEM, and (ii) to determine the influence of these solutions on the dentinal penetration of CeraSeal sealer with CLSM.

The null hypotheses proposed were that the effectiveness of $Ca(OH)_2$ removal would not differ among the tested chelators and that bioceramic sealer penetration would remain unaffected by either the chelating agent used or the level of the root canal.

# MATERIALS AND METHODS

The present in-vitro study was carried out in the Department of Conservative Dentistry and Endodontics at GITAM Dental College and Hospital, Visakhapatnam, from October 2024 to April 2025. Before commencement, consent was procured and clearance pertaining to ethical issues was obtained from the Institutional Ethical Committee and Dr NTR University of Health Sciences under reference number D210050125.

**Inclusion criteria:** The current study included premolars which were extracted due to periodontal weakening or as part of orthodontic treatment. Teeth which were non carious, completely root-formed teeth without the absence of restorations or fractures, were also included in the study.

**Exclusion criteria:** Teeth were excluded if caries, prior fillings, crack lines, or fractures were present on the crown or root surfaces.

**Sample size calculation:** A sample size of 120 extracted mandibular premolar teeth was selected after consultation with a statistician, using G*Power software (version 3.1) guided by reference data from previous in-vitro studies by Dias-Junior LCL et al., and Jaiswal S et al., for statistical estimation [6,7].

## Study Procedure

The collected samples were cleaned (ultrasonic scaler-Cavitron) after taking preoperative radiographs and stored in physiological saline at 4°C for 3 days, as it maintains moisture, structure, mechanical properties, and cooler temperature slows growth of any contaminants [3,5]. Conventional access preparations were created, and the patency of the canal was confirmed. ProTaper Next rotary instruments (Dentsply Maillefer) were used to prepare the canals mechanically up to # F3, using sodium hypochlorite (2.5%) irrigant. Following canal shaping, irrigation was performed three times using 2 mL of EDTA (17%) for one minute per cycle. This was followed by a final rinse with 5 mL of deionised water. Moisture was removed from the canals using F3-sized absorbent paper points.

**Intracanal placement of calcium hydroxide:** Calcium hydroxide medicament (Ultradent Products Inc., South Jordan, USA) was introduced into the root canals of all the experimental groups, excluding the control group. Radiographic imaging was used to verify complete canal filling. Cavit-G (3M ESPE, MN, USA) was used to seal the access cavities, and specimens were stored at 37°C in an environment with 100% humidity for seven days.

**Calcium hydroxide elimination and irrigation protocol:** Following removal of provisional restorative material, canals were initially rinsed with normal saline (10 mL) and re-instrumented up to the working length using # F3 file. Teeth were assembled into four experimental groups, and a control group (n=24 each) based on the type of chelating agent employed.

- Group 1 (n=24): Control, in which $Ca(OH)_2$ dressing was not placed.

The removal of $Ca(OH)_2$ medicament was carried out using the following methods [7,8,9]:

- Group 2 (n=24): EDTA (17%) irrigation.
- Group 3 (n=24): Etidronic acid 9%.
- Group 4 (n=24): Maleic acid (7%) was used for irrigation.
- Group 5 (n=24): Fumaric acid (0.7%) irrigation.

The root canals were irrigated 3 times in all sample teeth, using 2 mL of the respective irrigant per cycle, ensuring a standardised total volume of 6 mL. Ultrasonic activation was carried out using an ultrasonic unit (Satelec P5 Newtron, France) equipped with a stainless steel ultrasonic K-file tip (Satelec/Acteon, Merignac, France) of size 20/0.2, operated at a power setting of 3. The ultrasonic tip was positioned 2 mm short of the working length and stimulated with gentle vertical strokes. To neutralise the effect of the irrigants, a final rinse with saline solution was performed.

**Evaluation of root canal cleanliness with SEM analysis:** One-half of the sample in every group (n=12 each) was split using a mallet and chisel longitudinally, and then the tooth fragments were observed under ULTRA 55, Field Emission SEM with Energy-Dispersive X-ray Spectroscopy (EDS)(Karl Zeiss, Germany). The micrograph images were recorded for apical, middle and coronal root sections i.e. 2 mm, 6 mm and 10 mm from the apical end, at 3000X magnification [Table/Fig-1].

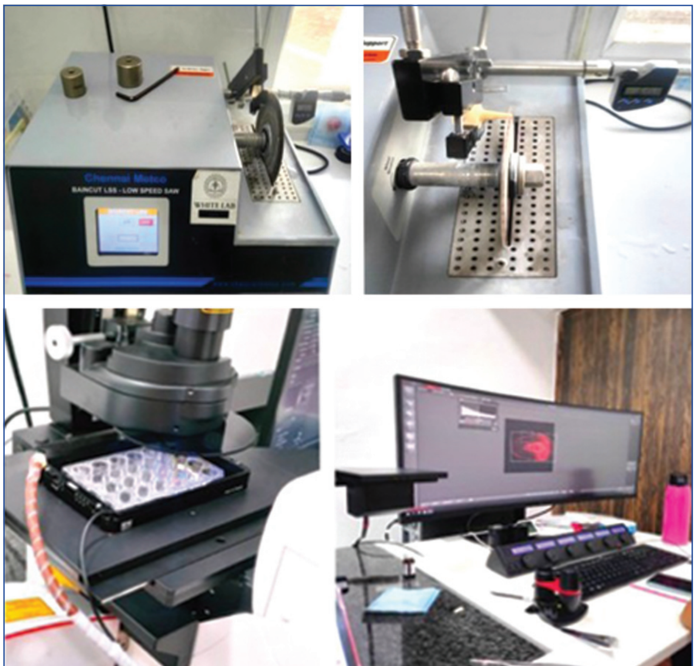
**[Table/Fig-1]:** Composite image of sectioned tooth sample and SEM equipment with image processing.

The percentage of patent tubules has been calculated by using the formula:

$$\text{Percentage of open tubules} = \frac{\text{Number of open tubules}}{\text{Total number of tubules in the photomicrograph per } 50\ \mu m^2} \times 100\ [10].$$

**Sealer penetration assessment:** The remaining half of the specimens (n=12 per group) underwent obturation using F3 gutta-percha cones (ProTaper, Dentsply) in combination with CeraSeal bioceramic sealer (Meta Biomed Co., South Korea). The sealer was dispensed onto a mixing pad using an automix tip, and a small quantity of 0.1% fluorescent Rhodamine B dye (Sigma Aldrich Corp., USA) was incorporated. The volume of the dye-sealer mixture was standardised to approximately 0.05 mL per canal, and the obturation was performed with the cold lateral compaction technique.

Subsequently, horizontal sectioning of the roots at 2, 6 and 10 mm from the apex was done. The depth to which the sealer penetrated root dentin was assessed using CLSM (Leica DMi8, Leica Microsystems GmbH, Germany), operated at an excitation/emission wavelength of 540/590 nm [Table/Fig-2].



**[Table/Fig-2]:** Composite image of Microtome with tooth attached for sectioning and Confocal laser scanning microscope equipment with image processing.

To determine the extent of penetration, from the canal surface to the farthest point at which the sealer infiltrated, was measured. Measurements were taken in buccal, mesial, lingual and distal directions of the canal. The average of these four measurements was then calculated for each specimen.

## STATISTICAL ANALYSIS

Statistical evaluation was executed using Statistical Package for Social Sciences (SPSS) version 23.0 (IBM Corp., Armonk, NY, USA). One-way ANOVA was applied to assess the number of open dentinal tubules within and between the experimental groups across the coronal, middle, and apical regions. To analyse variations in penetration of sealer among the groups and at different root levels, two-way ANOVA was used. Tukey's post-hoc test was conducted for pairwise assessments. A p-value below 0.05 was regarded as statistically significant.

| Group | Region | Coronal | Middle | Apical |
|---|---|---|---|---|
| Control group | Mean % of open dentinal tubules | 100.00 | 99.58 | 99.34 |
| | SD | 0 | 0.62 | 1.05 |
| | Coronal | - | | |
| | Middle | p=1.0000 | - | |
| | Apical | p=0.9998 | p=1.0000 | - |
| EDTA group | Mean % of open dentinal tubules | 95.31 | 93.04 | 89.01 |
| | SD | 2.43 | 2.91 | 2.52 |
| | Coronal | - | | |
| | Middle | p=0.0838 | - | |
| | Apical | p=0.0001* | p=0.0001* | - |
| Etidronic acid group | Mean % of open dentinal tubules | 94.72 | 91.66 | 87.19 |
| | SD | 1.27 | 1.53 | 1.94 |
| | Coronal | - | | |
| | Middle | p=0.0014* | - | |
| | Apical | p=0.0001* | p=0.0001* | - |
| Maleic acid group | Mean % of open dentinal tubules | 99.28 | 98.41 | 96.38 |
| | SD | 0.99 | 1.32 | 2.18 |
| | Coronal | - | | |
| | Middle | p=0.9965 | - | |
| | Apical | p=0.0037* | p=0.2070 | - |
| Fumaric acid group | Mean % of open dentinal tubules | 99.02 | 98.24 | 95.67 |
| | SD | 1.14 | 1.55 | 1.95 |
| | Coronal | - | | |
| | Middle | p=0.9990 | - | |
| | Apical | p=0.0002* | p=0.0215* | - |

**[Table/Fig-3]:** Intragroup pair-wise comparison of percentage of open tubules at different regions in all the five groups by Tukey's multiple post-hoc test.
p<0.05* indicate significant difference

## RESULTS

**Dentinal tubule patency:** The mean percentage of open dentinal tubules for all groups was presented in [Table/Fig-3]. Overall, maleic acid and fumaric acid exhibited significantly superior cleaning efficacy at the middle and apical third compared to EDTA and etidronic acid (p-value=0.0001). Although maleic acid demonstrated a higher percentage of open dentinal tubules than fumaric acid at the apical third, a significant difference was not noticed statistically (p-value=0.9996) [Table/Fig-4].

All experimental groups exhibited a significant difference from the control overall (p-value=0.0001). In the apical third, etidronic acid performed similarly to 17% EDTA (p-value=0.3811), while maleic acid and fumaric acid showed no significant difference in efficacy (p-value=0.9996). In all the groups, a gradual reduction of open dentinal tubules was noted from coronal to apical root regions [Table/Fig-3-5].

A statistically significant difference was identified in the coronal third between the control group and both the EDTA and etidronic acid groups (p-value=0.0001). In the middle third of the root, no significant difference was found between the control group and those treated with maleic acid (p-value=0.9473) or fumaric acid (p-value=0.8587). The EDTA and etidronic acid groups demonstrated notably lower effectiveness compared to the others [Table/Fig-4].

**Sealer penetration depth:** The average sealer penetration depths for all groups are summarised in [Table/Fig-6]. Among the tested chelating agents, maleic acid exhibited deeper infiltration of sealer into root dentin, followed by fumaric acid. The difference in penetration depth between the maleic acid and the other groups was significant (p-value=0.0001*). Etidronic acid resulted in the lowest mean sealer penetration [Table/Fig-7-9].

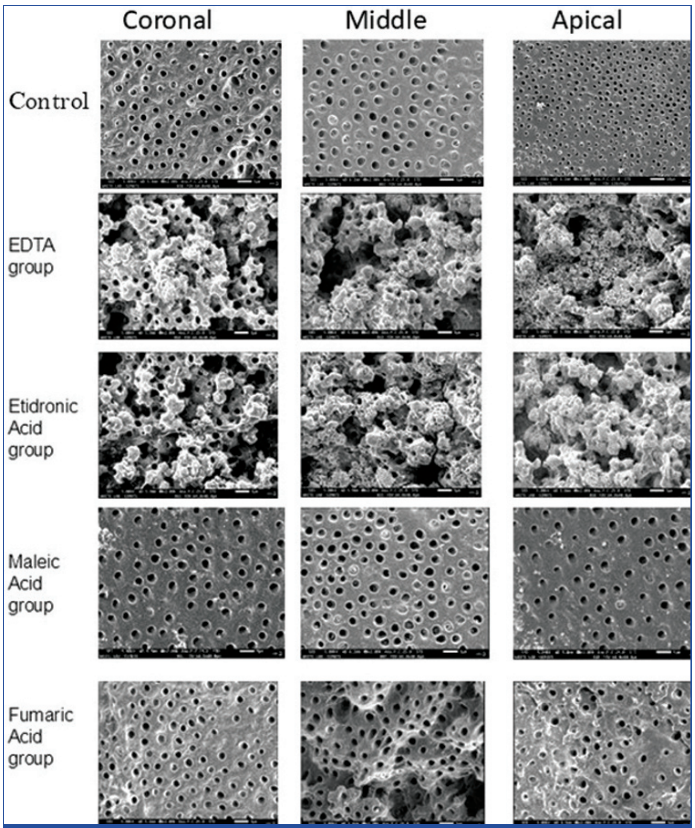
Additionally, the depth of sealer penetration was influenced significantly depending on root region in all groups (p-value=0.0001*), with the greatest penetration observed in the coronal part, whereas the least penetration was seen in the apical region [Table/Fig-8].

## DISCUSSION

Effective elimination of intracanal medicaments such as $Ca(OH)_2$ is a prerequisite for ensuring reliable adhesion, adaptation, and long-term sealing of the endodontic sealer to root dentin. Residual

| | Groups | Control | EDTA | Etidronic acid | Maleic acid | Fumaric acid |
|---|---|---|---|---|---|---|
| Coronal third | Control | --- | p=0.0001* | p=0.0001* | p=0.9996 | p=0.9887 |
| | EDTA | p=0.0001* | --- | p=1.0000 | p=0.0001* | p=0.0001* |
| | Etidronic acid | p=0.0001* | p=1.0000 | --- | p=0.0001 | p=0.0001 |
| | Maleic acid | p=0.9996 | p=0.0001* | p=0.0001* | --- | p=1.0000 |
| | Fumaric acid | p=0.9887 | p=0.0001* | p=0.0001 | p=1.0000 | --- |
| Middle third | Control | --- | p=0.0001* | p=0.0001* | p=0.9473 | p=0.8587 |
| | EDTA | p=0.0001* | --- | p=0.8287 | p=0.0001* | p=0.0001* |
| | Etidronic acid | p=0.0001* | p=0.8287 | --- | p=0.0001* | p=0.0001* |
| | Maleic acid | p=0.9473 | p=0.0001* | p=0.0001* | --- | p=1.0000 |
| | Fumaric acid | p=0.8587 | p=0.0001* | p=0.0001* | p=1.0000 | --- |
| Apical third | Control | --- | p=0.0001* | p=0.0001* | p=0.0027* | p=0.0001* |
| | EDTA | p=0.0001* | --- | 0.3811 | p=0.0001* | p=0.0001* |
| | Etidronic acid | p=0.0001* | 0.3811 | --- | p=0.0001* | p=0.0001* |
| | Maleic acid | p=0.0027* | p=0.0001* | p=0.0001* | --- | p=0.9996 |
| | Fumaric acid | p=0.0001* | p=0.0001* | p=0.0001* | p=0.9996 | --- |

**[Table/Fig-4]:** Intergroup comparison between all the five groups at each level (coronal, middle and apical third) in percentage of open tubules with p-values.
p-value (p)<0.05* indicate significant difference




**[Table/Fig-5]:** Composite image of representative scanning electron microscopic images from each group at coronal (12 mm), middle (8 mm) and apical (4 mm) sections.

| Groups | Control | EDTA | Etidronic acid | Maleic acid | Fumaric acid |
|---|---|---|---|---|---|
| Coronal third | 1458.83 ± 9.38 | 1162.45 ± 9.42 | 914.12 ± 14.22 | 1861.48 ± 15.83 | 1660.56 ± 10.10 |
| Middle third | 868.22 ± 10.71 | 760.45 ± 9.59 | 645.26 ± 9.27 | 1039.77 ± 16.63 | 1023.94 ± 17.08 |
| Apical third | 505.55 ± 6.90 | 488.98 ± 6.90 | 271.49 ± 13.56 | 629.23 ± 36.40 | 607.65 ± 8.11 |

**[Table/Fig-6]:** Descriptive data for intergroup comparison in respective to depth of sealer penetration.

| Groups | Control | EDTA | Etidronic acid | Maleic acid | Fumaric acid |
|---|---|---|---|---|---|
| Mean(µm) | 944.20 | 803.96 | 610.29 | 1176.83 | 1097.38 |
| SD | 398.54 | 280.71 | 267.53 | 520.14 | 439.28 |
| Control | - | | | | |
| EDTA | p=0.0001* | - | | | |
| Etidronic acid | p=0.0001* | p=0.0001* | - | | |
| Maleic acid | p=0.0001* | p=0.0001* | p=0.0001* | - | |
| Fumaric acid | p=0.0001* | p=0.0001* | p=0.0001* | p=0.0001* | - |

**[Table/Fig-7]:** Inter-group pair-wise comparison of mean depth of sealer penetration.
Tukeys multiple post-hoc test , p-value <0.05* indicate significant difference

$Ca(OH)_2$ can interfere with the penetration of the sealer, compromise bonding, and negatively affect the long-term prognosis of endodontic treatment [8].

In the present study, none of the tested chelating agents were able to eliminate $Ca(OH)_2$ from the canal walls, which necessitated the rejection of the first null hypothesis. This finding is consistent with earlier literature by Ballal NV et al., that even after thorough irrigation protocols, traces of $Ca(OH)_2$ persist in canal irregularities, isthmuses, and the apical third [8].

When the different irrigants were compared, maleic acid and fumaric acid demonstrated significantly greater efficacy in removing $Ca(OH)_2$ residues compared with the more commonly employed agents, namely 17% EDTA and 9% etidronic acid. Several chemical and physical factors explain the superior performance of these organic acids [8,9].

First, their stronger acidic nature plays a crucial role. Maleic acid (pKa≈1.9) and fumaric acid (pKa≈3.0) create a more acidic environment than EDTA (pKa≈2.6, but weaker chelation at low concentrations) and etidronic acid (pKa≈4.3), facilitating enhanced dissolution of $Ca(OH)_2$ [7,8,9]. Calcium hydroxide is only sparingly soluble in water but reacts readily in acidic conditions to release free calcium ions, which can then be bound or chelated by the irrigant [9]. This dual action dissolution followed by chelation enables maleic and fumaric acids to perform more effectively than EDTA and HEBP, which act mainly by chelation alone.

Second, molecular size and structural configuration contribute to their effectiveness. Both maleic and fumaric acid have relatively small molecular dimensions, allowing them to diffuse more easily into dentinal tubules and canal irregularities. This property increases the surface area of contact between the irrigant and residual $Ca(OH)_2$, enabling improved dissolution and removal. Larger molecules, such as EDTA, may experience steric hindrance in narrow canal spaces, thereby reducing their penetration depth and efficiency [11].

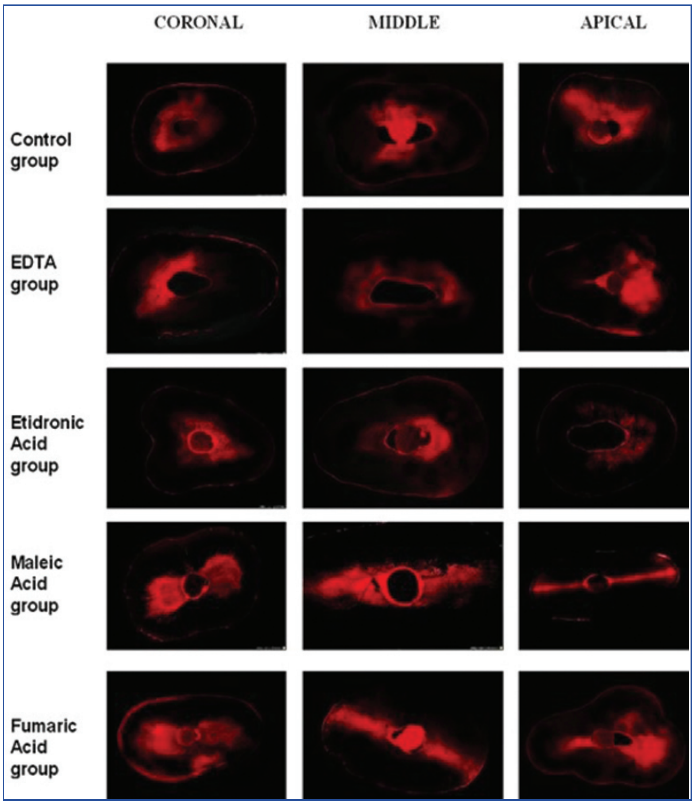
Another important factor is the surface tension characteristics of the irrigants. Maleic and fumaric acids are known to have lower surface tension values than EDTA and etidronic acid. A reduced surface tension enhances wettability, allowing the solution to spread along dentinal walls and penetrate finer canal ramifications. Improved contact between the irrigant and dentinal surfaces results in more efficient displacement of debris and medicament residues [12].

Collectively, these properties—acid strength, molecular size, and favourable surface tension—explain the superior $Ca(OH)_2$ removal efficacy observed in the present study for maleic and fumaric acids. In contrast, the reduced performance of EDTA and etidronic acid can be attributed to their predominant reliance on ion exchange and chelation, which is less effective when high alkalinity from $Ca(OH)_2$ residues is present [13]. Furthermore, EDTA is known to have a relatively slower calcium complexation rate, while etidronic acid, though biocompatible and less aggressive to dentin, exhibits milder chelating capacity compared to other organic acids [13-16].

| | Groups | Control | EDTA | Etidronic acid | Maleic acid | Fumaric acid |
|---|---|---|---|---|---|---|
| Coronal third | Control | --- | p=0.001* | p=0.001* | p=0.001* | p=0.001* |
| | EDTA | p=0.001* | --- | p=0.001* | p=0.001* | p=0.001* |
| | Etidronic acid | p=0.001* | p=0.001* | --- | p=0.001* | p=0.001* |
| | Maleic acid | p=0.001* | p=0.001* | p=0.001* | --- | p=0.001* |
| | Fumaric acid | p=0.001* | p=0.001* | p=0.001* | p=0.001* | --- |
| Middle third | Control | --- | p=0.001* | p=0.001* | p=0.001* | p=0.001* |
| | EDTA | p=0.001* | --- | p=0.001* | p=0.001* | p=0.001* |
| | Etidronic acid | p=0.001* | p=0.001* | --- | p=0.001* | p=0.001* |
| | Maleic acid | p=0.001* | p=0.001* | p=0.001* | --- | p=0.001* |
| | Fumaric acid | p=0.001* | p=0.001* | p=0.001* | p=0.001* | --- |

| | | | | | | |
|---|---|---|---|---|---|---|
| Apical third | Control | --- | p=0.001* | p=0.001* | p=0.001* | p=0.001* |
| | EDTA | p=0.2741 | --- | p=0.001* | p=0.001* | p=0.001* |
| | Etidronic acid | p=0.001* | p=0.001* | --- | p=0.001* | p=0.001* |
| | Maleic acid | p=0.001* | p=0.001* | p=0.001* | --- | p=0.001* |
| | Fumaric acid | p=0.001* | p=0.001* | p=0.001* | p=0.0261* | --- |

**[Table/Fig-8]:** Intergroup comparison between all five groups at each level (coronal, middle and apical third) in respect to sealer penetration
p-value <0.05* indicates significant difference




**[Table/Fig-9]:** Composite image of Representative confocal laser scanning microscopic images from each group at coronal (2 mm), middle (6 mm) and apical (10 mm) sections.

The findings of the present study are in agreement with Arslan H et al., [17], who observed that 10% citric acid and 7% maleic acid outperformed 1% NaOCl and 17% EDTA in removing $Ca(OH)_2$ combined with chlorhexidine. Their results, like ours, underscore the advantage of stronger organic acids in eliminating intracanal medicaments.

Some of the recent studies [7,18] have demonstrated that 0.7% fumaric acid provides superior smear layer removal in the apical third compared to 17% EDTA and 7% maleic acid. Its effectiveness is further enhanced when combined with rotary systems, yielding improved canal cleanliness [18]. Additionally, fumaric acid has been shown to improve post-endodontic fracture resistance relative to conventional chelators [19].

Regarding sealer penetration, maleic acid and fumaric acid groups demonstrated the highest mean values across all root levels, which led to the rejection of the second null hypothesis. The superior sealer penetration observed in these groups can be directly correlated with their improved cleaning ability and smear layer removal.

By eliminating more $Ca(OH)_2$ residues and exposing dentinal tubules more effectively, these agents facilitated deeper penetration of CeraSeal bioceramic sealer. Bioceramic sealers rely on both micromechanical interlocking into dentinal tubules and chemical interaction with dentin; thus, the extent of tubule exposure directly influences their performance [20].

Among all groups, the maleic acid group achieved the most favorable results, especially in the apical third. This can be attributed to its high acidic potential, smaller molecular size, and proven capacity to remove both the smear layer and residual $Ca(OH)_2$ [17]. Comparable results were reported by Alim Uysal BA et al., [20], who demonstrated greater sealer penetration in apical sections when maleic acid was used as a final irrigant compared with EDTA and etidronic acid. Similarly, other authors have confirmed the superiority of maleic acid for smear layer removal in the apical region [21].

The present study also observed that mean sealer penetration was consistently greater in coronal and middle thirds than in apical regions across all groups. This finding aligns with previous research by Ozasir T et al., [22], Yilmaz A et al., [23], and Kourti E and Pantelidou O [24], who emphasised that anatomical variations in the apical third pose challenges for irrigant effectiveness.

The apical region typically exhibits reduced dentinal tubule density, smaller tubule diameters, and a higher prevalence of sclerotic or atubular dentin, which together hinder irrigant penetration and sealer adaptation [23]. Furthermore, the apical third is more difficult to access mechanically and hydrodynamically due to reduced canal taper and restricted irrigant flow, all of which contribute to the lower penetration observed.

### Limitation(s)

As the study was conducted on premolars with single roots and minimal curvature, it may not accurately represent the complexity of multirooted teeth commonly encountered in clinical practice. Additionally, further research is needed to assess the biocompatibility, antimicrobial activity of different concentrations of fumaric acid and its effect on dentin microhardness before considering its clinical use.

## CONCLUSION(S)

The present study demonstrated that 7% maleic acid and 0.7% fumaric acid can be considered as effective chelating agents for calcium hydroxide medicament removal and to enhance the depth of penetration of a bioceramic sealer into root dentin.

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