RESEARCH PAPER

Effect of Herbal Irrigants with and without Laser Activation on Microhardness of Root canal dentin - A Systematic Review with Meta-Analysis

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ABSTRACT

Root canal irrigants are crucial for disinfection but can reduce dentin microhardness, raising the risk of fractures. With increasing in advancement various herbal irrigants and laser activation have been emerged as root canal irrigants for Endodontic treatment. This systematic review and meta-analysis examines the effect of these methods on root canal dentin microhardness evaluated in invitro studies. Adhering to PRISMA 2020 guidelines, we conducted a search in PubMed, Scopus, Google Scholar, and Science Direct databases up to February 2025, focusing on invitro studies that compared herbal irrigants (with or without laser activation) to conventional ones like 2.5% NaOCl and 17% EDTA regarding their effects on dentin microhardness.

The study protocol was registered in PROSPERO, and PRISMA guidelines were followed. The research question was: "How the effect of herbal irrigants, by activation using lasers or alone affects the dentin microhardness in Endodontic treatment ? Two reviewers independently screened and evaluated the articles. A total of 65 articles were identified across all databases, with 43 selected for full-text analysis. After applying eligibility criteria, 21 studies were included in this review. The findings revealed that traditional irrigants, such as 17% EDTA and NaOCl, significantly reduced dentin microhardness through demineralization and proteolysis, with EDTA alone causing reductions up to 20.21%. In contrast, herbal alternatives like Neem, Miswak, and Green Tea were found to be biocompatible, maintaining microhardness levels comparable to saline, particularly Triphala and 6.5% Grape Seed Extract demonstrated exceptional preservation of mechanical properties.

However, the study noted that laser activation (e.g., diode or Er:YAG lasers),accelerated microhardness loss when increasing the duration of time from 10 to 40 seconds.The review concludes that while herbal irrigants offer a safer profile for preserving tooth structural integrity, laser parameters must be optimized for preservation of dentinal strength.
Keywords: *Sodium Hypochlorite, Triphala, Neem, Green tea, Diode, Er:YAG, Nd:YAG*
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INTRODUCTION

Maintaining the structure and integrity of dentin during Endodontic procedures is essential for the long-term durability of teeth.Root canal irrigants are crucial in eliminating the smear layer, dissolving organic matter, and disinfecting the canal system. However, their interaction with dentin's inorganic and organic components can impact its microhardness, which is vital for mechanical

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strength and bonding capabilities. Traditional chemical irrigants like sodium hypochlorite (NaOCl) and Ethylenediaminetetraacetic acid (EDTA) are known for their antimicrobial properties and ability to remove the smear layer, but they have been found to negatively affect dentin microhardness, potentially weakening structural integrity. Recently, herbal irrigants have gained attention as viable alternatives due to their biocompatibility, antioxidant properties, and antimicrobial effectiveness. Substances such as Morinda citrifolia, Green tea polyphenols, Triphala, and Neem extract have shown antimicrobial affects similar to conventional irrigants while being less cytotoxic[1]. Despite the growing interest, the evidence regarding their impact on the physical properties of root dentin, particularly microhardness, is varied and inconclusive. Additionally, the use of laser activation for irrigants, including diode, Er:YAG, and Nd:YAG lasers, has also been suggested to improve penetration, smear layer removal, and chemical reactivity within the root canal system.Laser activation might change the dynamics and interaction of irrigants with dentinal substrates, potentially affecting dentin microhardness .However, Currently, there is an absence of a comprehensive synthesis that systematically evaluates and contrasts the affects across existing invitro studies. A systematic review based on this criteria is important for the clinicians and researchers about the relative impact of herbal irrigants and laser- assisted irrigation protocols on dentin microhardness, an essential biomechanical property implicated in root strength and adhesive dentistry. Consequently, the combined use of herbal irrigants with laser activation may exert synergistic, neutral, or adverse effects on dentin microhardness. Microhardness assessment, commonly performed using Vickers or Knoop hardness tests, provides quantitative insight into mineral loss and structural changes in dentin following irrigation protocols.[2-4]A synthesis of this evidence is necessary to determine whether herbal alternatives preserve dentinal integrity better than conventional irrigants and whether laser activation modifies these effects. Therefore, this systematic review aims to evaluate the evidence regarding the impact of herbal irrigants, alone and in combination with laser activation, on root canal dentin microhardness.

2. MATERIALS AND METHODS

The protocol for this systematic review was registered with PROSPERO (Version 1.0) under the registration number CRD420250650711.This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (https://www.prisma-statement.org/prisma-2020).

2.1. Eligibility Criteria

The research included invitro studies of single-rooted human permanent teeth subjected to irrigation with herbal irrigants or laser activation (Er:YAG, Nd:YAG, or diode) during endodontic treatment. Review articles, letters, clinical studies, case reports/series, and studies involving deciduous or bovine teeth were excluded.

2.2. Literature Search

The search strategy followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (Table 2). An electronic literature search was performed across four prominent databases (PubMed, Google Scholar, Science Direct, and Scopus) until February 1, 2025. The search was restricted to articles published in English only.

Table 2.

Database	Search Strategy
PubMed	("Root Canal Therapy"[MeSH] OR "Endodontics"[MeSH] OR "root canal" OR "endodontic treatment") AND ("Phytotherapy"[MeSH] OR "Plant Extracts"[MeSH] OR "herbal irrigant*" OR "plant extract*" OR "natural irrigant*") AND ("Lasers"[MeSH] OR "laser activation" OR "Er:YAG" OR "Nd:YAG" OR "diode laser") AND ("Dentin"[MeSH] OR "dentin microhardness" OR microhardness)
Scopus	TITLE-ABS-KEY (("root canal" OR "endodontic treatment") AND ("herbal irrigant*" OR "plant extract*" OR "natural irrigant*") AND ("laser activation" OR "Er:YAG" OR "Nd:YAG" OR "diode laser") AND ("dentin microhardness" OR microhardness))
Science Direct	("root canal" OR endodontic) AND ("herbal irrigant*" OR "plant extract*") AND ("laser activation" OR "Er:YAG" OR "Nd:YAG" OR "diode laser") AND ("dentin microhardness" OR microhardness)
Google Scholar	"herbal irrigants" AND "laser activation" AND "dentin microhardness"

The search results were imported into Rayyan (web-based systematic review software) and Zotero(7.0.32), where duplicates were removed by S. A. and L. M. The titles and abstracts were then reviewed based on the inclusion criteria, and studies meeting the criteria were subjected to full-text screening for qualitative synthesis.

2.3. Data Extraction

Data extraction included study characteristics, methodology, and outcomes related to root canal dentin microhardness.The study identification focused on the design, including the sample size, tooth type, type of irrigants used, its contact time, and methods for sectioning. Protocols compared herbal irrigants with irrigants like NaOCl or EDTA, specifying concentration, volume, contact time, and an laser activation parameters such as type and power. Microhardness was recorded in VHN or KHN as a mean ± SD for the cervical, middle, and apical thirds, based on the specific load and dwell time used.

The quality assessment tool for invitro studies (QUIN tool) checklist was used to evaluate the internal methodological quality (risk of bias) of the included studies. Each of the 12 parameters considered was assessed for individual studies and then the percentage was calculated as (score × 100) / (2 × number of criteria applied).The Risk of bias assessment was done in (Revmann version 5.1.4).

Results

The initial electronic database search found 64 articles. After removing duplicates, the total was reduced to 43.Subsequent screening based on abstracts and titles resulted in a further assessment of 34 articles. Finally, 21 full-text articles met the eligibility criteria were included in the study (Figure 1).

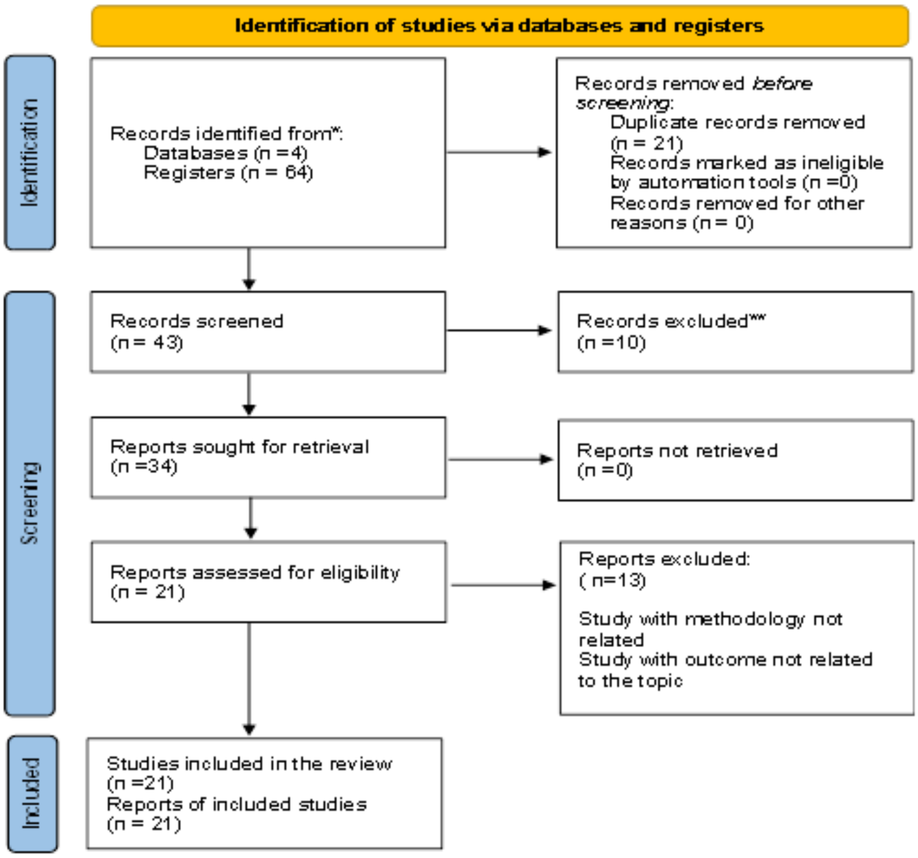

Figure 1. PRISMA flow diagram of literature search and selection process

3.1 Irrigating Solution Evaluation

Various irrigating solutions were evaluated for their impact on dentin microhardness (Table 4), such as sodium hypochlorite, EDTA ,herbal irrigants and laser activation .They were tested at different concentrations and at contact time time intervals.

Table 4: Root dentin microhardness after contact with different irrigating solutions - Data Extraction from Included studies

Sl. No	Author (Year)	Sample Size, Type of Teeth, and Section Used in Each Group	Groups / Intervention Contact Time (Minutes)	Load (g) Given during Testing and Dwell Time	Outcome Measure test	Statistical Analysis	MHV
1	Viapiana et al. (2012)	72 single-canal,canines (LS)	Main Group (Irrigant) Distilled Water 1% NaOCl 1% NaOCl + 17% EDTA	25 gm (30, 90, 150, and 300 µm) 10 sec	Knoop Microhardness (KHN)	ANOVA + Tukey	Dentin Depth / Irrigant Solution / Mean ± SD 300 µm: NaOCl + EDTA 54.0 ± 11.7; Distilled water 51.7 ± 10.9; NaOCl 51.1 ± 11.6 150 µm: NaOCl + EDTA 51.0 ± 11.6; NaOCl 48.8 ± 9.8; Distilled water 47.6 ± 11.7 90 µm: NaOCl + EDTA 49.5 ± 11.0; NaOCl 47.5 ± 9.2; Distilled water 46.7 ± 12.3 30 µm: NaOCl + EDTA 46.7 ± 10.5; NaOCl 43.4 ± 8.8; Distilled water 43.0 ± 12.0
2	Prabhakar et al. (2013)	16 premolars LS	Group I: 0.2% CHX; Group II: 6% MCJ; 15 min Group III: 6% MCJ + 0.2% CHX; Group IV: Saline.	300 g and dwell time of 10 s.	Vickers	Paired t-test + ANOVA	Group / Irrigant / Baseline (Mean ± SD) / After 15 Min (Mean ± SD) / Mean Diff. / P-value (Significance) I / 0.2% CHX / 51.59 ± 8.98 / 53.15 ± 8.20 / 1.56 / 0.74 (NS) II / 6% MCJ / 54.40 ± 8.42 / 57.38 ± 6.10 / 2.98 / 0.44 (NS) III / MCJ + CHX / 58.94 ± 8.80 / 59.14 ± 7.34 / 0.20 / 0.96 (NS) IV / Saline / 52.70 ± 8.15 / 55.68 ± 6.86 / 2.98 / 0.41 (NS)
3	Prince Maria PHILIP et al. (2021)	60 canines (120 specimens) (LS)	Group 1: 2.5% Sodium hypochlorite, Group 2: Miswak stick extract, Group 3: Cashew leaves extract. Group 4: Mango leaves extract and Group 5: Normal saline (control). (n = 20) , 5 ml of each irrigant for 10 min.	200 g 20 s dwell time.	Vickers + Flexural test	ANOVA + t-test	Microhardness and Flexural Strength Groups / Microhardness (Cervical) / Microhardness (Middle) / Microhardness (Apical) / Flexural Strength / P-value Normal Saline / 0.31 ± 0.02^ab / 0.30 ± 0.02^ab / 0.30 ± 0.01^ab / 1.77 ± 0.44^A / 0.067- Cashew / 0.28 ± 0.02^a / 0.28 ± 0.03^a / 0.29 ± 0.01^a / 4.61 ± 0.11^B / 0.167 Mango / 0.27 ± 0.01^a / 0.28 ± 0.01^a / 0.28 ± 0.02^a / 4.54 ± 0.12^B / 0.121 Miswak / 0.28 ± 0.01^a / 0.27 ± 0.01^a / 0.14 ± 0.02^a / 4.43 ± 0.27^B / 0.047* NaOCl / 0.11 ± 0.02^b / 0.10 ± 0.01^b / 0.13 ± 0.02^b / 3.27 ± 0.23^B / <0.001
4	Arslan et al. (2015)	42 Single-rooted, maxillary anterior teeth (LS)	Group / Protocol (Primary Irrigant + Agita 1 Distilled water (Control) 2 17% EDTA (5 ml) 3 17% EDTA (5 ml) + 60s Ultrasoni 4 17% EDTA (5 ml) + 10s Laser 5 17% EDTA (5 ml) + 20s Laser 6 17% EDTA (5 ml) + 30s Laser 7 17% EDTA (5 ml) + 40s Laser	50-g load for 15 s	Vickers	ANOVA	Procedure / Mean (%) / SD (%) / Range (Min–Max %) Distilled water / 4.40ᵃ / 4.39 / 0.00 – 12.67 17% EDTA / 20.21ᵇ / 3.37 / 14.19 – 26.65 Ultrasonic agitation / 23.63ᵇᶜ / 4.91 / 18.80 – 33.79 Laser agitation (10s) / 18.62ᵇ / 7.66 / 7.46 – 30.81 Laser agitation (20s) / 21.14ᵇ / 5.24 / 12.58 – 33.68 Laser agitation (30s) / 23.20ᵇᶜ / 5.09 / 15.97 – 28.34 Laser agitation (40s) / 27.85ᶜ / 5.26 / 13.92 – 34.70
5	Eskandarinezhad et al. (2015)	88 single rooted mandibular premolars (TS)	Group I: distilled water (control group) Group II: Triphala (15 min) Group III: 2% chlorhexidine, Group IV: 5.25% hypochlorite sodium.	200g 15s	Vickers	ANOVA + Tukey	Endodontic Irrigation Solution / Mean / SD / Statistical Significance Distilled water (Control) / 45.27 / 7.25 / Baseline / Comparison Triphala / 44.96 / 7.15 / No sig. difference from control 2% Chlorhexidine / 41.62 / 5.23 / - 5.25% Sodium Hypochlorite / 38.12 / 6.71 / Significantly decreased (p < 0.05$)
6	Bhagwat et al. (2016)	100 Single rooted teeth (LS)	Group I: distilled water for 20 min ,(control group) Group II: 1.3% NaOCl for 20 min 17% EDTA for 1 min Group III: 1.3% NaOCl for 20 min followed by 2% CHX digluconate for 5 min Group IV: 1.3% NaOCl for 20 min followed by 18% HEBP for 5 min Group V:1.3% NaOCl up to 20 min followed by 4% Propolis up to 5 min.	100 g 30 s.	Vickers	ANOVA + Tukey	Mean Micro-hardness of Dentin by Irrigating Solution Irrigating Solution / Mean (VHN) / Standard Deviation (SD) Distilled water / 55.996 / 2.990 HEBP / 55.643 / 6.945 Propolis / 49.769 / 4.078 CHX / 42.878 / 1.983 EDTA / 41.498 / 2.928
7	Zaki et al. (2017)	40 mandibular premolar teeth.(LS)	Group / Treatment / Substance i 0.5% *Z. officinale* Roscoe oil ii 2.5% Sodium hypochlorite (NaOCl) iii 17% Ethylenediaminetetraacetic ac iv Normal saline (5 min)	50 g , 10s	Vickers	ANOVA) with repeated measures and post hoc Tukey's HSD test to	Irrigant / Baseline / Final / Mean Diff. / Significance (p-value) 0.5% *Z. officinale* (Ginger) / 34.24 / 25.89 / 8.35 / < 0.001 (Significant) 17% EDTA / 33.75 / 26.04 / 7.72 / < 0.001 (Significant) 2.5% NaOCl / 31.47 / 26.34 / 5.13 / < 0.001 (Significant) 0.9% Normal Saline / 29.82 / 26.04 / 2.21 / 0.014 (Significant)
8	Saha et al. (2017)	80 mandibular premolars. (LS)	(n = 20, 15 Minutes Duration) Treatment / Substance Sodium Hypochlorite (NaOCl) Ethylenediaminetetraacetic Acid (EDTA)* Chitosan Morinda citrifolia Juice (MCJ)	300 gm load and dwell time of 20 seconds	Vickers	ANOVA + Tukey	Mean Micro-hardness of Dentin by Irrigant Irrigating Solution / Mean (VHN) ± SD 6% *Morinda citrifolia* juice (MCJ) / 56.91 ± 2.11 3% Sodium Hypochlorite (NaOCl) / 55.15 ± 1.86 0.2% Chitosan / 44.65 ± 3.19 17% EDTA / 43.12 ± 2.51
9	Ladhani et al. (2017)	75 mandibular premolars (LS)	**Experimental Groups and Irrigation Protocols** Group / Activation Method / Irrigant Subgroups / Procedure 1 / Negative Control / Distilled Water / 60s irrigation; dried with paper points. 2 / No Activation (Positive Control) / (1) 5.25% NaOCl(2) 17% EDTA(3) 2% CHX / 60s irrigation with respective irrigant; 60s final saline rinse. 3 / Manual Dynamic Activation / (1) 5.25% NaOCl(2) 17% EDTA(3) 2% CHX / 60s irrigation and agitation with F3 GP point; 60s final saline rinse. 4 / Diode Laser Activation / (1) 5.25% NaOCl(2) 17% EDTA(3) 2% CHX / 2W continuous waves for 10s (repeated 5x, total 50s); 60s final saline rinse. 5 / Nd:YAG Laser Activation / (1) 5.25% NaOCl(2) 17% EDTA(3) 2% CHX / 2W continuous waves for 10s (repeated 5x, total 50s); 60s final saline rinse.	coronal, middle and apical third, 100g load	Vickers	ANOVA + Tukey	Group / NaOCl / EDTA / CHX Group I / 67 / 68 / 69 Group II / 60 / 62 / 65 Group III / 58 / 60 / 64 Group IV / 42 / 48 / 53 Group V / 44 / 47 / 54
10	Khallaf et al. (2020)	Out of 100 freshly extracted teeth with single root. (**Fifty teeth were used**) (LS)	group 1, saline; group 2, Moringa oleifera; group 3, Moringa oleifera + chlorhexidine; group 4, chlorhexidine; group 5, sodium hypochlorite	300 g load for 15 s	Vickers	Repeated measures ANOVA	Mean Hardness by Canal Third and Group Group / Apical (Mean ± SD) / Middle (Mean ± SD) / Coronal (Mean ± SD) / Intragroup p-value 1 / 60.57 ± 3.16 / 63.73 ± 2.85 / 73.10 ± 12.74 / 0.292 (NS) 2 / 83.90 ± 5.01 / 79.03 ± 9.92 / 71.30 ± 3.02 / 0.304 (NS) 3 / 95.60 ± 7.61 / 65.33 ± 5.10 / 87.33 ± 7.15 / 0.025* 4 / 99.17 ± 2.36 / 89.23 ± 6.22 / 82.87 ± 12.97 / 0.295 (NS) 5 / 61.37 ± 2.95 / 72.30 ± 2.15 / 76.77 ± 3.24 / 0.028* Intergroup p / < 0.001* / 0.002* / 0.228 (NS)

<table>
<tr><td>11</td><td>Abuldris et al. (2020)</td><td>Out of 64 , 20 mandibular single rooted premolars used</td><td>5 ml of each irrigant for 5 minutes.
Group I: 2% Turmeric extract. Group II: 2% Thymus vulgaris oil. Group III: 5.25% NaOCl. Group IV: 2% CHX</td><td>200 gm 15 s</td><td>Vickers</td><td>ANOVA + Kruskal-Wallis</td><td>
<table>
<tr><td>Time Interval</td><td>2% Turmeric (Group I)</td><td>2% Thymus (Group II)</td><td>5.25% NaOCl (Group III)</td><td>2% CHX (Group IV)</td><td>P-Value</td></tr>
<tr><td></td><td>Mean ± SD</td><td>Mean ± SD</td><td>Mean ± SD</td><td>Mean ± SD</td><td></td></tr>
<tr><td>Day 1</td><td>39.56 ± 5.88</td><td>44.24 ± 19.90</td><td>42.13 ± 9.37</td><td>34.49 ± 5.55</td><td>0.599</td></tr>
<tr><td>Day 3</td><td>60.58 ± 2.12</td><td>44.65 ± 7.77</td><td>57.89 ± 7.73</td><td>61.81 ± 5.53</td><td>0.002*</td></tr>
<tr><td>Day 7</td><td>48.63 ± 9.61</td><td>54.39 ± 6.22</td><td>46.83 ± 11.54</td><td>61.87 ± 16.72</td><td>0.209</td></tr>
</table></td></tr>
<tr><td>12</td><td>Alsayed et al. (2021)</td><td>30 premolars (LS)</td><td>(n = 10);
Group I (90% Aloe vera), Group II (4% propolis) and Group III (2.6% NaOCl).</td><td>200-g load for 15</td><td>Vickers</td><td>ANOVA + Tukey</td><td>
<table>
<tr><td>Root section</td><td>Propolis (mean±SD)</td></tr>
<tr><td>Coronal</td><td>24.13±2.55</td></tr>
<tr><td>Middle</td><td>20.09±3.14</td></tr>
<tr><td>Apical</td><td>19.94±3.99</td></tr>
<tr><td>Overall</td><td>19.08±4.70</td></tr>
</table></td></tr>
<tr><td>13</td><td>Gondi et al. (2021)</td><td>60 single rooted teeth (LS)</td><td>Group I (n =12): Normal saline (negative control group)
Group II (n = 12): 2.5% Sodium hypochlorite (positive control group).

Group III (n = 12): A. indica (AI). Group IV (n = 12): M. citrifolia (MC). Group V (n = 12): Green tea (GT).
5 mL of irrigating solution for 5 min.</td><td>50-g ,10 seconds</td><td>Vickers</td><td>Wilcoxon + Mann-Whitney</td><td>Comparison of Microhardness Values (Wilcoxon Signed-Ranks Test)
<table>
<tr><td>Groups</td><td>Initial Microhardness (mean ± SD)</td><td>Posttreatment Microhardness (mean ± SD)</td><td>Change (mean ± SD)</td></tr>
<tr><td>Group I: Saline</td><td>59.49 ± 5.12</td><td>58.76 ± 4.64</td><td>0.73 ± 4.15</td></tr>
<tr><td>Group II: 3% NaOCl</td><td>61.61 ± 5.29</td><td>56.43 ± 5.52</td><td>5.18 ± 5.06</td></tr>
<tr><td>Group III: AI</td><td>60.21 ± 4.63</td><td>60.18 ± 4.63</td><td>0.03 ± 4.63</td></tr>
<tr><td>Group IV: MCJ</td><td>57.28 ± 2.77</td><td>52.08 ± 3.07</td><td>5.20 ± 2.77</td></tr>
<tr><td>Group V: Green tea</td><td>59.63 ± 4.52</td><td>59.28 ± 4.51</td><td>0.35 ± 4.52</td></tr>
</table>
*p < 0.05 (significant), **p > 0.05 (not significant)</td></tr>
<tr><td>14</td><td>Elika et al. (2021)</td><td>40 single rooted teeth (LS)</td><td>Four groups (n=20), follows:
Group 1 - Saline; (15 min)
Group 2 - 5% NaOCl +17% EDTA;
Group 3 - Triphala; 5%
Group 4 – Chloroquick</td><td>200 g load 20 s</td><td>Vickers</td><td>ANOVA , post hock tukey (intercomparision)</td><td>
<table>
<tr><td>Groups</td><td>Timepoint</td><td>Frequency</td><td>Mean ± S.D.</td><td>P-value</td></tr>
<tr><td>Group 1</td><td>Pre-treatment</td><td>20</td><td>55.98 ± 3.94</td><td>0.447**</td></tr>
<tr><td></td><td>Post-treatment</td><td>20</td><td>55.07 ± 4.15</td><td></td></tr>
<tr><td>Group 2</td><td>Pre-treatment</td><td>20</td><td>54.03 ± 5.88</td><td><0.001*</td></tr>
<tr><td></td><td>Post-treatment</td><td>19</td><td>48.00 ± 5.32</td><td></td></tr>
<tr><td>Group 3</td><td>Pre-treatment</td><td>20</td><td>47.40 ± 5.53</td><td><0.001*</td></tr>
<tr><td></td><td>Post-treatment</td><td>20</td><td>43.60 ± 5.95</td><td></td></tr>
<tr><td>Group 4</td><td>Pre-treatment</td><td>20</td><td>43.46 ± 4.43</td><td><0.001*</td></tr>
<tr><td></td><td>Post-treatment</td><td>20</td><td>38.80 ± 4.90</td><td></td></tr>
</table></td></tr>
<tr><td>15</td><td>Kilden (2022)</td><td>100 premolars (LS)</td><td>
<table>
<tr><td>Group</td><td>Treatment / Photosensitizer</td><td>Irrigation / Pre-Treatment Protocol</td><td>Activation Source (Wavelength)</td><td>Application Parameters</td></tr>
<tr><td>NE</td><td>NaOCl (2.5%) & EDTA (17%)</td><td>5 ml NaOCl (1 min), 5 ml EDTA (1 min), Distilled Water (1 min)</td><td>N/A</td><td>N/A</td></tr>
<tr><td>MB</td><td>Methylene Blue (50 mg/l)</td><td>5 min dark incubation; 1 min ultrasonic agitation</td><td>Diode Laser (660 nm)</td><td>100 mW, 90 s, 9J total dose, 320 J/cm² energy density</td></tr>
<tr><td>CUR</td><td>Curcumin (500 mg/l)</td><td>5 min dark incubation; 1 min ultrasonic agitation</td><td>Blue LED (480 nm)</td><td>1000 mW/cm², 60 s</td></tr>
<tr><td>TBO</td><td>Toluidine Blue O (100 mg/l)</td><td>5 min dark incubation; 1 min ultrasonic agitation</td><td>Diode Laser (660 nm)</td><td>220 mW, 60 s, 13.2J total dose</td></tr>
<tr><td>DS</td><td>None</td><td>No disinfection protocol used</td><td>N/A</td><td>N/A</td></tr>
</table></td><td>300 g of (15 seconds)</td><td>Vickers</td><td>one-way ANOVA and Dunnett's post hoc tests</td><td>Distilled water - 49,06
NaOCl/EDTA - 44,30
Toluidine blue O - 51,69
Curcumin - 53,23
Methylene blue - 51,91</td></tr>
<tr><td>16</td><td>Farooq et al. (2022)</td><td>90 single rooted teeth (LS)</td><td>
<table>
<tr><td>Group</td><td>Treatment / Material</td><td>Type</td></tr>
<tr><td>Group A</td><td>Ethanolic extract of Sapindus mukorossi</td><td>Experimental</td></tr>
<tr><td>Group B</td><td>17% Ethylenediamine tetraacetic acid (EDTA)</td><td>Experimental</td></tr>
<tr><td>Group C</td><td>Distilled Water</td><td>Control</td></tr>
</table></td><td>300 gm, 10 seconds</td><td>Vickers</td><td>Wilcoxon</td><td>Table 1: Before and After Irrigation Microhardness Comparison
(Wilcoxon Signed Rank)
<table>
<tr><td>Group</td><td>Before [Mean (SD)]</td><td>After [Mean (SD)]</td><td>p-value</td></tr>
<tr><td>A: Sapindus mukorossi</td><td>60.14 (0.63)</td><td>60.07 (0.49)</td><td>0.54</td></tr>
<tr><td>B: 17% EDTA</td><td>60.26 (0.74)</td><td>56.62 (0.72)</td><td>< 0.0001*</td></tr>
<tr><td>C: Distilled Water</td><td>60.46 (0.40)</td><td>60.45 (0.35)</td><td>0.88</td></tr>
</table>
Table 2: Post-Irrigation Inter-Group Comparison
(Mann-Whitney)
<table>
<tr><td>Comparison</td><td>Mean (SD)</td><td>p-value</td></tr>
<tr><td>Group A vs Group B</td><td>60.07 (0.49) vs 56.62 (0.72)</td><td>< 0.001*</td></tr>
<tr><td>Group A vs Group C</td><td>60.07 (0.49) vs 60.45 (0.35)</td><td>0.002*</td></tr>
<tr><td>Group B vs Group C</td><td>56.62 (0.72) vs 60.45 (0.35)</td><td>< 0.001*</td></tr>
</table></td></tr>
<tr><td>17</td><td>Koparde et al. (2024)</td><td>8 Mandibular premolars (LS)</td><td>Group 1 –5 mL of 6.5% GSE for 2 min
Group 2 –5 mL of distilled water for 2 min</td><td>300 g load 20 s</td><td>Vickers</td><td>t-test + Kruskal</td><td>
<table>
<tr><td>Sample</td><td>1)Coronal</td><td>Middle</td><td>Apical</td><td>Average</td><td>Sample</td><td>(2)Coronal</td><td>Middle</td><td>Apical</td><td>Average</td></tr>
<tr><td>1</td><td>76</td><td>80</td><td>80</td><td>79</td><td>1</td><td>42</td><td>32</td><td>27</td><td>34</td></tr>
<tr><td>2</td><td>82</td><td>87</td><td>84</td><td>84</td><td>2</td><td>44</td><td>45</td><td>43</td><td>44</td></tr>
<tr><td>3</td><td>80</td><td>82</td><td>85</td><td>82</td><td>3</td><td>46</td><td>47</td><td>48</td><td>47</td></tr>
<tr><td>4</td><td>89</td><td>87</td><td>88</td><td>88</td><td>4</td><td>48</td><td>50</td><td>50</td><td>49</td></tr>
<tr><td>5</td><td>80</td><td>80</td><td>83</td><td>81</td><td>5</td><td>52</td><td>54</td><td>51</td><td>52</td></tr>
<tr><td>6</td><td>81</td><td>78</td><td>77</td><td>79</td><td>6</td><td>55</td><td>57</td><td>55</td><td>56</td></tr>
<tr><td>7</td><td>81</td><td>81</td><td>77</td><td>80</td><td>7</td><td>57</td><td>59</td><td>55</td><td>57</td></tr>
<tr><td>8</td><td>94</td><td>92</td><td>96</td><td>94</td><td>8</td><td>59</td><td>59</td><td>57</td><td>58</td></tr>
</table></td></tr>
<tr><td>18</td><td>Patil et al. (2025)</td><td>30 mandibular premolars (LS)</td><td>G1 – 3% NaOCl, G2 – 3% NaOCl + diode laser irradiation, G3 – 17% EDTA, G4 – 17% EDTA solution + diode laser irradiation, G5-Normal saline (control group). 5 min , 2ml</td><td>50 g for 10 s</td><td>Vickers</td><td>ANOVA + Tukey</td><td>Table 1: Microhardness Values of the Groups
<table>
<tr><td>Group</td><td>n</td><td>Mean</td><td>SD</td></tr>
<tr><td>3% NaOCl</td><td>6</td><td>64.47</td><td>6.88</td></tr>
<tr><td>3% NaOCl + Diode Laser</td><td>6</td><td>63.12</td><td>7.75</td></tr>
<tr><td>17% EDTA</td><td>6</td><td>56.65</td><td>4.61</td></tr>
<tr><td>17% EDTA + Diode Laser</td><td>6</td><td>53.4</td><td>3.99</td></tr>
<tr><td>Normal Saline (Control)</td><td>6</td><td>67.53</td><td>3.11</td></tr>
</table>
Table 2: Pairwise Comparison of Hardness
<table>
<tr><td>Comparison</td><td>Mean Difference</td><td>P-value</td></tr>
<tr><td>NaOCl vs. NaOCl + Laser</td><td>1.35</td><td>0.993</td></tr>
<tr><td>NaOCl vs. EDTA</td><td>7.82</td><td>0.139</td></tr>
<tr><td>NaOCl vs. EDTA + Laser</td><td>11.07</td><td>0.016*</td></tr>
<tr><td>NaOCl vs. Saline</td><td>-3.07</td><td>0.872</td></tr>
<tr><td>NaOCl + Laser vs. EDTA</td><td>6.47</td><td>0.288</td></tr>
<tr><td>NaOCl + Laser vs. EDTA + Laser</td><td>9.72</td><td>0.041*</td></tr>
<tr><td>NaOCl + Laser vs. Saline</td><td>-4.42</td><td>0.648</td></tr>
<tr><td>EDTA vs. EDTA + Laser</td><td>3.25</td><td>0.847</td></tr>
<tr><td>EDTA vs. Saline</td><td>-10.88</td><td>0.018*</td></tr>
<tr><td>EDTA + Laser vs. Saline</td><td>-14.13</td><td>0.001*</td></tr>
</table></td></tr>
</table>

19	Pandia et al. (2025)	24 single rooted Premolars (LS)	(n=20) Group / Treatment Material / Concentration / Duration Group 1: Propolis, 8%, 5 minutes Group 2: Propolis, 20%, 5 minutes Group 3: Propolis, 30%, 5 minutes Group 4: NaOCl, 2.5%, 5 minutes Group 5: NaOCl, 5%, 5 minutes Group 6: 17% EDTA, 17%, 5 minutes	200g ,20 s	Vickers	ANOVA / Kruskal	Group / Percentage Decrease (Mean ± SD) 800 Propolis 3.68 ± 0.48 2000 Propolis 7.17 ± 0.53 3000 Propolis 10.27 ± 1.09 2.500 NaOCl 9.00 ± 0.35 500 NaOCl 11.48 ± 0.49 1700 EDTA 14.63 ± 0.58 Group / n / Mean / P-Value (ANOVA) 800 Propolis 8 3.68 0.001* 2000 Propolis 8 7.17 3000 Propolis 8 10.27 2.500 NaOCl 8 9.00 500 NaOCl 8 11.48 1700 EDTA 8 14.63
20	Murali et al	20 premolars	Groups - 5 mL 3% NaOCl 7% EDTA (Smear Clear) (Fig. 6) for 1 minute (PTN) - 5 mL green tea polyphenol (K Patel Pvt Ltd, India) (60 mg/mL in 10% DMSO) for 1 minute (PTN) - 5 mL 3% NaOCl - 5 mL green tea polyphenol)	50 g force for 14 second	Vickers		1.PTN 2.5% NaOCl + 17% EDTA 5 51.11 42.82 6.64002 5.81287 - - 2. PTN-Green tea 5 51.04 47.23 7.13822 7.02765 3.TF-2.5% NaOCl + 17% EDTA 5 8.94 40.37 1.06196 1.00912 4. TF-Green tea 5 48.78 43.59 3.31881 2.99811
21.	Mostaz T et al 2024	45 mandibular premolars	Group 1: saline (negative control), group 2: 5.25 % NaOCl (positive control), group 3: 10 % Miswak, group 4: 15 % Miswak, group 5: 20 % Miswak]	50 gm 10 sec	Vickers	One way Anova,post hock tukey	Groups / Before (Mean ± SD) / After (Mean ± SD) / P-value (ψ) Saline 62.736 ± 3.312 / 62.364 ± 2.921 (a) / 0.125 Sodium Hypochlorite 62.594 ± 2.953 / 54.937 ± 3.936 (b) / 0.002* 1000 Miswak 62.016 ± 2.035 / 60.782 ± 1.943 (a) / 0.327 1500 Miswak 62.082 ± 2.027 / 61.753 ± 1.946 (a) / 0.427 2000 Miswak 62.304 ± 2.638 / 62.264 ± 2.737 (a) / 0.570 P-value (V) 0.942 / 0.046*

Methodological quality assessment via the QUIN tool (figure 2,) revealed that while most studies maintained a low risk of bias in objectives, methodology, randomization, and statistical reporting. An unclear risk was frequently observed regarding sample size calculations, sampling techniques, and operator details due to incomplete reporting across several trials. Most significantly, the highest risk of bias was identified in blinding and outcome assessor details, which potentially introduces performance and detection bias, thereby limiting the internal validity of the findings.Although the included studies were suitable for synthesis with quality scores between 79% and 92%, the reliability of their effect estimates is assessed by these specific reporting deficiencies and a general lack of rigorous blinding protocols.

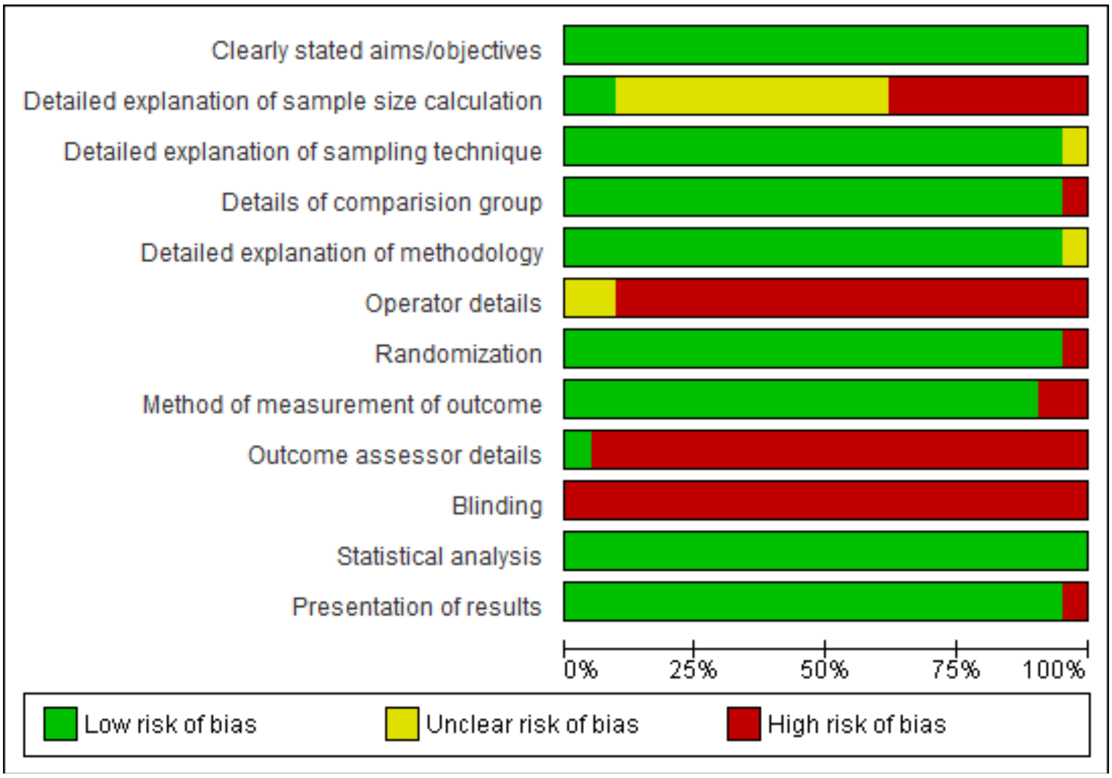

Figure 2 Quality assessment of included in vitro studies using the QUIN tool.

Risk of Bias in Included Studies

The risk of bias assessment using the QUIN tool with 12 items categorized twelve studies as low risk, medium risk , unclear risk.(Figure 3).

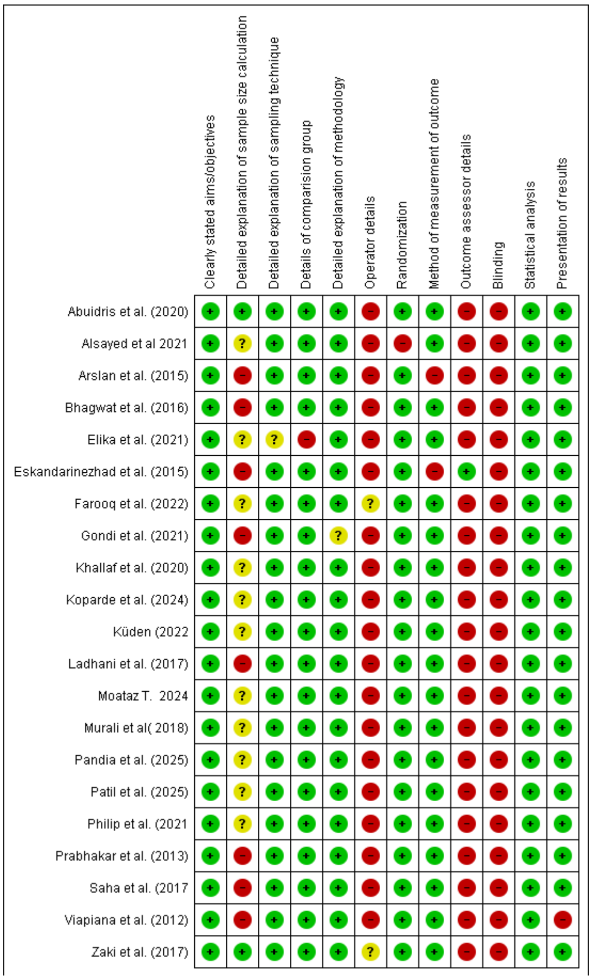

Figure 3: Risk of bias for each included study using the QUIN tool

3.3 Meta-analysis

Meta analysis was performed by sequentially excluding individual studies to explore sources of heterogeneity. Standardized mean difference (SMD) was used as the effect measure to account for variations in measurement scales and baseline values across studies. A random-effects model was used to account for expected heterogeneity.

Methodological variability : Considerable methodological variability was observed among the included studies, particularly in terms of applied load (ranging from 100 g to 300 g), type of microhardness indenter (Vickers), irrigation duration, and laser activation protocols. These variations may influence the measured dentin microhardness and contribute to heterogeneity.

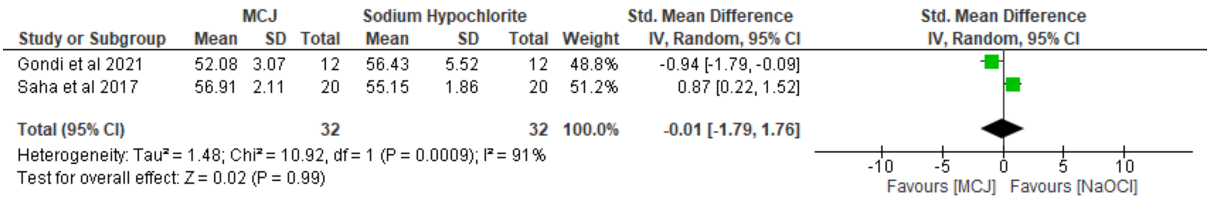

Figure 1: Forest plot of comparison: 1 MCJ vs Sodium hypochlorite, outcome: 1.1 MCJ vs Sodium hypochlorite on Dentin Microhardness.

Forest plot showing the comparison of Morinda citrifolia juice (MCJ) and sodium hypochlorite (NaOCl) on dentin microhardness using a random-effects model across included studies.

Inference: The meta-analysis showed **no statistically significant difference** between MCJ and sodium hypochlorite in terms of dentin microhardness (MD = −1.06; 95% CI: −7.02 to 4.91; P = 0.73).However, **high heterogeneity (I^2 = 90%)** indicates substantial variability between the included studies, suggesting that results should be interpreted with caution.

Forest plot showing the comparison of Triphala and sodium hypochlorite (NaOCl) on dentin microhardness using a random-effects model across included studies.

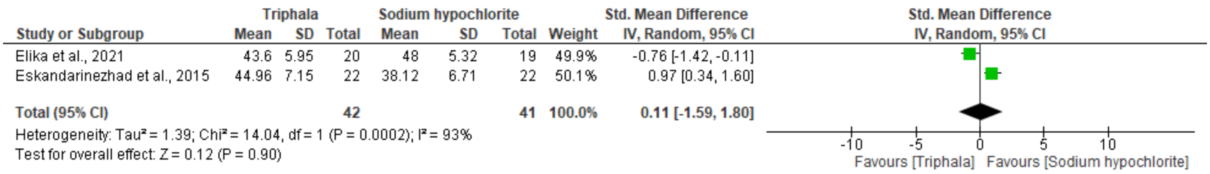

Figure 2: Forest plot of comparison: 2 Triphala vs Sodium hypochlorite, outcome: 2.1 Triphala vs Sodium hypochlorite on Dentin Microhardness.

Inference: The meta-analysis showed **no statistically significant difference** in dentin microhardness between Triphala and sodium hypochlorite (MD = 1.17; 95% CI: −9.84 to 12.19; P = 0.83). However, **very high heterogeneity (I² = 94%)** indicates considerable variability between studies, and the results should be interpreted with caution.

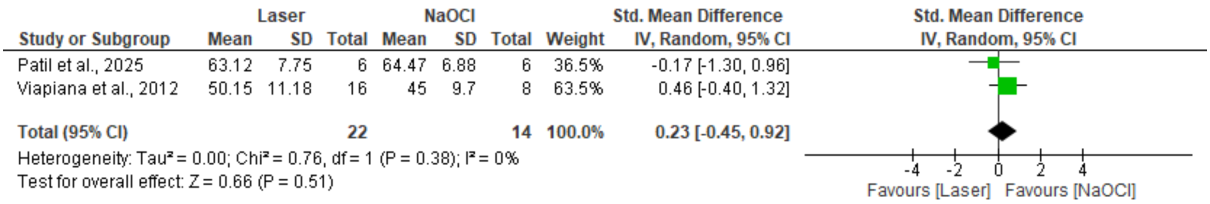

Figure 3: Forest plot of comparison: 3 NaOCl vs LASER, outcome: 3.1 NaOCl vs LASER on Dentin Microhardness.

Forest plot showing the effect of laser-assisted irrigation compared to sodium hypochlorite (NaOCl) on dentin microhardness using a random-effects model across included studies with different laser wavelengths and activation protocols.

Inference: The meta-analysis showed **no statistically significant difference** in dentin microhardness between laser-assisted irrigation and sodium hypochlorite (MD = 1.77; 95% CI: −4.59 to 8.14; P = 0.59).Low heterogeneity (I² = 11%) indicates good consistency between studies, suggesting reliable but non-significant findings.

DISCUSSION

4.1. Sample Selection and Preparation

To ensure consistency, the inclusion criteria across the studies focused almost exclusively on human extracted permanent teeth, with a strong preference for single-rooted teeth such as premolars and canines[1-4].This selection is clinically relevant because single-rooted teeth provide straight forward canal anatomy, reducing the variables associated with complex root configurations during irrigation and laser activation. For instance, Viapiana et al. (2012) and Philip et al. (2021) utilized single-canal canines, while several authors, including Saha et al. (2017) and Patil et al. (2025), specifically have chosen mandibular premolars.[3,15,20]

4.2 Sample Size and Statistical Power

The total number of teeth used across the studies varied widely, from as few as eight specimens in smaller pilot studies (Koparde et al., 2024) to as many as 100 teeth in more comprehensive evaluations (Bhagwat et al., 2016; Küden et al., 2022). Common group sizes ranged from n=6 to n=20 per intervention.[12,24,25].Conversely, larger sample sizes, such as those by Farooq et al. (2022) with 90 teeth, provide more required data regarding the non-significant impact of natural extracts like Sapindus mukorossi compared to the aggressive demineralization of 17% EDTA.[25,30]

4.3 Specimen Preparation and Sectioning Techniques

Tooth sectioning is pivotal for exposing the dentin surface for microhardness testing. The sources indicated a clear preference for longitudinal sectioning (LS) over transverse sectioning (TS)..This approach allowed for the evaluation of microhardness along the entire length of the root canal, facilitating a regional analysis of the apical, middle, and coronal thirds. This is essential because the density and diameter of dentinal tubules vary by region, affecting how irrigants such as 5.25% NaOCl, herbal solutions, or lasers interact with the tooth structure.Only a few studies, such as Eskandarinezhad et al, utilized transverse sections to measure the hardness at specific horizontal planes[11]. The preparation protocols typically involved embedding the sections in self-curing acrylic resin, followed by sequential polishing to create a smooth, reflective surface necessary for accurate Vickers or Knoop indentation. By standardizing these preparation steps, researchers have minimized the risk of surface irregularities interfering with the outcome measures.

4.4 Microhardness Testing Tools

Dentin microhardness serves as a critical indicator of the structural integrity of the root canal system in the evaluation of Endodontic irrigants.These tools allow researchers to quantify the degree of demineralization or organic matrix degradation caused by synthetic chemicals,herbal extracts and laser activation.The Vickers hardness serves as the primary tool for broad comparative analysis while the Knoop hardness test provides the specialised depth dependent data.

4.4.1 Vickers Hardness Testing:

The outcomes are typically reported as Vickers hardness number[6,19,20,21]. It employed a square-based diamond pyramid indenter to create precise indentations on the dentin surface. The applied load in these studies ranged from 50 g (used by Arslan et al., Gondi, and Patil) to 300 g (used by Saha et al., Khallaf, and Koparde)[15,18,25]. The most common dwell times recorded were 10 seconds[5], 15 seconds[6], and 20 seconds[9].

4.4.2 Knoop Hardness Testing

Although less frequently reported in this review, the Knoop Hardness Test has been employed in studies focusing on depth-dependent microhardness. Viapiana et al. (2012) utilized Knoop Microhardness with a 25 g load and a 10-second dwell time to measure changes at precise depths of 30, 90, 150, and 300 µm from the canal surface[3].

4.4.3. Comparative Reliability and Outcome Measures

The tools used in this study helped compare traditional and herbal irrigants for teeth. They showed that 17% EDTA and 5.25% NaOCl had decrease in hardness values[1-3]. Arslan et al. found that using a laser with 17% EDTA for 40 seconds showed the reduced hardness ,when compared with herbal solutions such as Miswak did not alter the hardness of teeth[10,20].

5. EFFECT OF CONTACT TIME OF IRRIGATING SOLUTIONS ON DENTIN MICROHARDNESS

The contact time of irrigating solutions is a critical factor in endodontic treatment, as prolonged exposure to irrigants can significantly reduce dentin microhardness and compromise tooth structural strength and its mineral loss. The reduction in dentin integrity is a multi-variable function of chemical concentration and exposure duration. Synthesis of the data suggests that chemical concentration often outweighs contact time in the microhardness equation. For instance,increasing Propolis concentration

5.3 Laser and Activation Times

When laser activation is used within a total irrigation period (e.g., 2 min), the duration of agitation directly impacts the outcome. Arslan et al. (2015) demonstrated that while 10 seconds of laser agitation with EDTA caused an 18.62% reduction, increasing the agitation to 40

from 8% to 30% nearly tripled the microhardness reduction despite identical exposure times.Conversely, even short-duration exposure (120s) in resulted in a ~20% reduction when combined with EDTA, highlighting the aggressive nature of the chelating efficiency over time-dependent factors[14,18,27].

5.1Traditional and Synthetic Irrigants

Even a very short contact time of 1 min with 17% EDTA was sufficient to cause a significant reduction in microhardness (approximately 41.5 VHN).At a 5-minute interval,17% EDTA resulted in a 14.63% decrease based on Viapiana et al. (2012), and 5% NaOCl resulted in an 11.48% decreased in microhardness .Some protocols utilized NaOCl for up to 20 min, which consistently lead to significant demineralization and organic matrix degradation. Based on Eskandarinezhad et al., 15 min of 5.25% NaOCl also showed a significant decrease in microhardness compared to the baseline[11].

5.2 Herbal Irrigants

Many herbal irrigants maintained dentin integrity over longer durations. For instance, Miswak, Cashew, and Mango leaf extracts showed no significant reduction in microhardness after 10 min of contact. Philip et al. (2021) and Prabhakar et al. (2013) found no significant difference from the baseline after 15 min of contact with 6% Morinda citrifolia juice (MCJ) or 0.2% CHX[6,26]. However, other studies have found that a 15-minute exposure to 3% NaOCl or 6% MCJ significantly decreased microhardness, indicating that the impact can vary based on the specific concentration and study conditions.[24,25]After 2 min of contact with 6.5% Grape Seed Extract (GSE), microhardness levels were significantly higher than those after contact with distilled water[6,20].

seconds resulted in a much higher 27.85% reduction.Diode and Nd:YAG laser protocols often involve short, repeated bursts (e.g., five 10-second cycles for a total of 50 seconds), which are sufficient to cause the greatest reduction in microhardness across all canal thirds when compared to manual irrigation[10].

Table 5: Effect of Contact Time of Irrigating Solutions on Dentin Microhardness

Contact Time	Irrigant Example	Effect on Microhardness
50–60 Seconds	17% EDTA / NaOCl (Laser)	**Greatest reduction** compared to manual.
1 Minute	17% EDTA	**Significant reduction** (~41.5 VHN).
2 Minutes	6.5% GSE	**Maintained integrity** (Avg 82 VHN).
5 Minutes	17% EDTA / 5% NaOCl	**Significant decrease** (11–14%).
10 Minutes	Miswak / Mango / Cashew	**No significant change**.
15 Minutes	6% MCJ / 0.2% CHX	**No significant difference**
20 Minutes	1.3% NaOCl	**Significant reduction**.

Table 6: Effect of various activation methods on Dentin Microhardness

Irrigant Category	Activation Method	Impact on Microhardness
Herbal (Miswak, Neem)	Manual	**No significant change**; preserves integrity.
Herbal (Triphala, GSE)	Manual	**Significant preservation** compared to synthetic controls.
Herbal (Propolis, Ginger)	Manual	**Significant reduction** (3.6% – 10.2%).
Synthetic (EDTA, NaOCl)	Manual	**Significant reduction** (9% – 20%).
Synthetic (EDTA, NaOCl)	**Laser/Ultrasonic**	**Highest reduction** (up to 27.85%).
Herbal-PDT (Curcumin)	**Blue LED**	**Moderate reduction**; safer than EDTA/NaOCl.

6. EFFECT OF VARIOUS IRRIGANTS ON MICROHARDNESS OF ROOT CANAL DENTIN

6.1Effect of Sodium hypochlorite on microhardness of root canal dentin

Low Concentrations (1% – 2.5%) : Philip et al. (2021) and Gondi et al. (2021) reported that 2.5% NaOCl significantly decreased microhardness compared to herbal extracts and saline (p<0.001)[20,27].Pandia et al. (2025) discovered that using 2.5% NaOCl led to a 9.00% reduction in microhardness[27].

Moderate Concentrations (3%): Studies demonstrating that 3% NaOCl solutions significantly reduce hardness (p<0.05) reported by Patil et al[28].

High Concentrations (5% – 5.25%) : The 11.48% decrease in microhardness of 11.48% when using 5% NaOCl is based on Pandia et al. (2025) and Eskandarinezhad et al. (2015).The consistent reporting of significant decline in Vickers Hardness Numbers for 5.25% NaOCl, specifically a decrease from a baseline of 62.594 to 54.937 (p=0.002), is documented by Moataz T. Hekal (2024)[29].

6.2 NaOCl with Laser Activation

The addition of laser activation (such as Diode or Nd: YAG) to NaOCl irrigation can lead to a greater reduction in microhardness values across all canal thirds compared to manual irrigation. However, the synergistic effect may vary with 3% NaOCl ,Diode Laser activation resulted in a mean hardness of 63.12 VHN compared to 64.47 VHN for 3% NaOCl alone with a non-significant p-value of 0.993.The study further noted that while the NaOCl combinations were relatively conservative, the combination of 17% EDTA and Diode Laser resulted in significantly lower mean microhardness values (53.4 VHN) than both the NaOCl groups and the saline control[28].

6.3 Effect of EDTA on dentin microhardness

Bhagwat et al. (2016) found that the time dentin is exposed to 1 minute of contact with EDTA can lower dentin hardness to 41.498 VHN, compared to 55.996 VHN with distilled water.[12]The weakening is worse when with increased time to 5 minutes and also with agitation methods[27,28]. Pandia et al. reported that 17% EDTA reduced hardness by 14.63%, while 5% Sodium Hypochlorite (NaOCl) by 11.48%.[10,27] EDTA is significantly more detrimental to dentin hardness than most herbal alternatives, such as Miswak (Moataz T. et al., 2024), and Triphala (Eskandarinezhad et al., 2015), which generally showed no significant impact on the structural integrity of the tooth.

6.4 Herbal Irrigants

Several herbal extracts have been identified as "biocompatible" with dentin structure, maintaining microhardness levels comparable to saline or distilled water controls. Specific findings regarding 10%, 15%, and 20% concentrations of Miswak extract, as documented by Moataz T. Hekal (2024), indicated no significant impact on microhardness[29]. Additionally, Philip et al. (2021) evaluated Miswak stick extract and found it to be significantly less aggressive than sodium hypochlorite[20]. The "biocompatible" nature of Neem is supported by multiple studies, including Gondi et al. (2021), who reported no significant change (p = 0.109), between pre and post-treatment values. Evaluation by Gondi et al. (2021) showed that green tea extract did not cause a significant decrease in hardness compared to the baseline (p = 0.058)[22]. Farooq et al. (2022) reported that an ethanolic extract of Sapindus mukorossi showed no significant change (p = 0.34) between pre-irrigation (60.14 VHN) and post-irrigation (60.07 VHN) levels[22,30]. The herbal extracts of Mango and Cashew Leaf Extracts were found by Prince Maria Philip et al. (2021) to maintain dentin integrity over 10-minute contact periods, with p-values (0.121 and 0.167 respectively) indicating no significant reduction in microhardness[20].

6.5 Herbal Irrigants with Significant Impact

Pandia et al. (2025).evaluated the percentage decrease in microhardness and found that while lower concentrations (8% and 20%) had smaller impacts, 30% Propolis caused a significant 10.27% reduction over a 5-minute duration[27]. Zaki et al. in the experimental study, demonstrated that 0.5% *Z. officinale* Roscoe oil showed a baseline microhardness of 34.24 VHN and a final microhardness of 25.89 VHN, resulting in a statistically significant mean difference of 8.35 VHN (p<0.001)[13].

Prabhakar et al. (2013) reported that irrigation with 6% MCJ for 15 minutes resulted in a non-significant mean difference of 2.98 VHN (p = 0.44)[6]. In contrast, Gondi et al. (2021) observed a significant change of 5.20 VHN (p = 0.002), et al[22]. Saha et al. (2017) noted that although MCJ was less aggressive than EDTA, it still resulted in a final mean hardness of 56.91 VH[15]. The data regarding 6.5% Grape seed extract

maintaining a significantly higher average microhardness of 82.125 VHN compared to the distilled water control (46.875 VHN) were obtained from the study by Koparde et al.[25]

Table 7

Herbal Irrigant	Key Findings	Study Reference
Triphala	Showed no significant difference from distilled water control; preserved hardness better than 5.25% NaOCl.	Eskandarinezhad et al.
Propolis	Showed dose-dependent reduction: 8% Propolis (3.68% decrease) vs. 30% Propolis (10.27% decrease).	Pandia et al.
Neem (A.	No significant change in post-treatment microhardness compared to	Gondi et al.

indica)	baseline.	
Miswak	Results vary; some studies show significant apical microhardness reduction, while others show no significant difference from saline at 10-20% concentrations.	Philip et al.; Moataz T et al.
Green Tea	Minimal change in microhardness; not statistically significant compared to saline.	Gondi et al.
Sapindus mukorossi	No significant difference from baseline microhardness (60.14 vs 60.07 VHN).	Farooq et al.

SUMMARY

Effective Preservers : (Neem and Miswak)The Neem (A. indica) is supported by Gondi et al who reported no significant change in hardness[22].The findings for Miswak (S. persica) showing no significant impact at 10%–20% concentrations are from Moataz T. Hekal et al[29] and Philip et al[20].

Aggressive Herbals (Propolis and Ginger Oil):The aggressive nature of propolis (specifically a 10.27% decrease at 30% concentration) has been documented by Pandia et al.A significant reduction caused by ginger oil (mean difference of 8.35 VHN) was reported by Zaki et al[13],which are generally less aggressive than 17% EDTA (14.63%) based on Pandia et al[27] and Saha et al.[15]

6.6 The impact of laser activation can be detailed through the following key observations.

Ladhani et al. (2017) found that Diode and Nd: YAG laser activation of 5.25% NaOCl led to the greatest overall reduction in microhardness values across all canal thirds .Their research demonstrated that laser-assisted irrigation is significantly more aggressive compared to manual dynamic activation while laser-activated irrigation enhanced the irrigant's interaction with the dentin surface, facilitating deeper penetration into the tubules[16].

Kuden et al (2022) reported that when laser energy is used as an activation source for photodynamic therapy with photosensitizers like methylene blue or Toluidine blue the resulting microhardness values ranged from (51.69 to 51.91 VHN) are generally higher than those seen with traditional chemical sequences like NaOCl/EDTA (44.30 VHN).This suggests that laser use in PDT protocols may be less structurally damaging than laser-assisted activation[24]. Ladhani et al. (2017) showed the broad regional impact of laser-assisted irrigation.Patil et al. (2025) demonstrated that the combination of 17% EDTA and a Diode laser resulted in the lowest mean microhardness (53.4 VHN) recorded in their study[28].

LIMITATIONS

A primary concern is the high risk of bias in sample size calculation. Many studies lacked formal power analysis to justify specimen counts, particularly problematic in small pilot studies with as few as eight specimens. This omission affects determination of statistical power to detect microhardness changes. A significant limitation was the lack of complete numerical data across studies, precluding comprehensive meta-analysis for specific comparisons like laser-assisted irrigation versus sodium hypochlorite. Substantial experimental heterogeneity exists regarding laser parameters, irrigant concentrations, and exposure times. Different testing methodologies varying indentation loads (25g to 300g) and dwell times (10 to 30 seconds) complicated the direct result comparision. The focus on human permanent single-rooted teeth limits generalizability to multi-rooted configurations. As evidence comes from in vitro studies, findings may not fully replicate clinical conditions during Endodontic procedures.

RECOMMENDATIONS FOR FUTURE STUDIES

1. **Optimization of Herbal Concentrations**: Research should focus on optimizing the concentrations of herbal irrigants to enhance antimicrobial efficacy and smear layer removal while preserving dentin integrity.
2. **Standardization of Experimental Protocols**: It is imperative to standardize laser Parameters to establish optimal laser types, wavelengths, power settings, and durations, as exceeding 30-40 seconds results in increased microhardness loss. Testing Parameters should standardize indentation loads and dwell times for Vickers and Knoop hardness testing.
3. **Methodology and Statistical Power**: Research must report power analyses to justify the number of specimens to ensure sufficient sample sizes to detect changes in microhardness.
4. **Addressing Inconsistent Findings**: Further investigation is required for herbal irrigants and laser activation with inconsistent evidence, studies should aim to resolve conflicting results.
5. **Expansion of Study Scope**: Research should extend beyond single-rooted teeth to include, complex root configurations and multi-rooted teeth. Long-term mechanical evaluations, including sealer bonding and fracture resistance.

CONCLUSION

Traditional irrigants compromise the structural stability required for long-term tooth survival, while herbal irrigants such as Triphala, propolis, and Azadirachta indica exhibit a comparatively lesser reduction in the microhardness of root canal dentin when compared to conventional irrigants like sodium hypochlorite and EDTA. This suggests a potential advantage in preserving the structural integrity of dentin while maintaining acceptable biological properties.Herbal irrigants ,especially when used judiciously with controlled laser settings may represent a promising alternative in Endodontic irrigation protocols, though further standardized in vivo studies are required to establish definitive clinical recommendations.

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