Systematic Review
Herbal Agents versus Ethylene Diamine Tetra Acetic Acid on Removal of the Smear Layer—A Systematic Review of In Vitro Studies

Kavalipurapu Venkata Teja 1, Krishnamachari Janani 2,*, Abdullah Ali Alqahtani 3,*, Ali Robaian 3, Feras Alhalabi 3, Khalid A. Merdad 4, Mohammad Khursheed Alam 5,6,7,*, Deepti Shrivastava 5,*, Kumar Chandan Srivastava 9,*,

1 Department of Conservative Dentistry and Endodontics, Mamata Institute of Dental Sciences, Bachupally, Hyderabad 500090, Telangana, India; metejaendo@gmail.com
2 Department of Conservative Dentistry and Endodontics, SRM Institute of Science and Technology, SRM Dental College, Chennai 600089, Tamil Nadu, India
3 Department of Conservative Dental Sciences, College of Dentistry, Prince Sattam Bin Abdulaziz University, Alkharij 11942, Saudi Arabia; aa.alqahtani@psau.edu.sa (A.A.A.); ali.alqahtani@psau.edu.sa (A.R.); f.alhalabi@psau.edu.sa (F.A.)
4 Endodontic Department, Faculty of Dentistry, King Abdulaziz University, Jeddah 80200, Saudi Arabia; kmerdad@kau.edu.sa
5 Department of Preventive Dentistry, College of Dentistry, Jouf University, Sakaka 72345, Saudi Arabia; mkalam@ju.edu.sa
6 Center for Transdisciplinary Research (CFTR), Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600077, Tamil Nadu, India
7 Department of Public Health, Faculty of Allied Health Sciences, Daffodil International University, Dhaka 1205, Bangladesh
8 Private Practice, Ernakulam District, Aluva 683106, Kerala, India; jerryjosekavungal@gmail.com
9 Oral Medicine and Radiology, Department of Oral and Maxillofacial Surgery and Diagnostic Sciences, College of Dentistry, Jouf University, Sakaka 72345, Saudi Arabia

* Correspondence: jananik6@srmist.edu.in (K.J.); sdeepti20@gmail.com (D.S.); drkcs.omr@gmail.com (K.C.S.)

Abstract: This systematic review aimed to compare the efficacy of herbal agents with ethylene diamine tetraacetic acid (EDTA) in removing the smear layer during root canal instrumentation. The research question in the present study was to assess: “Is there a significant difference in reducing smear layer comparing EDTA and herbal agents?” Electronic databases (PubMed, Scopus, and Web of Science) were searched from their start dates to April 2022 using strict inclusion and exclusion criteria, and reviewed following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 guidelines. Only in vitro studies comparing herbal agents with EDTA were included in the current systematic review. Two reviewers independently assessed the included articles. A total of 625 articles were obtained from an electronic database. Eighteen papers were included for review of the full text, out of which, ten papers were excluded because they did not meet the inclusion criteria. Finally, eight articles were included in the systematic review. The present systematic review considered only in vitro studies; hence, the result cannot be completely translated to strict clinical conditions. The results of the present systematic review have shown that *quixabeira*, *morindacitrifolia*, *oregano* extract, and neem show better smear layer removal compared to other herbal agents, whereas they showed reduced smear layer removal when compared with EDTA. Although, it was seen that most of the included studies did not report a high quality of evidence. Hence, the present systematic review concludes that herbal agents have reported to show inferior smear layer removal when compared to EDTA. Thus, as far as herbal based alternatives are concerned, there is no highest level of evidence to state its real benefit when used as a chelating root canal irrigant.

Keywords: endodontics; disinfection; EDTA; herbal agents irrigants; smear layer; root canal treatment; natural components
1. Introduction

In endodontics, there is an enormous amount of literature on the use of conventional irrigants as root canal disinfectants. To target the complete elimination of microorganisms from the root canal, importance should be focused on the efficiency of chemo-mechanical disinfection. Eventually, during canal preparation, inadvertently, smear layer formation is bound to occur. The smear layer is considered an amorphous substance that consists of inorganic dentin, odontoblastic process, and the necrotic and viable pulp [1]. The smear layer can be of two types: it can either be forced on the superficial dentin or plugged into the dentinal tubules. The thickness of the superficial smear layer can be up to 2–5 \( \mu m \) [2–4].

Previous reports have shown that the smear layer tends to plug into dentinal tubules due to adhesive and capillary action, which hinders the efficiency of the irrigant and the sealing ability.

Moreover, these conventional antimicrobial agents, such as sodium hypochlorite, cannot remove the inorganic dentin layer from the root canal system. To overcome this, the use of chelating agents becomes mandatory, and for this purpose, various chelating agents, such as ethylene diamine tetra acetic acid (EDTA), maleic acid, and citric acid, have been employed [5,6]. EDTA is considered a conventional irrigant, as it is most commonly employed to eliminate the smear layer, but over the recent decade, there is literature stating that maleic acid possesses better clinical efficiency than EDTA [6]. Apart from chelating agents, studies have also shown that sodium hypochlorite can remove the smear layer [7].

To date, there has been no systematic review to assess the efficiency of smear layer removal of herbal agents in comparison with EDTA and sodium hypochlorite. Therefore, this systematic review was undertaken to investigate the effect of herbal irrigants compared to routinely used irrigants. This systematic review intends to address the research question on the efficiency of smear layer removal of herbal agents with conventional agents (EDTA and sodium hypochlorite).

2. Materials and Methods

This systematic review was conducted following the PRISMA 2020 (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. PICOS was based on: population: human extracted teeth infected with E. Faecalis; intervention: experimental studies using herbal root canal irrigants for smear layer removal; comparison: experimental studies using conventional root canal irrigant NaoCl, EDTA for smear layer removal; outcome: removal of smear layer efficiency; study type: in vitro study.

2.1. Inclusion Criteria and Exclusion Criteria

The included in vitro studies were performed on extracted teeth. Herbal agents in comparison with NaoCl, EDTA for smear layer removal were considered inclusion criteria, and animal studies, review articles, case reports, and case series were excluded from this current systematic review.

2.2. Search Strategy

A detailed search strategy was performed for the identification of included studies. The combination of vocabulary and free text search was performed in a PubMed search up to April 2022. Search terms related to root canal dentin; endodontic treatment; irrigants; herbal agents; smear layer removal; and NaOCl, EDTA were used to search for potential articles. Other databases used for the search were from Scopus and the Cochrane Library. Full-text articles in the English language were only applied for the initial phase of the article search (Table 1).
Table 1. Search strategy.

| Database       | Criteria                                                                 |
|----------------|---------------------------------------------------------------------------|
| PubMed         | (((extracted teeth) AND (root canal treatment [MeSH Terms])) OR (endodontic treatment [MeSH Terms]) AND (herbal irrigants [MeSH Terms])) OR (herbal root canal irrigants)) OR (EDTA [MeSH Terms]) OR (Ethylenediaminetetraaceticacid [MeSH Terms]) OR (sodium hypochlorite solution [MeSH Terms])) OR (sodium hypochlorite root canal irrigant)) AND (smear layer removal) |
| Scopus         | (TITLE-ABS-KEY (root canal therapy) OR TITLE-ABS-KEY (endodontic treatment) OR TITLE-ABS-KEY (root canal irrigants) AND TITLE-ABS KEY (sodium hypochlorite irrigant) OR ALL (ethylenediaminetetraaceticacid) OR ALL (edta) AND TITLE-ABS-KEY (smear layer removal)) |
| Cochrane library | #1 Endodontic treatment  
#2 Root canal treatment  
#3 Root canal irrigant  
#4 Sodium Hypochlorite irrigant  
#5 EDTA  
#6 Herbal irrigants  
#7 Smear layer removal |

2.3. Selection of Studies

Based on exclusion and inclusion criteria, the studies were included with the aid of a software manager (Rayyan Systems Inc., Cambridge, MA 02142, USA), and the data were reviewed independently by two reviewers (K.J., K.V.T.), and in case of disagreement, a third reviewer (J.J.) sorted the consensus.

2.4. Data Extraction

Data extraction was performed by (K.J., K.V.T.), and the eligibility of the studies was assessed following full-text articles. Articles were included that compared conventional smear layer removal agents (EDTA, NaOCL) with herbal agents. Variables, such as teeth selection, positive and negative control, canal preparation size, irrigation protocol, choice of irrigants, volume and concentration of irrigant, choice of the needle, irrigant activation, method of evaluation, magnification, and scoring criteria, were assessed.

2.5. Risk of Bias

Risk of bias was performed to assess the specific issues about the review’s potential bias. The risk of bias was scored as “low” when the details of the parameters mentioned above were mentioned with no ambiguity, but when there was ambiguity, they were scored as unclear. When no details were mentioned, it was scored as “high”.

3. Results

The search resulted in 625 papers from PubMed, Scopus, and Web of Science. Duplicates were removed, resulting in 587 papers. A total of 569 papers were excluded because they were out of scope. Eighteen papers were included for review of the full text. Hand searching and reference linkage did not result in any additional papers. Ten papers were excluded because they did not meet the inclusion criteria. Eight articles were included for further analysis to inform this review (Table 2). A summary of the article selection is presented as a flowchart, based on PRISMA guidelines (Figure 1). General characteristics of the included articles were tabulated for eight studies on smear layer removal (Table 3).
Table 2. List of included articles.

| Author and Year | Title of Included Article |
|-----------------|----------------------------|
| Lahijani et al. 2006 [8] | The effect of German chamomile (*Matricaria recutita* L.) extract and tea tree (*Melaleuca alternifolia* L.) oil used as irrigants on removal of smear layer: a scanning electron microscopy study |
| Murray et al. 2008 [9] | Evaluation of Morinda Citrifolia as an Endodontic Irritant |
| Candeiro et al. 2011 [10] | A comparative scanning electron microscopy evaluation of smear layer removal with apple cider vinegar and sodium hypochlorite associated with EDTA |
| Costa et al. 2012 [11] | In vitro evaluation of the root canal cleaning ability of plant extracts and their antimicrobial action |
| Chabbra et al. 2015 [12] | Smear layer removal efficacy of combination of herbal extracts in two different ratios either alone or supplemented with sonic agitation: An in vitro scanning electron microscope study |
| Evren OK et al. 2015 [13] | Antibacterial and smear layer removal capability of oregano extract solution |
| Kumar A et al. 2018 [14] | Comparative Evaluation of Antibacterial and Smear Layer Removal Efficacy of Two Different Herbal Irrigants: An in vitro Study |
| Susan et al. 2019 [15] | Intra radicular Smear Removal Efficacy of Triphala as a Final Rinse Solution in Curved Canals: A Scanning Electron Microscope Study |

Table 3. Smear layer assessment.

| Author and Year | Selection of Teeth | Sample Size | Herbal Irrigant | Positive Control | Negative Control | Other Irrigant |
|-----------------|--------------------|-------------|----------------|-----------------|-----------------|---------------|
| Lahijani et al. 2006 [8] | Single rooted permanent teeth | N = 40 | Group C: hydroalcoholic extract of German chamomile group D: tea tree oil | Group B: 2.5% NaOCl with 17% EDTA | Group A: sterile distilled water | Group E: 2.5% NaOCl alone |
| Murray et al. 2008 [9] | Permanent Single Rooted Premolar | N = 60 | Group 1: 6% MorindaCitrifolia Juice (MCJ) with a flush of 17% EDTA, followed by a final flush of MCJ Group 2: 6% MCJ mixed equally with 2% CHX with a flush of EDTA and final flush of MCJ/2% CHX Group 3: 6% MCJ with a flush of saline, followed by a final flush of MCJ | Group 4: 6% NaOCl with a flush of 17% EDTA, followed by a final flush of 6% NaOCl | Group 6: Sterile Saline | Group 5: 2% Chlorhexidine |
| Candeiro et al. 2011 [10] | Maxillary and mandibular molars | N = 40 | Group A—Apple vinegar Group B—Apple vinegar and 17% EDTA as final rinse | Group C—1% NaOCl and 17%EDTA as a final rinse | Group D: Saline | - |
| Costa et al. 2012 [11] | Single rooted permanent teeth | N = 20 | Group 1: 50% Aroeira-Da-Praia Group 2: 50% Quixabeira | Group 3: 2.5% NaOCl with 17% EDTA | Not mentioned | - |
| Author and Year  | Selection of Teeth | Sample Size | Herbal Irrigant | Positive Control | Negative Control | Other Irrigant |
|------------------|---------------------|-------------|----------------|------------------|------------------|---------------|
| Evren OK et al. 2015 [13] | Permanent maxillary central incisors | N = 180 | Group 9: 1% OES + distilled water  
Group 10: 2% OES + distilled water  
Group 11: 5% OES + distilled water  
Group 12: 1% OES + 17% EDTA + distilled water  
Group 13: 2% OES + 17% EDTA + distilled water  
Group 14: 5% OES + 17% EDTA + distilled water  
Group 8: 5.25% NaOCl + 17% EDTA + distilled water  
Group 15: sterile saline + 17% EDTA + distilled water | - | - |
| Chabbra et al. 2015 [12] | Single canal teeth | N = 50 | Group C—Combination of Citrus aurantifolia and Sapindus mukorossi in 1:1 ratio  
Group D—Combination of Citrus aurantifolia and Sapindus mukorossi in 1:1 ratio supplemented with sonic agitation  
Group E—Combination of Citrus aurantifolia and Sapindus mukorossi in 2:1 ratio  
Group F—Combination of Citrus aurantifolia and Sapindus mukorossi in 2:1 ratio supplemented with sonic agitation | Group B—17% ethylenediaminetetraacetic acid  
Group A—Distilled water | - |
| Kumar A et al. 2018 [14] | Maxillary central incisors | N = 120 | Antimicrobial efficacy (n = 60), smear layer removal efficacy (n = 60).  
Group IIB: 25% Neem extract (n = 20)  
Group IIC: 25% Tulsi extract (n = 20)  
Group IIA: 17% EDTA (n = 20) | Not mentioned | - |
| Susan et al. 2019 [15] | Mandibular first molar | N = 74 | Group 3: Triphala premixed  
Group 4: Triphala premixed (Sonic activation)  
Group 5: Triphala premixed (Ultrasound activation)  
Group 6: 3% Triphala in 10% DMSO  
Group 7: 5% Triphala in 10% DMSO  
Group 8: 10% Triphala in 10% DMSO  
Group 9: 10% citric acid  
Group 10: 10% DMSO  
Group 2: 17% EDTA  
Group 1: normal saline | - | - |
3.1. Assessment of Smear Layer Removal

A. Size of apical preparation

Three studies used the hand-filing method with a minimum of 30 k file apical preparation of the canal [8,10,14]. The remaining studies used a rotary system for canal preparation [9,12,13,15]. Apical preparation size was prepared up to 25.06 in one study [15], 50.06 in one study [13], and 35.06 in two studies [9,12] (Table 4).

---

**Figure 1.** PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only. * Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers). **If automation tools were used, indicate how many records were excluded by a human, and how many were excluded by automation tools.**

---

| Identification of studies via databases and registers |
|-----------------------------------------------------|
| Records identified from 3 Databases: 625* |
| • Pubmed (n=577) |
| • Scopus (n=47) |
| • Cochrane (n=1) |
| Records removed before screening: |
| • Duplicate records removed (n =38 ) |
| • Records marked as ineligible by automation tools (n =0 ) |
| • Records removed for other reasons (n = 0) |
| Records screened (n = 587) |
| Records excluded** (n =569 ) |
| Reports sought for retrieval (n =18 ) |
| Reports not retrieved (n =0 ) |
| Reports assessed for eligibility (n = 18) |
| Reports excluded: (n =10 ) |
| Reason: the reason for exclusion is that, the objective of the study was not according to the PICO of our systematic review. |
| Studies included in review (n =8 ) |
| Reports of included studies (n = 8) |

---

**Int. J. Environ. Res. Public Health 2022, 19, x FOR PEER REVIEW 6 of 16**
Table 4. Methodology assessment for smear layer.

| Author and Year          | Root Canal Preparation (Instruments Used and Size of Preparation) | Irrigation Protocol | Volume of Irrigant | Time of Irrigation | Needle Used for Irrigation | Irrigant Activation Devices Used |
|--------------------------|------------------------------------------------------------------|---------------------|-------------------|-------------------|---------------------------|---------------------------------|
| Lahijani et al. 2006 [8] | K file up to 30 apical preparation                               | No protocol was mentioned | Intra instrumentation—2 mL. Final flush—10 mL. | Intra instrumentation 10 s Final flush—2 min | Not mentioned in the study | Nil |
| Murray et al. 2008 [9]   | Protaper up to 35.06                                             | No protocol was mentioned | Not mentioned in the study | Not mentioned in the study | Not mentioned in the study | Nil |
| Candeiro et al. 2011 [10]| Up to 45 K file                                                 | No protocol was mentioned | 2 ml of irrigating solution at every change of file | Not mentioned | Not mentioned | Not mentioned |
| Costa et al. 2012 [11]   | Preparation size was not mentioned                              | No protocol was mentioned | Group 1 and 2—3 mL | Not mentioned in the study | Not mentioned in the study | Nil |
| Evren OK et al. 2015 [13]| Protaper up to 50 0.6                                           | No protocol mentioned Following the canal preparation, irrigation was performed with the respective irrigant | Group 8 3 ml 5.25% Naocl 3 ml 17% EDTA 5 ml distilled water 1 min | Not mentioned in the study | Not mentioned in the study | Nil |
| Chabbra et al. 2015 [12] | Apical size 35, 0.06 taper using nickel titanium files            | No protocol was mentioned | During instrumentation, each root canal irrigated using 2 mL and final rinse 3 mL of solution corresponding to its group | 5 min | 30 gauge Side vented | Sonic activation performed in group D and F |
Table 4. Cont.

| Author and Year     | Root Canal Preparation (Instruments Used and Size of Preparation) | Irrigation Protocol | Volume of Irrigant | Time of Irrigation | Needle Used for Irrigation | Irrigant Activation Devices Used |
|---------------------|---------------------------------------------------------------|---------------------|-------------------|-------------------|--------------------------|----------------------------------|
| Kumar et al. 2018 [14] | K file up to 30 size apical preparation                      | No protocol mentioned (following the canal preparation, irrigation was performed with respective irrigant) | 6 ml | 45 s | 25-gauge needle | Nil |
| Susan et al. 2019 [15] | Up to apical size 25, 0.06 taper using rotary nickel-titanium files | 1 mL of the irrigant was used for canal irrigation after each instrument | No protocol was mentioned | 8 mL during biomechanical preparation, and 5 mL for final rinse | 3 min | 28-gauge side vented needle | Not mentioned |

B. Irrigation protocol

No standard irrigation protocol was performed about removing the smear layer amongst all the included studies.

C. Volume and time of irrigation

One study did not mention the volume of root canal irrigant used [9], and three studies did not mention the time of irrigation [9–11]. Detailed information regarding the volume and time of irrigation is provided in Table 4.

D. Choice of irrigation needle and irrigant activation

Out of the included studies, five studies did not mention the gauge of the needle used for the disinfection of the root canal [8–11,13]. Only three studies mentioned the gauge of the needle [12,14,15]. Kumar et al. [14] used a 25-gauge needle, Susan et al. [15] used 28-gauge, and a 30-gauge needle was used in Chhabra et al. [12] amongst all the studies, and only two studies mentioned the vent of the needle used [12,15]. When considering the irrigant activation, none of the studies used irrigant activation devices for smear layer removal, apart from Chhabra et al. [12] (Table 4).

E. Method of smear layer assessment

All the included studies performed Scanning Electron Microscopy (S.E.M.) analysis to assess smear layer removal. Five studies reported the smear layer assessment at all three levels of the root canal system [8,9,11,12,15]. Two studies did not mention the levels of assessment [13,14] (Table 4).

F. Magnification level and scoring criteria

Two studies did not mention the level of magnification at which the smear layer was assessed [12,14]. Two studies did not mention the scoring criteria they used for smear layer removal assessment [11,13]. One study used Romes et al. criteria [14], whereas the other two studies used Hulsmann et al. criteria [8,12]. The results of this present systematic review for smear layer removal efficiency have been tabulated (Table 5).
Table 5. Result assessment on smear layer.

| Author and Year | Smear Layer Evaluation Method | Assessment of Level of Root Canal | Magnification | Scoring Criteria | Statistical Analysis | % of Open Dentinal Tubules | Outcome |
|----------------|-------------------------------|----------------------------------|--------------|-----------------|---------------------|---------------------------|---------|
| Lahijani et al. 2006 [8] | SEM analysis | Cervical, middle, and apical level of canal | 2000× and 5000× | Hulsmann et al. criteria | Kruskal–Wallis and Mann–Whitney U tests | 2.5% Naocl with 17% EDTA—no smear layer detected 2.5% Naocl—moderate smear layer, mainly in apical third Chamomile—moderate to heavy smear layer in apical third. Moderate to thin in middle and coronal sections | 2.5% Naocl followed by 17% EDTA showed better results in smear layer when compared to chamomile extract. The least effective was tea tree oil |
| Murray et al. 2008 [9] | SEM analysis | Cervical, middle, and apical level of canal | 2000× | Modified semi-quantitative visual criterion by Madison and Hokett criteria | X² statistical test | % of complete smear layer removal 6% Naocl with 17% EDTA final flush—40% smear removed (middle and coronal area) MCJ with 17% EDTA final flush—70% smear removed (middle and coronal area) MCJ with saline—20, 30, 20% smear removed (apical, middle, coronal, respectively) | 6% MCJ was equally effective as 6% Naocl when 17% EDTA was used as final flush. |
| Candeiro et al. 2011 [10] | SEM analysis | Middle and apical third | ×1000 | Vale et al. criteria | Kruskal–Wallis and Dunn’s test Wilcoxon test | Middle third less smear layer removal than apical third | Apple cider vinegar with EDTA showed better smear layer removal, followed by apple cider vinegar and NaCl/EDTA |
| Costa et al. 2012 [11] | SEM analysis | Cervical, middle, and apical level of canal | Not mentioned | Not mentioned | Kruskal–Wallis analysis | Naocl with EDTA—more accumulation of smear layer in apical third than middle and coronal Aneira-Da-Praia—more accumulation of smear layer in apical third and middle than coronal Quixabeira—less accumulation of smear layer than Naocl with EDTA | Quixabeira was found to be more effective in apical smear layer removal than Naocl with EDTA |
| Evren OK et al. 2015 [12] | SEM analysis | Levels not mentioned | ×8000 | Not mentioned | Kruskal–Wallis and Mann–Whitney U tests | 1 or 2 or 5% oregano extract solution followed by 17% EDTA showed maximum removal of smear layer, whereas 1 or 2 or 5% oregano extract alone failed to remove the smear layer | 5.25% Naocl followed by 17% EDTA showed similar effect on smear layer removal as that of 1 or 2 or 5% oregano extract solution |
| Chabbra et al. 2015 [13] | SEM analysis | Coronal, middle, and apical third | ×1000 | Hulsmann et al. criteria | One-way analysis of variance. Tukey’s post hoc test | Mean score of smear layer 17% EDTA (Group B) Coronial = 1.4 Middle = 2.2 Apical = 1.8 Group F (Citrus aurantifolia and Sapindusmukorossi in 2:1 ratio with sonic agitation) Coronial = 1.6 Middle = 2.6 Apical = 2.1 | 17% EDTA and Combination of Citrus aurantifolia and Sapindusmukorossi in 2:1 ratio with sonic agitation showed maximum removal of smear layer |
| Kumar A et al. 2018 [14] | SEM analysis | Levels not mentioned | Not mentioned | Rome et al. criteria | One-way analysis of variance. Tukey’s post hoc test | Mean of smear layer EDTA = 1.20 Neem leaf extract = 1.90 Tulsi extract = 2.70 | EDTA showed maximum removal of smear layer followed by neem extract. The least was observed with tulsi. |
| Susan et al. 2019 [15] | SEM analysis | Coronal, middle, and apical third | ×2000 | Caron et al. | Not mentioned | Mean score of smear layer Group 5: Triphala premixed (Ultrasonic activation) = 1.6 ± 0.63 | Triphala showed least amounts of smear in all thirds of the root canal, with mean values of 1.6 ± 0.63, similar to that of EDTA |

Discussion of results of the included studies, among the studies that mentioned the level assessment, five studies performed the evaluation at all three levels, namely...
coronal, middle, and apical [8,9,11,12,15], whereas Candeiro et al. [10] evaluated only middle and apical. Three studies evaluated at 2000× [8,9,15] and two studies at 1000× magnification [10,12]. Only one study used a magnification of 8000× [13]. Out of the eight included studies, five studies reported herbal agents as inferior to conventional agents [8,12–15] (Table 4).

3.2. Risk of Bias

The Joanna Briggs Institute (J.B.I.) criteria for risk of bias was modified according to in vitro studies by evaluating the domains of the present review, such as experimental condition (control groups, sampling methods), evidence on ethical approval, incomplete data (needle size, design, and time and volume of irrigation), blinding, standardization, and reporting of data. Blinding in these studies implies blinding of the evaluator.

Figure 2 depicts the risk of the bias plot. On reviewing the smear layer removal studies, the authors opine that a few essential parameters need to be addressed. Parameters, such as smear layer assessment at different levels in the sample, the range of magnification for the evaluation, standardized protocol, volume of the irrigant, and choice of the needle, were not disclosed in the studies. None of the studies obtained ethical committee approval, apart from Murray et al. [9]. Only lahijani et al. [8] reported blinding in their study, whereas this was missing in the other included studies (Table 6).

| Author and Year | Experimental Condition | Evidence on Ethical Approval | Incomplete Data | Blinding | Standardization | Reporting Data |
|----------------|------------------------|-----------------------------|-----------------|----------|----------------|----------------|
| Lahijani et al 2006 | Low High | Unclear | (type of needle and needle design not mentioned) | High | Low | Unclear | (percentage or mean value of remaining smear layer adherent not mentioned) |
| Murray et al 2008 | Low Low | High | (volume of irrigant, time of irrigation, and type of needle used not mentioned) | High | Low | Low |
| Candeiro et al 2011 | Low High | High | (time and needle gauge and design not mentioned) | High | Low | Low |
| Costa et al 2012 | Low High | High | High | High | Low | Low |
| Evren OK et 2015 | Low High | High | High | High | Low | Low |
| Chabbarra et al 2015 | Low High | High | High | High | Low | Low |
| Kumar A et al 2018 | Low High | High | High | High | Low | Low |
| Susan et al 2019 | Low High | High | High | High | Low | Low |

Figure 2. Risk of bias.
Table 6. Risk of bias assessment on smear layer (predetermined criteria based on JBI criteria).

| Author and Year | Experimental Condition (Control Groups, Sampling Methods) | Evidence on Ethical Approval | Incomplete Data (Needle Size, Design, Time & Volume of Irrigation) | Blinding | Standardization | Reporting Data |
|-----------------|-----------------------------------------------------------|-------------------------------|---------------------------------------------------------------|---------|-----------------|-----------------|
| Lahijani et al. 2006 [8] | Low                                                       | High                          | Unclear (type of needle and needle design not mentioned)       | High    | Low             | Unclear (percentage or mean value of remaining smear layer adherent not mentioned) |
| Murray et al. 2008 [9] | Low                                                       | Low                           | High (volume of irrigant, time of irrigation, and type of needle used not mentioned) | High    | Low             | Low             |
| Candeiro et al. 2011 [10] | Low                                                       | High                          | High (time and needle gauge and design not mentioned)         | High    | Unclear (irrigation protocol not mentioned) | Low             |
| Costa et al. 2012 [11] | Unclear (negative control not mentioned)                 | High                          | High (volume of irrigant, type of needle used not mentioned) | High    | High (range of magnification for SEM analysis not mentioned) | Unclear (percentage or mean value of remaining smear layer adherent not mentioned) |
| Evren OK et al. 2015 [13] | Low                                                       | High                          | Low                                                          | High    | High (irrigation protocol not mentioned) | Low             |
| Chabbra et al. 2015 [12] | Low                                                       | High                          | Unclear (gauge and needle design not mentioned)               | High    | Unclear (smear layer assessment at different levels and criteria for assessment, type of needle used not mentioned) | Unclear (percentage or mean value of remaining smear layer adherent not mentioned) |
| Kumar A et al. 2018 [14] | Unclear (negative control not mentioned)                 | High                          | Low                                                          | High    | High (irrigation protocol was not mentioned, smear layer assessment at different levels, and range of magnification not mentioned) | Unclear (percentage or mean value of remaining smear layer adherent not mentioned) |
| Susan et al. 2019 [15] | Low                                                       | High                          | Low                                                          | High    | Low             | Unclear (statistical test not reported) |

4. Discussion

The success of endodontic treatment depends on the three-dimensional seal of the root canal system. To achieve this, optimal canal preparation, the use of disinfectant with antimicrobial efficacy and pulp-dissolving ability, along with the use of chelating agents are needed. The smear layer formed following mechanical canal preparation can plug into the dentinal tubules to a depth ranging from 40 to 110 µm [1]. Hence, it is of utmost importance to remove this smear layer to allow optimal infiltration of the irrigant to the intricacies of the root canal system, facilitating the adequate penetration of intracanal medicament.
There are still two schools of thought on removing or maintaining the smear layer. Some reports proposed that maintaining the smear layer intact prevents the further ingress of microorganisms [16], but the authors of this systematic review opine that complete removal of the smear layer is mandatory to attain the three-dimensional seal. This can be achieved by maximum disinfection with the appropriate use of chelators, such that it aids in the penetration of the sealer into the dentin, and enhanced adhesion to the obturating material [17]. Failure to attain this three-dimensional seal may lead to microleakage, eventually leading to endodontic treatment failure.

Over the years, the widely used agents for the disinfection of the root canal include sodium hypochlorite as the primary root canal irrigant, and EDTA as a chelating agent. Sodium hypochlorite is a non-specific proteolytic agent possessing antibacterial properties, and dissolves the remnant pulp tissue [18]. Apart from this, it causes the dissolution of organic components of dentin. It has been already proved in the endodontic literature three decades ago that the combination of sodium hypochlorite and EDTA enhanced the removal of smear layer [19]. When EDTA is combined with sodium hypochlorite, the resulting solution’s activity spreads throughout the rest of the pulp tissues, along with antibacterial properties [20]. A study by landolo et al. reported that heating the hypochlorite to 180 °C was sufficient enough to provide a clean canal without the use of EDTA [21]

The thickness of the smear layer depends on the type of instrument used for preparation. Compared to rotary cutting instruments, hand instruments produced less smear layer formation [22]. When rotary and the reciprocating cutting instruments are compared, studies have shown rotary instruments to form a minimal smear layer [23]. This is because rotary instruments’ configuration is designed to be strenuous to attain contact of the instrument in the long oval and non-rounded canals, which eventually leads to reduced smear layer formation in rotary instruments [24]. The depth and packing density of the smear layer also varies greatly depending on whether the dentin is cut dry or wet, the amount and composition of the irrigating solution used, and the kind and speed of the instrument employed, according to a study by Pashley in 1984 [25]

As we know that the use of EDTA plays a significant role in the removal of the smear layer from the root canal system, it was also reported to tend to induce dentinal erosion, and increase the chance of perforation during instrumentation depending on the concentration volume and contact time [26]. Several studies have shown that EDTA usage for a contact time of 1 min was required to remove the smear layer [27–29]. Among the included article in this systematic review, Kumar et al. [9] used the contact time of 45 s, and also, the volume of the irrigant used differed, ranging from 3 mL to 8 mL.

The choice of the needle gauge and design of the needle vent do influence the irrigant to reach the intricacies of the root canal system [30]. In order to achieve this, it is mandatory to use no less than a 28- or 30-gauge needle with a side vent to avoid inadvertent extrusion of the irrigant. In some instances, conventional syringe needle irrigation fails to reach the third apical intricacy of the root canal system, which warrants the use of irrigation activation to increase the flow and distribution. Activation of the irrigant causes acoustic streaming and cavitation, enabling the irrigant to reach the inaccessible areas of the canal [31–33]. All the included studies used S.E.M. for analysis at different levels, namely coronal, middle, and apical third. All included studies showed that the remanence of the smear layer is present in the apical part, which is attributed to confounding factors. Studies showed that liquid EDTA promotes enhanced smear layer removal due to reduced surface tension [34]. Moreover, the addition of surfactant decreases the surface tension, and enhances the wettability [35,36].

Over the last two years, research has focused on the use of preheated chelating agents in their efficiency in the removal of the smear layer, and in improving the bond strength [37]. Better results were shown when preheated EDTA was used [37]. The above-mentioned was in corroboration with another study which had mentioned similar results [38]. It has been reported in the literature that preheating the irrigating solutions lowers the surface tension of the dentinal tubules, allowing for improved penetration into the tubules and efficient
smear layer removal [39,40]. The authors of this review make a standpoint that more clinical trials are required to uncover the symbiotic effects of preheated hypochlorite and EDTA. Moreover, all the details of the study need to be mentioned and focused on keeping the risk of bias low. Furthermore, the authors of this review believe that the experiments were performed with standardized protocol, but might not have reported the intricate details since these are in vitro studies.

4.1. Quantitative Review

Assessing the smear layer removal, 6% Morinda showed a similar result to 6% NaoCl with 17% EDTA [11]. Quixbeira and Neam [15] also showed a similar result as compared with conventional irrigating agents. The authors of this review opine contradictory results in the included studies; there could possibly be a difference in the volume of the irrigant used and the contact time in those studies. The smear layer removal efficiency of oregano extract was similar to that of the conventional agent [12]. The other agents, such as chamomile and tulsi, showed lesser efficiency than the comparison group [8,9].

Hence, it is impossible to conclude that one herbal agent is a better alternative irrigant for smear layer removal, as there are variables in the included studies.

4.2. Qualitative Review

Meta-analysis could not be performed due to the heterogeneity of the included articles and variation in the included studies.

4.3. Inference

Regarding smear layer removal efficiency, herbal agents (tea tree oil, tulsi, chamomile) showed lesser efficiency than EDTA. Quixabeira, Morinda Citrifolia, oregano extract, and neem showed better smear layer removal. The authors of this review infer that the herbal agents cannot be a substitute to conventional agents, as the included studies report incomplete data and a lack of standardization.

4.4. Limitation and Future Inference

The present systematic review was at the in vitro level of analysis; therefore, the result cannot translate the exact clinical conditions. Studies should concentrate on the irrigant activation, concentration, type, volume, and contact time of these herbal agents, and concentrate on the precipitate formation when used with conventional irrigating solutions.

5. Conclusions

This systematic review concludes that despite quixabeira, morinda citrifolia, oregano extract, and neem showing better smear layer removal compared to other herbal agents, reduced smear layer removal was evident when compared with EDTA. Hence, the present systematic review concludes that herbal agents have reported to show inferior smear layer removal when compared to EDTA.

Author Contributions: Conceptualization, K.V.T., K.J., J.J. and K.C.S.; methodology, K.V.T., K.J.; D.S., J.J. and K.C.S.; software, K.V.T.; A.A.A., A.R., F.A., K.A.M. and K.C.S.; validation, K.V.T., K.J., M.K.A., D.S. and J.J.; writing—original draft preparation, K.J., D.S., J.J. and K.C.S.; writing—review and editing, K.V.T., K.J., A.A.A., A.R., F.A., K.A.M., M.K.A., D.S., J.J. and K.C.S.; supervision, K.C.S.; funding acquisition, K.V.T., A.A.A., A.R., F.A., K.A.M., M.K.A. and K.C.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.
References

1. Alamoudi, R.A. The smear layer in endodontic: To keep or remove—An updated overview. Saudi Endod. J. 2019, 9, 71.

2. Mader, C.L.; Baumgartner, J.C.; Peters, D.D. Scanning electron microscopic investigation of the smeared layer on root canal walls. J. Endod. 1984, 10, 477–483. [CrossRef]

3. Goldman, L.B.; Goldman, M.; Kronman, J.H.; Lin, P.S. The efficacy of several irrigating solutions for endodontics: A scanning electron microscopic study. Oral Surg. Oral Med. Oral Pathol. 1981, 52, 197–204. [CrossRef]

4. Brännström, M.; Johnson, G. Effects of various conditioners and cleaning agents on prepared dentin surfaces: A scanning electron microscopic investigation. J. Prosthod. Dent. 1974, 31, 422–430. [CrossRef]

5. Machado, R.; Garcia, L.D.; da Silva Neto, U.X.; Cruz Filho, A.D.; Silva, R.G.; Vansan, L.P. Evaluation of 17% EDTA and 10% citric acid in smear layer removal and tubular dentin sealer penetration. Microsc. Res. Tech. 2018, 81, 275–282. [CrossRef]

6. Ballal, N.V.; Kandian, S.; Mala, K.; Bhat, K.S.; Acharya, S. Comparison of the efficacy of maleic acid and ethylenediaminetetraacetic acid in smear layer removal from instrumented human root canal: A scanning electron microscopic study. J. Endod. 2009, 35, 1573–1576. [CrossRef]

7. Silveira, L.F.; Silveira, C.F.; Martos, J.; de Castro, L.A. Evaluation of the different irrigation regimens with sodium hypochlorite and EDTA in removing the smear layer during root canal preparation. J. Microsc. Ultrastruct. 2013, 1, 51–56. [CrossRef]

8. Sadr Lahijani, M.S.; RaoofKateb, H.R.; Heady, R.; Yazdani, D. The effect of German chamomile (Matricaria recutita L.) extract and tea tree (Melaleuca alternifolia L.) oil used as irrigants on removal of smear layer: A scanning electron microscopy study. Int. Endod. J. 2006, 39, 190–195. [CrossRef]

9. Murray, P.E.; Farber, R.M.; Namenov, K.N.; Kutletter, S.; Garcia-Godoy, F. Evaluation of Morinda citrifolia as an endodontic irrigant. J. Endod. 2008, 34, 66–70. [CrossRef]

10. Candere, G.T.; Matos, I.B.; Costa, C.F.; Fonteles, C.S.; Vale, M.S. A comparative scanning electron microscopy evaluation of smear layer removal with apple vinegar and sodium hypochlorite associated with EDTA. J. Appl. Oral Sci. 2011, 19, 639–643. [CrossRef]

11. de Costa, B.E.M.M.; de Evangelista, A.A.P.; de Medeiros, A.C.D.; Dametto, F.R.; de Carvalho, R.A. In Vitro evaluation of the root canal cleaning ability of plant extracts and their antimicrobial action. Braz. Oral Res. 2012, 26, 215–221. [CrossRef] [PubMed]

12. Chhabra, N.; Gyanani, H.; Kamatagi, L. Smear layer removal efficacy of combination of herbal extracts in two different ratios either alone or supplemented with sonic agitation: An in vitro scanning electron microscope study. J. Conserv. Dent. 2015, 18, 374–378. [CrossRef] [PubMed]

13. Ok, E.; Adanir, N.; Ozturk, T. Antibacterial and smear layer removal capability of oregano extract solution. Eur. J. Dent. 2015, 9, 20–24. [CrossRef] [PubMed]

14. Kumar, A.; Sarthaj, A.S.; Antony, S.M. Comparative Evaluation of Antibacterial and Layer Removal Efficacy of Two Different Herbal Irrigants—An in vitro Study. Int. Health Res. J. 2018, 1, 350–354. [CrossRef]

15. Susan, A.C.; Bharathraj, A.R.; Praveen, M.; Kumar, N.S.M.; Karunakaran, J.V. Intraradicular Smear Removal Efficacy of Triphala as a Final Rinse Solution in Curved Canals: A Scanning Electron Microscope Study. J. Pharm. Bioalied Sci. 2019, 11 (Suppl. S2), S420–S428.

16. Shahranavan, A.; Haghdoost, A.-A.; Adl, A.; Rahimi, H.; Shadifar, F. Effect of smear layer on sealing ability of canal obturation: A systematic review and meta-analysis. J. Endod. 2007, 33, 96–105. [CrossRef]

17. Jhamb, S.; Nihal, V.; Singh, V. An in vitro study to determine the sealing ability of sealers with and without smear layer removal. J. Conserv. Dent. 2009, 12, 150. [CrossRef]

18. Byström, A.; Sundqvist, G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. Oral Surg. Oral Med. Oral Pathol. 1983, 55, 307–312. [CrossRef]

19. Abbott, P.V.; Heijkoop, P.S.; Cardaci, S.C.; Hume, W.R.; Heithersay, G.S. An S.E.M. study of the effects of different irrigation sequences and ultrasonics. Int. Endod. J. 1991, 24, 308–316. [CrossRef]

20. Baumgartner, J.C.; Cuenin, P.R. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. J. Endod. 1992, 18, 605–612. [CrossRef]

21. Iandolo, A.; Amato, M.; Dagna, A.; Poggio, C.; Abdellatif, D.; Franco, V.; Pantaleo, G. Intracanal heating of sodium hypochlorite: Scanning electron microscope evaluation of root canal walls. J. Conserv. Dent. 2018, 21, 569. [CrossRef]

22. Khademi, A.; Saatchi, M.; Shokouhi, M.M.; Baghaei, B. Scanning Electron Microscopic Evaluation of Residual Smear Layer After Preparation of Curved Root Canals Using Hand Instrumentation or Two Engine-Driven Systems. Iran. Endod. J. 2015, 10, 236–239.

23. Prati, C.; Foschi, F.; Nucci, C.; Montebugnoli, L.; Marchioni, S. Appearance of the root canal walls after preparation with NiTi rotary instruments: A comparative S.E.M. investigation. Clin. Oral Investig. 2004, 8, 102–110. [CrossRef] [PubMed]

24. Preparation of Oval-shaped Root Canals in Mandibular Molars Using Nickel-Titanium Rotary Instruments: A Micro-Computed Tomography Study—Science Direct. Available online: https://www.sciencedirect.com/science/article/abs/pii/S0099239909010723 (accessed on 30 November 2021).

25. Ashley, D.H. Smear layer: Physiological considerations. Oper. Dent. Suppl. 1984, 3, 13–29. [PubMed]

26. Sen, B.H.; Ertürk, O.; Pişkin, B. The effect of different concentrations of EDTA on instrumented root canal walls. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 2009, 108, 622–627. [CrossRef]

27. Crompton, B.J.; Goodell, G.G.; McClanahan, S.B. Effects on smear layer and debris removal with varying volumes of 17% REDTA after rotary instrumentation. J. Endod. 2005, 31, 536–538. [CrossRef]
28. Spanó, J.C.E.; Silva, R.G.; Guedes, D.F.C.; Sousa-Neto, M.D.; Estrela, C.; Pécora, J.D. Atomic absorption spectrometry and scanning electron microscopy evaluation of concentration of calcium ions and smear layer removal with root canal chelators. *J. Endod.* **2009**, *35*, 727–730. [CrossRef]

29. Teixeira, C.S.; Felippe, M.C.S.; Felippe, W.T. The effect of application time of EDTA and NaOCl on intracanal smear layer removal: An S.E.M. analysis. *Int. Endod. J.* **2005**, *38*, 285–290. [CrossRef] [PubMed]

30. Teja, K.V.; Janani, K.; Srivastava, K.C.; Shrivastava, D.; Jose, J.; Marya, A.; Karobari, M.I. Comparison of Herbal Agents with Sodium Hypochlorite as Root Canal Irrigant: A Systematic Review of in vitro Studies. *Evid.-Based Complementary Altern. Med.* **2021**, *25*, 2021. [CrossRef]

31. Orlowski, N.B.; Schimdt, T.F.; da Teixeira, S.C.; da Garcia, L.F.R.; Savaris, J.M.; Tay, F.R.; Bortoluzzi, E.A. Smear Layer Removal Using Passive Ultrasonic Irrigation and Different Concentrations of Sodium Hypochlorite. *J. Endod.* **2020**, *46*, 1738–1744. [CrossRef]

32. Tashkandi, N.; Alghamdi, F. Effect of Chemical Debridement and Irrigant Activation on Endodontic Treatment Outcomes: An Updated Overview. *Cureus* **2022**, *23*, 14. [CrossRef]

33. Walsh, L.J.; George, R. Activation of Alkaline Irrigation Fluids in Endodontics. *Materials* **2017**, *10*, 1214. [CrossRef]

34. Chen, G.; Chang, Y.-C. Effects of liquid- and paste-type EDTA on smear-layer removal during rotary root-canal instrumentation. *J. Dent. Sci.* **2011**, *6*, 41–47. [CrossRef]

35. Mohan, R.P.; Pat, A.R. The comparison between two irrigation regimens on the dentine wettability for an epoxy resin based sealer by measuring its contact angle formed to the irrigated dentine. *J. Conserv. Dent.* **2015**, *18*, 275–278. [CrossRef]

36. Zancan, R.F.; Hadis, M.; Burgess, D.; Zhang, Z.J.; di Maio, A.; Tomson, P.; Duarte, M.A.H.; Camilleri, J. A matched irrigation and obturation strategy for root canal therapy. *Sci. Rep.* **2021**, *11*, 4666. [CrossRef]

37. Pradhan, P.K.; Dipallini, S.; Sahoo, K.C.; Patri, G.; Lata, S. Effect of temperature and activation techniques of irrigating solutions on push-out bond strength of fiber post. *J. Conserv. Dent.* **2020**, *23*, 295–298. [CrossRef]

38. Çiçek, E.; Keskin, Ö. The effect of the temperature changes of EDTA and MTAD on the removal of the smear layer: A scanning electron microscopy study. *Scanning* **2015**, *37*, 193–196. [CrossRef]

39. Dioguardi, M.; Gioia, G.D.; Illuzzi, G.; Laneve, E.; Cocco, A.; Troiano, G. Endodontic irrigants: Different methods to improve efficacy and related problems. *Eur. J. Dent.* **2018**, *12*, 459–466. [CrossRef]

40. Uzunoglu, E.; Turker, S.A.; Karahan, S. The Effect of Increased Temperatures of QMix and EDTA on the Push-out Bond Strength of an Epoxy-resin Based Sealer. *J. Clin. Diagn Res.* **2015**, *9*, ZC98–ZC101. [CrossRef]