REVIEW PAPER

PROPOLIS EFFICIENCY AGAINST *ENTEROCOCCUS FAECALIS* COMPARED WITH OTHER INTRA-CANAL MEDICAMENTS: A SYSTEMATIC REVIEW WITH META-ANALYSIS

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ABSTRACT

Introduction: Appropriate disinfection of root system is crucial for the success in endodontics. The most common bacterial species detected in root canals in both deciduous and permanent teeth is *Enterococcus faecalis*. Experimental reports evaluating anti-bacterial effectiveness of natural remedies, such as propolis, are increasing.

Objectives: This systematic review was designed to answer a question: "Is propolis extract more effective against *Enterococcus faecalis* compared with other intra-canal medicaments?".

Material and methods: Following inclusion and exclusion criteria, thirteen studies from PubMed, Scopus, and Web of Science databases were included in this systematic review, according to preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement guidelines. Study protocol was registered in the international prospective register of systematic reviews (PROSPERO), number: CRD42023445364.

Results: Based on meta-analysis, bactericidal activity against *E. faecalis* for propolis extract was significant compared with control samples (saline). Of the selected intra-canal medicaments, chlorhexidine demonstrated the most bactericidal effect against *E. faecalis*. In contrast, calcium hydroxide was less effective compared with propolis.

Conclusions: Propolis extract seems to be more effective against *E. faecalis* compared with saline and calcium hydroxide. However, further research is necessary to establish its reliability in endodontic clinical scenarios.

Key words: disinfection, propolis, intra-canal medicament, endodontics, *Enterococcus faecalis*.

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INTRODUCTION

Many factors influence the success of endodontic therapy. One of the most essential factors is the removal of contamination from root canal system, including remaining pulp tissue, micro-organisms, and toxins [1]. For this reason, the reduction and elimination of infection as well as disinfection of root canal system must be optimized [2]. The most common bacterial species detected in root canals in both deciduous and permanent teeth is *Enterococcus faecalis* [3]. *E. faecalis* is really resistant; it can survive in difficult conditions and must be removed in endodontic procedures, which is critical to long-term success of endodontic treatment [4]. The basis of root canal treatment is instrumentation and irrigation, but due to anatomic complexity, chemo-mechanical

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instrumentation has limitations. However, the type and quantity of used canal irrigation agents may affect the frequency of post-operative pain related to root canal treatment, especially in case of apical extrusion of sodium hypochlorite (NaOCl) [5, 6].

Consequently, the use of intra-canal medication can help eliminating the remaining bacteria after the first step of endodontic procedures [7]. Calcium hydroxide, as a gold standard, has been used in the disinfection of root canal system for almost a century [8, 9]. Its wide anti-microbial activity against common endodontic pathogens has been proven, but is less effective against *E. faecalis* and *Candida albicans*. These drawbacks initiated searching for new solutions [10].

Propolis, also known as bee glue, is a natural flavonoid-rich resinous product of honeybees that is known for its valuable properties, such as anti-bacterial and anti-fungal effects as well as anti-oxidative, anti-inflammatory, and healing properties [11-13]. Propolis consists of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% other substances (flavonoids, vitamins, minerals, polyphenols, amino acids, ethanol, etc.); its content depends on climate, season, and flora of the propolis region [12, 14]. The broad biological activity of a substance is determined by a combination of its various chemical components. The mixture of natural substances has anti-microbial properties, due to pinocembrin, galangin, and caffeic acid phenethyl ester content. Its mechanism of action is probably based on the inhibition of bacterial RNA-polymerase [13]. One of the investigated applications of propolis is its use in endodontic treatment, especially as ethanol extracts solution [14].

OBJECTIVES

This systematic review was designed to answer the question: "Is propolis extract more effective against *Enterococcus faecalis* compared with other intracanal medicaments?". In order to formulate the research question and to facilitate literature review, PICO (population, intervention, comparison, and outcome) framework was applied.

MATERIAL AND METHODS

SEARCH STRATEGY AND DATA EXTRACTION

Based on preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement guidelines [15], the present systematic review was conducted using PubMed, Scopus and Web of Science databases, up till June 23, 2023. The search queries included: for PubMed, propolis AND endodonti*; for Scopus, TITLE-ABS-KEY(propolis AND endodonti*); and for Web of Science, TS = (propolis AND endodonti*).

Titles, abstracts, and full texts of papers were screened by two independent investigators. Studies included in this review matched all the pre-defined criteria of PICOS (population, intervention, comparison, outcomes, and study design), as reported in Table 1. Study protocol was registered in the international prospective register of systematic reviews (PROSPERO), with number: CRD42023445364.

The results of meta-analysis were presented in forest plots using MedCalc Statistical Software, version 19.5.3 (MedCalc Software Ltd., Ostend, Belgium). Pooled standardized mean differences for colony forming units counts of *Enterococcus faecalis* after application of propolis extract and other intra-canal medicaments were calculated. Meta-analysis of propolis anti-microbial efficiency was performed separately, compared with other intra-canal medicaments, including chlorhexidine (CHX), NaOCl, triantibiotic mixture (TAM), $Ca(OH)_2$, and saline.

QUALITY ASSESSMENT AND CRITICAL APPRAISAL FOR SYSTEMATIC REVIEW OF INCLUDED STUDIES

Risk of bias in each study was assessed using study quality assessment tool issued by the National Heart, Lung, and Blood Institute of the National Institute of Health [16]. Questionnaires were answered by two independent investigators, and any disagreement was resolved by their discussion. The summarized quality assessment is reported in Figure 1. The most frequently encountered risks of bias

TABLE 1. Inclusion and exclusion criteria according to PICOS framework

Parameter	Inclusion criteria	Exclusion criteria
Population	Extracted human teeth infected with *Enterococcus faecalis*	Other study models (e.g., animal teeth, *Candida* infection)
Intervention	Propolis extract application	Application of propolis mixed with other substances (e.g., chitosan)
Comparison	Applications of other intra-canal medicaments	
Outcomes	Colony forming units counts of *Enterococcus faecalis* after applications	Only other anti-microbial measures (e.g., MIC, MBC, zone inhibition)
Study design	*Ex vivo*/*in vitro* studies	Literature reviews, case reports, expert opinions, letters to editors, conference reports
	Published until June 23, 2023	Not published in English

MIC – minimum inhibitory concentration, MBC – minimum bactericidal concentration

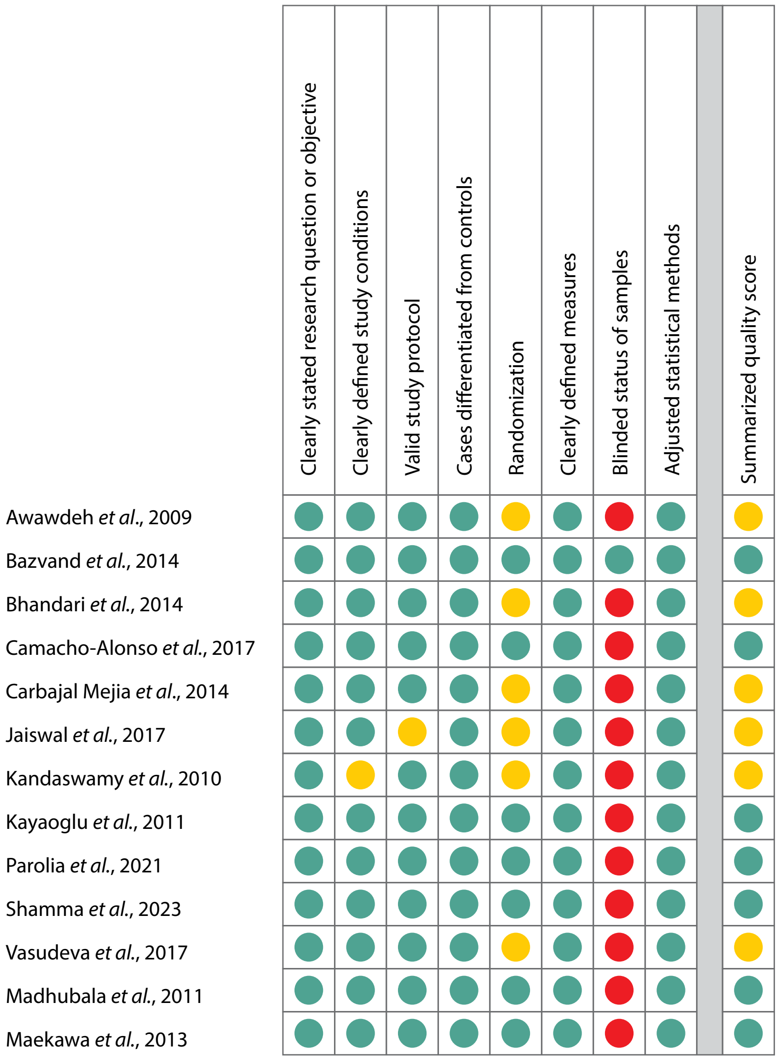

FIGURE 1. Quality assessment with main potential risk of bias (risk level: green – low, yellow – unspecified, red – high; quality score: green – good, yellow – intermediate, red – poor)

were the absence of data on blinding (twelve studies) and randomization (six studies). Critical appraisal was summarized by adding up the points for each criterion of potential risk (points: 1 – low, 0.5 – unspecified, 0 – high). Seven studies (53.8%) were classified as good quality (≥ 85% total score), and six (46.2%) were classified as intermediate (≥ 65% total score).

Level of evidence was assessed using a classification of the Oxford center for evidence-based medicine criteria [17]. All of the included studies had the fourth level of evidence, according to the five-graded scale.

RESULTS

Following the search criteria, the current systematic review included thirteen studies, with a total data from 1,648 samples (including 404 propolis samples). Figure 2 shows the detailed selection strategy of the records. The inclusion and exclusion criteria are presented in Table 1.

In Table 2, data about the detailed characteristics of investigated studies are presented, including authors and year of publication, test and control sample size, sample medium, test and control medicaments, duration of application, outcome evaluation methods, and main findings of the studies. All the studies examined extracted human teeth infected with *E. faecalis*, which were analyzed microbiologically using colony forming units counts after applications of different intra-canal medicaments.

In the meta-analysis, nine studies reporting the values of colony-forming unit (CFU) counts were included [18-24, 27, 28]. Pooled standardized mean differences in CFU counts of *Enterococcus faecalis* for propolis extract and other intra-canal medicaments (CHX, NaOCl, TAM, $Ca(OH)_2$, and saline) are shown in Figure 3. Table 3 presents the calculated standardized mean differences in CFU counts of *E. faecalis* for all the studies included in meta-analysis, separately for each intra-canal medicament.

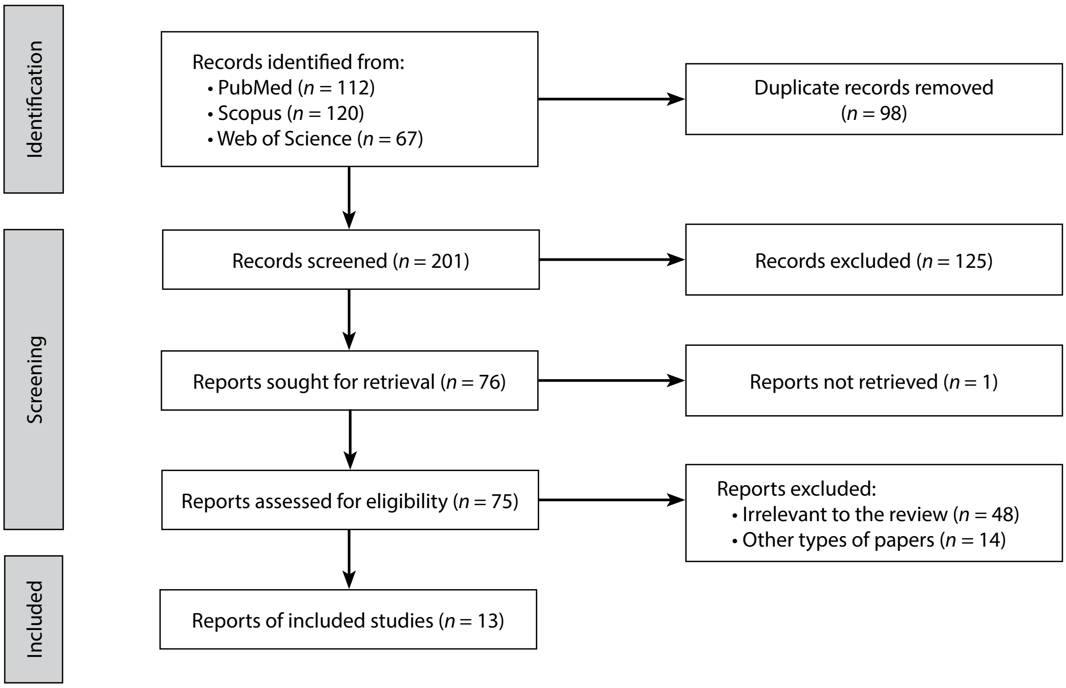

FIGURE 2. PRISMA flow diagram presenting search strategy

The bactericidal activity against *E. faecalis* for propolis extract was significant compared with the control (saline). Of the tested intra-canal medicaments, CHX was the most significantly effective compared with propolis. Propolis was probably less effective than TAM and NaOCl, although only two studies described these comparisons. In contrast, propolis extract showed a significantly more effective bactericidal effect against *E. faecalis* compared with calcium hydroxide.

DISCUSSION

The included studies reviewed the significance of effective root canal treatments, and highlighted the need for thorough debridement and disinfection of the root canal system. They addressed the limitations of traditional methods and explored the potential of intra-canal medicaments for reducing bacterial load and facilitating tissue repair. The most common bacterium in endodontic infections is *E. faecalis*, and the studies primarily focused on its anti-microbial control. Different intra-canal medicaments were tested for their effectiveness against *E. faecalis*, including propolis, CHX, calcium hydroxide, and other natural substances.

As of 2009, researchers have become interested in the effectiveness of non-standard intra-canal medicaments against *E. faecalis*, such as propolis. Awawdeh *et al.* [18] reported a laboratory study investigating the potential of anti-microbial efficacy of a Jordanian propolis-based intra-canal medicament, in comparison with non-setting calcium hydroxide paste, used as short-term medications in *ex vivo* model in a 1-2 days treatment time. The researchers concluded that propolis is more effective than non-setting calcium hydroxide as an intra-canal medicament in rapidly eliminating *E. faecalis ex vivo*.

E. faecalis poses many challenges in endodontic infections, with its ability to penetrate dentinal tubules, surviving in root-filled teeth, and adhering to collagen matrix in dentin. In 2017, Camacho-Alonso *et al.* [21] evaluated the anti-bacterial effect of photodynamic therapy (PDT) and 2% CHX. Several treatment strategies against *E. faecalis* were examined, including the use of root canal irrigants, such as NaOCl and CHX as well as TAM, propolis, ozone, and PDT. The study found that several treatment methods, including NaOCl, PDT, CHX, TAM, propolis, and ozone, significantly reduced bacterial counts compared with the positive control group. The authors concluded that the application of PDT, 2% CHX, TAM, propolis, and ozone, showed anti-bacterial potential similar to 2.5% NaOCl against endodontic infection. However, no statistically significant differences were observed between the treatment groups in terms of the area contaminated by bacteria and debris.

Moreover, Jaiswal *et al.* [23] investigated effective debridement and disinfection of root canals. The study employed different solutions, including NaOCl, CHX, propolis, and various concentrations of chitosan (a natural polysaccharide) mixed with CHX, to determine their effectiveness in eradicating *E. faecalis* biofilm. The study was performed on 90 extracted mandibular premolars, and a standardized testing methodology was

TABLE 2. Detailed characteristics of included studies

Author(s), year, [Ref.]	Test group	Control group	Control group (substance)	Sample medium	Test medicaments	Duration of application	Outcome measure	Outcome evaluation methods	Propolis findings against E. faecalis
Awawdeh *et al.*, 2009 [18]	40	10	Saline	Extracted single-rooted teeth	Propolis ($n = 20$) $Ca(OH)_2$ ($n = 20$)	2 days	CFU count	Colonies counted	Significant difference between means of CFU of propolis and $Ca(OH)_2$, after 1 and 2 days of medication application. The mean CFU of dentine discs treated with propolis was 0 after both 1 and 2 days of application. The means CFU of dentine discs treated with non-setting $Ca(OH)_2$ after 1 and 2 days of application was higher than propolis
Bazvand *et al.*, 2014 [19]	75	15	Saline	Extracted single-rooted teeth	Propolis ($n = 15$) 0.2% CHX ($n = 15$) TAM ($n = 15$) Aloe vera ($n = 15$)	7 days	CFU count	Colonies counted and recorded by blinded microbiologist	The Aloe vera group had significantly higher mean CFU than those in the other three experimental groups. No significant differences between the means CFUs of the CHX, TAM, and propolis groups were found
Bhandari *et al.*, 2014 [20]	90	30	Saline	Extracted anterior single-rooted teeth	Propolis ($n = 30$) CHX ($n = 30$) $Ca(OH)_2$ ($n = 30$)	5 days	CFU count	Digital colony counter	2% CHX had a significantly lower mean CFU than propolis; propolis had a significantly lower mean CFU than $Ca(OH)_2$, but only on day 1. There was no significant difference in their anti-microbial activities on day 3 and day 5
Camacho-Alonso *et al.*, 2017 [21]	120	20	Saline	Extracted single-rooted teeth	Propolis ($n = 20$) CHX ($n = 20$) NaOCl ($n = 20$) TAM ($n = 20$) PDT ($n = 20$) Ozone ($n = 20$)	7 days	CFU count	Colonies counted	The propolis group obtained higher values of mean CFU than the 2.5% NaOCl and 2% CHX groups. No significant differences in mean CFU between PDT, 2% CHX, TAM, propolis and ozone compared with 2.5% NaOCl were observed
Carbajal Mejía *et al.*, 2014 [22]	45	15	Saline	Extracted single-rooted teeth	Propolis ($n = 15$) CHX ($n = 15$) $Ca(OH)_2$ ($n = 15$)	14 days	CFU count	Colonies counted	No significant difference in mean CFU between CHX and propolis anti-bacterial activity at 100 and 200 µm was found
Jaiswal *et al.*, 2017 [23]	160	20	Saline	Extracted mandibular premolars single-rooted, type I Vertucci's classification, vertically sectioned	Propolis ($n = 20$) 2% CHX ($n = 20$) 5% NaOCl ($n = 20$) 1% acetic acid ($n = 20$) 0.2% chitosan ($n = 20$) 0.2% chitosan + 2% CHX ($n = 20$) 1% chitosan + 1% CHX ($n = 20$) 2% chitosan + 2% CHX ($n = 20$)	3 weeks	CFU count	Digital colony counter	Propolis exhibited significant anti-microbial activity
Kandaswamy *et al.*, 2010 [24]	150	30	Saline	Extracted single-rooted teeth	Propolis ($n = 30$) CHX ($n = 30$) $Ca(OH)_2$ ($n = 30$) MCJ ($n = 30$) POV-I ($n = 30$)	5 days	CFU count	Colonies counted	2% CHX had a significantly lower mean CFU than other four experimental groups. There was no significant difference between propolis and MCJ

TABLE 2. Cont.

Author(s), year, [Ref.]	Test group	Control group	Control group (substance)	Sample medium	Test medicaments	Duration of application	Outcome measure	Outcome evaluation methods	Propolis findings against E. faecalis
Kayaoglu *et al.*, 2011 [25]	64	32	Ethanol/ phosphate-buffered saline	Extracted single-rooted teeth – dentinal blocks	Propolis ($n = 32$) CHX ($n = 16$) $Ca(OH)_2$ ($n = 16$)	7 days	CFU count	Colonies counted	The anti-bacterial activity of propolis samples was between $Ca(OH)_2$ and CHX
Parolia *et al.*, 2021 [26]	180	30	Saline	Extracted anterior teeth – dentinal blocks	P100 ($n = 30$) P300 ($n = 30$) PN100 ($n = 30$) PN300 ($n = 30$) 2% CHX ($n = 30$) 6% NaOCl ($n = 30$)	10 minutes	CFU count	Colonies counted	PN300 had a significantly lower mean CFU than saline, P100, P300, and PN100, but there was no significant difference between PN300 and 6% NaOCl and 2% CHX at all time intervals and both depths
Shamma *et al.*, 2023 [27]	72	24	Saline	Extracted primary second molars	Propolis ($n = 24$) chitosan ($n = 24$) $Ca(OH)_2$ ($n = 24$)	7 days	CFU count	Colonies counted	No significant difference found in mean CFU between propolis, chitosan, and $Ca(OH)_2$ after 1 week of application, but after 72 h, propolis CFU was significantly lower than $Ca(OH)_2$ and chitosan
Vasudeva *et al.*, 2017 [28]	180	30	Saline	Extracted mandibular first premolars	Propolis ($n = 30$) CHX ($n = 30$) $Ca(OH)_2$ ($n = 30$) Honey ($n = 30$), Aloe vera ($n = 30$) Curcuma longa ($n = 30$)	5 days	CFU count	Colonies counted	Significant differences were observed between propolis/Curcuma longa, honey, Aloe vera, and $Ca(OH)_2$, where propolis had much lower CFU than honey, Aloe vera, and $Ca(OH)_2$. There was no significant difference between mean CFUs of 5th day on 200 µm between propolis and Curcuma longa
Madhubala *et al.*, 2011 [29]	96	24	Saline	Extracted permanent incisors	Propolis ($n = 24$) TAM ($n = 24$) $Ca(OH)_2$ ($n = 24$) Saline ($n = 24$)	7 days	%RCC	Colonies counted	Propolis exhibited significantly higher bacterial reduction compared with TAM at day 1 and day 2. Propolis and TAM presented the maximum %RCC compared with $Ca(OH)_2$, where anti-bacterial activity was gradually increased, with a maximum of 59.4% at day 7
Maekawa *et al.*, 2013 [30]	84	12	Saline	Extracted single-rooted teeth	Propolis ($n = 12$) Propolis + Ca $(OH)_2$ ($n = 12$) $Ca(OH)_2$ ($n = 12$) Ginger ($n = 12$) Ginger + $Ca(OH)_2$ ($n = 12$) CHX ($n = 12$) CHX + $Ca(OH)_2$ ($n = 12$)	7 days	%RCC	Colonies counted	After 14 days of incubation, the $Ca(OH)_2$ group was similar to CHX + $Ca(OH)_2$, propolis + $Ca(OH)_2$, GIN and GIN + $Ca(OH)_2$ groups, presenting the highest rates of %RCC, being different from the CHX, propolis, and GIN groups. After 21 days, there were resemblances between groups containing $Ca(OH)_2$, which were similar to the propolis group

CFU – colony-forming unit. %RCC – percentage reduction in colony counts, $Ca(OH)_2$ – calcium hydroxide, CHX – chlorhexidine, TAM – triantibiotic mixture, NaOCl – sodium hypochlorite, PDT – photodynamic therapy, MCJ – Morinda citrifolia juice, POV-I – povidone iodine, PN100 – propolis nano-particle 100 µg/ml, PN300 – propolis nano-particle 300 µg/ml, P100 – propolis 100 µg/ml, P300 – propolis 300 µg/ml, GIN – ginger

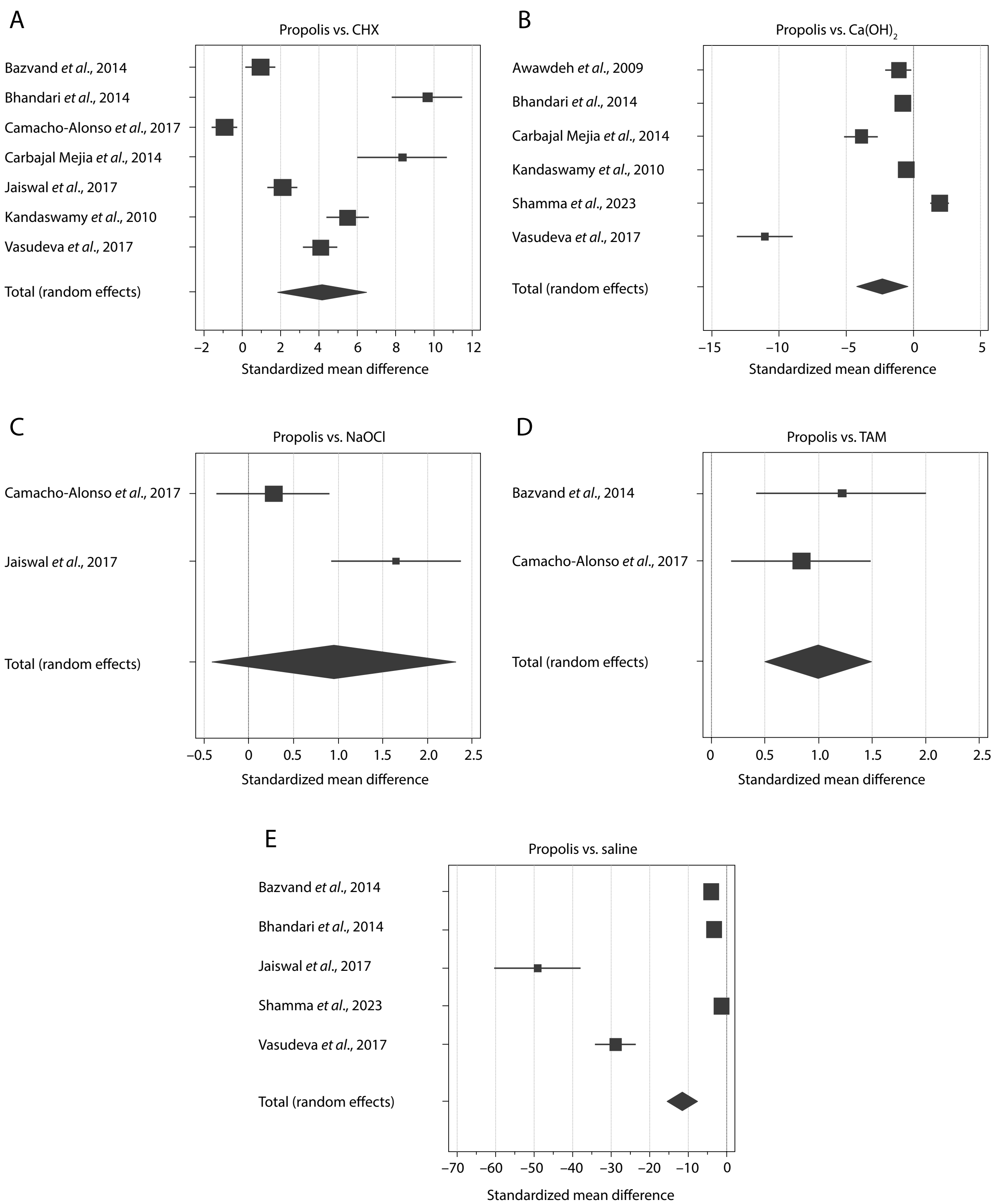

FIGURE 3. Pooled standardized mean differences in CFU counts for *Enterococcus faecalis* shown separately for each intra-canal medicaments (CHX, NaOCl, TAM, $Ca(OH)_2$, saline) in comparison with propolis extract

used involving tooth samples, inoculation with bacteria, and subsequent application of the tested irrigants. The results indicated the following key findings: combination of chitosan and CHX exhibited high anti-bacterial efficacy comparable with NaOCl and 2% CHX alone; propolis, a natural substance collected by bees, demonstrated anti-microbial activity comparable with 0.2% chitosan, suggesting its potential as an endodontic irrigant; NaOCl and 2% CHX performed similarly in terms of anti-bacterial efficacy; chitosan alone at 0.2% concentration showed promising anti-microbial activity against *E. faecalis* biofilm; 1% acetic acid used to pre-

TABLE 3. Detailed results of meta-analysis comparing anti-microbial efficiency of propolis extract compared with other intra-canal medicaments, i.e., CHX, NaOCl, TAM, $Ca(OH)_2$, and saline

Study	SMD	95% CI	*p*-value	Weight
Propolis vs. CHX				
Bazvand *et al.*, 2014 [19]	0.962	0.191 to 1.733	< 0.001	14.71
Bhandari *et al.*, 2014 [20]	9.673	7.833 to 11.512		13.67
Camacho-Alonso *et al.*, 2017 [21]	–0.923	–1.585 to –0.262		14.77
Carbajal Mejía *et al.*, 2014 [22]	8.367	6.038 to 10.697		13.10
Jaiswal *et al.*, 2017 [23]	2.106	1.318 to 2.894		14.70
Kandaswamy *et al.*, 2010 [24]	5.507	4.379 to 6.636		14.44
Vasudeva *et al.*, 2017 [28]	4.103	3.196 to 5.010		14.61
Total (random effects)	4.128	1.823 to 6.434		
Propolis vs. NaOCl				
Camacho-Alonso *et al.*, 2017 [21]	0.278	–0.352 to 0.909	0.169	50.88
Jaiswal *et al.*, 2017 [23]	1.653	0.922 to 2.383		49.12
Total (random effects)	0.954	–0.415 to 2.322		
Propolis vs. TAM				
Bazvand *et al.*, 2014 [19]	1.216	0.420 to 2.012	< 0.001	41.01
Camacho-Alonso *et al.*, 2017 [21]	0.839	0.183 to 1.494		58.99
Total (random effects)	0.993	0.497 to 1.490		
Propolis vs. $Ca(OH)_2$				
Awawdeh *et al.*, 2009 [18]	–1.100	–2.071 to –0.129	0.016	16.93
Bhandari *et al.*, 2014 [20]	–0.794	–1.324 to –0.264		17.38
Carbajal Mejía *et al.*, 2014 [22]	–3.861	–5.115 to –2.607		16.45
Kandaswamy *et al.*, 2010 [24]	–0.545	–1.065 to –0.0252		17.39
Shamma *et al.*, 2023 [27]	1.959	1.260 to 2.658		17.22
Vasudeva *et al.*, 2017 [28]	–11.007	–13.082 to –8.932		14.63
Total (random effects)	–2.327	–4.223 to –0.431		
Propolis vs. saline				
Bazvand *et al.*, 2014 [19]	–4.111	–5.419 to –2.803	< 0.001	24.29
Bhandari *et al.*, 2014 [20]	–3.264	–4.049 to –2.479		24.67
Jaiswal *et al.*, 2017 [23]	–49.196	–60.348 to –38.043		8.81
Shamma *et al.*, 2023 [27]	–1.391	–2.030 to –0.752		24.74
Vasudeva *et al.*, 2017 [28]	–28.918	–34.227 to –23.610		17.49
Total (random effects)	–11.543	–15.552 to –7.534		

pare chitosan solutions did not contribute significantly to anti-microbial activity observed, indicating that anti-microbial efficacy was primarily due to chitosan itself. It is important to note that the study had various limitations, such as its *in vitro* nature and the fact that it evaluated only anti-bacterial efficacy without considering potential cytotoxic effects on host tissues. Additionally, the study did not assess long-term effects and the potential for clinical application. The study suggests that chitosan-based solutions, particularly in combination with CHX, show promise as alternative root canal irrigants with significant anti-microbial activity against *E. faecalis* biofilm.

Bazvand *et al.* [19] conducted *ex vivo* experiment investigating the anti-bacterial efficacy of different intra-canal medicaments against *E. faecalis* in deep dentin, including TAM, CHX gel, propolis, and *Aloe vera* against root canal system infection. TAM group exhibited the least bacterial growth; however, the rate of bacterial growth between CHX and propolis groups showed no significant differences. *Aloe vera* presented anti-bacterial effects on *E. faecalis*, but in comparison with other substances, it was less effective. Natural substances, such as propolis, could have potential benefits over traditional synthetic medicaments, eliminating bacteria from the root canal system, and promoting tissue repair and healing in endodontic treatments. The findings are specific to the experimental conditions and the tested medicaments.

Bhandari *et al.* [20] conducted *in vitro* study evaluating and comparing the anti-microbial efficacy of different intra-canal medicaments at three time intervals (1, 3, and 5 days), specifically focusing on their effectiveness against *E. faecalis*. The researchers used a CHX gel and propolis, just like Bazvand *et al.* [19], but also another gold standard in endodontics, calcium hydroxide. The study employed a standardized methodology involving the use of 120 human extracted teeth, contamination of dentine with *E. faecalis*, and application of various intra-canal medicaments. The controlled approach adds to the reliability of the findings. The results indicated that 2% CHX gel consistently showed the highest anti-microbial activity, while propolis demonstrated better efficacy than calcium hydroxide initially, but exhibited similar effectiveness in subsequent days.

The efficiency of the same intra-canal medicaments, i.e., calcium hydroxide, 2% CHX gel, and propolis, was investigated by Carbajal Mejía *et al.* [22]. However, in this study, their anti-microbial effects were investigated against both *E. faecalis* and *C. albicans*. This *in vitro* study was carried out on 120 chemo-mechanically prepared roots infected with *E. faecalis* (n = 60) and *C. albicans* (n = 60). Each group was divided into four sub-groups for intra-canal medicament applications. The results showed that all experimental substances reduced *E. faecalis*. Propolis was effective against *E. faecalis*, but showed resistance against *C. albicans*. Only CHX presented anti-fungal efficacy. The authors concluded that both CHX and propolis are the most effective agents against *E. faecalis*, whereas only CHX shows the highest anti-fungal activity on *C. albicans*.

Kandaswamy *et al.* [24] study aimed to evaluate the disinfection of dentinal tubules contaminated with *E. faecalis*. The researchers investigated the anti-microbial effectiveness of various intra-canal medicaments, including propolis, Morinda citrifolia juice (MCJ), and povidone-iodine (POV-I) as well as gold standards in endodontic treatment, such as 2% CHX gel and calcium hydroxide. One hundred and eighty extracted human teeth were infected for 21 days with *E. faecalis*. The authors used various references to support their claims and methods, which lends credibility to their scientific basis. The results showed that 2% CHX gel exhibited the highest anti-microbial efficacy, achieving 100% inhibition of *E. faecalis* at both 200 μm and 400 μm depths of dentine from day 1 to day 5. POV-I, propolis, and MCJ demonstrated varying levels of inhibition, with propolis and MCJ showing similar efficacy. Calcium hydroxide exhibited the lowest inhibition.

Moreover, a study by Vasudeva *et al.* [28] evaluated the efficacy of various intra-canal medicaments, including 2% CHX, propolis, curcuma longa, honey, *Aloe vera*, and calcium hydroxide in disinfecting dentinal tubules. In their study, 210 human mandibular first premolars were infected with *E. faecalis* for 21 days. The researchers assessed the anti-microbial efficacy of medicaments at the end of 1, 3, and 5 days., which allows for replication of the study by others. The results showed that 2% CHX gel was the most effective. Among the natural extracts, propolis and curcuma longa hold a promising future, but further studies are necessary.

Additionally, Kayaoglu *et al.* [25] investigated the issue of anti-bacterial activity of propolis extracts against *E. faecalis*, and compared their effectiveness with established endodontic disinfectants, such as calcium hydroxide and CHX, using methodically prepared dentinal blocks. The authors discussed the complex and variable chemical composition of propolis, including its phenolic compounds, flavonoids, terpenes, and other organic compounds based on geographical and botanical factors. This diversity of compounds contributes to potential anti-bacterial properties of propolis. In the results, propolis extracts showed anti-bacterial activity against *E. faecalis*, with effectiveness comparable with calcium hydroxide, but slightly inferior with CHX. The anti-bacterial activity of propolis is attributed to its flavonoid content and other compounds. This conclusion aligns with existing research highlighting the anti-microbial properties of propolis. The study limitations include a focus on cultivable bacteria and a lack of discussion on potential cytotoxicity, tissue response, and sealing ability of the tested agents. In conclusion, propolis extracts could be considered as potential alternatives or adjuncts to traditional disinfectants in endodontic treatments.

A study by Madhubala *et al.* [29] focused on evaluating the effectiveness of calcium hydroxide, a traditional intra-canal medicament, compared with newer anti-microbial agents, called propolis and TAM. The study used 120 freshly extracted human incisors with standardized canal preparations, simulating a clinical scenario. The selection of *E. faecalis* as the tested organism and comparison of both traditional and newer medicaments, enhance the clinical relevance of the research. The anti-bacterial effects of the medicaments were evaluated over a short duration (1, 2, and 7 days). Propolis and TAM were found to be more effective in reducing *E. faecalis* counts compared with calcium hydroxide. In conclusion, the authors suggested that propolis could be a promising alternative intra-canal medicament due to its high anti-bacterial efficacy and various beneficial properties. Maekawa *et al.* [30] evaluated the action of glycolic propolis, ginger extracts, calcium hydroxide, and CHX gel, either individually or in combination, against *C. albicans*, *E. faecalis*, *Escherichia coli*, and bacterial endotoxins. The study considered the polymicrobial nature of endodontic infections, which often involve a combination of bacterial and fungal species, such as *C. albicans*. The findings of this study suggested that while calcium hydroxide has limitations in eliminating certain micro-organisms, combinations of medications, e.g., calcium hydroxide and CHX gel, show promising results. Additionally, the anti-microbial potential of natural extracts, including glycolic propolis and ginger, was highlighted indicating a possible avenue for developing alternative treatments in endodontics.

Parolia *et al.* [26] investigated the anti-bacterial effects of propolis nano-particles as an endodontic irrigant against *E. faecalis* biofilm within the root canal system. The study employed 210 extracted human teeth, and investigated various concentrations of propolis, propolis nano-particles, NaOCl, and CHX, as potential irrigants. The researchers used multiple methods, including microbiological analysis, SEM, confocal laser scanning microscopy (CLSM), and molecular docking studies, to assess the anti-bacterial efficacy of the tested agents. The results showed that propolis nano-particles were equally effective compared with 6% NaOCl and 2% CHX in reducing *E. faecalis* biofilms.

In the most recent study, Shamma *et al.* [27, 31] also focused on primary teeth. The authors conducted *in vitro* research evaluating the effectiveness of different intracanal medicaments against *E. faecalis* bacteria in primary root canal systems. The study aimed to compare the anti-bacterial efficacy of chitosan, propolis, and calcium hydroxide over three periods (24 hours, 72 hours, and 7 days) of application on 96 extracted primary second molars. The study measured bacterial colony reduction as a proxy for efficacy, but did not directly assess clinical outcomes or other important factors influencing the success of endodontic treatments. As results showed, chitosan and propolis medicaments were as effective as calcium hydroxide against *E. faecalis* in primary root canal treatment, and might be considered alternative irrigants between treatment sessions. While the study presents valuable information about the anti-bacterial effects of chitosan, propolis, and calcium hydroxide in an *in vitro* setting, it is important to note that the results should be interpreted cautiously due to the limitations of study design.

In addition to the discussed drawbacks of the studies included, the limitations of the present review should also be mentioned. The studies demonstrated various research models, used different types of teeth, and diverse application protocols. Furthermore, the composition and concentration of propolis extracts varied between studies. Similarly, bacterial counts were assessed in different ways and at different depths. Also, the sample sizes of the studies were not the largest, and the studies were conducted under experimental rather than clinical conditions. Further clinical research is necessary to determine the practical implications of these findings in endodontic treatment.

CONCLUSIONS

The included studies collectively highlighted the potential of propolis and other natural substances as alternatives to traditional intra-canal medicaments for effective root canal treatment. Based on the meta-analysis, propolis extract seems to be more effective against *E. faecalis* compared with calcium hydroxide and saline. While these findings are promising, further research, including clinical trials, is required to establish their practicality, safety, and effectiveness in real-world endodontic scenarios. This is of particular importance, since medicine is increasingly returning to natural preparations.

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