Article

Antimicrobial Efficacy of Propolis in Comparison to Chlorhexidine against *Enterococcus faecalis*: A Systematic Review and Meta-Analysis

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Abstract: Propolis is proposed to possess antibacterial and anti-inflammatory properties, which can be used in endodontic applications. However, evidence on its efficacy in comparison to chlorhexidine against *Enterococcus faecalis* (*E. faecalis*) is controversial. The aim of the current study was to compare the antibacterial efficacy of Propolis and chlorhexidine as an intracanal medicament against *E. faecalis* in extracted human permanent teeth. The focused question was, “Does Propolis show better antibacterial efficacy than Chlorhexidine (CHX) as an intracanal medicament against *E. faecalis* in extracted human permanent teeth?”. Databases including PubMed/Medline, Scopus, EMBASE, ISI-Web of Science were searched from 1990 to August 2020 using different combinations of the following keywords: “Propolis”, “Intracanal medicament”, “*E. faecalis*”, “Antibacterial activity” and “Chlorhexidine”. Ten studies fulfilling inclusion criteria were considered for qualitative analysis, followed by quantitative analysis of eight studies. Heterogeneity was calculated for colony forming units (CFU) of *E. Faecalis* using the Chi-square test and I^2 statistics. Forest plots were computed reporting standard mean difference (SMD) of outcomes and 95% confidence intervals. The overall mean difference for CFU of *E. faecalis* showed a statistically significant difference between the antibacterial efficacy of Propolis and CHX (SMD = 3.20 [1.70, 4.69] Z = 4.20; $p < 0.001$). CHX showed superior antibacterial efficacy against *E. faecalis* compared to Propolis.

Keywords: propolis; polyphenolic compound; chlorhexidine; intracanal medicament; *E. faecalis*; systematic review

1. Introduction

One of the most prevalent bacteria isolated from the infected root canal of failed obturation is *Enterococcus faecalis* [1,2]. *Enterococcus faecalis* (*E. faecalis*) is a facultative anaerobic gram-positive bacterium. It is considered as one of the most resistant bacterium that is difficult to eliminate due to its ability to invade dentinal tubules, compete with other bacteria and survive in harsh environments for a considerable long time without nutrition [3].

Intracanal medicament has a pivotal role in the eradication of microorganisms from an infected root canal [4,5]. Chlorhexidine is bis-biguanide and possesses prolong substantivity. It is bacteriostatic in low concentration while bactericidal in high concentration [6]. The efficacy of chlorhexidine against *E. faecalis* is established by various studies [6–8]. De Lucena

et al. [7] reported high efficacy of chlorhexidine in contrast to calcium hydroxide against *E. faecalis*. Similarly, Savitha et al. [8] found significant inhibition of *E. faecalis* by 2% chlorhexidine medicament gel in endodontic re-treatment procedure using RT-qPCR.

In recent years, a paradigm shift from synthetic to natural medicament has been noticed. Various researches have focused on natural intracanal medicament that is deleterious to all bacteria and free from the unwanted effect of synthetic medicaments [9]. One of the newly emerging natural intracanal medicaments is Propolis. Propolis is a wax cum resin substance that contains 58 to 78% polyphenolic compounds prepared by honeybees to protect their honey from contamination of microorganisms [10]. It has an array of antibacterial, antioxidant, and anti-inflammatory properties that render its use for multiple indications in dentistry [11]. Numerous studies have recommended the use of Propolis as an intracanal medicament due to its effectiveness against *E. faecalis* [11–13]. Awawdeh et al. [13] and Victorino et al. [12] showed that Propolis was more efficient than calcium hydroxide against *E. faecalis*, while Madhubala et al. [14] reported the efficacy of Propolis as 100% against *E. faecalis* following a 7-day application.

In light of existing literature, controversy exists in the efficacy of Propolis and chlorhexidine against *E. faecalis*. It is hypothesized that Propolis exhibits comparable antimicrobial efficacy in comparison to CHX against *E. faecalis*. Therefore, the aim was to perform a systematic review that can evaluate the antibacterial activity of Propolis in comparison to chlorhexidine against *E. faecalis* in extracted human teeth.

2. Materials and Methods

2.1. Protocol Registration

The study protocol was registered on the PROSPERO International prospective register of systematic reviews with the registration no. CRD42020201052. The PRISMA guideline was followed for reporting this systematic review and meta-analysis.

2.2. Focused Question

“Does Propolis show better antibacterial activity than chlorhexidine as an intracanal medicament against *E. faecalis* in extracted human permanent teeth?”

2.3. Search Strategy

An extensive literature search was made, including Pubmed/Medline (National Library of Medicine, Bethesda, 74 Maryland), Scopus/EMBASE/ISI Web of Science using different combinations of the following keywords: Propolis; intracanal medicament; *E. faecalis*; antibacterial activity. All papers published in the last 20 years, from June 2000 to June 2020, were retrieved. The title and abstract of all papers were read and matched with inclusion criteria. All papers fulfilling inclusion criteria were considered relevant (Figure 1).

Figure 1. Schematic representation of the PRISMA flowchart for literature search performed in this study.

2.4. Inclusion Criteria

Studies performed in extracted human permanent teeth comparing the antibacterial activity of Propolis and chlorhexidine as an intracanal medicament against *E. faecalis* were included.

2.5. Exclusion Criteria

The following studies were excluded, (a) all in vivo studies, (b) studies performed on animal or bovine teeth, (c) studies involving human primary extracted teeth (deciduous teeth), (d) case reports, review articles, commentaries, letter to the editor, unpublished articles, and (e) studies performed on less than 10 samples.

2.6. Study Selection and Data Extraction

Studies were selected according to the inclusion criteria by the principal investigator (MAA), then re-checked and confirmed by the second investigator (FV), if there were disagreements between the judgments of these two investigators, then a third investigator's opinion was used to resolve the issue. The principal investigator searched through ClinicalTrials.gov, International Clinical Trials Registry Platform (ICTRP), Dissertation and Theses Global, and conference proceedings at Embase and Scopus for Gray literature. In addition, articles were manually searched, and an assessment of references of the selected articles was undertaken. However, no new studies were found or included based on the inclusion criteria.

The data were extracted from included studies and transferred to a data form containing general information about the studies (Table 1). In Table 2, the total number of samples,

test group and control group, medicament used, medicament duration, inoculation period, outcome measure, and outcome evaluation method were noted.

Table 1. General characteristics of included studies.

Study Number	Authors	Year	Country	Test Group	Control Group	Outcome
Group 1. CFU count as outcome measure						
1	Agrima Vasudeva et al.	2017	India	Propolis, Chlorhexidine, Honey gel, Calcium hydroxide, Curcuma longa gel, Aloe vera gel,	Saline	2% Chlorhexidine gel was most effective followed by Propolis and Curcuma longa
2	Jeison B. Carbajal Mejía	2014	Peru	CHX, Propolis, CaOH	Saline	There was no significant difference between CHX and Propolis reducing <i>E. faecalis</i>
3	Sonam Bhandari et al.	2014	India	CHX, Propolis, CaOH	Saline	2% chlorhexidine produced 100% antimicrobial efficacy as compared to Propolis (66.37%)
4	Leila Bazvand et al.	2014	Iran	Triantibiotic, CHX, Propolis, Aloe vera	Saline	There were no significant differences between the means of CFUs for the TAM, CHX, and Propolis groups ($p > 0.05$). The mean CFU for the Aloe vera group was significantly more than those in the three other experimental groups ($p < 0.05$).
5	Maekawa et al.	2013	Brazil	Propolis, CHX, CaOH, glycolic ginger extracts	Saline	All ICMs(including Propolis) were able to eliminate <i>E. faecalis</i> in the root canals, except for CH paste, which did not eliminate completely <i>E. faecalis</i> .
6	Güven Kayaoglu et. al	2011	Turkey	2%CHX, Propolis, CaOH	Ethanol/ Phosphate-Buffer saline	Propolis samples were not superior to CHX in terms of bacterial elimination, although a significant reduction in the cultivable numbers of bacteria was achieved
7	D. Kandaswamy et al.	2010	India	Propolis, MCJ(morinda citrifolia juice), CaOH, Povidone Iodine, CHX.	Saline	Two percent chlorhexidine demonstrated significant inhibition against <i>E. faecalis</i> followed by POV-I, Propolis, MCJ, and Ca(OH) ₂
8	Nagesh Bolla et al.	2012	India	Odontopaste, CHX, Propolis	No medicament- Negative control	Odontopaste has better antibacterial efficacy against <i>Enterococcus faecalis</i> followed by Chlorhexidine, Propolis shows partial antifungal efficacy against <i>Candida albicans</i> .

Table 1. Cont.

Study Number	Authors	Year	Country	Test Group	Control Group	Outcome
Group 2. Optical density as outcome measure						
9	Shruti Saha et al.	2015	India	Propolis, CHX+metronidazole, Curcuma longa CaOH,	Saline	Propolis showed the least value of optical density (0.33 ± 0.62) indicating it as the best antibacterial medicament while CHX and metronidazole combination and <i>Curcuma Longa</i> also showed better efficiency than calcium hydroxide.
Group 3. Culture medium turbidity as outcome measure						
10	Juliana Ferreira Piovesani	2012	Brazil	Copaiba oil, Propolis extracts, CHX, propylene glycol, CaOH	+ve control group = propylene glycol –ve control group = No inoculation	None of these medicaments proved to be considerably bactericidal.

Table 2. Outcomes of studies included in systematic review.

Article Number	Total Number of Samples	Test Group	Control Group	Permanent Teeth Studied	Medicaments	Medicament Duration	Inoculation Period	Outcome Measure	Outcome Evaluation Method	CFU Count Difference
Group 1. Studies performed on anterior/single rooted teeth										
1	50	40	10	Single rooted teeth	Odontoposte, CHX, Propolis	24–48 h	24 h	CFU count and Zones of inhibition	Colonies Counted	Chlorhexidine 1.60 ± 0.52 CfU/mL Propolis: 9.10 ± 0.74 CfU/mL Mean Zone of inhibition (in mm): 24 h > CHX = 4.80 Propolis = 11.9 48 h > CHX = 1.60 Propolis = 9.10
2	120	90	30	Single rooted teeth	CHX, Propolis, CaOH, saline	14 days	21 days	CFU count	Colonies counted	Chlorhexidine = 0 Propolis = 0.86 CfU/mL
3	120	90	30	Anterior teeth	CHX, Propolis, CaOH, Saline	1, 3, 5 days	21 days	CFU count	Digital colony counter	Chlorhexidine = 0 Propolis = 1.13 CfU/mL
4	90	60	30	Single rooted teeth	Triantibiotic, CHX, Propolis, Aloe Vera	7 days	21 days	CFU count	Colonies counted and recorded by blinded microbiologist	Chlorhexidine = 0.88 ± 0.57 CfU/mL Propolis = 2.93 ± 2.88 CfU/mL
5	96	84	12	single-rooted teeth	Propolis, ginger extracts, CHX, CaOH	14 days	21 days	CFU count	Serial dilution and plated method	Chlorhexidine = 0.87 ± 0.18 CfU/mL Propolis = 0.98 ± 0.03 CfU/mL
6	90	72	18	single-rooted teeth	Propolis, CHX+metronidazole, Curcuma longa CaOH,	1, 2 and 5 days	21 days	Optical Density	Optical density calculated using colorimeter	Value of Optical Density: Propolis: 0.33 ± 0.02 CHX + metronidazole: 0.34 ± 0.03
7	96	64	32	Extracted single-rooted human teeth	2%CHX, Propolis, CaOH	1 day/7 days	14 days	CFU count	Colonies counted	Percent Reduction in CFU: CHX: 10% Propolis: 71%
8	50	40	10	Single rooted teeth	Copaiba oil, Propolis extracts, CHX, propylene glycol, CaOH	7 days	48 h	Culture medium turbidity	Unique calibrated examiner analyzed culture medium ranking.	Culture medium turbidity Chlorhexidine = +++ Propolis = +++
Group 2. Studies performed on mandibular premolar teeth										
9	210	180	30	Mandibular first premolar	Propolis, Chlorhexidine, Honey gel, Calcium hydroxide, Curcuma longa gel, Aloe vera gel, saline	1, 3, 5 days	21 days	CFU count	Colonies counted	Chlorhexidine = 0.10 ± 0.31 CfU/mL Propolis = 2.80 ± 0.63 CfU/mL
10	180	150	30	single-rooted human mandibular premolar teeth	Propolis, MCJ(morinda citrifolia juice), CaOH, Povidone Iodine, CHX	1, 3 and 5 days	21 days	CFU count	Colonies Counted	Chlorhexidine = 0 ± 0 Propolis = 2.1 ± 0.56 CfU/mL

+++ : presence of bacteria.

2.7. Quality Assessment of the Studies

The quality assessment of the studies was performed through the revised Cochrane Risk of Bias tool [RoB 2.0, Cochrane Methods, London, UK] (Higgins et al., 2016). A slight modification of this tool was made in order to include content used in the methodology of all included in-vitro studies. The quality of all studies was thoroughly evaluated based on multiple factors including, tooth specimen preparation protocol, smear layer removal protocol prior to *E. faecalis* inoculation, specimen sterilization before inoculation, growth of *E. faecalis* verified, purity of the culture checked, randomly divided samples, medicament placement protocol, and confirmation of bacterial identity after medicament removal (Table 3).

Table 3. Risk of bias assessment for included studies, based on standardization of reported factors. These included specimen preparation, smear layer removal, specimen sterilization, verification of *E. faecalis* growth, purity of the culture, randomized sampling, medicament placement protocol, and confirmation of bacterial identity after medicament removal.

S. No	Authors	Year	Country	Tooth Preparation Protocol	Smear Layer Removal	Specimen Sterilization before Inoculation	Growth of <i>E. faecalis</i> Verified	Purity of Culture	Randomization	Medicament Placement Protocol	Confirmation of Bacterial Identity	Overall Score (8)
1	Agrima Vasudeva et al.	2017	India	Yes	Yes	Yes	Yes	Yes	No	Yes	No	6
2	Jeison B. Carbajal Mejia	2014	Peru	Yes	Yes	Yes	Yes	Yes	No	Yes	No	6
3	Sonam Bhandari et al.	2014	India	Yes	Yes	Yes	Yes	Yes	No	Yes	No	6
4	Leila Bazvand et al.	2014	Iran	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	7
5	Maekawa et al.	2013	Brazil	No	Yes	Yes	Yes	No	Yes	Yes	No	5
6	Shruti Saha et al.	2015	India	Yes	Yes	Yes	Yes	No	No	Yes	No	5
7	Guvem Kayaoglu et. al	2011	Turkey	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	7
8	D. Kandaswamy et al.	2010	India	Yes	Yes	Yes	Yes	Yes	No	Yes	No	6
9	Nagesh et al.	2011	India	No	Yes	No	No	No	No	Yes	No	2
10	Juliana et al.	2012	Brazil	No	No	Yes	Yes	No	Yes	No	No	3

2.8. Meta-Analysis

Heterogeneity was calculated for colony forming units (CFU) of *E. faecalis* using the Chi-square test and I^2 statistics. For meta-analyses, if the I^2 was higher than 50%, a random-effects model was used; otherwise, a fixed-effects model for $I^2 \leq 50\%$ was applied. A P -value of <0.05 was set for significant heterogeneity. Forest plots were computed reporting standard mean difference (SMD) of outcomes and 95% confidence intervals (CI).

3. Results

3.1. Study Selection

The PRISMA flow chart for the selection of studies is shown in Figure 1. A total of 63 studies were retrieved after the initial search from four databases. No additional record was found by manual search. 38 studies were selected after duplicate studies removal. Selected articles were screened thoroughly, and 28 were excluded based on predetermined criteria. The remaining 10 studies [15–24] were included in qualitative analysis, and meta-analysis was performed on eight articles only [15,18–24].

3.2. General Characteristics of the Studies Included

All studies included in the review were published between July 2000 and July 2020. Test medicaments include chlorhexidine and Propolis specifically, along with other medicaments, e.g., honey gel, calcium hydroxide, Curcuma longa, aloe vera gel, tri-antibiotic paste, glycolic ginger extracts, calcium hydroxide plus metronidazole, etc. Control groups used saline in all studies, although propylene glycol [18] and ethanol [16] were used as controls in two studies. Most of the studies used CFU (Colony Forming Units) count as an outcome measure, however, the outcome in three studies was assessed by optical density [23], culture medium turbidity [18], and bacterial zones of inhibition [17].

3.3. Main Outcomes of the Study

Five out of ten included studies concluded that chlorhexidine showed better antibacterial properties than Propolis against *Enterococcus faecalis* [15–17,21,24]. In three out of ten studies included in the review, Propolis was equally effective against *E. faecalis* as an

intracanal medicament [19,20,22]. However, only one study presented contrary results [23]. Finally, one study conducted by Piovesani et al. [18] assessed the bactericidal activity of multiple medicaments and showed that none of the medicaments was considerably bactericidal.

3.4. Quality Assessment of the Studies Included

The risk of bias was assessed using the Cochrane Risk of Bias Tool [25]. Studies were categorized as high risk (Score 0–3), moderate risk (Score 4–6), and low risk (Score 7–8). The risk of bias assessment was performed on the basis of tooth specimen preparation protocol (by Haapasalo and Orstavik Model) [26], smear layer removal protocol prior to *E. faecalis* inoculation, specimen sterilization before inoculation, verification of growth of *E. faecalis*, purity of culture assessed, random division of samples, medicament placement protocol, and confirmation of bacterial identity after medicament removal. Only two of the studies included had a high risk of bias [Score 0–3] [17,18]. Six out of ten studies had a moderate risk of bias [Score 4–6] [15,19,21–24], however, two studies showed a low risk of bias [Score 7–8] [16,20].

3.5. Meta-Analysis

There was significant heterogeneity ($\text{Chi}^2 = 357.26$, $P < 0.0001$, $I^2 = 98.04\%$) for the mean CFU count of *E. Faecalis* bacteria, therefore, a random-effect model was utilized between the groups. A total of eight studies were included for the meta-analyses [15,18–24]. The overall mean difference for *E. Faecalis* CFU between antibacterial effect of Propolis and CHX (control) was statistically significant (SMD = 3.20 [1.70, 4.69] $Z = 4.20$; $p < 0.001$). Although Propolis showed a significant reduction of *E. Faecalis* as compared to no treatment, CHX exhibited a significantly higher antimicrobial effect on *E. Faecalis* in comparison to Propolis ($p < 0.001$) (Figure 2).

Figure 2. Forest plot showing standard mean difference between Propolis and CHX (control group) treatment in the reduction of *E. Faecalis* Colony forming units.

4. Discussion

The present study was based on the hypothesis that Propolis exhibits comparable antimicrobial efficacy in comparison to CHX against *E. faecalis*. The outcomes of the present systematic review and meta-analysis showed that the antibacterial efficacy of Propolis as an intracanal medicament against *E. faecalis* was not comparable or superior to chlorhexidine. Therefore, based on these observations, the hypothesis was rejected.

Since absolute disinfection of the root canal system is not achievable with instrumentation unaided by intracanal medicament. The use of medicaments is of particular importance to remove remaining microbes and create an environment favorable for periradicular tissue repair. In endodontic treatment, chlorhexidine, iodine potassium iodide, and their combinations with calcium hydroxide have been found effective in reducing the numbers of *E. faecalis* in the root canal system. However, none of these are considered to ensure complete eradication of *E. faecalis* [20]. *E. faecalis* is a gram-positive, facultative

anaerobe that is present in 22–77% of cases of endodontic failure and is recovered from previously root canal-treated teeth with peri-radicular infection [27,28]. It can survive as a single organism or as a major part of the endodontic flora even in ecologically demanding conditions [29]. It can penetrate dentinal tubules and has the remarkable ability to adapt to lethal changes. The capability of the microbe to invade dentinal tubules and adhere to collagen in the presence of human serum can be considered as a virulence factor responsible for its presence in failed endodontically treated teeth [17,30,31]. *E. faecalis* forms biofilms, which even prolongs its survival by making it resistant to phagocytosis, antibodies, and antimicrobial agents.

In the present systematic review, five out of ten included studies [15–17,21,24] concluded that chlorhexidine was more effective than Propolis against *E. faecalis* as an intracanal medicament. Chlorhexidine is a bis-biguanide and effective intracanal medicament in endodontic, with substantivity. It is active against gram-positive as well as gram-negative organisms, particularly *E. faecalis* [24]. Vasudeva et al. [24] evaluated the antibacterial efficacy of Propolis and 2% CHX along with other medicaments, showing maximum microbial inhibition of up to 200–400 micrometers depth, while Propolis exhibited the second highest antibacterial efficacy against *E. faecalis* among all medicaments. Other studies also reported similar results and mentioned chlorhexidine as a more effective intracanal medicament against *E. faecalis* [15,16,21]. Interestingly, Arsalan et al. [32] assessed the minimum inhibitory concentrations of CHX and Propolis along with other irrigants, concluding that Propolis was more effective in lower concentrations (MBC = 0.150 mg/mL compared to 0.512 mg/mL of CHX) against *Candida albicans*, while CHX was more effective in lower concentrations against *E. faecalis* (0.256 mg/mL compared to 0.6 mg/mL of Propolis). Although the study was not performed on extracted teeth, it reported that chlorhexidine is bactericidal to *E. faecalis* in lower concentrations as compared to Propolis. However, since *E. faecalis* entraps in dentinal tubules and the study did not assess bacterial inhibition in extracted teeth, possibly contrasting outcomes could have resulted if extracted teeth were used.

In the present study, three studies also concluded that the antibacterial efficacy of Propolis is comparable to CHX against *E. faecalis* [19,20,22]. Propolis has been used as an antibacterial agent in traditional medications for a very long time. Its antibacterial efficacy is attributed to its concentration and flavonoid contents. Kayaoglu et al. [16] compared two ethanolic extracts of Propolis, ART (Artvin, northeast Turkey) and TM (a mixed sample containing Propolis collected from hives from four different areas in Tekirda). They showed comparable antibacterial outcomes; however, TM samples contained 1.7 times more flavonoids. In another study, it was observed that flavonoid-rich Propolis extracts also inhibit bacterial growth at lower concentrations [33]. Therefore, the authors hypothesize that the difference in flavonoid contents may be the reason why Propolis exhibited inferior antibacterial properties than chlorhexidine in other includes [15–17,21,24].

On the contrary, one study conducted by Saha et al. [23] concluded that Propolis was a better intracanal medicament than chlorhexidine against *E. faecalis*. These findings among the included studies may be attributed to the different methodological techniques utilized for assessing microbial inhibition. The different techniques used included optical density and CFU counts. Another study conducted by Piovesani et al. [18] employed culture medium turbidity, concluding that none of the medicaments assessed proved to be considerably bactericidal. A number of studies assessed the role of Propolis as an intracanal medicament or irrigant due to its remarkable antibacterial properties [16,24]. Propolis possesses remarkable antibacterial and anti-inflammatory properties due to its flavonoids contents (quercetin, galangin, pinocembrin) and caffeic acid [24]. Numerous studies observed good antimicrobial activity of Propolis against *E. faecalis*, suggesting its use as an intracanal medicament in lieu of conventional medicaments [15,29]. All the studies included in the review confirm the bactericidal properties of Propolis against *E. faecalis* and other microbes of endodontic origin.

5. Limitations

Although the findings suggest superior antimicrobial activity of CHX, in comparison to Propolis in inhibiting *E. faecalis*, it is pertinent to mention that the studies included were in-vitro, with an inherent limitation when simulating in-vivo environment; therefore, results should be interpreted cautiously. In addition, the quality assessment of the included studies reflected a low to moderate body of available evidence on which conclusions were based. Therefore, further in-vivo randomized controlled trials investigating the efficacy of Propolis as a root canal medicament against *E. faecalis* are recommended.

6. Conclusions

As an intracanal medicament, Propolis displayed bactericidal ability against *E. faecalis*. However, chlorhexidine showed significantly higher antibacterial efficacy towards *E. faecalis* than Propolis. Further randomized controlled trials are recommended to validate the bactericidal potential of Propolis intracanal medicament against *E. faecalis*.

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