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Yasmeen T. Aly

Mohammed H. Mostafa

Yomna O. Mourad

ORIGINAL STUDY

Evaluation of Antimicrobial Efficacy of Propolis and Chamomile as Root Canal Irrigant on *Enterococcus faecalis*

Yasmeen T. Aly [a,*], Mohammed H. Mostafa [b], Yomna O. Mourad [b]

[a] Department of Pedodontics, Faculty of Oral and Dental Medicine, Badr University in Cairo, Egypt
[b] Department of Pedodontics and Oral Health, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt

Abstract

Purpose: To evaluate the antibacterial effectiveness of Propolis and Chamomile as root canal irrigants on *Enterococcus faecalis* for Primary Teeth. **Patients and methods:** 45 extracted primary anterior teeth were selected. Access cavity and mechanical preparation of root canals were done; teeth were sterilized in an autoclave at 121° for 20 min. These teeth were inoculated with *Enterococcus faecalis* with reference strain ATCC 19433. Then, grouped into three groups which were irrigated with 2% chlorohexidine, Propolis, and German chamomile, respectively. Samples were cultured on bile auscline agar and then incubated at 37 °C for 24 h. Counting of colony-forming units before and after irrigation was performed. **Results:** Chamomile had a higher antimicrobial effect than Propolis and both showed an antimicrobial effect lower than chlorohexidine. Analysis of variance test and Bonferroni post hoc test revealed that chlorohexidine recorded a barely significantly greater percentage of bacterial reduction in comparison with other two groups ($P = 0.05$). The percentage reduction in Propolis and Chamomile groups was not significantly different. **Conclusion:** Propolis and chamomile can be utilized as a natural alternative for chlorhexidine in root canal irrigation, even if its concentration might need to be higher or be applied for an extended amount of time inside the canals.

Keywords: Chlorohexidine, *Enterococcus faecalis*, German chamomile, Propolis

1. Introduction

Considering rapid development of caries in deciduous teeth, and the resulting damage to pulp due to contamination of pulpal tissue by bacteria and their toxins, so, endodontic treatment is inevitable. The goal of the treatment of deciduous teeth with large caries lesions surrounding the pulp is to preserve the teeth in the dental arch, restore the healthy condition of the tissues affected by pulpal infection, and maintain the normal development of the permanent successor teeth [1].

An additional goal of endodontic therapy for infected root canals is to increase the chances of complete root canal system disinfection and sustain asepsis throughout the tooth's lifetime [2].

Although bacteria are the major microorganisms found in initial endodontic infections, *Enterococcus faecalis* may be considered a major pathogen in post-treatment diseases or a survivor of the chemo-mechanical steps, gaining an advantage with its virulence factors that promote its adhesion to host cells. It can resist nutrient deprivation in endodontically treated teeth and attach to the collagen present in the dentin, showing resistance to chemo-mechanical procedures [3].

A root canal is a complicated confined region with anatomical and microbiological challenges, additionally, standard instrumentation misses more than 35% of the root canal surface. Since traditional instrumentation methodologies gather contents in isthmus areas, the ideal irrigating solution should be a biocompatible, potent antimicrobial agent, tissue solvent, lubricant, and smear layer cleanser, capable of physically discharging debris with prolonged impact but without altering the physical characteristics of dentin [4].

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* Corresponding author at: Al-Azhar University, Faculty of Dental Medicine for Girls, Nasr City, Cairo, 11754, Egypt.
E-mail addresses: yasmeentantawi@gmail.com, YasmeenAhmed.8521@azhar.edu.eg (Y.T. Aly).

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Due to its long-lasting broad-spectrum effect and low toxicity, the broad-spectrum antibacterial agent chlorhexidine (CHX) has been utilized as an irrigant. The positive charge of CHX, a synthetic cationic biguanide, combines with the negative charge of the phosphate group on the microbial wall to change the osmotic balance of the cell. This interaction is what gives CHX its efficiency [5].

For canal disinfection, many herbal irrigants with anti-inflammatory, antibacterial, and antiseptic characteristics have been examined and suggested [6].

Propolis is a naturally, non-toxic beehive product that contains different compounds from various geographic regions including resin, pollen, vitamins, flavonoids, and phenols. Flavonoids are one of the most significant components because they have anti-inflammatory, antiviral, antiallergic, anti-cancer, antibacterial, and antioxidant properties. As a result, it can be utilized as an intracanal medication as well as a canal irrigation solution. It can both stimulate the synthesis of tubular dentin and reduce pulp inflammation during vital pulp therapy. Thus, propolis has a bright future in both medicine and dentistry [7].

Asteraceae family member *Matricaria chamomilla*, sometimes known as German chamomile, is a popular therapeutic flowering plant that thrives in temperate parts of Europe, Asia, America, and Africa [8].

It contains anti-inflammatory, bacteriostatic, antibacterial, sedative, antiseptic, anticatarrhal, and spasmolytic effects and is widely used over the world. The chemical composition of essential oils and extracts of chamomile contains over 120 constituents including terpenoids, chamazulene, phenolic compounds, coumarins and flavonoids. Chamomile contains a wide range of biological effects, including analgesic, antiallergic, antipyretic, antibacterial, antifungal, anticancer, antidiabetic, antiparasitic, anti-inflammatory, and antioxidant properties [9].

In addition to its superior efficacy of the smear layer removal [10]. So, the antibacterial efficacy of these herbal agents is evaluated to be used as root canal irrigants in primary molars.

2. Material and methods

2.1. Teeth selection

A total of 45 single rooted freshly extracted teeth were used in this study, the teeth were taken from children patients (between 2 and 8 years) who lost their teeth due to trauma which results in avulsion or intrusion or extraction or due to delayed exfoliation of primary teeth. These teeth were collected anonymously.

Ethical approval was obtained in accordance with guidelines from research ethics committee of faculty of Dental Medicine for Girls, Al-Azhar University (REC-PE-22-06).

Inclusion criteria: All teeth were deciduous anterior teeth, single-rooted, free of caries and with completely formed root.

Exclusion criteria: Permanent, posterior, multi-rooted, carious teeth were discarded.

Enterococcus faecalis reference strain (ATCC 19433) was supplied from Microbiological Resources Centre (Cairo MIRCEN) faculty of Agriculture, Ain-Shams University to be used in this study. Its name is *Enterococcus faecalis*, while its synonym is Streptococcus faecalis and it was supplied as actively growing culture on slope agar.

2.2. Teeth preparation

The teeth were cleaned from outside to eliminate any soft or hard tissue debris by scaler, and then washed with water; the teeth were disinfected with 1% NaOCl and stored in thymol solution to preserve them until their use.

Then gaining access in all teeth was performed and all pulp tissue debris were removed with H file then the root canals of all teeth were prepared mechanically with K files starting with size 15 up to size 40 to the full working length. 1 ml of 1% NaOCl was used as an irrigant to remove the organic debris of pulpal tissue after each use with K file and 17% ethylene diamine tetraacetic acid (EDTA) solution was used to remove smear layer (inorganic) of root dentin after each file size [11].

All teeth were then, decoronated below the level of cementoenamel junction until having a standardized root length with 8 mm [12] using diamond disc in low-speed straight hand piece under coolant water spray. Then all teeth were sterilized in autoclave (at 121 °C, for 20 min) [13].

2.3. Teeth grouping

The specimens were categorized into three groups as the following:

Group I: included 15 samples irrigated with 2% chlorohexidine solution.

Group II: included 15 samples irrigated with ethanolic extract of propolis.

Group III: included 15 samples irrigated with German chamomile solution.

2.4. Preparation of ethanolic extract of propolis

Extraction of the Propolis was carried out by maceration with 96% ethanol. Three-hundred grams of propolis were measured by digital electronic scale and were combined with 300 ml 96% ethanol at 37 °C to achieve 100% (w/v) extract. The mixture was then stored in a bottle closed tightly for 1 week. Then the supernatant was filtrated using chromafil filter to eliminate impurities [14].

2.5. Preparation of German chamomile extract

A 150 gm of German chamomile powder were measured by digital electronic scale and were held at room temperature while being soaked in 300 ml of 96% ethanol. The solution was filtered using filter paper after 72 h (Fig. 1). A rotating flask evaporator was used to evaporate the ethanol and concentrate the extract. The extract was kept at 4 °C in refrigerator until necessary [15].

2.6. Selection and preparation of bacterial microorganisms

The bacteria were inoculated in Brain Heart Infusion broth and incubated for 24 h at 37 °C before work [16]. Few separate colonies on the bile auscline media were inoculated inside the brain heart infusion broth by sterile bacteriologic loop to produce the bacterial suspension. The turbidity of this suspension was corrected to a 0.5 McFarland standard, by adding more organism if the suspension was too clear or diluting it with the brain heart infusion broth if the suspension was too heavy. After adjustment, the suspension was ready for use [3,17].

Fig. 1. Filtration of German chamomile.

One ml of bacterial suspension was injected inside each root canal by sterile plastic syringe under pressure to make sure that it reached the full working length, and then these samples were placed individually inside Eppendorf tubes and submerged with 2 ml of brain heart infusion broth, closed and inserted inside a rack, then incubated at 37 °C for 24 h for allowing bacteria to multiply and proliferate [18].

2.7. Estimation of bacterial counting

Three sterile absorbent paper points size #30, #35, and #40 were inserted inside each root canal to take the bacterial sample and left for 1 min for each paper point to be saturated with the bacterial suspension and this was the first microbial sample (S1).

The paper point specimens were removed from the canal using sterile tweezer and placed in a sterile falcon tube containing 1 ml saline [19]. Prepare serial 10 fold dilution of bacterial suspension in sterile saline (1/10, 1/100, 1/1000, 1/10 000, 1/100 000) using micropipette, 0.1 ml from each dilution was plated on the bile auscline agar using the bacteriologic loop then aerobically incubated at 37 °C for 24 h. The colony forming unit was counted by multiplication of number of-colonies/plate by the dilution and volume factor ($10'10^4 \times 2$) 200 000/organisms/ml.

2.8. Application of irrigant solution

2.8.1. Group I

After incubation of the first 15 root canals with the bacterial suspension, irrigation with 1 ml of 2% chlorohexidine were applied. 5 min later, three sterile paper points were inserted to take the second sample (S2a).

2.8.2. Group II

After incubation of the second 15 root canals with the bacterial suspension, irrigation with 1 ml of ethanolic extract of propolis solution were applied. 5 min later, three sterile paper points were inserted canals to take the second sample (S2b).

2.8.3. Group III

After incubation of the third 15 root canals with the bacterial suspension, irrigation with 1 ml of concentrated extract of German chamomile were applied. 5 min later, three sterile paper points were inserted inside the root canals to take the third

sample (S2c), Then the bacterial counting was performed.

2.9. Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 18 was used for data management and statistical analysis. The mean, standard deviation, median, range, and confidence intervals were used to summarize numerical data. By examining the data distribution and performing the Kolmogorov–Smirnov and Shapiro–Wilk tests, data were examined for normality. Analysis of variance test and Bonferroni post hoc analysis were used to compare groups based on normally distributed numerical variables. Comparison between different observations was performed using paired *t* test. The percent change was calculated by the formula: (Value after-value before)/value before X100.

All *P* values are two-sided. *P* values less than or equal to 0.05 were considered significant.

3. Results

The highest mean reduction percentage was recorded in chlorohexidine group (−22.3 ± 2.62), followed by chamomile group (−20.46 ± 2.16), with the least value was recorded in propolis group (−20.35 ± 2.33). Analysis of variance test and Bonferroni post hoc test revealed that, chlorohexidine recorded a barely significantly greater percentage of bacterial reduction in comparison to other groups ($P = 0.05$). The percentage reduction in propolis and chamomile groups was not significantly different (Table 1, Fig. 2).

4. Discussion

The eradication of bacteria, bacterial biofilm, and smear layer using chemo-mechanical instruments, as well as the use of different irrigants to remove or dissolve organic and inorganic debris, is critical to the effectiveness of root canal treatment., thus preventing chances of reinfection [20].

In this study *Enterococcus faecalis* was chosen because it is the most prevalent species of bacteria in deciduous root canals, especially cases with reinfection and more resistant to endodontic treatment [21]. In addition, Faecalis was detected in 55% of necrotic primary teeth and referred to etiology of chronicity of periapical lesion [22].

E. faecalis can cause extra-radicular infection by either directly secreting toxins or indirectly inducing inflammation. It can also acquire and transfer extrachromosomal elements as well as encode virulence characteristics, which aid in colonization and competition with other bacteria, resistance to host defense mechanisms, and pathogenic alterations. *E. faecalis* can also form a well-organized

Table 1. Descriptive statistics and comparison between groups regarding Reduction percent of bacteria colony forming units (%).

	Mean	Std. Dev	Median	95% Confidence Interval for Mean		Min	Max	F	*P*
				Lower Bound	Upper Bound				
Chlorohexidine	−22.30	2.62	−21.69	−23.76	−20.85	−28	−19	3.19	.05*
Propolis	−20.35	2.33	−19.60	−21.64	−19.06	−24	−17		
Chamomile	−20.46	2.16	−20.19	−21.66	−19.26	−25	−17		

Significance level *P* less than or equal to 0.05, *significant.

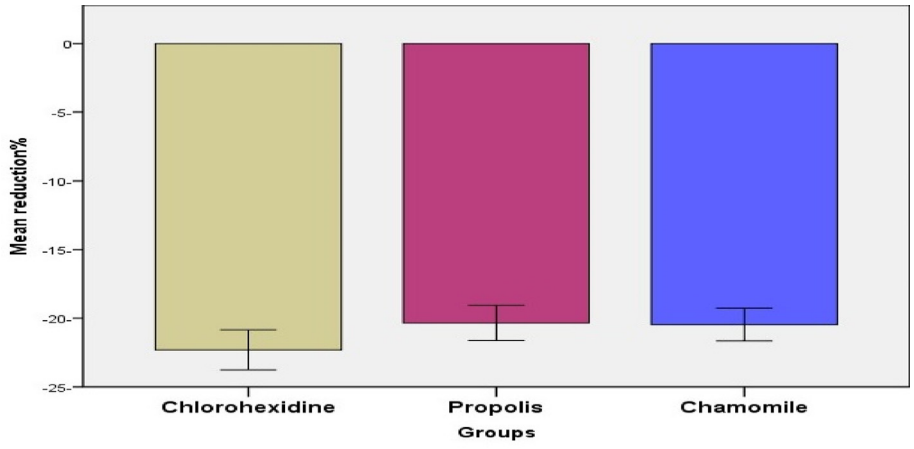

Fig. 2. Bar chart illustrating mean percentage of reduction (%) of bacteria colony forming units.

biofilm that can withstand the healing process. It can cause hydroxyapatite precipitation in mature biofilms, resulting in calcified biofilms [23].

E. faecalis has capabilities to live in various environments, such as the capacity to withstand various disinfection techniques, the ability to form biofilms, the capacity to live in areas beyond the reach of chemo-mechanical debridement of a root canal, and the synergistic interaction of various strains [24]. In the current study the teeth samples used were anterior primary teeth instead of posterior ones, because of the many root canals, it would be difficult to obtain standardization of root canals for more accurate results [25].

CHX 2% in gel formulation was selected as first group irrigant in this study due to its substantivity broad spectrum antimicrobial effect in eradication of *Enterococcus faecalis* [5]. To overcome CHX's negative impacts of tooth staining, bad taste, dry mouth, and overall inadequate antibacterial features in relation to NaOCL, safer herbal agents with antibacterial activities were utilized, furthermore the herbal's widespread availability, higher cost-effectiveness, increased storage life, and reduced amount of toxic impacts [10,26].

Ethanolic extract of Propolis was used as a natural alternative irrigant for the second group in the current study. Because ethanol is from the best extraction solvent, which makes propolis very rich in flavonoids and phenolics content with a higher antioxidant activity than water extract of propolis [27]. The variable biological characteristics as antibacterial and anti-inflammatory effects were due to mainly flavonoid, esters and phenolic compounds content [28].

German chamomile was the last herbal irrigant used in the present study because it is a chelating agent that cleans the smear layer in a better than sodium hypochlorite [29]. It is also well-known by its anti-inflammatory, analgesic and antimicrobial effects due to the capric acid, chamazolene, caprylic acid, chlorogenic acid and flavonoid components [30].

In the current study, chlorhexidine group showed the highest antibacterial effect against *E. faecalis* (-22.3 ± 2.62), with a significantly greater percentage of bacterial reduction in comparison to other groups ($P = 0.05$), followed by German chamomile group (-20.46 ± 2.16) and propolis group (-20.35 ± 2.33), respectively. There was a statistically significant difference between propolis and German chamomile.

The study results were in agreement with a recent study which considered chlorhexidine to be more effective than propolis and there was enhanced antibacterial capacity with increasing concentrations [31]. Also, the study results correspond to a previous study which displayed that propolis gel was not as effective as CHX in bacterial reduction [32].

Chamomile showed the second highest reduction of bacterial colonies among other groups and that was in accordance with a prior study which demonstrated that, Chamomile provided a good alternative as an antimicrobial agent in dental practice [33], as well as another study which showed that, Miswak and chamomile had a good efficacy against *E. faecalis*, however, when compared with NaOCl, the findings were not statistically significant [15].

While the propolis showed an antibacterial effect but with the least mean value among the experimented irrigants. The results were in accordance with other studies which indicated that, propolis samples had antibacterial action, although not to the level of CHX [12,28,32]. On the other hand, the current study results were in disagreement with previous studies which showed that, the Ethanolic extract of propolis was more effective against *E. faecalis* in comparison with chlorhexidine [34,35].

Also, another study reported that chamomile had no antimicrobial activity on *Candida albicans* and *E. faecalis* at a concentration of 150 mg/ml [31], adding to the previous study which stated that compared with 5% Sodium hypochlorite and Propolis, the antibacterial activity of Liquorice and German chamomile was much lower [36]. The discrepancies in extract processing conditions, German chamomile and propolis quality, and culture times may account for the discrepancies between the previous findings and those of this investigation.

5. Conclusions

Within the parameters of this study. The following was determined: German chamomile solution has slightly stronger antibacterial effect than propolis and both show an antimicrobial effect less than 2% chlorohexidine. This method considered an effective line for bacterial eradication in primary root canals and needs to be clinically confirmed due to the presence of other microorganisms.

5.1. Recommendations

Further *in vivo* studies and also longer follow ups periods are needed to evaluate possible risks or undesirable effects of propolis and German chamomile on the developing permanent teeth.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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