IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 22, Issue 6 Ser.13 (June. 2023), PP 01-07
www.iosrjournals.org

Evaluation Of Anti-Microbial Efficacy Of Garcinia Extract With Garcinol, Calcium Hydroxide And Chlorhexidine Gluconate As An Intra Canal Medicament Against Enterococcus Faecalis - An In Vitro Study

Arthanarieswaran Andamuthu Sivakumar[1], M.Chittrarasu[2], P.Denisha[3], Jambai Sampath Kumar Sivakumar[4], Saravanapriyan Soundappan[5] , P.V.Shyamala[6]
[1-6](Department of Conservative Dentistry & Endodontics, Vivekanandha Dental College for Women, India)

Abstract:
Background*: Microorganisms Are The Etiologic Factor For Pulpal And Periradicular Diseases To Occur. However When The Infection Progresses It Leads To Necrosis And Apical Periodontitis Furthermore The Whole Root Canal Will Be Infected By Microorganisms. Complete Debridement And Reduction Of The Bacterial Infection From The Root Canal Space Is Necessary For Long-Term Success Of Endodontic Treatment. In Addition To Thorough Cleaning And Shaping, The Placement Of Intracanal Medicament Is Necessary To Prevent Secondary Infection Of The Root Canal. This Study Was Conducted To Evaluate The Better Intracanal Medicament Against The Most Virulent Root Canal Microorganism.*
Materials And Methods*: Sixty Human Mandibular Premolar Teeth Extracted For Therapeutic Reasons Were Collected, Cleaned And Stored In Distilled Water. The Teeth Were Decoronated 1 To 2mm Below The Cemento-Enamel Junction. Cleaning And Shaping Of The Canals Were Done With Rotary Files In A Sequential Manner Using Pro Taper Gold System. Irrigation Was Performed With 5% Sodium Hypochlorite Solution And Final Irrigation With 17% EDTA Solution And Chlorhexidine Gluconate. Bacterial Samples Of Enterococcus Faecalis (ATCC 29212 Strain) Was Used In This Study. Each Root Canal Was Inoculated With A Bacterial Solution And Was Incubated In A Closed Container At 37°C For A Period Of 21 Days. The Teeth Were Then Be Randomly Divided Into Three Experimental Groups Of 20 Samples Each (N=20) And The Test Medicaments Were Placed In The Corresponding Group Using Lentulo Spiral Of 25size. Group 1(N=20) – Garcinia Extract With Garcinol, Group 2(N=20) – Calcium Hydroxide, Group 3(N=20) – 2% Chlorhexidine Gluconate. After 24 Hours Of Incubation, Number Of Colony Forming Units (CFU's) Were Assessed With Colony Counter And The Data Were Recorded For Each Group Respectively.*
Results*: Garcinia Extract Shows Increased Antimicrobial Activity Than CHX And Calcium Hydroxide (P<0.05).*
Conclusion: *The Results Showed The Potential Of Garcinia Extract To Be Used As An Intracanal Medicament. They Have Superior Antimicrobial Action On The E. Faecalis In The Root Canal System.*
Key Word*: Antimicrobial Efficacy, Colony Forming Unit, Garcinia, Intra Canal Medicaments.*

Date of Submission: 17-06-2023 Date of Acceptance: 27-06-2023

I. Introduction

Root canal infections are mainly initiated by the microorganisms that can invade through the dental pulp and colonized the entire root canal system. These microbial colonies and their toxins, by products rapidly get an access to the periapical area and forming a sequence of inflammatory responses leads to apical periodontitis. In general root canal diseases are prompted by polymicrobial infections[1]. Enterococcus faecalis (E. Faecalis) is the main bacteria amenable for the failure of endodontic treatment and they have the ability to survive in various environments, its capability to resist distinct measures of disinfection, to produce a biofilm, to remain stagnant in surfaces unreachable to the chemical and mechanical debridement of a root canal system, and the antagonistic reaction of different strains, all these makes E. faecalis as an strong and highly resistant microbe[2]. The exact objectives of the intracanal medication are restriction of bacterial re-growth, provide continuous disinfection and forms a physical barrier[3] . For the past several years, various molecules have been isolated from different species of Garcinia, which mainly include xanthones and xanthone derivatives. However, the isolation of hydroxycitric acid [HCA] from a certain species of Garcinia and its biological properties were attracted the attention from most of the biochemists and health practitioners[4]. Garcinia extract has the possible bacteriostatic effect due to its low pH. With concern to toxicity or safety, it is important to account that G. cambogia has traditionally been used in

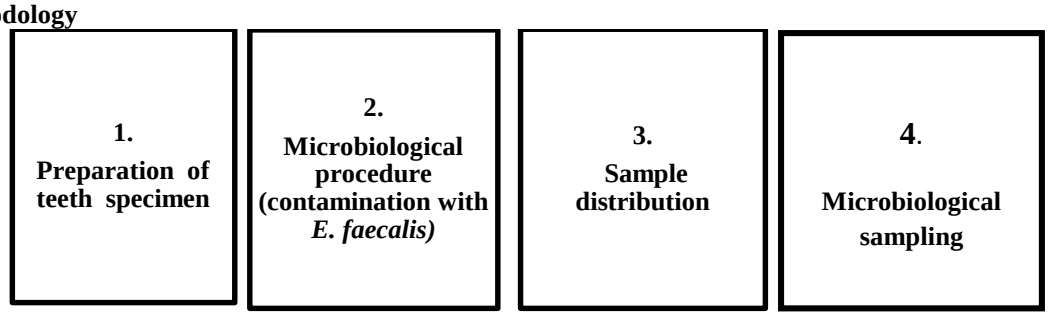
human diet or as a supplement (as a therapeutic preparation) without any evidence of adverse effects from its use[5]. Calcium hydroxide has enhanced antibacterial effects of biomechanical procedures during endodontic treatment 10. CHX gel maintains essentially all the dentinal tubules open because its viscosity makes the debris in suspension and reducing the formation of smear layer. Moreover, the gel formulation maintains the "active principle" of CHX in contact with the microorganisms for a longer time, thereby inhibiting the growth[6]. The current study opens a new approach of trying herbal extracts as intracanal medicaments to eliminate the microorganism in the root canal system and aid in long term success in endodontics.

II. Material And Methods

Inclusion criteria:
Human mandibular single rooted premolars with single canal confirmed using radiograph were included in this study

Exclusion criteria:
Root canal treated teeth, teeth with root caries, root with multiple canals, calcifications, internal resorptions and teeth with open apex were excluded from the study.

Methodology



PREPARATION OF TEETH SPECIMEN:

Sixty extracted human permanent mandibular single rooted premolars were collected and stored in distilled water. The teeth were decoronated 1 to 2mm below the cemento-enamel junction using a diamond disc with a water coolant spray to a standardized root length of 12mm. Canal patency were established using a 10 size K-file and working length will be determined, 0.5mm short of the apex. Radiographs will be taken to confirm the same. Cleaning and shaping of the canals were done with rotary files in a sequential manner using Sx, S1, S2, F1, and F2 Pro Taper gold system.

Irrigation was performed with 5% sodium hypochlorite solution using sterile 2ml syringe during instrumentation and the canals were finally irrigated with 17% EDTA solution and chlorhexidine gluconate. The canals were dried with sterile absorbent paper points. The external root surfaces were coated with three layers of clear nail varnish. The specimens were autoclaved at 121^0C under 15 lbs pressure for 15 minutes.

MICROBIOLOGICAL PROCEDURE (CONTAMINATION WITH *ENTEROCOCCUS FAECALIS*):

Bacterial samples of *Enterococcus faecalis* (ATCC 29212 strain) was used in this study. Bacteria were grown in Muller-Hinton agar for 24 hours and the culture was suspended in 5ml of Brain Heart Infusion (BHI) broth and incubated for 4 hours at 37^0C and its turbidity adjusted to 0.5 McFarland-standard. Each root canal was inoculated with a bacterial solution up to the canal orifice using sterile syringe. Roots were mounted in the 96 well titre plates containing 2% sterile agar media which was allowed to solidify so that the root specimens can be stabilized. Each canal was sealed with dental wax and was incubated in a closed container at 37°C for a period of 21 days. The canals were re-inoculated with fresh bacterial samples once in every 3days. To check the cell viability and purity of culture, samples were taken from each canal using sterile paper point and sub cultured in Muller Hinton agar plate.

Fig 1: Root canal inoculated with a bacterial solution & Incubated for 21 days

SAMPLE DISTRIBUTION:

After 21 days, the canal contents were aspirated aseptically and each canal was rinsed with 5ml distilled water using a 5ml syringe and then dried with sterile paper points. The teeth were then be randomly divided into three experimental groups of 20 samples each (n=20) and the test medicaments were placed in the corresponding group using lentulo spiral of 25size.

Group 1(n=20) – Garcinia extract with Garcinol
Group 2(n=20) – Calcium hydroxide
Group 3(n=20) – 2% Chlorhexidine Gluconate

The samples were sealed at both ends with dental wax and then incubated for 24hours at 37°C temperature.

Fig 2: Garcinia extract with Garcinol, Calcium hydroxide and 2% Chlorhexidine

MICROBIOLOGICAL SAMPLING:

Microbiological samples were obtained by placing a sterile paper point into the canal for 60 seconds. The paper point is then transferred into a micro test tube containing 1ml of physiological saline solution. Each sample were mixed for 30s on a vortex mixer. Then 0.1ml aliquot of the microbial suspension was plated on a Muller-Hinton agar plate respectively. After 24 hours of incubation, number of colony forming units (CFU's) were assessed with colony counter and the data were recorded for each group respectively.

Fig 3: Colony forming units

Statistical analysis

The statistical analysis was performed using IBM SPSS version 26 (IBM, Armonk, USA). The normality of the data was tested using Shapiro Wilk test. As the data was found to be skewed, intergroup comparison was performed using Kruskal wallis test followed by post hoc Dunn's test. For all comparisons, $p<0.05$ was considered to be statistically significant.

III. Result

Descriptive statistics such as mean, standard deviation, standard error, 95% confidence interval, minimum and maximum values for "CFU" with respect to the different groups are represented in Table.1.

Intergroup comparison performed using Kruskal Wallis test showed that there was a statistically significant difference between the compared groups ($p<0.0001$) (Table 2). Dunn's post hoc analysis showed that there was a statistically significant difference with respect to all pairwise comparisons ($p<0.0001$) except group 1 versus group 3 ($p=1.000$) with highest CFU units shown by Group 2 (92.400±24.1103) (Table 1 and 3). The lowest CFU units was reported with group 1 (38.1±12.2) and group 3 (39±11.43) with no statistically significant difference between both the groups ($p=1.000$) (Table 1 and 3). The mean CFU with respect to different groups is illustrated using figure 1. The median, interquartile range, minimum and maximum CFU values with respect to different groups is illustrated using figure 2. The median (p50) is represented using the thick line in the vertical box, the first quartile (p25) and third quartile (p75) are represented by the upper and lower limit of the box. Maximum and minimum values are presented using upper and lower limit of whiskers and the outliers as dots above and below the whiskers.

The findings of this study demonstrated that Group 1 showed maximum antimicrobial efficacy followed by Group 3 and Group 2 against E. faecalis.

I.CFU/ml

TABLE 1: Descriptive statistics

Descriptive statistics								
CFU								
	N	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**
					Lower Bound	**Upper Bound**		
Group 1	**20**	**38.100**	**12.1997**	**2.7279**	**32.390**	**43.810**	**18.0**	**60.0**
Group 2	**20**	**92.400**	**24.1103**	**5.3912**	**81.116**	**103.684**	**54.0**	**132.0**
Group 3	**20**	**39.000**	**11.4340**	**2.5567**	**33.649**	**44.351**	**18.0**	**54.0**
Total	**60**	**56.500**	**30.5401**	**3.9427**	**48.611**	**64.389**	**18.0**	**132.0**

***p<0.05 is statistically significant**

****p<0.001 is statistically highly significant**

TABLE 2: Intergroup performed using Kruskal Wallis test

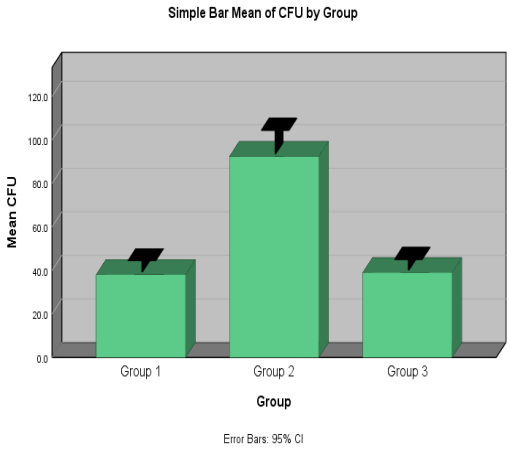
Hypothesis Test Summary using Kruskal Wallis test				
	Null Hypothesis	**Test**	**Sig.**	**Decision**
1	**The distribution of CFU is the same across categories of Group.**	**Independent-Samples Kruskal-Wallis Test**	**.000**	**Reject the null hypothesis.**
Asymptotic significances are displayed. The significance level is .050.				

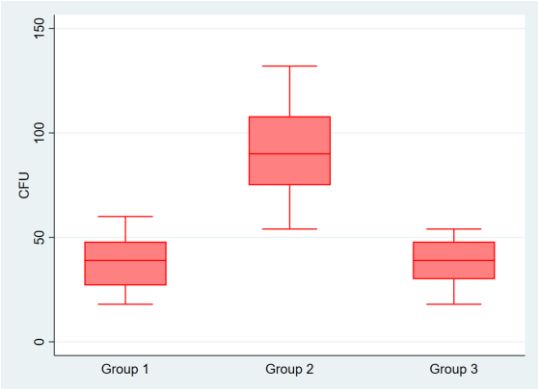
***p<0.05 is statistically significant**
****p<0.001 is statistically highly significant**

TABLE 3: Intergroup performed using Dunn's post hoc analysis

Pairwise Comparisons of Group using Dunn's post hoc analysis					
Sample 1-Sample 2	**Test Statistic**	**Std. Error**	**Std. Test Statistic**	**Sig.**	**Adj. Sig.[a]**
Group 1-Group 3	**-.900**	**5.499**	**-.164**	**.870**	**1.000**
Group 1-Group 2	**-29.850**	**5.499**	**-5.429**	**.000**	**.000**
Group 3-Group 2	**28.950**	**5.499**	**5.265**	**.000**	**.000**
Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05.					
a. Significance values have been adjusted by the Bonferroni correction for multiple tests.					

***p<0.05 is statistically significant**
****p<0.001 is statistically highly significant**





IV. Discussion

To encourage healing of apical periodontitis, microorganisms within the root canal system must be eliminated. Most of the studies have indicated that the prognosis of apical periodontitis after root canal treatment is poorer if viable microorganisms are present in the canal at the time of the root filling and it also leads to failure of the treatment. Successful elimination of the microbial agents in the root canal system is the indispensable way to improved prognosis. The essential elements in the control of endodontic infections include systemic antibiotic therapy, instrumentation and irrigation, locally used intracanal medicaments between appointments, root canal filling, and coronal restoration.

The infection may be purely anaerobic, but the anaerobes are, in many cases, accompanied by micro-aerophilic and facultative bacteria, such as Actinomyces species, Lactobacillus species and streptococci. In previously root-filled teeth with apical periodontitis, the ecology may be quite different, and in many cases the environment no longer supports the dominance of anaerobic bacteria. The most frequently isolated species by far in previously root-filled teeth with apical periodontitis is Enterococcus faecalis, but several other facultative and even anaerobic bacteria are often isolated. While monoinfections are not detected in primary apical periodontitis,

E. faecalis is often found in pure culture in previously root-filled teeth with apical periodontitis. However, E. faecalis is often found together with streptococci, lactobacilli, other facultative bacteria, and also with anaerobic bacteria. Gram-negative enteric rods (coliforms and Pseudomonas species) and yeasts are found almost entirely only in previously root-filled teeth with apical periodontitis[7].

E. faecalis is a normal intestinal organism and may inhabit the oral cavity and gingival sulcus. In its intestinal environment, it is considered a commensal organism that contributes to carbohydrate, amino acid, and vitamin metabolism. However, the subsets of this species appear to be pathogenic because they have acquired a number of genes conferring infectivity and virulence, including resistance to multiple antibiotics.

It is feasible that if inoculation of enterococci occurs during initial root canal therapy or because of subsequent microleakage these microorganisms will persist in the root canal far after other bacteria have died, because of their fastidious nature. E. faecalis was shown to be capable of withstanding high pH changes as well as prolonged periods of little to no nutrients[8].

Due to the concern related to the accuracy of in vitro research and the different patterns of virulence and resistance genes of E. faecalis strains, their monitoring should be encouraged for establishing the most effective drug.

Zancan RF et.al., in 2018 stated that TAP had the better antimicrobial result than CH/DAP against E. Faecalis strains and a higher prevalence of live cells was found for ATCC 29212 when the two bacteria were compared regarding the antibiotic pastes and this strain is more resistant[9]. Hence E. faecalis was chosen.

It is generally recommended that the root canal should be filled with an antibacterial dressing, e.g. calcium hydroxide, between appointments to secure the sterility of the canal space, until it is filled at the next appointment. A variety of intracanal medicaments have been used between appointments to complete disinfection of the root canal[7].

When a tooth does not respond to root canal treatment, bacteriological sampling may be needed to determine the bacteria present in the root canal system and therefore to aid the choice of intracanal medication.

Calcium hydroxide has antimicrobial effectiveness against microorganisms found in root canal infections. Its antimicrobial mechanism of action involves the speed of dissociation into calcium and hydroxyl ions in a high pH (12.6) environment that inhibits enzymatic activities metabolism, growth and cellular division - essential to microbial life. It can change the integrity of the cytoplasmic membrane by means of chemical injuries to organic components and transport of nutrients, or by means of destruction of phospholipids or unsaturated fatty acids of the cytoplasmic membrane, observed in the lipidic peroxidation process, which is a saponification reaction. Estrela et al in 2003 studied that the antimicrobial effect on the cultures of S. aureus, E. faecalis, P. aeruginosa, B. subtilis, C. albicans and one mixed culture in infected canals by CH Paste occurred in 60 days, length of time necessary for microbial inactivation[10].

Morrier et al in 2003 resulted that the various calcium hydroxide medications differ in their antimicrobial activity as indicated by zones of inhibition. Calcium hydroxide mixed with glycerin was the most effective against the reference bacterial strains. The sensitivity of the three obligate anaerobes to the antimicrobial activity also varied with the same cement[11].

According to Sonali et al[12] in 2017 Oil paste containing calcium hydroxide compounds should be used for a minimum of 7 days to achieve maximum therapeutic effectiveness and it can be formulated that non-setting premixed calcium hydroxide pastes are better in antimicrobial activity as compared to calcium hydroxide powder and calcium hydroxide points.

In our experiment calcium hydroxide with barium sulphate formulation in a paste form available as RC Cal is used. After 24 hrs CH were effective against E. faecalis. These differences may be occurs by the different methodologies used.

Regarding the spectrum of activity, CHX is bactericidal and effective against Gram-positive and Gram-negative bacteria, facultative and strict anaerobes and 2% CHX has been found to be highly active against E. faecalis. CHX seems to act by adsorbing on to the cell wall of microorganisms and causing the leakage of intracellular components. In the liquid presentation, CHX kills microorganisms in 30 s or less, while in the gel formulation it takes from 22s (2% CHX gel) to 2h (0.2% CHX gel)[6].

Endo et al in 2013, stated that in re-treatment gutta percha removal and chemomechanical preparation with 2% CHX gel, an effective root canal disinfectant, are effective for root canal disinfection, whereas additional intra canal dressing did not improve disinfection**[13]**.

As a root canal irrigant and intracanal medicament, CHX has an antibacterial efficacy comparable to that of sodium hypochlorite (NaOCl). In addition, it is also effective against strains resistant to $Ca(OH)_2$[14]. However, CHX has good efficacy against E.faecalis. The agar diffusion test suggested that the efficacy is concentration-dependent as 2% CHX gel is more efficient.

Herbal plant extract is an inherent part of our ancient culture and is being used in ayurveda and siddha systems of medicine for decades. Currently, a number of herbs are also being used in oral health care. Root canal infections are the microbial disease affecting the tooth predominantly caused by the E.faecalis. Today various

conventional antimicrobial agents are available, but they have numerous side effects also.The antibacterial activity of garcinia extract revealed potential inhibitory activity against E. faecalis. Hence, the plant mediated intra canal medicaments can be used as good therapeutic agent against microorganisms and also for the successful outcome of the root canal treatment.

According to the findings of the present study, none of the intra canal medicaments obtained 100% elimination of E. Faecalis within 24 hrs. Even though endodontic microflora is polymicrobial in nature, in our study single species of Enterococcus faecalis was used. which can be a limitation. Therefore, further studies have to be done using various species of microorganisms.

Within certain limitations, our results indicating the efficacy of garcinia extract justify its use as an intracanal medicament in endodontics. It can be considered as a natural medicament and cost effective. So, further clinical trials are needed to support this result which is obtained and further investigations regarding the efficacy of these intracanal medicaments in vitro and in vivo are needed for better understanding and for clinical applications.

V. Conclusion

Results of this study indicate that E. faecalis present in the root canal system can be eliminated or reduced with intracanal medicaments. The herbal extracts of Garcinia with garcinol have the potential to be used as intracanal medicaments, as the already established 2% of CHX. They have superior antimicrobial action on the E. faecalis in the root canal system. Thus, this study opens a new approach of trying herbal extracts as intracanal medicaments to eliminate the microorganism in the root canal system and aid in long term success in endodontics.

References

[1]. Wong J, Manoil D, Nasman P, Belibasakis GN, Neelakantan P. Microbiological aspects of root canal infections and disinfection strategies: an update review on the current knowledge and challenges. Frontiers in Oral Health. 2021;2:672887.
[2]. Alghamdi F, Shakir M. The influence of Enterococcus faecalis as a dental root canal pathogen on endodontic treatment: A systematic review. Cureus. 2020;12(3): 7257.
[3]. Berman LH, Hargreaves KM. Cohen's pathways of the pulp.12TH ed. Elsevier Health Sciences.2020;976-984.
[4]. Jena BS, Jayaprakasha GK, Singh RP, Sakariah KK. Chemistry and biochemistry of (−)-hydroxycitric acid from Garcinia. Journal of agricultural and food chemistry. 2002 Jan 2;50(1):10-22.
[5]. Marquez F, Babio N, Bullo M, Salas-Salvado J. Evaluation of the safety and efficacy of hydroxycitric acid or Garcinia cambogia extracts in humans. Critical reviews in food science and nutrition. 2012;52(7):585-94.
[6]. Gomes BP, Vianna ME, Zaia AA, Almeida JF, Souza-Filho FJ, Ferraz CC. Chlorhexidine in endodontics. Brazilian dental journal. 2013 Mar;24:89-102.
[7]. Haapasalo M, Endal U, Zandi H, Coil JM. Eradication of endodontic infection by instrumentation and irrigation solutions. Endodontic topics. 2005;10(1):77-102.
[8]. Kaufman B, Spangberg L, Barry J, Fouad AF. Enterococcus spp. in endodontically treated teeth with and without periradicular lesions. Journal of endodontics. 2005;31(12):851-6.
[9]. Zancan RF, Canali LC, Tartari T, Andrade FB, Vivan RR, Duarte MA. Do different strains of E. faecalis have the same behavior towards intracanal medications in in vitro research?. Brazilian oral research. 2018;32.
[10]. Estrela C, Estrela CR, Pécora JD. A study of the time necessary for calcium hydroxide to eliminate microorganisms in infected canals. Journal of Applied Oral Science. 2003;11:133-7.
[11]. Review Morrier JJ, Benay G, Hartmann C, Barsotti O. Antimicrobial activity of Ca (OH) 2 dental cements: an in vitro study. Journal of Endodontics. 2003;29(1):51-4.
[12]. Sonali GA, Rohit P, Manoj H, Ajay N. Comparative evaluation of calcium ion, hydroxyl ion release and pH levels between nano calcium hydroxide and other calcium hydroxide based intracanal medicaments: an in vitro study. University J Dent Scie 2017; 3(1):50-56.
[13]. Endo MS, Ferraz CC, Zaia AA, Almeida JF, Gomes BP. Quantitative and qualitative analysis of microorganisms in root-filled teeth with persistent infection: monitoring of the endodontic retreatment. European journal of dentistry. 2013;7(03):302-9.
[14]. Basrani B, Tjaderhane L, Santos JM, Pascon E, Grad H, Lawrence HP, Friedman S. Efficacy of chlorhexidine-and calcium hydroxide–containing medicaments against Enterococcus faecalis in vitro. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2003;96(5):618.