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A COMPARATIVE EVALUATION OF THE ANTIBACTERIAL EFFICACY OF VARIOUS ROOT CANAL IRRIGANTS AGAINST E. FECALIS : AN IN-VITRO STUDY

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ABSTRACT

The aim of endodontic treatment is to clean the root canal system including all vital and necrotic tissues, microorganisms, and its byproducts. The root canal system anatomy is extremely complex and diverse making it difficult to clean and shape it effectively. So, irrigation and disinfection of root canals plays a very important role in the success of endodontic treatment. The aim of this study was to evaluate & compare antimicrobial efficacy of 3.8% Silver Diamine Fluoride, 5.25% Sodium Hypochlorite, 100mg/ml Vitex Negundo Linn , 0.2 % Nano Chitosan , 0.9% Normal Saline and 2% Chlorhexidine against Enterococcus faecalis as root canal irrigant.

Sixty single rooted human permanent teeth were taken and decoronated to standardize the canal length. After biomechanical preparation, teeth were inoculated with E.faecalis & randomly divided into 6 groups and the final irrigation was carried out with tested irrigants. Group A(n = 10): 0.9% Normal Saline(control); Group B(n = 10): 3.8% Silver Diamine Fluoride; Group C(n = 10): 5.25% Sodium hypochlorite; Group D(n = 10): 100mg/ml vitex negundo linn; Group E(n=10): 0.2% Nano Chitosan and Group F(n=10): 2% Chlorhexidine.

The obtained constituent was cultured on agar plates & the number of CFUs (Colony forming units) per plate was determined using a digital colony counter, and statistically analysed using one-way ANOVA followed by post hoc Tukey test. 5.25% Sodium Hypochlorite showed maximum antimicrobial activity against E. faecalis when compared to 3.8% Silver Diamine Fluoride, 100mg/ml Vitex Negundo Linn , 0.2 % Nano Chitosan , 0.9% Normal Saline and 2% Chlorhexidine.

The present in-vitro study indicates that all the tested have exhibited effective antimicrobial efficacy against E. faecalis, with 5.25% Sodium Hypochlorite(Group C) found to be most effective against E. faecalis followed by the other irrigants.

Introduction

An endodontic infection is beckoned by a hive of bacterial species. Enterococcus faecalis is a non-fastidious, gram-positive facultative anaerobe that can infiltrate dentinal tubules.[1] This viable bacteria withstand biomechanical preparation and serve as a persistent source of infection or inflammation.[2]

The amount of bacteria in the canal is reduced by about 50% when conventional mechanical equipment is used alone.[3] As a result, endodontic irrigants are required to remove microbes from the root canal inaccessible regions.[4] Because of its great tissue dissolving ability, sodium hypochlorite is a commonly utilized intracanal irrigant in a variety of concentrations.[4] The clinically approved sodium hypochlorite concentration is 5.25% w/v.[5] Chlorhexidine, which has a 2% w/v concentration and an intrinsic substantivity feature, is another commonly used irrigation solution.[6] Although CHX is excellent as a final irrigant, it is not recommended to use it as the primary endodontic irrigant of the canal since it cannot destroy necrotic remains.[7]

A recently developed substance called chitosan is a biopolymer that is non-toxic.[8] Due to its chelating properties and broad-spectrum antibacterial action, it is used in endodontic treatment.[9] Chitosan nanoparticles are more biocompatible with tissues due to these characteristics.[10]

Due to its excellent anti-bacterial characteristics, silver has been utilized in dentistry from its inception. It is used in many areas for disinfection.[11] In an effort to be a successful irrigant in endodontic therapy, silver diamine fluoride, an anticariogenic substance with a high fluoride release capacity, is used.[12]

Numerous herbal extracts with antibacterial properties, including as neem, triphala, aloe vera, propolis, green tea, and morinda citrifolia, have also gained popularity in the field of endodontics.[13] The monoterpene molecule found in the ayurvedic plant Vitex negundo linn, also known as Nirgundi, which is rich in glycosides, alkaloids, and tannins, causes cell membrane lysis, which accounts for the antibacterial activity against bacteria in both aqueous and alcoholic extracts.[14] Till date, there has been no ideal endodontic irrigant which makes the quest for an ideal root canal irrigant still on. This invitro study aims to evaluate the antibacterial efficacy of 3.8% Silver Diamine Fluoride, 5.25% Sodium Hypochlorite, 0.2 % Nano Chitosan, 2% Chlorhexidine , 0.9% Normal Saline and an ayurvedic extract Vitex Negundo Linn against Enterococcus fecalis.

Materials and method

In the current investigation, single-rooted permanent human teeth that had been removed for periodontal or orthodontic reasons were gathered. The number of samples for this study was sixty after the collected samples were examined using inclusion and exclusion criteria. Included single-rooted teeth had patent 1:1 canal arrangement and were devoid of any morphological flaws, fractures, resorption, calcification, or trauma. Excluded from the study were teeth with varied canal structure, repaired teeth, and carious teeth. With hand and ultrasonic scalers, the sample teeth were selected and checked for any tissue remains, plaque, or calculus on the roots. Six groups overall were created from the chosen sixty samples, five of which were based on the irrigants to be

studied and one of which served as the control group. Each set had ten teeth in it.

Using an ISO #10K file and a digital radiograph through PSP, the working length was calculated. Using a HyFlex CM rotary file and the crown-down procedure, a root canal was prepared till size 25/6% taper file. Each canal was shaped and then irrigated with 5.25% sodium hypochlorite and 17% EDTA solution.

All of the samples were autoclaved for 15 minutes at 121°C and 15 lbs of pressure after root canal preparation. After sterilization, root canal apices of all the samples were sealed with light-cure GIC using instrument and light cure gun. The samples were then mounted on a wax block with measurements of 12 mm x 12 mm x 17 mm, and two coats of nail polish were applied to them. After the samples were injected with Enterococcus fecalis, the teeth were separated into six experimental groups, one of which was a control group, at random. From the inoculated root canals, samples of Enterococcus fecalis were taken prior to irrigation. Using the appropriate irrigant designated for each of the tested groups, the following irrigation protocol was followed: The sample's root canal received 0.5 ml of testing irrigant over a two-minute period using a sterile 2 mL syringe with a 24 gauge needle. To avoid any potential carryover of the tested irrigant, all experimental teeth were then cleansed with distilled water. A level around 1 mm below the root apex was reached by circumferentially filing with an endodontic hand file. Then, to absorb the canal contents, a 6%, ISO size 25 paper point was inserted into each canal up to the working length. After that, paper points were put into eppendorf tubes with 1 mL of saline and stirred for a minute in a vortex mixer. Blood agar plates were used to cultivate aliquots of 500µl dilutions. All plates were cultivated for 48 hours at 37°C in a 5% CO2 microaerophilic atmosphere. The quantity of CFUs (Colony Forming Units) per plate was then calculated using a digital colony counter.

Using SPSS software Version 19.0 (IBM Corporation, Chicago, USA), statistical analysis was carried out after obtaining the mean colony forming units of each group. The Student's paired t-test, one-way ANOVA, and Post-hoc Tukey Test were used for descriptive and inferential statistics during the statistical study.

Results

The mean CFU counts were compared among the six groups of ten samples each. The analysis done by one-way ANOVA showed statistically significant differences ($p<0.001$) in mean CFUs counts. The Group C (5.25% Sodium Hypochlorite) had the greatest mean reduction in CFU count followed by Group F (2% Chlorhexidine) Group E (0.2% Nanochitosan) Group D (100mg/ml vitex negundo linn) and Group B (3.8% Silver Diamine Fluoride). The Control Group A (0.9% normal saline) had the lowest mean reduction in CFU count.

Output Table:

Table 1: Comparison of reduction in mean CFU counts among the groups

Groups	Max.	Min.	Mean	S.D.	P value
Group A	17	9	12.10	2.46	0.01
Group B	26	19	21.80	2.34	
Group C	67	46	56.00	8.45	
Group D	27	19	23.40	3.09	
Group E	31	21	26.90	3.17	
Group F	37	26	31.20	3.55	

Discussion

This study made a sincere attempt to evaluate and compare antimicrobial efficacy of 3.8% Silver Diamine Fluoride, 5.25% Sodium Hypochlorite, 100mg/ml Vitex Negundo Linn , 0.2 % Nano Chitosan , 0.9% Normal Saline and 2% Chlorhexidine against Enterococcus fecalis as root canal irrigant.

In the present study, single-rooted teeth with in vitro closed-end canal models with the apex sealed with GIC were chosen for standardization in order to more accurately mimic in vivo root canal conditions.[15]

The current study's design was comparable to that employed by Nourzadeh et al. in 2017, who measured bacteria decrease following chemomechanical debridement.[16]

Because it was readily available, blood agar was employed in the current investigation as a medium for Enterococcus fecalis growth that was collected before and after irrigation.[17]

In the current investigation, E fecalis colony forming units were measured to examine the reduction in microbial growth before and post irrigation. Because of its greatest resistance to treatment and recurrence virulence, Enterococcus fecalis was chosen as the test organism for the current investigation.[18]

In endodontics, the irrigants make it easier to remove bacteria, debris and toxins from root canals both during and after instrumentation.[19] Although none of the most often used irrigants in dental practice including sodium hypochlorite, chlorhexidine, EDTA, hydrogen peroxide, and normal saline can be regarded as optimum or optimal, they are the most widely utilized.[20]

Group C in the current study consisted of the 5.25% sodium hypochlorite solution. In water, sodium hypochlorite ionizes to form Na+ and hypochlorite (HCO-) ions, bringing hypochlorous acid (HOCl) into equilibrium. The antimicrobial effects are caused by this hypochlorous acid.[21] In the present study, 5.25% NaOCl was utilized as an irrigant because of its superior antibacterial activity and tissue-dissolving ability.

Chlorhexidine is another often used antibacterial root canal irrigant. The interaction between the positive charge of the molecule and the negatively charged phosphate groups on the microbial cell walls is what gives the irrigant its antibacterial effectiveness.[22]

2% Chlorhexidine was considered in this study as one of the endodontic irrigants defined as group F since it is relatively non-toxic and has a greater substantivity rate.[23]

Chitosan has arisen in the field of dentistry as an alternative to root canal irrigants, a natural antibacterial agent.[24] Because of their larger surface area and higher charge density (Positive charge), chitosan nanoparticles (CNPS) have strong antibacterial action, which causes the bacterial cells they interact with to die. [25] 0.2% Nanochitosan was examined in the current investigation and assigned to group E.

Because of their antibacterial activity and the deposition of silver compounds, silver diamine fluoride regimens have been proven to be successful in preventing the formation of cariogenic bacteria.[26] This gave justification for testing SDF's antibacterial effectiveness against enterococcus fecalis in the current trial, where it was assigned group B.

Herbal alternatives may be suggested to counteract the negative effects of traditional chemical irrigants.[27] Vitex negundo Linn. colloquially, Nirgundi, an ayurvedic plant aqueous and alcoholic extracts show antibacterial effects on bacteria.[28] Out of all the Vitex negundo Linn. concentrations tested by Deogade et al., 100 mg/ml demonstrated the strongest antibacterial effect.[29] As a result, group D in the current investigation consisted of Vitex negundo Linn. at a concentration of 100mg/ml.

In the present study, the results found showed that 5.25% Sodium Hypochlorite irrigant (Group C) most effective against E. fecalis followed by 2% Chlorhexidine irrigant (Group F), 0.2% Nano-chitosan irrigant (Group E), 100mg/ml Vitex negundo linn irrigant

(Group D) and least effective for 3.8% Silver Diamine Fluoride irrigant (Group B).

After irrigation with sodium hypochlorite (Group C), the mean CFU/ml for Enterococcus fecalis decreased from 106.30±27.43 to 50.30±32.47. Because of its capacity to denature endotoxins, disintegrate organic tissue, and break up bacterial biofilms, sodium hypochlorite exhibits the strongest antibacterial action.[5] The findings of the current study agreed with those of Reyhani et al. and Oliveira DP et al.[30,31] The results of the study conducted by Abu AlTiman were different from those of the current study.[32] The findings of the study conducted by Dametto et al, on the other hand, were different from those of the current investigation.[33] The mean CFU/ml for Enterococcus fecalis in Group F (2% chlorhexidine) samples decreased from 109.60±16.98 before irrigation to 78.40±18.99 after irrigation. The mean CFU/ml for Enterococcus fecalis in the Vitex negundo Linn. Group (Group D) before irrigation was 113.60±21.62 which reduced to 90.20±20.81 after irrigation in the current study. While Vitex negundo Linn extract (Group D) has antibacterial activity due to its secondary metabolites, which cause cytoplasmic membrane degradation, chlorhexidine (Group F) has a higher antibacterial efficacy than Vitex negundo Linn extract (Group D) due to its ability to denature the bacterial cell wall as observed by Nagarsekar et al.[34] This outcome where sodium hypochlorite has more antibacterial efficacy compared to chlorhexidine can be validated due to presence of cationic bisbiguanides in Chlorhexidine which becomes inactivated on coming in contact with organic biofilm. In addition, it has limited penetration into matrix of bacterial biofilm.[35]

In the present study, 2% chlorhexidine was found to be more efficient against Enterococcus fecalis than chitosan nanoparticles. Contrary to the present study, a study conducted by Yadav P et al showed that Chitosan nanoparticles had antibacterial efficacy equivalent to that of 2% chlorhexidine.[36]

The CFU/ml of Enterococcus fecalis reduced from 116.40±29.46 to 89.50±28.68 after irrigation with 0.2% chitosan nanoparticles. In comparison to Vitex negundo Linn extract (Group D) and silver diamine fluoride (Group B), nanochitosan particles (Group E) were found to have higher antibacterial efficacy. This observation may be explained by interactions between the positively charged CNP and the negatively charged bacterial cell membrane, causing leakage of intracellular components and bacterial death.[37] In order to increase penetration, chitosan was also employed in nanoparticle form, which may have contributed to the substance's antibacterial action against E fecalis.

The CFU/ml of Enterococcus fecalis reduced from 105.50±29.68 to 83.70±30.24 after irrigation with 3.8% Silver Diamine Fluoride (Group B) which makes SDF the least effective antibacterial irrigant in the present study. In the present study, SDF was the least effective irrigant against E fecalis which may be explained by the fact that 3.8% SDF contains fewer number of silver and fluoride ions compared to the traditionally used 38% SDF.

Conclusion

The present in-vitro study evaluated & compared antimicrobial efficacy of 3.8% Silver Diamine Fluoride, 5.25% Sodium Hypochlorite, 100mg/ml Vitex Negundo Linn , 0.2 % Nano Chitosan , 0.9% Normal Saline and 2% Chlorhexidine against Enterococcus faecalis as root canal irrigants. Within the limitations of this study the following conclusions were drawn: All irrigants were effective against E. faecalis when compared to the control 0.9% Normal Saline, thus can be used as an effective root canal irrigant. 5.25% Sodium Hypochlorite showed maximum antimicrobial activity against E. faecalis when compared to 3.8% Silver Diamine Fluoride, 100mg/ml Vitex Negundo Linn , 0.2 % Nano Chitosan , 0.9% Normal Saline and 2% Chlorhexidine. 5.25% Sodium Hypochlorite(Group C) was found to be most effective against E. faecalis followed by 2% Chlorhexidine (Group F), 0.2% Nano-chitosan(Group E), 100mg/ml Vitex negundo linn (Group D) and least effective for 3.8% Silver Diamine Fluoride (Group B). However, further in-vivo studies are required to evaluate which of the various recently introduced test irrigants are more appropriate as root canal irrigants, not just against E. faecalis, but also for other persistent endodontic pathogens.

REFERENCES

1. Love, R. M. (2001). Enterococcus faecalis–a mechanism for its role in endodontic failure. International endodontic journal, 34(5), 399-405.
2. Stuart, C. H., Schwartz, S. A., Beeson, T. J., & Owatz, C. B. (2006). Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. Journal of endodontics, 32(2), 93-98.
3. Saber, S. E. D. M., & El-Hady, S. A. (2012). Development of an intracanal mature Enterococcus faecalis biofilm and its susceptibility to some antimicrobial intracanal medications; an in vitro study. European journal of dentistry, 6(01), 043-050.
4. Siqueira Jr, J. F., Lima, K. C., Magalhães, F. A., Lopes, H. P., & de Uzeda, M. (1999). Mechanical reduction of the bacterial population in the root canal by three instrumentation techniques. Journal of Endodontics, 25(5), 332-335.
5. Torabinejad, M., Cho, Y., Khademi, A. A., Bakland, L. K., & Shabahang, S. (2003). The effect of various concentrations of sodium hypochlorite on the ability of MTAD to remove the smear layer. Journal of endodontics, 29(4), 233-239.
6. Basrani, B., Tjäderhane, L., Santos, J. M., Pascon, E., Grad, H., Lawrence, H. P., & Friedman, S. (2003). Efficacy of chlorhexidine-and calcium hydroxide–containing medicaments against Enterococcus faecalis in vitro. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 96(5), 618-624.
7. Naenni, N., Thoma, K., & Zehnder, M. (2004). Soft tissue dissolution capacity of currently used and potential endodontic irrigants. Journal of endodontics, 30(11), 785-787.
8. Shrestha, A., Friedman, S., & Kishen, A. (2011). Photodynamically crosslinked and chitosan-incorporated dentin collagen. Journal of dental research, 90(11), 1346-1351.
9. Ma, E., MM, R., & Elmesellawy, M. Y. (2022). Comparative Evaluation of the Antibacterial Effect of Different Combinations of Etidronate, Nanochitosan and NaOCl on E. Faecalis Biofilm. Journal of International Dental & Medical Research, 15(4).
10. Bashir, S. M., Ahmed Rather, G., Patrício, A., Haq, Z., Sheikh, A. A., Shah, M. Z. U. H., ... & Fonte, P. (2022). Chitosan nanoparticles: a versatile platform for biomedical applications. Materials, 15(19), 6521.
11. Mathew, V. B., Madhusudhana, K., Sivakumar, N., Venugopal, T., & Reddy, R. K. (2012). Anti-microbial efficiency of silver diamine fluoride as an endodontic medicament–An ex vivo study. Contemporary clinical dentistry, 3(3), 262.
12. Chu, C. H., Lo, E. C. M., & Lin, H. C. (2002). Effectiveness of silver diamine fluoride and sodium fluoride varnish in arresting dentin caries in Chinese pre-school children. Journal of dental research, 81(11), 767-770.
13. Jain, P., & Ranjan, M. (2014). Role of herbs in root canal irrigation-A review. IOSR J Pharm Biol Sci, 9(2), 06-10.
14. Paria, S., Chaki, S., Ghosdastidar, S., & Mookerjee, M. (2011). Evaluation of antimicrobial activity and antioxidant property of V. Negundo Leaf Extract. IJDFR, 2(4), 337-45.
15. Takeda, F. H., Harashima, T., Kimura, Y., & Matsumoto, K. (1999). A comparative study of the removal of smear layer by three endodontic irrigants and two types of laser. International Endodontic Journal, 32(1), 32-39.
16. Nourzadeh, M., Amini, A., Fakoor, F., Raoof, M., & Sharififar, F. (2017). Comparative antimicrobial efficacy of Eucalyptus galbie and Myrtus communis L. extracts, chlorhexidine and sodium hypochlorite against Enterococcus faecalis. Iranian endodontic journal, 12(2), 205.
17. Berber, V. B., Gomes, B. P. F. A., Sena, N. T., Vianna, M. E., Ferraz, C. C. R., Zaia, A. A., & Souza-Filho, F. J. (2006). Efficacy of various concentrations of NaOCl and instrumentation techniques in reducing Enterococcus faecalis within root canals and dentinal tubules. International endodontic journal, 39(1), 10-17.
18. Siqueira Jr, J. F., & Rôças, I. N. (2004). Polymerase chain reaction–based analysis of microorganisms associated with failed endodontic treatment. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 97(1), 85-94.
19. Haapasalo, M., Shen, Y., Qian, W., & Gao, Y. (2010). Irrigation in endodontics. Dental Clinics, 54(2), 291-312.
20. Kandaswamy, D., & Venkateshbabu, N. (2010). Root canal irrigants. Journal of conservative dentistry: JCD, 13(4), 256.
21. Barrette Jr, W. C., Hannum, D. M., Wheeler, W. D., & Hurst, J. K. (1989). General mechanism for the bacterial toxicity of hypochlorous acid: abolition of ATP production. Biochemistry, 28(23), 9172-9178.
22. Athanassiadis, B., Abbott, P. V., & Walsh, L. J. (2007). The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics. Australian dental journal, 52, S64-S82.
23. Tanomaru Filho, M., Leonardo, M. R., Silva, L. A., Anibal, F. F., & Faccioli, L. H. (2002). Inflammatory response to different endodontic irrigating solutions. International endodontic journal, 35(9), 735-739.
24. Kmiec, M., Pighinelli, L., Tedesco, M. F., Silva, M. M., & Reis, V. (2017). Chitosan-properties and applications in dentistry. Adv Tissue Eng Regen Med Open Access, 2(4), 00035.
25. Shi, Z., Neoh, K. G., Kang, E. T., & Wang, W. (2006). Antibacterial and mechanical properties of bone cement impregnated with chitosan nanoparticles. Biomaterials, 27(11), 2440-2449.
26. Thibodeau, E. A., Handelman, S. L., & Marquis, R. E. (1978). Inhibition and killing of oral bacteria by silver ions generated with low intensity direct current. Journal of dental research, 57(9-10), 922-926.
27. Pujar, M., & Makandar, S. D. (2011). Herbal usage in endodontics-A review. International journal of contemporary dentistry, 2(1).
28. Gill, B. S., & Mehra, R. (2018). Navgeet; Kumar, S. Vitex negundo and its medicinal value. Mol. Biol. Rep, 45(6), 2925-2934.
29. Deogade, M. S., Pandya, T., Prasad, K. S., Kale, K., & Tankhiwale, N. (2018). Antimicrobial activity of Vitex negundo Linn.(Nirgundi) leaves extract. Journal of Research in

Traditional Medicine, 2(4), 99-99.
30. Reyhani, M. F., Rezagholizadeh, Y., Narimani, M. R., Rezagholizadeh, L., Mazani, M., Barhaghi, M. H. S., & Mahmoodzadeh, Y. (2017). Antibacterial effect of different concentrations of sodium hypochlorite on Enterococcus faecalis biofilms in root canals. Journal of dental research, dental clinics, dental prospects, 11(4), 215.
31. Oliveira, D. P., Barbizam, J. V., Trope, M., & Teixeira, F. B. (2007). In vitro antibacterial efficacy of endodontic irrigants against Enterococcus faecalis. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 103(5), 702-706.
32. Al-Timan, J. A. A., Al-Huwaizi, H. F., & Abed, H. H. (2020). Evaluating the effect of adding Chitosan nanoparticles on the disinfection properties of Glyde material: An in vitro study. Research Journal of Pharmacy and Technology, 13(1), 255-258.
33. Dametto, F. R., Ferraz, C. C. R., de Almeida Gomes, B. P. F., Zaia, A. A., Teixeira, F. B., & de Souza-Filho, F. J. (2005). In vitro assessment of the immediate and prolonged antimicrobial action of chlorhexidine gel as an endodontic irrigant against Enterococcus faecalis. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology, 99(6), 768-772.
34. Nagarsekar, K. S., Nagarsenker, M. S., & Kulkarni, S. R. (2010). Evaluation of composition and antimicrobial activity of supercritical fluid extract of leaves of Vitex negundo. Indian journal of pharmaceutical sciences, 72(5), 641.
35. Echeverri, D., Alderete, D., ECHEVERRI, D., & ALDERETE, D. (2015). In vitro antibacterial effect of 2% chlorhexidine against Enterococcus faecalis in dentin previously irrigated with 5% sodium hypochlorite. Int. J. Odontostomat, 9(1), 25-29.
36. Yadav, P., Chaudhary, S., Saxena, R. K., Talwar, S., & Yadav, S. (2017). Evaluation of Antimicrobial and Antifungal efficacy of Chitosan as endodontic irrigant against Enterococcus Faecalis and Candida Albicans Biofilm formed on tooth substrate. Journal of clinical and experimental dentistry, 9(3), e361.
37. del Carpio-Perochena, A., Bramante, C. M., Duarte, M. A. H., de Moura, M. R., Aouada, F. A., & Kishen, A. (2015). Chelating and antibacterial properties of chitosan nanoparticles on dentin. Restorative dentistry & endodontics, 40(3), 195-201.