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Comparative evaluation of antibacterial efficacy of nonantibacterial drugs - Diclofenac Sodium, Omeprazole and Cetirizine against E. faecalis when used as an intracanal medicament: An in vitro study

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ABSTRACT

Aim: This study aimed to evaluate the antibacterial efficacy of non-antibacterial drugs—diclofenac, omeprazole, and cetirizine—against *Enterococcus faecalis* when used as intracanal medicaments at time interval of 24 hr and 7 days in endodontic treatments.

Settings and Design: An *in-vitro* comparative study

Materials and Method: The study comprised of four test groups- Group – 1 [Ca $(OH)_2$ (16mg/ml)], Group – 2 [Diclofenac sodium (400 mg/ml)], Group – 3 [Omeprazole (4 mg/ml)], Group – 4 [Cetirizine (2 mg/ml)], each group contained 10 samples, which were evaluated for antibacterial efficacy using agar well diffusion test. The experimental specimens were then placed on agar plates inoculated with *E. faecalis*, and the area of inhibition was calculated.

Statistical analysis: Antibacterial efficacy was evaluated using multiple group comparison using One-way Anova, Posthoc Tukey test and Paired t-test.

Results: All the experimental groups exhibited bacterial inhibition; however, group 2 exhibited highest antibacterial activity compared to group 1,3 and 4.

Conclusion: This study demonstrates the promising antibacterial efficacy of non-antibacterial drugs, particularly diclofenac sodium, when used as intracanal medicament against Enterococcus faecalis, highlighting its potential as an effective alternative in endodontic treatments.

INTRODUCTION

Microorganisms play a key role in the development of periapical and pulpal disorders.[1] Sometimes complete disinfection of canals is very difficult to achieve because of the complex anatomical variations of root canal. The most frequent survivors present in root canal failure cases are Enterococcus faecalis in very high proportion. It can survive in extreme conditions and has the ability to form biofilms.[1-3] Hence, the use of antibacterial agents in the form of intracanal medicament is inevitable to eliminate endodontic microflora.[4,5]

Common choices include calcium hydroxide, chlorhexidine, double antibiotic paste, triple antibiotic paste, iodine-based solutions, etc., which are widely used for their antimicrobial properties, though each has limitations like resistance, staining or weakening of root canal dentine structure on prolonged use.

Among the various intracanal medicaments available for use, calcium hydroxide (Ca $[OH]_2$) is the most frequently used medicament since many years. But it has a drawback that it does not destroy the biofilm of E. faecalis, subsequently resulting in newer antibacterial compounds being proposed as a substitute.[6,7]

In recent years, the rise of antibiotic resistance has prompted the exploration of non-antibacterial drugs for their potential antibacterial properties when used as an intracanal medicament. They include some antihistamines, antipsychotics, tranquillizers, anti-hypertensives, local anesthetics and even NSAIDs etc. All of these drugs with moderate to powerful anti-microbial properties have been classified under the common term “non antibiotics”.[8]

Diclofenac sodium (DCS) is an NSAID that belongs to the phenylacetic acid family. In endodontics, effective pain management is sometimes challenging. The efficacy of Diclofenac sodium using different delivery routes for preventing post-endodontic pain has been studied with favorable results. Diclofenac sodium (DS), part of the NSAID family, has demonstrated a high bactericidal action on Gram-positive and Gram-negative bacteria by suppressing bacterial DNA synthesis.[9]

Recently, proton pump inhibitors (PPIs) were proposed as an adjuvant to intracanal medicaments. Although the role of proton pump in the survival of resistant endodontic pathogens is known, limited research is carried out to evaluate the antibacterial efficacy of PPIs.[10] Cetirizine, an antihistamine commonly used to alleviate allergy symptoms, has garnered attention for its potential role in managing root canal infections. Emerging studies suggest that cetirizine's anti-inflammatory properties may help to mitigate pain and swelling associated with dental infections, offering a complementary approach to traditional analgesics.[11]

That is why need for the study arises to compare these non-antibacterial drugs for their antibacterial efficacy against *E. faecalis* when used as an intracanal medicament. The null hypothesis of the study was that there is no difference in antimicrobial efficacy of Diclofenac Sodium, Ca $(OH)_2$, Cetirizine and Omeprazole against *E. faecalis* when used as an intracanal medicament at interval of 24hr and 7 days evaluation.

Material and Methodology

Bacterial strains and media

The American-type culture collection 29212 of E. faecalis was obtained and nourished in brain-heart infusion broth. The agar diffusion test method was used to evaluate the antibacterial activity of materials. To adjust for turbidity, inoculum density was set at 0.5 McFarland (1.58 × 108 bacteria/ml).[13]

Preparation of medicament

All the medicaments were precured in powder form and mixed with distilled water.

Group – 1 [Ca $(OH)_2$ (16mg/ml)]: One milliliter of distilled water was mixed with 16 mg powder to attain a concentration of 16 mg/ml.

Group – 2 [Diclofenac sodium (400 mg/ml)]: One milliliter of distilled water was mixed with 400 mg powder to attain a concentration of 400 mg/ml.

Group – 3 [Omeprazole (4 mg/ml)]: Ten milliliter of distilled water was mixed with 40 mg powder to attain a concentration of 4 mg/ml.

Group – 4 [Cetirizine (2 mg/ml)]: 250 milliliter of distilled water was mixed with 500 mg powder to attain a concentration of 16 mg/ml.

Agar well diffusion assay

The experiment required a total of 8 Mueller–Hinton agar plates, 2 plates for each group. The bacteria were seeded on agar plates. Cotton swabs were used to ensure an even distribution of bacteria cultivated on the agar plates. Wells of 7 mm in diameter and 4 mm in depth were punched out. 5 wells per 1 Mueller–Hinton agar plate. About 0.1 ml of the test material was filled into each well. Incubation of the plates was done aerobically at 37°C for 24 hours. After incubation, a blinded examiner measured the zone of bacterial inhibition around each well as the shortest distance (mm) from the initial point of bacterial growth to the outer margin of the well with an inhibition zone. measuring scale.

Evaluation of inhibition zone were done after 24 h and 7 days interval.

Results

On comparing the medicaments, a notable difference in the diameter of the growth inhibition zones was observed between the four medicaments, with the greatest diameter seen for Diclofenac sodium followed by Ca $(OH)_2$, cetirizine and omeprazole. On comparison of the parameter of Diameter of inhibition zone (in mm) at 24 h, the highest mean is seen in Group 2 (6.8) followed by Group 1 (4), Group 4 (3.4), and the least value in Group 3 (1.4). This parameter is statistically significant with a test value of 67.818 and p value of <0.001.

Table 1: Diameter of inhibition zone (in mm) at 24 H and 7 days evaluation

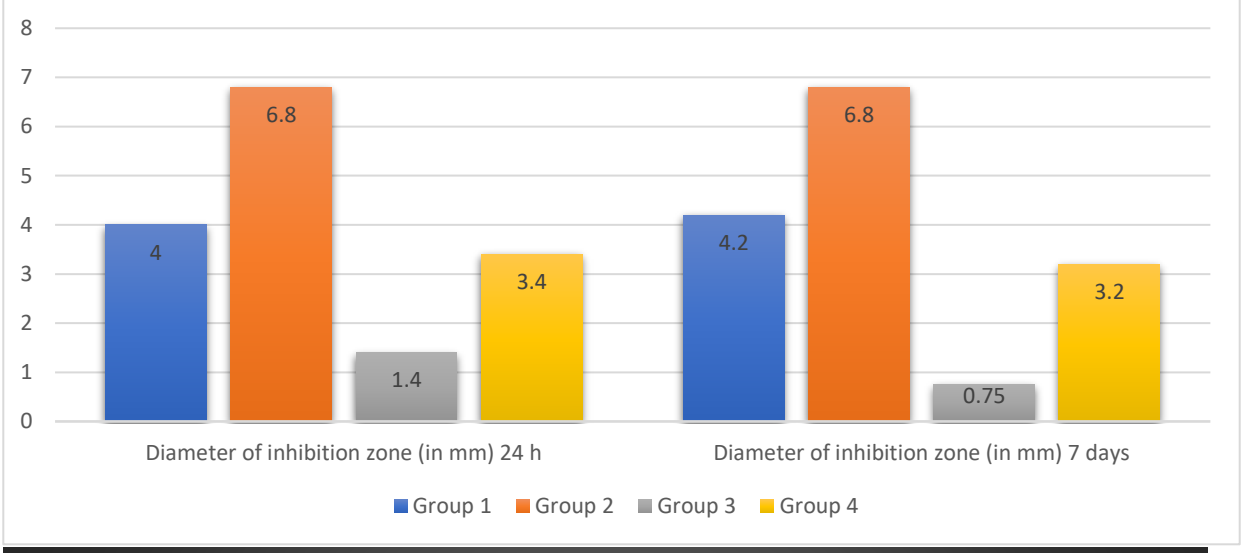
Diameter of inhibition zone (in mm)		
GROUPS	**24 h**	**7 days**
Ca(OH)2	40 mm	42 mm
Diclofenac sodium	68 mm	68 mm
Omeprazole	13 mm	7.5 mm
Cetirizine	34 mm	32 mm

On comparison of the parameter of Diameter of inhibition zone (in mm) at 7 days the highest mean is seen in Group 2 (6.8) followed by Group 1 (4.2), Group 4 (3.2), and the least value in Group 3 (0.75). This parameter is statistically significant with a test value of 210.174* and p value of <0.001.

On comparison of the parameter of difference between 24 hours and 7 days the highest mean is seen in Group 3 (0.65) followed by Group 4 (0.2), Group 2 (0), and the least value in Group 1 (-0.2). This parameter is statistically significant with a test value of 11.272 and p value of <0.001. Posthoc analysis shows that the significant differences are seen between Group 1 and Group 3, Group 2 and Group 3, Group 3 and Group 4.

Figure 2: Posthoc Turkey test showing difference between the 24 hours and 7 days values

	Group 1	Group 2	Group 3	Group 4	F /welch	P value
Diameter of inhibition zone (in mm) 24 h	4±1.027	6.8±0.789	1.4±0.516	3.4±0.994	67.818	<0.001
Diameter of inhibition zone (in mm) 7 days	4.2±0.753	6.8±0.789	0.75±0.264	3.2±1.033	210.174*	<0.001
Difference between 24 hours and 7 days	-0.2±0.35	0±0	0.65±0.474	0.2±0.35	11.272	<0.001



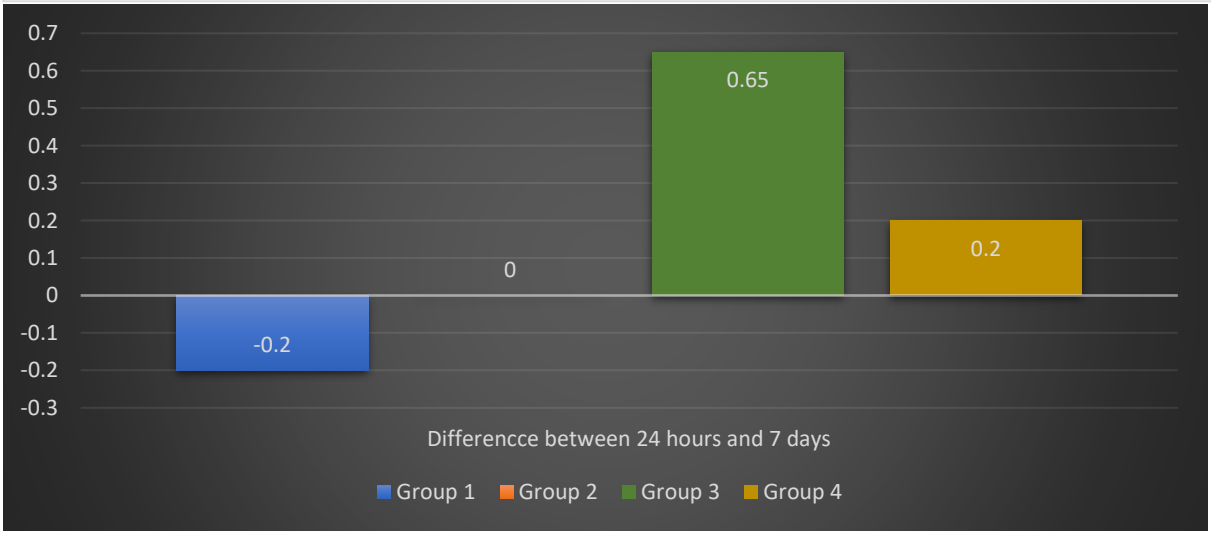


Table 2: One way Anova test showing difference between the 24 hours and 7 days values

Table 3: Paired t-test to compare the 24 hours and 7 days values

		N	Mean ± SD	Mean difference ± SD	t	P VALUE
Group 1	Diameter of inhibition zone (in mm) 24 h	10	4±1.03	-0.2±0.35	-1.81	0.104
	Diameter of inhibition zone (in mm) 7 days	10	4.2±0.75			
Group 2	Diameter of inhibition zone (in mm) 24 h	10	6.8±0.79			
	Diameter of inhibition zone (in mm) 7 days	10	6.8±0.79			
Group 3	Diameter of inhibition zone (in mm) 24 h	10	1.4±0.52	0.65±0.47	4.33	**0.002**
	Diameter of inhibition zone (in mm) 7 days	10	0.75±0.26			
Group 4	Diameter of inhibition zone (in mm) 24 h	10	3.4±0.99	0.2±0.35	1.81	0.104
	Diameter of inhibition zone (in mm) 7 days	10	3.2±1.03			

DISCUSION

The development of antibiotic resistance has led to the evolution of alternative methods for the disinfection of root canals. In light of this, the antimicrobial efficacy of non-steroidal anti-inflammatory drugs (NSAIDs), proton pup inhibitors (PPI), antihistaminines were compared with Ca $(OH)_2$ to analyze their effectiveness as an intracanal medicament against E. faecalis in this study. [10,12-14]

In this study, concentration of 400 mg/ml for Diclofenac sodium was used as the study aimed to determine the maximum efficacy of the drug against *E. faecalis*, particularly given the bacteria's known resistance to many conventional treatments. A concentration of 16 mg/ml was chosen for Ca $(OH)_2$ as it is consistent with the typical formulations used in endodontic practice. This concentration reflects what is commonly used in clinical settings as an intracanal medicament. A study conducted by **Maji et al.** in 2017 on antimicrobial activity of cetirizine dihydrochloride against many pathogens, it was concluded that antimicrobial spectrum of the drug ranged from 200 - 2000 µg/ml for tested Gm +ve and Gm –ve microorganisms. E. faecalis being highly resistant microorganism the concentration of 2 mg/ml was chosen to evaluate maximum potential antibacterial activity of the drug.[11]

The concentration of 4mg/ml was chosen for Omeprazole as it is low enough to minimize potential toxicity to surrounding tissues, which is critical when evaluating a drug not primarily intended for antibacterial use. The concentration has been selected based on previous research that examined omeprazole's effects in different contexts, providing a foundation for comparison and ensuring methodological consistency with recognized practices.[15]

Enterococcus faecalis was chosen for this study due to its prevalence as a resistant bacterial species commonly found in persistent endodontic infections. *E. faecalis* is known for its intrinsic resistance to many conventional antibacterial agents, making it an important target for evaluating the efficacy of alternative treatments.[16]

The agar well diffusion test was used in this study to assess the antibacterial efficacy of the non-antibacterial drugs against *Enterococcus faecalis as it* provides clear visual results in the form of inhibition zones around the wells, making it easy to measure the effectiveness of each drug against the bacterial strain.[17] The results of this study indicate a notable antibacterial efficacy of non-antibacterial drugs against Enterococcus faecalis. Specifically, diclofenac sodium emerged as the most effective agent, surpassing the efficacy of both calcium hydroxide (Ca $(OH)_2$) and the other non-antibacterial drugs tested, namely cetirizine and omeprazole.[18]

Diclofenac sodium, a non-steroidal anti-inflammatory drug (NSAID), demonstrated a significant ability to inhibit the growth of E. faecalis. This finding is intriguing, considering its primary use is not in antibacterial treatment. Diclofenac sodium's superior antibacterial properties can be attributed to its ability to penetrate bacterial cell membranes and disrupt cellular functions.[10] This non-steroidal anti-inflammatory drug (NSAID) may inhibit the synthesis of bacterial proteins or affect the bacterial metabolism, leading to reduced viability of *E. faecalis*. **Salem Milani et al. (**2013) and **Chockattu et al. (2018)** also showed superior results of diclofenac sodium when compared to Ca $(OH)_2$ in his study in.[19,20]

The drug's anti-inflammatory properties may also contribute to creating a more favorable environment for the healing process in endodontic treatments. Higher concentrations of diclofenac sodium can enhance its antibacterial effects.[19] Calcium hydroxide, known for its antimicrobial properties, served as a standard comparison in this study. Its alkaline pH is effective in neutralizing the acidic environment that many bacteria, including *E. faecalis*, thrive in. The slightly lower efficacy of calcium hydroxide compared to diclofenac in this study might suggest that while both agents are effective, the multifaceted action of diclofenac may provide a more potent antibacterial effect in this context.[21,22]

This study's results indicate that while cetirizine may contribute to overall treatment, its efficacy as a standalone intracanal medicament is limited.

Cetirizine, an antihistamine, showed some antibacterial activity, albeit less pronounced than that of diclofenac and calcium hydroxide. Its mechanism may involve interference with bacterial adhesion or modulating the immune response, but further research is needed to clarify its exact antibacterial properties. Although cetirizine is primarily an antihistamine, some studies have suggested it may have modulating effects on bacterial growth or biofilm formation.[11,23]

Omeprazole, primarily a proton pump inhibitor used for gastric acid management, showed the least antibacterial activity in this context.[10] A study conducted by **Wagner et al.** (2011) showed synergistic effect of omeprazole in combination with Ca $(OH)_2$ which was superior to Ca $(OH)_2$ when used alone.[24,25] But omeprazole does not have enough antimicrobial efficacy when used alone.[26] Thus, it showed its antibacterial efficacy at 24h evaluation but it gradually decreased at 7-day evaluation as E. faecalis might have developed resistance against omeprazole.

The results of this study underscore the potential of repurposing non-antibacterial drugs for endodontic treatment. While traditional antibacterial agents have their place, incorporating effective alternatives like diclofenac sodium could enhance therapeutic outcomes. The use of these drugs as adjuncts to standard treatments might help in managing infections more effectively, especially in cases resistant to conventional therapies.

CONCLUSION

In conclusion, this study demonstrates the promising antibacterial efficacy of non-antibacterial drugs, particularly diclofenac sodium, when used as intracanal medicaments against Enterococcus faecalis. The superior performance of diclofenac sodium, followed by calcium hydroxide, cetirizine, and omeprazole, highlights its potential as an effective alternative in endodontic treatments. These findings suggest that the repurposing of non-antibacterial agents may offer valuable therapeutic options in combating resistant strains of bacteria commonly found in root canal infections. Further research is warranted to explore the underlying mechanisms and to assess the clinical implications of these results in endodontic practice.

REFERENCES

- Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral surgery, oral medicine, oral pathology. 1965 Sep 1;20(3):340-9.
- Distel JW, Hatton JF, Gillespie MJ. Biofilm formation in medicated root canals. J Endod 2002;28:689-93
- Alghamdi F, Shakir M. The influence of Enterococcus faecalis as a dental root canal pathogen on endodontic treatment: A systematic review. Cureus. 2020 Mar;12(3).
- Chong BS, Pitt Ford TR. The role of intracanal medication in root canal treatment. Int Endod J 1992;25:97-106
- Ordinola-Zapata R, Noblett WC, Perez-Ron A, Ye Z, Vera J. Present status and future directions of intracanal medicaments. International endodontic journal. 2022 May;55:613-36.
- Momenijavid M, Salimizand H, Korani A, Dianat O, Nouri B, Ramazanzadeh R, et al. Effect of calcium hydroxide on morphology and physicochemical properties of Enterococcus faecalis biofilm. Sci Rep 2022;12:7595
- Mohammadi Z, Dummer PM. Properties and applications of calcium hydroxide in endodontics and dental traumatology. International endodontic journal. 2011 Aug;44(8):697-730.
- Lagadinou M, Onisor MO, Rigas A, Musetescu DV, Gkentzi D, Assimakopoulos SF, Panos G, Marangos M. Antimicrobial properties on non-antibiotic drugs in the era of increased bacterial resistance. Antibiotics. 2020 Mar 2;9(3):107.
- Morsy D, Nageh M. Evaluation of the antibacterial activity of ibuprofen, triple antibiotic paste and calcium hydroxide as intracanal medicaments against Enterococcus faecalis in single rooted teeth. A

Comparative In vitro study. Egypt Dent J 2022;68:1867-74
- Tilokani A, Agrawal P, Pradhan PK, Patri G, Karmakar N, Sinha Y. Comparative evaluation of antibacterial efficacy of nonsteroidal anti-inflammatory medications and proton-pump inhibitor against Enterococcus faecalis: An in vitro study. J Conserv Dent 2023;26:79-82.
- Maji HS, Maji S, Bhattacharya M. An Exploratory Study on the Antimicrobial Activity of Cetirizine Dihydrochloride. Indian Journal of Pharmaceutical Sciences. 2017 Sep 1;79(5).
- Silva AA, Silva PM. Non-antibiotic compounds: the activity of the NSAID diclofenac on bacteria—a review. Int. J. Curr. Microbiol. Appl. Sci. 2018;7:340-51.
- Koparkar T, Kalyan S, Mandke L, Vandekar M. Efficacy of various proton-pump inhibitors and calcium hydroxide as intracanal medicaments against Enterococcus Faecalis: An: in vitro: study. Endodontology. 2022 Oct 1;34(4):259-64.
- Barbarossa A, Rosato A, Corbo F, Clodoveo ML, Fracchiolla G, Carrieri A, Carocci A. Non-antibiotic drug repositioning as an alternative antimicrobial approach. Antibiotics. 2022 Jun 17;11(6):816.
- Mehta S, Verma P, Tikku AP, Chandra A, Bains R, Banerjee G. Comparative evaluation of antimicrobial efficacy of triple antibiotic paste, calcium hydroxide, and a proton pump inhibitor against resistant root canal pathogens. European journal of dentistry. 2017 Jan;11(01):053-7
- Salem-Milani A, Balaei-Gajan E, Rahimi S, Moosavi Z, Abdollahi A, Zakeri-Milani P, Bolourian M. Antibacterial effect of diclofenac sodium on Enterococcus faecalis. Journal of dentistry (Tehran, Iran). 2013;10(1):16.
- Jorgensen JH. MJ Ferraro Antimicrobial susceptibility testing: A review of general principles and contemporary practices., 2009, 49. DOI: https://doi.org/10.1086/647952.:1749-55.
- Salem-Milani A, Balaei-Gajan E, Rahimi S, Moosavi Z, Abdollahi A, Zakeri-Milani P, Bolourian M. Antibacterial effect of diclofenac sodium on Enterococcus faecalis. Journal of dentistry (Tehran, Iran). 2013;10(1):16.
- Mazumdar K, Dastidar SG, Park JH, Dutta NK. The anti-inflammatory non-antibiotic helper compound diclofenac: an antibacterial drug target. European journal of clinical microbiology & infectious diseases. 2009 Aug;28:881-91
- Chockattu SJ, Deepak BS, Goud KM. Comparison of anti-bacterial efficiency of ibuprofen, diclofenac, and calcium hydroxide against Enterococcus faecalis in an endodontic model: An: in vitro: study. Journal of Conservative Dentistry and Endodontics. 2018 Jan 1;21(1):80-4.
- Janani K, Teja KV, Ajitha P, Sandhya R. Evaluation of tissue inflammatory response of four intracanal medicament–An animal study. Journal of Conservative Dentistry and Endodontics. 2020 May 1;23(3):216-20
- Prabhakar AR, Hadakar SG, Raju OS. Comparative evaluation of pH and antibacterial effect of various calcium hydroxide combinations on E. faecalis and its effect on root strength: An: in vitro: study. Contemporary Clinical Dentistry. 2012 Jan 1;3(1):42-7.
- Carmona-Orozco ML, Echeverri F. Induction of biofilm in extended-spectrum beta-lactamase Staphylococcus aureus with drugs commonly used in pharmacotherapy. Microbial Pathogenesis. 2024 Oct 1;195:106863.
- Wagner C, Barth VC, de Oliveira SD, Campos MM. Effectiveness of the proton pump inhibitor omeprazole associated with calcium hydroxide as intracanal medication: an in vivo study. Journal of endodontics. 2011 Sep 1;37(9):1253-7
- Cogo DM, Oliveira SD, Antunes FC, Kopper PM, Nasário JS, Vier-Pelisser FV. Potentiation of the action of calcium hydroxide on Enterococcus faecalis by proton pump inhibitor omeprazole. Revista odonto ciência. Porto Alegre. Vol. 30, no. 3 (2015), p. 76-80. 2015.
- Vivekanandhan P, Abraham TA, Anuradha B. Antibacterial efficacy of Nisin and calcium hydroxide with a proton pump inhibitor as an intracanal medicament. World Journal of Dentistry. 2016 Oct 1;9:111-6.