Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/19866
DOI URL: http://dx.doi.org/10.21474/IJAR01/19866

RESEARCH ARTICLE

"CAN ANTI-OXIDANTS REPLACE CALCIUM HYDROXIDE AS INTRA CANAL MEDICAMENT" : AN IN VITRO STUDY

R.Rajasekhar Naik[1], S. Anitha Rao[2], J. Arun Rathod[3], S. Srinivas[4]

Manuscript Info

Manuscript History
Received: 08 September 2024
Final Accepted: 17 October 2024
Published: November 2024

Key words:-
ICM, Intracanal Medicament, CFUs-Colony Forming Units, CGA-Chlorogenic Acid

Corresponding Author:-
R.Rajasekhar Naik,
Conservative dentistry and Endodontics, Mamata dental college,Khammam ,Telangana

Abstract

Introduction: Endodontics has several main goals, one of which is disinfecting the root canal system. Even though the bacterial load can be effectively reduced by cleaning, shaping, and using antimicrobial medications, some bacteria still survive and proliferate, leading to reinfection. Herbal alternatives have been introduced to counter the inefficacy, possible side effects, and safety concerns associated with synthetic drugs. Herbal irritants are safe and to contain active ingredients with advantageous physiological effects. So, the aim of the study is to evaluate and compare the antimicrobial efficiency of Antioxidants, i.e., green coffee, Lemon, and Honey with Calcium hydroxide as intra-canal medicaments.

Methodology: A total of 156single-rooted teeth were decorated at the cement-enamel junction. The apical part of the roots was sealed with composite resin to prevent leakage, followed by instrumentation up to the F3 Protaper universal rotary system and bacterial inoculation. The samples weredivided into 5 groups based on intracanal medicament used $Ca(OH)_2$,lemon extract,honey,green coffee extract and saline, respectively. These samples were incubated for 24 hrs at 37°C, and then scrapings from these teeth were collected in a test tube containing BHI broth and incubated for 24 hrs at 37°C. samples were streaked on agar plates and observed for E.Faecalis colonies. The One-way ANOVA and Bonferroni Multiple Comparison tests were used to analyse the data statistically.

Results: Calcium hydroxide has shown the highest antimicrobial activity compared to other groups. There is no significant difference between lemon and honey. Green coffee has the least antimicrobial property.

Conclusion: Antioxidants have a potential to be used as intracanal medicament if its antibacterial activity could be enhanced.

Introduction:-

Hippocrates, the father of medicine, advocated "Let food be your medicine and let medicine be your food" more than 2,000 years ago.[1]The basis for successful endodontic treatment is elimination of microorganisms from the root canal [2]. Due to the complex nature of the root canal system, cleaning and shaping is often not enough to reduce the bacterial count. A combination of instrumentation, irrigation and intracanal medication is used [3]. Intracanal medicaments are used in Endodontics to complete the work started by instrumentation and irrigation to render the

root canal free of microorganisms[4]. Though instrumentation and irrigation reduce the bacterial count in the root canal to a great extent, some bacteria, mainly E faecalis, will stay back and continue to multiply. E. faecalis is the most persistent bacteria that may cause flare-ups or reinfections.As complete or near complete elimination of microorganisms from the root canal is a challenge, there is a need to identify the best intracanal medicament available.[5]

Calcium hydroxide is the most commonly used intracanal medicament. Its antimicrobial action is related to its high pH, which results in the inactivation of bacterial membrane enzymes [6]But Calcium hydroxide has some disadvantages in that calcium hydroxide is ineffective against E. faecalis. It resists calcium hydroxide for about 10 days[2]. Studies show that calcium hydroxide causes weakening of root dentin by 23-43.9% following root canal filling [7].Another disadvantage is that the removal of calcium hydroxide from the root canal is difficult.[8]

There is a growing trend to seek natural dental treatment remedies to overcome chemical agent drawbacks.[1] In Previous studies, antioxidants like lycopene (koppulu et al., 2015) and turmeric (Jay Chamele et al., 2014; AR Prabhakar et al., 2013) are used as intracanal medicaments.[9,10,11]

Antioxidants interact and stabilise free radicals produced by bacterial species, thereby protecting cells from the damage caused by free radicals.Antioxidants are substances that stop other molecules from oxidising, which prevents free radicals from forming. These free radicals can start hazardous chain reactions that harm or destroy cells, eventually resulting in the development of cancer. Antioxidants neutralise these radicals by giving up their electrons and stopping the electron-taking reaction. In everyday practice, antioxidants are frequently used for a variety of clinical scenarios.[12,13]

So, the present study aims to evaluate and compare the antimicrobial efficiency of Antioxidants, i.e., Green coffee, Lemon, and Honey, with Calcium hydroxide as intracanal medicaments.

Materials and Methods:-

E. faecalis pure strains (ATCC 29212) were acquired from a microbiology laboratory.

Preparation of extracts

$Ca(OH)_2$

$Ca(OH)_2$ powder was mixed with sterile saline in the ratio of 1.5:1 (wt/vol) to obtain a paste.[14]

Lemon extract

Fresh lemon fruits were freshly squeezed just before use using an electric squeezer for lemon extract.The lemon extract was diluted by physiological saline to 50% and non diluted lemon solution (100%).[15]

Honey sample

The honey used in this study was the commercially available Honey (Dabur Pharmaceuticals). It was collected in sterile container and checked for purity on blood agar by streaked on blood agar plate by streaked on blood agar plate and incubated overnight. The honey sample was diluted by physiological saline to 50% and non diluted honey (100%) referred to as neat.[16]

Green coffee extract

Green coffee (Organic LRM Green Coffee Beans Decaffeinated & Unroasted Arabica Coffee) extract prepared using soxhelets extraction apparatus. The green coffee extract was diluted by physiological saline to 50% and non diluted green cofffee (100%).

Preparation of tooth specimens

156 recently extracted single-rooted teeth were thoroughly cleaned of debris using an ultrasonic scaler (NSK, Varios 750). The teeth were subsequently trimmed to a standardised length of 10 mm using a safe-sided diamond disc manufactured by SHOFU. The patency of the apical foramen was confirmed by inserting a size 15 K file (manufactured by Mani, Japan) until it became visible at the apical foramen. The working length was then set 0.5 mm shorter than the apex. The canals have been prepared to adopt rotary ProTaper Files (Dentsply India) up to an apical size of F3. The canals were irrigated using a 5.2% sodium hypochlorite solution from VIP, Vensons India. RC Help from Prime Dental products was utilised as a lubricant. The canals were irrigated with a solution of 17%

ethylenediaminetetraacetic acid (Fisher Scientific, Fair Lawn, NJ, USA) to eliminate the smear layer. To prevent environmental contamination, a double layer of nail varnish was applied to the outer surfaces of the teeth. The apical foramen was closed using Brilliant Coltene composite resin.

Block contamination

Before inoculation, the samples underwent sterilisation in an autoclave for two cycles. The initial cycle was conducted at a temperature of 121ºC, while the subsequent cycle involved submerging the samples in 1 ml of nutrient broth in separate microcentrifuges. To create a more favourable environment for bacterial growth in the dentinal tubules, the samples were subjected to agitation in an ultrasonic bath for 15 minutes. This was done to enhance the penetration of the nutrient medium into the dentinal tubules. Two control teeth were placed in nutrient broth to verify the sterility of the samples. The cultivated E. faecalis was resuspended in 5 ml of TS broth and subjected to a 4-hour incubation at 37º C. The turbidity of the suspension was then adjusted to a value of 0.5 on the McFarland standard scale. The teeth samples were immersed in culture media containing E. faecalis in a round bottom flask. They were then agitated for 10 minutes and incubated at a temperature of 37ºC for a duration of 21 days.

Antimicrobial assessment

Four teeth were subjected to the same treatment and intentionally infected with E. faecalis as experimental samples. They were then incubated at a temperature of 37ºC for a duration of 1 week, without the administration of any medication, in order to observe the growth of E. faecalis. Teeth were then assigned into five experimental groups based on ICM usedas group I (Ca(OH)2), group II (lime extract), group III (honey extract), group IV (green coffee) and group V (saline).The concentrated mixture was filtered and the clear extract was stored in an airtight bottle in the refrigerator for antimicrobial studies. The respective intracanal medicaments were injected into the canal with a syringe. The control group didnot receive any intracanal medicament. The canal orifice wassealed with paraffin wax. Teeth were placed in the Eppendorf tube and incubated at 37ºC for 1 week.

Culturing of samples

The antimicrobial assessment was conducted at the end of day 1, day 2, and day 7, using ten specimens for each time interval. The opening of the root canal orifice was re-established. The extraction of dentin was performed using a 30 size H file from Mani, a company based in Japan. The H file was inserted to the desired depth, and using a filling motion, the dentinal shavings were removed from the bottom third to the top third of the canal. The dentin shavings were subsequently gathered and placed into test tubes that contained nutrient broth. A serial 10-fold dilution was prepared using nutrient broth as the diluent. Following serial dilution, a volume of 1 ml was transferred and placed onto Mueller-Hinton agar. The agar was then incubated at a temperature of 37ºC for a duration of 24 hours. The number of colony forming units (CFUs) was subsequently determined.

Quantitative analysis

The statistical analysis was conducted using the analysis of variance technique. In order to assess the notable disparity between the groups, multiple comparisons were conducted utilising the Bonferroni test as a post hoc analysis. A P value is deemed significant when it is below 0.05.

Results:-

Table: 1 shows mean colony forming units CFU/ml for different intra canal medicaments at different time intervals.The difference in mean CFU among the groups was found to be statistically significant ($P < 0.001$). Saline shows highest colony forming units 3.36 ± 0.49, 3.41 ± 0.45, 3.33 ± 0.35 after 1, 2 and 7 days respectively when compared to all other groups.Calcium hydroxide has shown lowest colony forming units after 1 day (0.4 ± 0.05), 2days (0.4±0.0) and 7days (0.1±0.01) when compared to other groups. This showscalcium hydroxide has shown highest antimicrobial activity when compared to other groups.When compared to calcium hydroxide, lemon (1.26 ± 0.12,1.06 ± 0.09,0.98 ± 0.07) and honey (1.70 ± 0.15,1.55 ± 0.13, 1.28 ± 0.1) shows more colony forming units after after 1, 2 and 7 days respectively. But there is no significant difference between lemon and honey at all three intervals. Green coffee (2.36 ± 0.27,2.10 ± 0.24,1.90 ±0.22) has shown highest colony forming units when compared to calcium hydroxide, lemon and honey after 1, 2 and 7 days respectively. This shows green coffee has least antimicrobial property.

Table: 2 shows percentage efficacy of intra canal medicaments at different time intervals(%). Intergroup comparison between groups showed significant difference between $Ca(OH)_2$(88.09%, 97.02%, 88.26%) , lemon (62.50%,

70.57%, 68.91%), honey (49.40%, 61.56%, 54.54%) and green coffee (29.76%, 42.94%, 38.41%) on day 1,day 2 and day 3 respectively.

Discussion:-

Walton wrote that "Intra-canal medicaments have traditionally gone hand-in-glove with endodontics. They are generally considered to be an integral part of treatment and important to the success of root canal therapy".[17] Strong disinfection medical agents have commonly been used as intracanal medicaments in an attempt to 'sterilise' the intracanal space.[18]

Enterococcus faecalis is a resilient organism that, although it constitutes a small portion of the microorganisms in untreated canals, significantly contributes to the development of persistent and resistant periradicular lesions following root canal treatment. It is frequently present in a significant proportion of root canal failures and can persist in the root canal as either a single organism or a major constituent of the microbial community. Waltimo et al (2003) states that enterococcus faecalis is the root canal survivor and 'star' in posttreatment disease.[19]

In the present study CFU is taken as criteria. A colony forming unit is normally estimate the number of cells present based on their ability to give rise to colonies under specific conditions of nutrient medium, temperature and time.

Hermann introduced calcium hydroxide in 1920, and since then it has been extensively utilised in the field of endodontics. It is a highly basic substance with a pH of around 12.5. The antimicrobial activity of Ca(OH)2 is attributed to the liberation of hydroxyl ions upon exposure to aqueous fluids. Hydroxyl ions are potent oxidising agents that exhibit strong reactivity with biomolecules. The lethal impact on microorganisms has been ascribed to the following mechanisms. The bacteria's cytoplasmic membrane is harmed, proteins are denatured, and/or the DNA is damaged, resulting in the bacteria's demise.[20]

In the present study calcium hydroxide show highest antibacterial activity because its high pH (12.5-12.8) and prsecence of hydroxyl ions. The present study results also coincide with previous study done by Siqueira & Lopes et al (1999).[20]

Calcium hydroxide, has been shown to be ineffective at killing E. faecalis on its own, especially when a high pH is not maintained [21–23]. The following reasons have been proposed to explain why E. faecalis is able to survive intracanal treatment with calcium hydroxide: (a) E. faecalis passively maintains pH homeostasis. This occurs as a result of ions penetrating the cell membrane as well as the cytoplasm's buffering capacity. (b) E. faecalis has a proton pump that provides an additional means of maintaining pH homeostasis. This is accomplished by "pumping" protons into the cell to lower the internal pH. (c) At a pH of 11.5 or greater, E. faecalis is unable to survive [24, 25]. However, as a result of the buffering capacity of dentin, it is very unlikely that a pH of 11.5 can be maintained in the dentinal tubules with current calcium hydroxide utilization techniques [26]. Diverse components of dentin including dentin matrix, type-I collagen, hydroxyapatite, and serum are responsible for altering the antibacterial effects of these medicaments.[27]

Lemon solution inhibited the growth of E. Faecalis.The antimicrobial property of lemon is due to prescence of flavonoids ,alkaloids, volatile oil and citric acid. When it is used as an irrigant lemon solution able to remove smear layer. The present study results in agreement with the findings of Sawsan et al (2008), Abuzied et al (1999).[28]

Honey has a broad-spectrum antibacterial activity suggested by several mechanisms. The hydroxyl ions present in honey as similar to hydrogen peroxide is considered to be responsible for its antibacterial activity. In addition, the osmotic pressure exerted by honey affects the survival of the microbes. The non peroxide components such as complex phenols and organic acids are also responsible for the antibacterial activity [16,29,30].The present study results coincides with previous studies done by Divya , Narayanan et al (2016).[31]

Some components in Greencoffee such as caffeine, volatile and nonvolatile organic acids, phenols and aromatic compounds are reported to have antimicrobial activity. Chlorogenic acid (CGA) and caffeic acid, which are nonvolatile organic acids found in coffee, inhibit the growth of microorganisms.CGA, as the active ingredient present in the unroasted green coffee beans, is responsible for its antimicrobial property. Polyphenol is an organic compound found in this extract is responsible for its antioxidants property. All these factors provide antibacterial, antifungal, antiviral, antiphlogistic, antioxidant, chemopreventive property of green coffee.[32,33]

Till now no studies used green coffee as intracanal medicament.This is the first study to green coffee taken as intracanal medicament.Mona et al (2017) used green coffee as mouth rinses and showed a statistical significant reduction in Streptococcus mutans colony count.[34]

Calcium hydroxide exhibits a moderate antimicrobial effect. Yoldas et al. reported that Ca(OH)2 exhibited a decrease in microhardness, possibly attributed to the proteolytic activity of Ca(OH)2. This indicates that Ca(OH)2 has certain disadvantages. The pH elevation observed following exposure to Ca(OH)2 can diminish the organic foundation of the dentin matrix, leading to protein structure degradation and disruption of the connections between collagen fibres and hydroxyapatite crystals. This can have an adverse impact on the mechanical characteristics of dentin.[35]$Ca(OH)_2$ Remnants had a negative effect on the push out bond strength of a root canal sealer.[36]

To overcome this antioxidants are used in the present study. Antioxidants poses substantial antibacterial activity along with antioxidants have no effect on microhardness of root dentin (Swapnil et al 2013)[11] and improves the bond strength of root dentin (Manimaran et al 2011).[11]Hence, antioxidants have a potential to be used as intracanal medicament if its antibacterial activity could be enhanced.

Figure 1:-Shows E. Faecalis colonies on agar plates after 7 days.

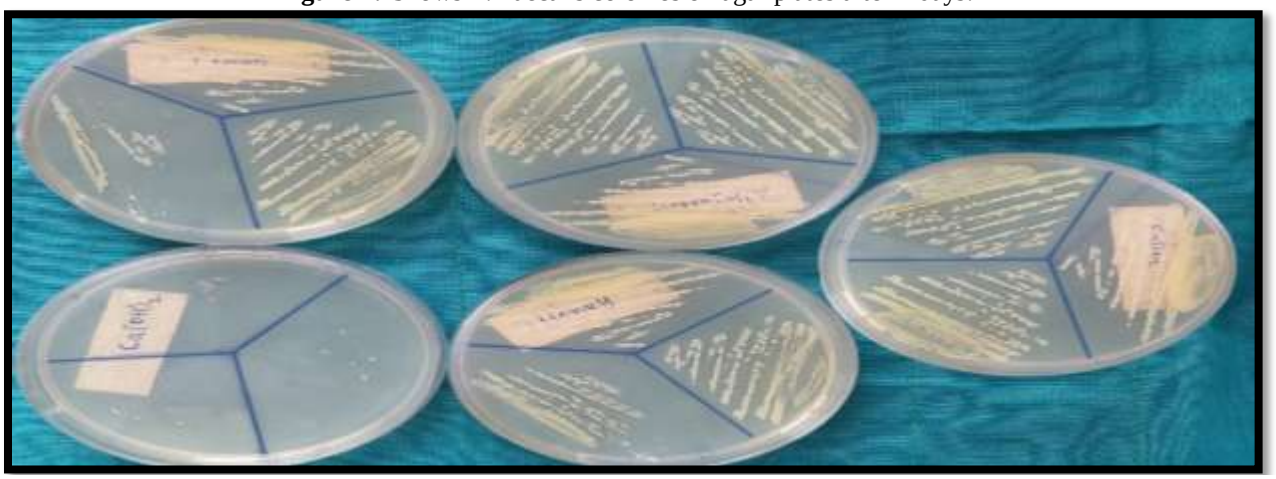


Table 1:-Shows Mean colony forming units CFU/ml for different intra canal medicaments at different time intervals , colony count (10^5).

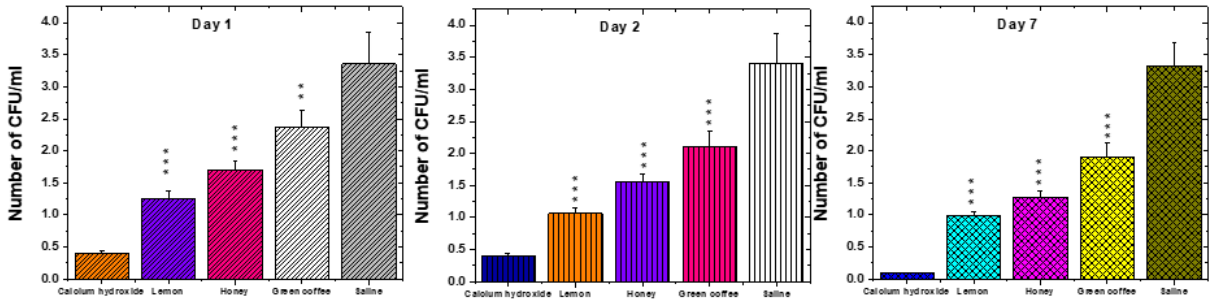
Sl.no	Intra canal medicament	Day -1 Mean ± SD	Day -2 Mean ± SD	Day-7 Mean ± SD
1	Calcium hydroxide	0.4 ± 0.05 ***	0.4±0.04 ***	0.1±0.01 ***
2	Lemon	1.26 ± 0.12 **	1.06 ± 0.09 **	0.98 ± 0.07 **
3	Honey	1.70 ± 0.15 **	1.55 ± 0.13 **	1.28 ± 0.1 **
4	Green coffee	2.36 ± 0.27 *	2.10 ± 0.24 *	1.90 ±0.22 *
5	Saline	3.36 ± 0.49	3.41 ± 0.45	3.33 ± 0.35

Table 2:-Shows percentage efficacy of intra canal medicaments at different time intervals(%).

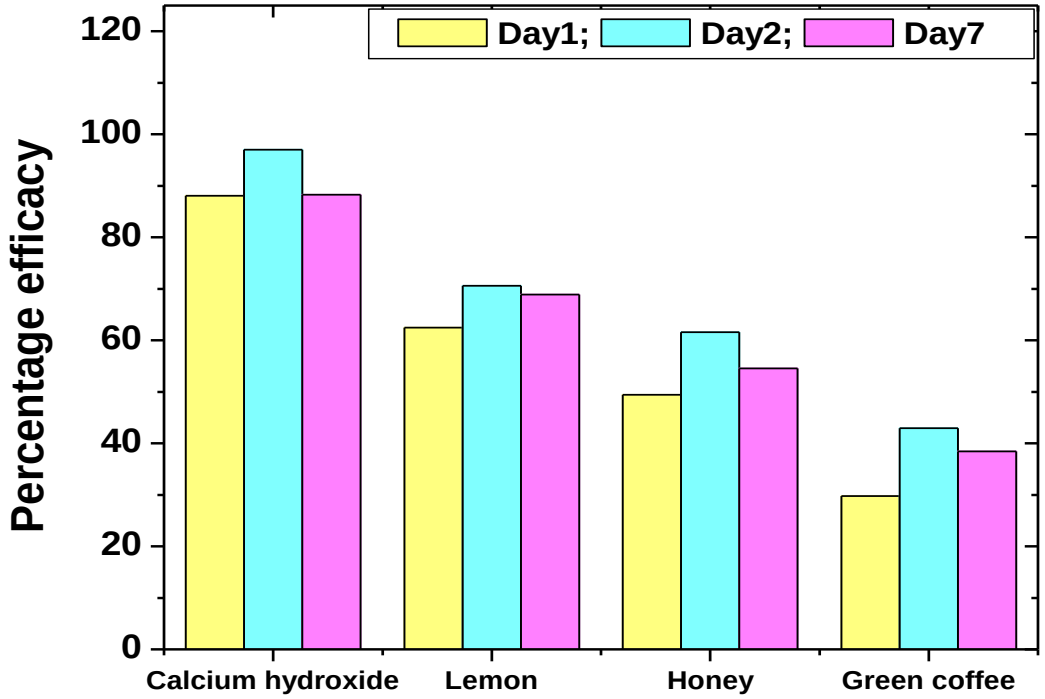
Sl.no	Intra canal medicament	Day -1	Day -2	Day-7
1	Calcium hydroxide	88.09***	97.02***	88.26***
2	Lemon	62.50**	70.57**	68.91**
3	Honey	49.40**	61.56**	54.54**

4	Green coffee	29.76*	42.94*	38.41*

Graph 1,2,3:-Shows mean number of colony forming units after 1 day ,2days and 7days respectively.



Graph 4:-Shows percentage efficacy of intra canal medicaments at different time intervals(%).



Conclusion:-

Calcium hydroxide has shown highest antimicrobial activity when compared to other groups followed by lemon and green coffee. There is no significant difference between lemon and honey. Green coffee has least antimicrobial property.Every moring our day starts with lemon, honey and green coffee. Hippocrates also states that “Let food be your medicine and let medicine be your food”. So why not we use these proven natural remedies in root canal treatment. Go green keep the root canal clean.

References:-

1. Ambareen Z, Chinappa A. Go green-keep the root canal clean. Int J Dent Sci Res. 2014 Dec;2(6B):21-5.
2. Kumar H. An in vitro evaluation of the antimicrobial efficacy of Curcuma longa, Tachyspermumammi, chlorhexidine gluconate, and calcium hydroxide on Enterococcus faecalis. Journal of Conservative Dentistry. 2013 Mar 1;16(2):144-7.
3. Adl A, Shojaee NS, Motamedifar M. A comparison between the antimicrobial effects of triple antibiotic paste and calcium hydroxide against Entrococcus faecalis. Iranian endodontic journal. 2012;7(3):149.
4. Ingle’s Endodontics 6. 2008.Page 992.

5. Yaduka P, Sharma S. Novel intracanal medicaments and its future scope. Int J Pharm Bio Sci. 2014 Jul;4(3):65-9.
6. Hamidi MR, Mahmoudi E, Moghadamnia AA, Zahedpasha S. Effect of calcium hydroxide and chlorhexidine medicaments on the apical seal. Iranian endodontic journal. 2012;7(1):15.
7. Rosenberg B, Murray PE, Namerow K. The effect of calcium hydroxide root filling on dentin fracture strength. Dental Traumatology. 2007 Feb;23(1):26-9.
8. Sinha N, Patil S, Dodwad PK, Patil AC, Singh B. Evaluation of antimicrobial efficacy of calcium hydroxide paste, chlorhexidine gel, and a combination of both as intracanal medicament: An: in vivo: comparative study. Journal of conservative dentistry. 2013 Jan 1;16(1):65-70.
9. Madhusudhana K, Archanagupta K, Suneelkumar C, Lavanya A, Deepthi M. Effect of addition of lycopene to calcium hydroxide and chlorhexidine as intracanal medicament on fracture resistance of radicular dentin at two different time intervals: An: in vitro: study. Journal of Conservative Dentistry. 2015 May 1;18(3):205-9.
10. Chamele J, Bhat C. Efficacy of turmeric extract as an intracanal medicament in deciduous teeth against Enterococcus faecalis: An in vitro study. Int. J.Curr. Microbio App.Sci.2014;3:17-25
11. Prabhakar AR, Taur S, Hadakar S, Sugandhan S. Comparison of antibacterial efficacy of calcium hydroxide paste, 2% chlorhexidine gel and turmeric extract as an intracanal medicament and their effect on microhardness of root dentin: an in vitro study. International journal of clinical pediatric dentistry. 2013 Sep;6(3):171.
12. Aksakalli S. Antioxidants in dentistry: Review of literature. Dentistry. 2013;4(1):181.
13. Mufeed A, Reshma VJ, Bharadwaj P, Kaushik PC. Role of antioxidants in dentistry. J Dent Panacea. 2014;1:62-7.
14. Cwikla SJ, Bélanger M, Giguère S, Progulske-Fox A, Vertucci FJ. Dentinal tubule disinfection using three calcium hydroxide formulations. Journal of endodontics. 2005 Jan 1;31(1):50-2.
15. Radwan IN, Randa B, Hend AN, Camilia G. Evaluation of antimicrobial efficacy of four medicinal plants extracts used as root canal irrigant on Enterococcus faecalis: An in-vitro study. International Dental & Medical Journal of Advanced Research. 2015;1(1):1-8.
16. Mittal L, Kakkar P, Verma A, Dixit KK, Mehrotra M. Anti Microbıal Activity of Honey Against Various Endodontic Micro Organisms-An In Vıtro Study. Journal of International Dental and Medical Research. 2012;5(1):9-13.
17. Walton RE. Intracanal medicaments. Dental Clinics of North America. 1984 Oct 1;28(4):783-96.
18. Peters OA, Peters CI. Cleaning and shaping of the root canal system. Cohen's Pathway of the Pulp ed. 2020 Dec 24;12:236-303.
19. Portenier I, Waltimo TM, Haapasalo M. Enterococcus faecalis–the root canal survivor and 'star'in post-treatment disease. Endodontic topics. 2003 Nov;6(1):135-59.
20. Siqueira Jr JF, Lopes H. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. International endodontic journal. 1999 Sep;32(5):361-9.
21. Lin Y, Mickel A, Chogle S. Effectiveness of selected materials against Enterococcus faecalis: Part 3. The antibacterial effect of calcium hydroxide and chlorhexidine on Enterococcus faecalis. J Endod2003;29:565– 6.
22. McHugh CP, Zhang P, Michalek S, Eleazer PD. pH required to kill Enterococcus faecalis in vitro. J Endod2004;30:218 –9.
23. Tronstad L, Andreasen J, Hasselgren G, Kristerson L, Riis I. pH changes in dental tissues after root filling with calcium hydroxide. J Endod1981;7:17–21.
24. Evans M, Davies JK, Sundqvist G, Figdor D. Mechanisms involved in the resistance of Enterococcus faecalis to calcium hydroxide. Int Endod J 2002;35:221– 8.
25. McHugh CP, Zhang P, Michalek S, Eleazer PD. pH required to kill Enterococcus faecalis in vitro. J Endod2004;30:218 –9.
26. Tronstad L, Andreasen J, Hasselgren G, Kristerson L, Riis I. pH changes in dental tissues after root filling with calcium hydroxide. J Endod1981;7:17–21.
27. Portenier I, Haapasalo H, Orstavik D, Yamauchi M, Haapasalo M. Inactivation of the antibacterial activity of iodide potassium iodide and chlorhexidine digluconate against Enterococcus faecalis by dentin, dentin matrix, type-I collagen, and heatkilled microbial whole cells. J Endod2002;28:634 –7.
28. Radwan IN, Randa B, Hend AN, Camilia G. Evaluation of antimicrobial efficacy of four medicinal plants extracts used as root canal irrigant on Enterococcus faecalis: an in-vitro study. Int Dent Med J Adv Res 2015 Dec;1:1-8.
29. Mandal MD, Mandal S. Honey: its medicinal property and antibacterial activity Asian Pac J Trop Biomed. 2011;1(2):154–60.

30. Osho, A, Bello O.O. Antimicrobial effect of honey produced by apismellifera on some common human pathogens. Asian J Exp Biol Sci. 2010;1(4):875-80.
31. Sundaram D, Narayanan RK, Vadakkepurayil KA. Comparative evaluation on antimicrobial effect of honey, neem leaf extract and sodium hypochlorite as intracanal irrigant: an ex-vivo study. J Clin Diagn Res 2016;10:ZC88-91
32. Bharath N, Sowmya NK, Mehta DS. Determination of antibacterial activity of green coffee bean extract on periodontogenic bacteria like Porphyromonasgingivalis, Prevotella intermedia, Fusobacterium nucleatum and Aggregatibacteractinomycetemcomitans: An in vitro study. Contemp Clin Dent 2015;6:166-9.
33. Yi TL, Shah M, Raulji D, Dave D. Comparative evaluation of antimicrobial efficacy of coffee extract and 0.2% chlorhexidine mouthwash on the periodontal pathogens Porphyromonasgingivalis, Prevotella intermedia, Fusobacterium nucleatum and Aggregatibacteractinomycetemcomitans: An in-vitro study. Adv Hum Biol. 2016;6:99-103.
34. Mona Yadav, Mamta Kaushik, Roshni Roshni, Pallavi Reddy. Effect of Green Coffee Bean Extract on Streptococcus mutans Count: A Randomised Control Trial. J Clin Diagn Res. 2017 May; 11(5): ZC68–ZC71.
35. Yoldas O, Dogan C, Seydaoglu G. The effect of two different calcium hydroxide combination on root dentin microhardness. Int Endod J 2004;37(12):828-831.
36. Tasdemir T, Celik D, Er K, Yildirim T, Ceyhanli KT, Yesilyurt C (2011) Efficacy of several techniques for the removal of calcium hydroxide medicament from root canals. International Endodontic Journal 44, 505–9.