ANTIMICROBIAL EFFICACY OF DIFFERENT HERBAL EXTRACTS AS INTRACANAL MEDICAMENT AGAINST ENTEROCOCCUS FAECALIS – AN IN VITRO STUDY

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfilment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

APRIL 2018

CERTIFICATE

This is to certify that this dissertation titled **"Antimicrobial efficacy of different herbal extracts as intracanal medicament against Enterococcus faecalis – An In Vitro Study"** is a bonafide record of work done by **Dr. VARSHINI R** under my guidance and to my satisfaction during her postgraduate study period, 2015 – 2018. This dissertation is submitted to **THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY**, in partial fulfilment for the award of the degree of Master of Dental Surgery in Conservative Dentistry and Endodontics, Branch IV. It has not been submitted (partially or fully) for the award of any other degree or diploma.

_____

Dr. Subha Anirudhan, MDS,

Guide, Professor,
Department of Conservative Dentistry and Endodontics,
Sri Ramakrishna Dental College and Hospital,
Coimbatore.

_____

Dr. V. Prabhakar, MDS,

Principal, Professor and HOD
Sri Ramakrishna Dental College and Hospital
Coimbatore

Date:

Place: Coimbatore

DECLARATION

NAME OF THE CANDIDATE	**Dr. Varshini R**
TITLE OF DISSERTATION	**Antimicrobial efficacy of different herbal extracts as intracanal medicament against Enterococcus faecalis– An In Vitro Study**
PLACE OF STUDY	**Sri Ramakrishna Dental College and Hospital**
DURATION OF THE COURSE	**2015-2018**
NAME OF GUIDE	**Dr. Subha Anirudhan**
HEAD OF THE DEPARTMENT	**Dr.V.Prabhakar**

I hereby declare that no part of the dissertation will be utilized for gaining financial assistance for research or other promotions without obtaining prior permission from the Principal, Sri Ramakrishna Dental College and Hospital. In addition, I declare that no part of this work will be published either in print or in electronics without permission from the guide who has been actively involved in the dissertation. The author solely has the rights for publishing the work with prior permission from the Principal, Sri Ramakrishna Dental College and Hospital, Coimbatore.

Head of the Department and Guide **Signature of the Candidate**

Urkund Analysis Result

Analysed Document: varshini.pdf (D34309608)
Submitted: 1/3/2018 12:53:00 PM
Submitted By: varshni.rs@gmail.com
Significance: 3 %

Sources included in the report:

The research proposal.docx (D24560574)
Sriramkumar. S.R.doc (D30228472)
INRODUCTION.docx (D34281621)
final plag.docx (D34243859)
Maria Granevik Master version 1 examinator .doc (D14202745)
https://www.researchgate.net/publication/303835726_Comparative_Evaluation_of_Antimicrobial_Activity_of_QMiX_25_Sodium_Hypochlorite_2_Chlorhexidine_Guava_Leaf_Extract_and_Aloevera_Extract_Against_Enterococcus_faecalis_and_Candida_albicans_-_An_in-vitro
http://www.pagepress.org/journals/index.php/mr/article/view/mr.2012.e22/6228
http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1806-83242004000300012

Instances where selected sources appear:

17

CERTIFICATE II

This is to certify that this dissertation work titled **"ANTIMICROBIAL EFFICACY OF DIFFERENT HERBAL EXTRACTS A INTRACANAL MEDICAMENT AGAINST ENTEROCOCCUS FAECALIS – AN IN VITRO STUDY"** of the candidate **Dr.Varshini R** with registration number **241517303** for the award of **Masters of Dental Surgery** in the branch of **Conservative Dentistry and Endodontics**. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **3** percentage of plagiarism in the dissertation.

Guide & Supervisor sign with Seal

ACKNOWLEDGEMENT

This thesis is the result of work done with immense support from many people and it is with immense pleasure that I express my heartfelt gratitude to all of them.

I devote my heartfelt thanks to **Dr. V. Prabhakar, MDS**, **Principal & Head of Department**, whose discipline and skills that run deep under his authoritative yet natural care during my post graduate period which enabled me to successfully conclude my thesis.

I would like to thank and acknowledge **Dr. Subha Anirudhan, MDS**, **Professor,** my Guide who has always been a source of support and encouragement at any moment, in and out of the department. I am grateful to her for her innovative ideas, constructive suggestions, valuable criticism and constant encouragement.

I take this opportunity to express my sincere gratitude to **Dr. Minu Koshy, MDS, Professor, Dr.Ambalavanan, MDS, Reader, Dr. Sriman Narayanan, MDS, Reader, Dr.Gayathri V, MDS, Senior Lecturer and Dr.Remya Verghese, MDS, Senior Lecturer** for their valuable guidance that enabled me to comprehend this dissertation and supported me at every juncture throughout my postgraduate curriculum.

This study wouldn't have come to existence without the effort and time of the faculties and residents at the **Department of Microbiology, Sri Ramakrishna Hospital, Coimbatore**. I sincerely acknowledge **Dr. Prasanna, Professor and Head, Department of Microbiology** and **Mr. Babu** for their valuable guidance, encouragement and support for the successful completion of the study.

I am thankful to **Dr.Malini** and **Dr.Suganya**, **Ramachandra Central Research Facility** for lending a helping hand with the sectioning of tooth samples and CLSM analysis for this study.

I am thankful to **Dr. Vipin jain**, for his guidance in the statistical works of this study.

I am thankful to my teachers **Dr.Sudhakar, MDS** and **Dr.Prabhu, MDS,** my seniors, my colleagues and my juniors, who have been together as friends and of great support throughout my period of study here. I am thankful to all other department staff members, my fellow colleagues in other departments, all UG staff members and non-clinical staffs of my department for their great support and encouragement.

I express my dearest gratitude to **my parents, my husband and my sister**, and the special people in my life who contributed in various ways towards my study and this dissertation.

Last but not the least, I am greatly indebted to **God the Almighty**, for blessing me with all the good things in my life and guiding me throughout.

Dr. VARSHINI .R

CONTENTS

INTRODUCTION

INTRODUCTION

Microorganisms are the source for the development of pulpal and periapical diseases. The goal of endodontic treatment is to control pulpal and periradicular infections and to promote healing. The success of endodontic treatment is directly influenced by the elimination of microorganisms in infected root canals. Chemomechanical instrumentation removes majority of bacteria, together with necrotic pulp debris[1]. However chemomechanical instrumentation should be supplemented by intracanal medicaments to eliminate the remaining bacteria, to prevent its regrowth and thereby making the environment conducive for periapical tissue repair [2].

Enterococcus faecalis is a part of the human normal flora and an important pathogen in opportunistic infections in humans. It is rarely present in primary apical periodontitis, but it is the dominant microorganism in root canal-treated teeth presenting with post treatment apical periodontitis [3]. Eradication of *Enterococcus faecalis* from the root canal remains a challenge, since it is resistant to a variety of antimicrobial agents [4].

The control and suppression of *Enterococcus faecalis* in the dental procedures are of primary importance in decreasing the penetration of bacteria inside the dentinal tubules and also limiting the formation of biofilms [5].

Calcium hydroxide is one of the most commonly used intra canal medicaments. Its antimicrobial efficacy is attributed to its high alkaline pH, antimicrobial effect and potential to stimulate healing of pulp and periapical tissues. Although it has antibacterial activity on a wide range of microflora present in the root

canal, it was found less effective against *Enterococcus faecalis* [1]. Chlorhexidine has a broad spectrum antimicrobial activity targeting both gram-positive and gram-negative microbes [6]. Hence, the combination of chlorhexidine and calcium hydroxide has been tried as an intracanal medicament to achieve the properties of both the medicaments. However it was found that the antimicrobial action of chlorhexidine was reduced [7].Various other intracanal medicaments have also been tried, with increased cytotoxicity. But till date there is no data to prove which medicament is effective than the other.

In last few decades, the use of alternative therapeutic agents has considerably increased and these agents which are derived from plants, insects, microorganisms, etc are a part of a growing trend to seek natural remedies in dental treatment .The use of natural derivatives may have a greater level of tolerance by the body with exhibition of fewer side effects. According to W.H.O., medicinal plants would be the best source to obtain a variety of drugs [8]. Many plants with biological and antimicrobiological properties have been studied since there has been a relevant increase in the incidence of antibiotic overuse [9].

Aloevera belongs to the Liliaceae family. Total leaf extracts contain anthraquinones, which have antibacterial properties [10]. **Anuj Bhardwaj et al**, in their study found that Aloe Vera gel has inhibitory effects on *S.pyogens* and *E.faecalis* in a biofilm model [32]. Ricinus communis is rich in ricinoleic acid, also known as castor acid, and can be used as an intracanal medicament [11]. In a study conducted by **Marcia Carneiro Valera et al**, in his culture study it was founded that Ricinus communis extract was able to completely eliminate *C.albicans* and also significantly reduced the amount of *E. faecalis* [12]. Lemon solution is considered as a natural source of citric

acid with low acidity. **Sawsan T et al**, found that fresh lemon solution was shown to have wide antibacterial efficiency including *E.faecalis* in a biofilm model and hence can be used as an intracanal medicament [46].

The purpose of the present study was to compare the antimicrobial efficacy of Calcium hydroxide, Aloevera, Ricinus communis and Lemon as intracanal medicament against *Enterococcus faecalis* in a tooth biofilm model using culture study and confocal microscopy.

AIM & OBJECTIVE

AIM AND OBJECTIVE

The purpose of this study was

1. In vitro testing of antimicrobial effects of intracanal medicaments (Aloevera, *Ricinus communis*, Lemon, Calcium hydroxide) against *Enterococcus faecalis* used in root canal treatment in tooth biofilm model.

2. To evaluate the effectiveness of Calcium hydroxide, Aloevera, *Ricinus communis* and lemon against *Enterococcus faecalis* by assessing the bacterial viability using confocal laser scanning microscope.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Verma et al (2017)[13] investigated the antibacterial efficacy of methanol extracts of leaves and roots of *Boerhavia diffusa, Eclipta alba(false daisy),Phyllanthus niruri* and *Ricinus communis* (castor oil) *a*gainst *Staphylococcus aureus, Bacteriodes subtilis, Escherichia coli, Salmonella typhii, Aspergillus niger and Candida albicans* by agar well diffusion methods. This study concluded that *Boerhavia diffusa* and *Phyllanthus niruri* leaf extract showed highest antibacterial activity against *S.aureus* and *S.typhii*. Leaf extract of *Phyllanthus niruri* and *Ricinus communis* showed highest antifungal activity against *A. niger* and *C. albicans.*

Mustafa (2016)[14] assessed the antimicrobial efficacy of neem (*Azadirachta indica*) extract ,2% chlorhexidine, 3% sodium hypochlorite against *Enterococcus faecalis* . Agar well diffusion test was used to study the antimicrobial efficacy with saline as control. This study concluded that neem leaf extract showed comparable zones of inhibition with that of chlorhexidine and sodium hypochlorite and therefore, neem leaf extract could be used as an alternative agent in root canal disinfection.

Wadhwa et al (2016)[15] evaluated the intracanal bacterial reduction promoted by chemomechanical preparation using three different herbal extracts named *Ocimum sanctum , Cinnamomum zeylanicum , Syzygium aromaticum* against *Enterococcus faecalis*. Samples taken before and after chemomechanical procedures were cultured, and the colony-forming units (CFUs) were counted. Bacterial identification was performed using Polymerase chain reaction technique. The 3 experimental groups

were less effective in terms of intracanal bacterial reduction as compared to NaOCl but more effective than distilled water.

Chandrappa et al (2015)[16] assessed the antimicrobial activity of herbal medicines (neem extract, tulsi extract) and chlorhexidine against *Enterococcus faecalis*. Agar diffusion method was used to evaluate the antimicrobial action of different medicines. This study concluded that significant antibacterial effect against *Enterococcus faecalis* was observed with chlorhexidine followed by neem extract and tulsi extract.

D.A. Attia et al (2015)[17] compared the antimicrobial effect of Calcium hydroxide paste (CaOH), Chlorhexidine gluconate (CHX) gel and Antibiotic-Corticosteroid paste against *Streptococcus mutans, Enterococcus faecalis* and *Candida albicans* in root canal lumen and radicular dentin for a period of 7 days using Mitis salivarius agar , Brain heart infusion blood agar and Sabouraud Dextrose agar .The authors concluded that CHX was the best medication used to eliminate the three different tested organisms at the two experimental sites, root canal lumen and radicular dentin. *Streptococcus mutans* was the most sensitive microorganism to the whole tested medications, whereas *Candida albicans* was the most resistant one. *Enterococcus faecalis* was more susceptible to CHX than the other medications.

Taneja et al (2015)[18] compared the antimicrobial efficacy of an oxazolidinone (linezolid[LZ]), lantibiotic (nisin), and calcium hydroxide against *Enterococcus faecalis* biofilm formed on tooth substrate after 2 and 7 days. The zones of inhibition were checked on Mueller-Hinton agar. They concluded that linezolid (LZ) showed maximum antimicrobial potential against *Enterococcus faecalis* biofilm followed by nisin after 2 and 7 days. Calcium hydroxide showed the least antimicrobial potential

against *E. faecalis* biofilm after 2 and 7 days. The antimicrobial effect of Linezolid and nisin was not affected with lapse of time, but that of calcium hydroxide decreased significantly with increasing time period.

Sanjay et al (2015)[19] evaluated the antibacterial activity of clove oil with intracanal medicaments (metronidazole, ciprofloxacin, clindamycin and calcium hydroxide) against *Enterococcus faecalis*. The antibacterial activity of the medicaments with and without clove oil was screened by agar well diffusion method. This study concluded that intra canal medicaments combined with clove oil showed greater antibacterial effects against *Enterococcus faecalis* than those intracanal medicaments without clove oil.

Upadhyay et al (2015)[20] determined the antibacterial efficacy of *Curcuma longa* (turmeric), calcium hydroxide, calcium hydroxide with turmeric oleoresin and metapex against root canal microorganisms such as *Staphylococcus aureus, Escherichia coli, Enterococcus faecalis* by agar well diffusion method. The authors found that calcium hydroxide with turmeric oleoresin showed maximum antibacterial efficacy against *Staphylococcus aureus, Escherichia coli , Enterococcus faecalis* followed by metapex and calcium hydroxide.

Murad et al (2014)[21] investigated the composition of the root canal microbiota in endodontic failures in order to identify and quantify the microorganisms. Microbiological samples were taken from 36 root canals with persistent endodontic infection. The presence, levels, and proportions of 79 bacterial species were determined by checkerboard DNA-DNA hybridization. The study concluded that the microbiota from teeth with persistent apical periodontitis presents a mixed and complex profile. *Enterococcus faecalis, Streptococcus epidermidis,*

Capnocytophaga sputigena and *Staphylococcus epidermidis* were found to be the most prevalent species.

Shaik et al (2014)[22] analyzed the antimicrobial efficacy and sustained release of Triple antibiotic paste (TAP) and calcium hydroxide as intracanal medicaments in root canals using chitosan as a carrier against *Candida albicans* and *Enterococcus faecalis* over a period of 2,7,21 days using Sabouraud's dextrose agar broth and brain heart infusion broth. They concluded that combining Triple antibiotic paste and calcium hydroxide with chitosan as a carrier had a good antimicrobial effect against *Candida albicans* and *Enterococcus faecalis.*

Abbaszadegan et al (2014)[23] synthesized and characterized silver nanoparticles (Ag NPs) with different surface charges and L929 fibroblasts in order to evaluate their cytotoxicity and antibacterial activity against *Enterococcus faecalis* in the absence and presence of dentine compared with sodium hypochlorite and chlorhexidine using Trypticase soy-agar plates and transmission electron microscopy. This study concluded that Ag NP surface charge was important in bactericidal efficacy against *Enterococcus faecalis*. The positively charged imidazolium-based ionic liquid-protected Ag NPs showed promising antibacterial results against *Enterococcus faecalis* and exhibited a high level of cytocompatibility to L929 fibroblasts.

J.B. Carbajal Mejía (2014)[24] evaluated the efficacy of calcium hydroxide, 2% chlorhexidine (CHX) gel, and propolis against both *Enterococcus faecalis* and *Candida albicans* using infected dentine models at two different depths (100 and 200 lm) after 14 days of application. He concluded that both chlorhexidine and propolis

were most effective against *Enterococcus faecalis,* whereas only chlorhexidine had the highest antifungal activity on *Candida albicans* in dentine of extracted teeth.

Gupta et al(2013)[25] evaluated the antimicrobial efficacy of *Ocimum sanctum, Cinnamomum zeylanicum, Syzygium aromaticum* and 3% sodium hypochlorite (NaOCl) against *Enterococcus faecalis* in planktonic suspension and biofilm phenotypes. The antibacterial efficacy was assessed using the agar well diffusion test, microdilution test and biofilm susceptibility assay (BSA) on cellulose nitrate membrane as well as in a tooth model. The authors concluded that *Cinnamomum zeylanicum* and *Syzygium aromaticum* had better antimicrobial efficacy than *Ocimum sanctum*. NaOCl had superior antimicrobial efficacy amongst all the groups.

Kandaswamy et al (2013)[26] compared the efficacy of garlic extract with 2% chlorhexidine (CHX) and calcium hydroxide $Ca(OH)_2$ in disinfection of dentinal tubules contaminated with *Enterococcus faecalis* by using real-time polymerase chain reaction (PCR). Agar diffusion test was done to evaluate the minimum inhibitory concentration of garlic extract against *Enterococcus faecalis.* This study concluded that 2% chlorhexidine showed the maximum antibacterial efficacy against *Enterococcus faecalis* followed by garlic extract and calcium hydroxide.

Ghonmode et al (2013)[27] compared the antimicrobial activity of neem leaf extracts, grape seed extracts and 3% sodium hypochlorite against *Enterococcus feacalis*. The agar diffusion test was performed in brain heart infusion media and broth and found that the neem leaf extract has a significant antimicrobial effect against *Enterococcus faecalis* followed by 3% NaOCl and grape seed extract. The results obtained in this study showed that neem leaf extract could be a viable medicament against *Enterococcus faecalis*.

Atila-Pektas et al (2013)[28] compared the antimicrobial activities of calcium hydroxide, 1% chlorhexidine gluconate gel, bioactive glass , calcium hydroxide plus Point (medicated gutta-percha with calcium hydroxide) and Activ Point (medicated gutta-percha with chlorhexidine diacetate) against *Enterococcus faecalis* and *Streptococcus mutans* for a period of 1 week using blood agar. This study concluded that chlorhexidine-impregnated medicaments were more efficient than alkaline-pH-acting medicaments.

Lee et al (2013)[29] evaluated the antibacterial efficacy of human b-defensin-3 (HBD3) peptide compared with calcium hydroxide and chlorhexidine on multispecies biofilms such as *Enterococcus faecalis , Actinomyces naeslundii , Lactobacillus salivarius , and Streptococcus mutans* by using confocal laser scanning microscopy. The results showed that HBD3 peptide exhibited more antibacterial activity against mature multispecies biofilms in vitro than either Calcium hydroxide or Chlorhexidine.

Thosar et al (2013)[30] assessed the antimicrobial efficacy of five essential oils (Tea tree oil, lavender oil, thyme oil, peppermint oil, and eugenol oil) against *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and *Candida albicans* by broth dilution method .This study concluded that apart from traditional use of eugenol, antibacterial effects of essential oils such as peppermint oil, tea tree oil and thyme oil can also provide an effective intracanal antiseptic solution against *Staphylococcus aureus, Enterococcus fecalis, Escherichia coli* and *Candida albicans*

Krishna et al (2012)[31] compared the antibacterial efficacy of 10% metronidazole gel, 2% chlorhexidine (CHX) gel, and a combination of calcium hydroxide and 2% CHX gel against *Enterococcus faecalis* using a culture technique . This study signifies that 2% CHX gel showed substantial antimicrobial activity

against *E. faecalis*. The combination of calcium hydroxide and 2% CHX gel also showed good antimicrobial activity. Hence, the efficacy of 2% CHX gel was greater than its combination with calcium hydroxide. The least effective drug against the microbes was metronidazole.

Bhardwaj et al (2012)[32] evaluated the antimicrobial activity of natural extracts of *Morinda citrifolia*, papain, and aloe vera (all in gel formulations), 2% chlorhexidine gel and calcium hydroxide, against *Enterococcus faecalis*. The antimicrobial efficacy was assessed using dentin shavings collected at 2 depths of 200 and 400 µm. The total colony forming units at the end of 1, 3, and 5 days were assessed. This study concluded that Chlorhexidine gel showed the maximum antimicrobial activity against *Enterococcus faecalis*, whereas calcium hydroxide showed the least. Among the natural intracanal medicaments, Morinda citrifolia gel consistently exhibited good inhibition upto the 5^{th} day followed by aloe vera gel and papain gel.

de Lucena et al(2012)[33] determined the viability of *Enterococcus faecalis* in infected human root dentine after exposure to root canal medicaments based on chlorhexidine and octenidine. The effect on *Enterococcus faecalis* viability was assessed by two fluorescent dyes (syto 9/ propidium iodide) to determine the 'proportion of viable bacteria' (PVB %) and number of colony-forming units (CFU).The proportion of viable bacteria was analysed by a fluorescence photomicroscope and the colony forming units was determined using Schaedler agar. In contrast to calcium hydroxide, both chlorhexidine and octenidine based intracanal medicaments were effective in decreasing the viability of *Enterococcus faecalis*.

Octenidine showed the most favourable results and may have potential as an endodontic medicament.

Miranda et al (2012)[34] evaluated the efficacy of the EndoVac® system and photodynamic treatment (PDT) as adjuncts to chemomechanical debridement associated with calcium hydroxide ($CaOH_2$) in reducing the levels of intracanal *Enterococcus faecalis*. Samples were obtained before (T1) and after the therapeutic procedures (T2) and, after intracanal medication (T3), plated onto Brain-heart infusion media and incubated to determine the colony-forming units (CFU mL1). The overall mean cell counts of *Enterococcus faecalis* were high at the initial contamination (T1). No significant change in bacterial counts from T2 to T3 was detected. This study concluded that the adjunctive use of the EndoVac® system and the photodynamic treatment, in combination or not, was as effective as the conventional chemomechanical debridement associated with $Ca(OH)_2$ on reducing the counts of intracanal *Enterococcus faecalis.*

Abd-Awn et al (2012)[35] assessed the effect of black seed oil extract on sensitivity of *Mutans streptococci* and the adherence to tooth surface in comparison to chlorhexidine gluconate. Four different concentrations of black seed oil extract (1%, 5%, 10%, and 20%) were prepared using ethanol as a solvent for the evaluation of the antimicrobial activity using agar diffusion test followed by determination of the Minimum Bactericidal Concentration (MBC) of the black seed oil extract. This study showed inhibition zones for black seed oil extract which were found to be increased as the concentration of the extract increased. The black seed oil extract has a bactericidal effect against *Mutans streptococci* at a concentration of 10%, and can inhibit the adherence of these microorganisms to the tooth surface.

Liu et al (2012)[36] tested a casein peptide in its glycosylated form (kappa-casein glycopeptide, KCGP) and its non-glycosylated form (kappa-casein peptide, KCP) for antibacterial efficacy against *Enterococcus faecalis* in planktonic and biofilm cultures. This study found that the casein-derived antimicrobial peptides KCGP and KCP inhibited growth of *Enterococcus faecalis* in the form of planktonic cells and also inhibited biofilm formation by the bacterium. These peptides, together with other antimicrobial agents, may have potential in the control of bacterial infection.

Badr et al (2011)[37] evaluated the antibacterial and cytotoxic effects of Liquorice as a root canal medicament and to compare its action to the commonly used root canal medicament calcium hydroxide $Ca(OH)_2$. Agar-well diffusion methods, broth microdilution tests and biofilm susceptibility assays were used to determine the antibacterial activity. Human periodontal ligament fibroblast tissue culture was used to assess the cytotoxicity of the preparations. Liquorice extract either separately or as Liquorice/$Ca(OH)_2$ mixture had a potent bactericidal effect against *Enterococcus faecalis* and retained compatibility with fibroblasts in tissue culture compared to the commonly used root canal medicament $Ca(OH)_2$.

Lima et al (2011)[38] evaluated the antimicrobial efficacy of calcium hydroxide-based intracanal medicaments such as calen, camphorated paramonochlorophenol (CMCP) and chlorhexidine (CHX) against *Enterococcus faecalis* using a tooth model. This study concluded that all calcium hydroxide-based medicaments were able to significantly reduce the presence of *Enterococcus faecalis* in the root canal system. Considering the methodology used, the associations Calen/CMCP and Calen/CHX were more effective in eliminating *Enterococcus faecalis.*

Vaghela et al (2011)[39] evaluated the disinfection of dentinal tubules using calcium hydroxide with propylene glycol and calcium hydroxide with iodoform in silicone oil, as compared to 2% chlorhexidine gel. The antimicrobial efficacy of the medicaments against *Enterococcus faecalis* and *Candida albicans* were assessed, using a dentinal tubule model at depths of 200 μm and 400 μm in tryptone soya (TS) broth and sabourauds dextrose (SD) broth. This study concluded that 2% chlorhexidine gel was effective against both *Enterococcus faecalis* and *Candida albicans*. Calcium hydroxide with propylene glycol was the most effective intracanal medicament along with 2% chlorhexidine against *Enterococcus faecalis*, whereas, calcium hydroxide with iodoform in silicone oil was the most effective intracanal medicament along with 2% chlorhexidine against *Candida albicans*.

Hemadri et al (2011)[40] evaluated the antimicrobial efficacy of Nisin against *Enterococcus faecalis* in solution and also evaluated and compared the effect of Nisin and Calcium hydroxide against *Enterococcus faecalis* in root canal system using Tripticase soy (TSY) blood Agar Plates. This study found that Nisin was effective at eradicating *Enterococcus faecalis* in pure culture and was more effective when compared to Calcium hydroxide in the elimination of this species within the root canal system.

Madhubala et al (2011)[41] evaluated and compared the antimicrobial activity of calcium hydroxide, triantibiotic mixture (TAM), and an ethanol extract of propolis as intracanal medicaments on *Enterococcus faecalis*–infected root canals at the end of 1, 2, and 7 days using brain/heart infusion broth. This study concluded that Propolis was more effective than triantibiotic mixture against *Enterococcus faecalis* at a 2-day time period, and both were equally effective at 7 days

Kandaswamy et al(2010)[42] investigated the antimicrobial activity of 2% chlorhexidine gel, propolis, *Morinda citrifolia* juice (MCJ), 2% povidone Iodine (POV-I), and calcium hydroxide on *Enterococcus faecalis*-infected root canal dentine at two different depths (200 lm and 400 lm) and three time intervals (day 1, 3 & 5). At the end of 1, 3, and 5 days, the remaining vital bacterial population was assessed. Dentine shavings were collected at two depths (200 lm and 400 lm), and total numbers of colony forming units were determined. The authors concluded that 2% chlorhexidine demonstrated significant inhibition against *Enterococcus faecalis* followed by Povidone Iodine, propolis, Morinda Citrifolia juice, and Ca $(OH)_2$. There was no significant difference between propolis and Morinda citrifolia juice and no significant difference between data at 200 lm and 400 lm.

Garcia et al (2009)[11] evaluated the antimicrobial activity of calcium hydroxide and *Ricinus communis* oil paste (Paste A) and a calcium hydroxide and propylene glycol paste (Paste B) as a root canal medicament. The Agar-well diffusion test was used to evaluate the antimicrobial activity of pastes A and B against *Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus mutans, Streptococcus sanguinis, and Candida albicans*. The results suggested that calcium hydroxide and *Ricinus communis* oil paste had greater antimicrobial activity than calcium hydroxide and propylene glycol paste in all the bacterial strains tested.

Ballal et al (2007)[43] investigated the antimicrobial efficacy of calcium hydroxide paste, 2% chlorhexidine gel and their combination against *Candida albicans* and *Enterococcus faecalis*. Inoculae of these organisms were used to make lawn cultures on Sabouraud's dextrose agar and blood agar plates. The results

zones of inhibition were checked on blood agar plates. The results showed that freshly minced garlic and campharated parachlorophenol represented statistically significant largest inhibitory zones followed by sodium hypochloride, lemon solution and citric acid.

Gomes et al (2003)[47] evaluated the effectiveness of 2% chlorhexidine gluconte gel and calcium hydroxide $Ca(OH_2)$ as intracanal medicaments at 1,2,7,15 and 30 days against *Enterococcus faecalis* using Brain heart infusion broth. Chlorhexidine gel alone completely inhibited the growth of *Enterococcus faecalis* after 1, 2, 7 and 15 days. The combination of chlorhexidine and $Ca(OH)_2$ was effective after 1 and 2 days and its antibacterial activity reduced between 7 and 15 days. This study concluded that 2% chlorhexidine gel alone was more effective against *Enterococcus faecalis* than calcium hydroxide.

Evans et al (2002)[48] studied the mechanisms that enable *Enterococcus faecalis* to survive the high pH of calcium hydroxide. *Enterococcus faecalis* strain JH2-2 was exposed to sub lethal concentrations of calcium hydroxide, with and without various pre-treatments. Blocking agents were added to determine the role of stress-induced protein synthesis and the cell wall-associated proton pump. No difference in cell survival was observed when protein synthesis was blocked during stress induction, however, addition of a proton pump inhibitor resulted in a dramatic reduction of cell viability of *Enterococcus faecalis* in calcium hydroxide. This study concluded that the survival of *Enterococcus faecalis* in calcium hydroxide appears to be unrelated to stress induced protein synthesis, but a functioning proton pump is critical for survival of *Enterococcus faecalis* at high pH.

Estrela et al (2001)[49] studied the antimicrobial effectiveness of calcium hydroxide plus saline, calcium hydroxide plus polyethylene glycol, calcium hydroxide plus camphorated paramonochlorophenol against *Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans.* The strains were inoculated in Brain Heart Infusion (BHI) broth .Two methods, the direct exposure test and the agar diffusion test were used to evaluate the antimicrobial effects. The authors found that calcium hydroxide plus saline was more effective than other groups against *Enterococcus faecalis, Bacillus subtilis* and *Candida albicans.* Calcium hydroxide plus camphorated paramonochlorophenol was more effective against *Pseudomonas aeruginosa* than other groups.

Love (2001)[50] studied the possible mechanism that would explain how *Enterococcus faecalis* could survive and grow within dentinal tubules and reinfect an obturated root canal. Cells of *Streptococcus gordonii, Streptococcus mutans* or *Enterococcus faecalis* were grown in brain heart infusion broth containing various amounts of human serum for 56 days. The ability of the three species to invade dentine and bind to immobilized Type 1 collagen in the presence of human serum was assessed by dentine invasion and microtitre well experiments. The results of the present study demonstrated that a virulence factor of *Enterococcus faecalis* in failed endodontically treated teeth may be related to the ability of *Enterococcus faecalis* cells to remain viable and maintain the capability to invade dentinal tubules and adhere to collagen in the presence of human serum.

Hancock et al (2001)[51] determined the composition of the microbial flora present in teeth after the failure of root canal therapy in a North American population. The samples were grown under aerobic and anaerobic conditions and then the

suggested that 2% chlorhexidine gel alone is more effective at 72 hours than calcium hydroxide paste alone or in combination with 2% chlorhexidine gel against both the organisms, even though calcium hydroxide showed better antifungal efficacy against *Candida albicans* at 24 hours.

Krithikadatta et al (2007)[44] evaluated the disinfection of dentinal tubules using 2% chlorhexidine gel, 2% metronidazole gel and bioactive glass in comparison with calcium hydroxide. The antibacterial efficacy against *Enterococcus faecalis* was assessed using tryptone soya agar plates, light microscopy and scanning electron microscopy. The authors concluded that 2% chlorhexidine gel was most effective against *Enterococcus faecalis* when compared to other medicaments tested. The overall percentage inhibition of bacterial growth was 100% with 2% chlorhexidine gel followed by 2% metronidazole gel, bioactive glass and calcium hydroxide.

Schafer and Bossmann (2005)[45] studied the antibacterial property of $Ca(OH)_2$ paste, chlorhexidine solution 2%(CHX), and a mixture of chlorhexidine and $Ca(OH)_2$ paste against *Enterococcus faecalis* and examined using agar diffusion method.This study found that chlorhexidine was significantly more effective against *Enterococcus faecalis* than $Ca(OH)_2$ paste or a mixture of chlorhexidine with $Ca(OH)_2$ paste. There was no increase in the efficiency of $Ca(OH)_2$ paste when chlorhexidine was added.

Sawsan et al (2004)[46] evaluated the antibacterial activity of two natural plants (freshly minced garlic extract and fresh lemon solution) with three traditional intracanal medications: 10% citric acid, 5.25% sodium hypochloride (NaOCl) and campharated parachlorophenol (CPCP) against *α -hemolytic Streptococci, Streptococci pyogens, Enterococcus faecalis and Pseudomonas aeruginosa.* The

bacterial growth was analyzed. The growth on agar plates was subcultured for identification on the basis of anaerobic, facultative anaerobic ,or aerobic growth and was found that the microbial flora was mainly of 1 to 2 strains of predominantly gram-positive organisms. *Enterococcus faecalis* was the most commonly recovered bacterial species and was found in 30% of the teeth with a positive culture.

Haapasalo et al (2000)[52] investigated the inactivation by dentine of the antibacterial activity of calcium hydroxide solution, 1% sodium hypochlorite, 0.5% and 0.05% chlorhexidine acetate, and 2/4% and 0.2/0.4% iodine potassium iodide as root canal medicaments. Purity of the cultures was checked by studying colony morphology under stereo microscopy and by gram stain. The effect of 0.05% chlorhexidine and 1% sodium hypochlorite on *Enterococcus faecalis* was reduced but not totally eliminated by the presence of dentine .The authors concluded that the dentine powder model appears to be an efficient tool for the study of interactions between local endodontic medicaments, dentine, and microbes.

Siqueira and Uzeda (1997)[53] evaluated the antibacterial activitiy of 0.12% chlorhexidine gel, 10% metronidazole gel, calcium hydroxide plus distilled water, calcium hydroxide plus camphorated paramono- chlorophenol (CPMC), and calcium hydroxide plus glycerine against *Porphyromonas endodontalis, Fusobacterium nucleatum, Enterococcus faecalis* and *Actinomyces viscosus* . An agar diffusion test was used, and the zones of bacterial inhibition around each medicament were recorded .The results revealed that calcium hydroxide/CPMC paste was effective against all bacterial strains tested. Chlorhexidine (0.12%) was also inhibitory against all strains but it was not more effective than calcium hydroxide mixed with CPMC. Metronidazole was effective than calcium hydroxide/CPMC paste against only

Porphyromonas endodontalis and *Fusobacterium nucleatum*. Calcium hydroxide mixed with distilled water or glycerin was ineffective against all bacterial strains used in this experiment.

Georgopoulou et al (1993)[54] compared the effectiveness of calcium hydroxide and paramonochlorophenol against anaerobic bacteria of root canal at 5, 15, 30 and 60 minutes using blood agar plates .The study revealed that paramonochlorophenol was less effective than calcium hydroxide against the total number of anaerobes .It was proved to be quickly and highly effective against some microorganisms such as *Bacteroides melaninigenicus*, *Porphyromonas gingivalis* as well as against *Actinomyces* species.

Sjogren et al (1991)[55] clinically evaluated the antibacterial effect of calcium hydroxide as a short term intracanal dressing by applying the medicament for 10 minutes or 7 days in root canals of teeth with periapical lesions. Sterile saline solution was introduced into the canal by means of a syringe. The fluid in the canal was absorbed with charcoaled paper points and transferred to a tube with 5 ml of anaerobic peptone yeast extract glucose (PYG) broth. The results showed that the 7-day dressing was effective in eliminating bacteria which survived biomechanical instrumentation of the canal, while the 10 minute application was ineffective.

Safavi et al (1985)[56] compared the antimicrobial effects of calcium hydroxide with that of iodine-potassium iodide as an intracanal medicament. Cultures taken from human root canals after the completion of canal preparation and before obturation were incubated and read periodically using a thioglycollate broth. This study concluded that calcium hydroxide was more effective against *Enterococcus faecalis* than iodine-potassium iodide.

MATERIALS & METHODS

MATERIALS AND METHODS

Armamentarium

- Diamond disc
- Endomotor(X-Smart,Dentsply Maillefer,Japan)
- Protaper files(Dentsply Maillefer,Switzerland)
- K files
- Disposable syringes
- Test tubes (Borosil 27 ml, Riviera 15 ml)
- Eppendorf tubes
- Incubator (NSW, India)
- Autoclave (Unique clave C-79, Confident)
- Paper points (Dentsply)
- Sterile swab
- Microcentifuge tube (1.5ml)
- Tweezer
- Hard tissue microtome(Leica,Germany)
- Confocal Laser Scanning Microscope(Zeiss,LSM 510 META,Germany)

Materials used

- Extracted human teeth – single canal premolars
- Herbal medicaments – Aloevera, *Ricinus communis* , Lemon
- Calcium hydroxide (Apexcal,Ivoclar Vivadent)
- Saline (0.9% w/v sodium chloride injection, NS, Baxter, India)

- Sodium hypochlorite (Prime dental,India)
- Ethylene Diamine Tetra Acetic acid(Dentsply Maillefer,USA)
- *Enterococcus faecalis*(ATCC 29212)
- Thioglycollate broth
- Blood Agar (Himedia,India)
- Muller Hinton agar (Himedia,India)
- Sticky wax
- Fluorescent dyes-Fluorescein diacetate and Propidium iodide(Sigma,USA)

EXPERIMENTAL DESIGN

Teeth selection and Standardization of Working Length:

Eighty single-rooted human mandibular premolars with closed apices, extracted for orthodontic reasons were used in this study. The teeth were cleaned of superficial debris, calculus, and tissue tags and stored in normal saline to prevent dehydration before use. Each tooth was radiographed to confirm the presence of a single patent canal. The tooth specimens were sectioned below the cementoenamel junction with a diamond disc to obtain a standardized tooth length of 13 mm (Fig. 1). The canals were accessed, and initially a size #10 Stainless Steel (SS) K file was inserted into the canal until the file tip was just visible at the apical foramen. The working length (WL) was kept 1mm short of the apical foramen.

Fig 1: Decoronated teeth

Standardization of Apical Canal Dimension:

The root canals were prepared using protaper files (Dentsply Maillefer, Switzerland) upto size F3.The canals were irrigated with sodium hypochlorite between each instrument and 17% EDTA as a final irrigant to remove the smear layer. All the roots were then washed and stored in saline.

Sterilization of teeth:

All the prepared teeth were packed in suitable autoclave pouches and autoclaved at 121° C

Inoculation of enterococcus faecalis:

Each root canal was inoculated with 24 h old cultured broths of bacterial solution of *Enterococcus faecalis* (Fig. 2, 3) using a sterile endodontic needle in a microbiological safety cabinet. After inoculation, the samples were kept in a closed eppendorf tube and incubated at 37°C for 21 days under aseptic conditions (Fig. 4, 5). The canals were re-inoculated with fresh bacterial samples at every 3 days interval to ensure viability of bacteria.

Fig 2: Enterococcus faecalis

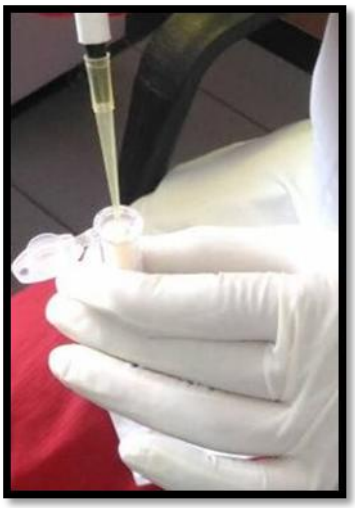


Fig 3: Inoculation of bacterial sample

Fig 4: Tooth samples in eppendorf tubes.

Fig 5: Incubator

Root canal medication:

The canal contents were aspirated after 21 days of incubation, then rinsed with 5 mL saline and patted dry with sterile paper points. The specimens were then randomly divided into five groups (*n* = 16 each) (Fig.6-9) for intracanal medicaments:

- Group I : Normal saline(control)
- Group II : Calcium hydroxide mixed with saline
- Group III : Aloevera
- Group IV : *Ricinus communis* (castor oil)
- Group V : Fresh lemon solution

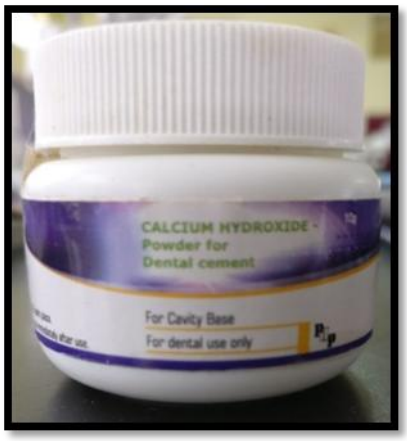


Fig 6: Cacium hydroxide

Fig 7: Aloevera

Fig 8: Lemon

Fig 9: Ricinus communis

Obtaining the Intracanal medicaments:

Calcium hydroxide was mixed with saline to obtain paste like consistency. Aloevera leaves contain clear gel and green part of the leaf that surrounds the gel produced juice or dried substance which is obtained manually. The aloevera in gel form is used as a medicament. Lemon solution (pH 2.21) is a natural source of citric acid (pH 1.68) with lower acidity. Fresh lemon solution is used as root canal medicament because of its wide antibacterial efficiency against *Enterococcus faecalis.*

Ricinus communis rich in ricinoleic acid, also known as castor oil plant .The castor oil is obtained by pressing the seeds of the *Ricinus communis* using a manual machine extractor. The herbal extracts were collected from the gardens of Agricultural College and Research Institute, Coimbatore (Tamilnadu Agricultural University).

Placement of Intracanal medicaments:

In all the samples, the prepared medicaments of 5 µL were injected in the root canals and completely filled (Fig.10). The canals were then sealed with sticky wax (Pyrax, India) and incubated at 37°C for 7 days. After 7 days of incubation, the wax was removed from each of the canals and irrigated with normal saline for 2 minutes.

Sterile paper points were inserted into the root canals. After adsorption of the canal contents for 1min, the points were dipped into sterile thioglycollate broth, incubated overnight under appropriate condition (Fig.11). After incubation, they were placed onto sterile Muller Hinton agar for count of *Enterococcus faecalis* in terms of CFU/ml.

Fig 10: Placement of intracanal medicament

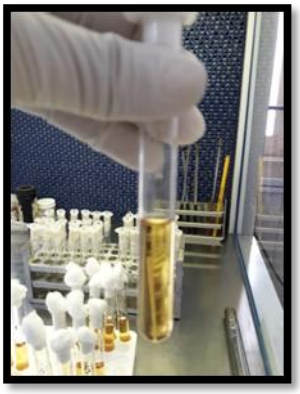


Fig 11: Thioglycollate broth

Antibacterial efficacy

The antibacterial efficacy of the intracanal medicaments against *Enterococcus faecalis* was compared using Culture Study (Colony Counting) and confocal microscopy.

Culture study:

After the placement of medicaments, absorbent paper points were used to take samples from the root canals. These absorbent paper points were introduced into the test tube containing sterile thioglycollate broth and incubated at 37°C for 24 hours. After incubation, the samples were plated onto sterile Muller Hinton agar and incubated for another 24 hours. Colony counting was done to determine the antibacterial efficacy (Fig.12-16). The number of colonies is directly proportional to the amount of residual bacteria present in the root canals after placement of medicament.

Colony counting:

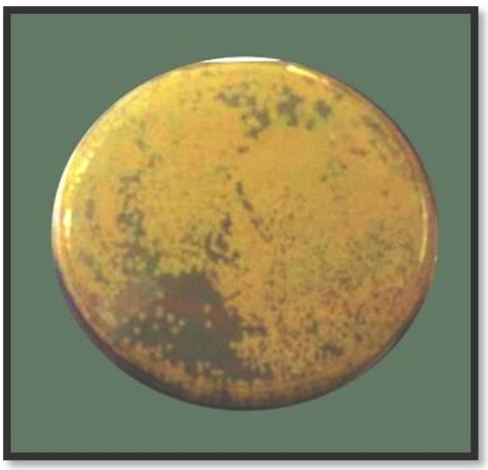


Fig 12: Saline

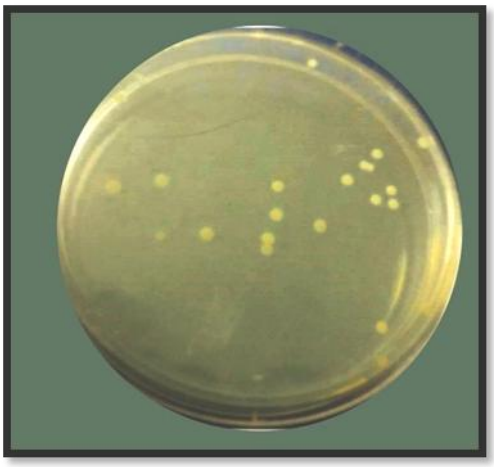


Fig 13: Calcium hydroxide

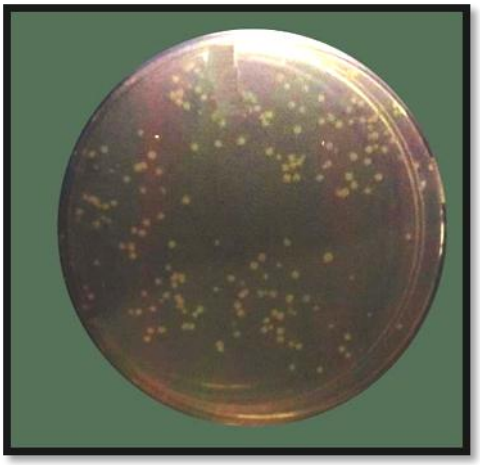


Fig 14: Aloevera

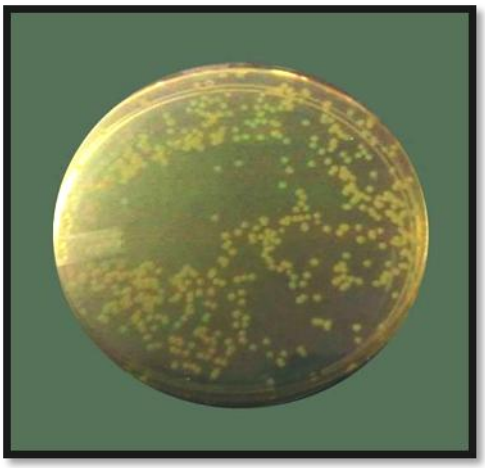


Fig 15: Lemon

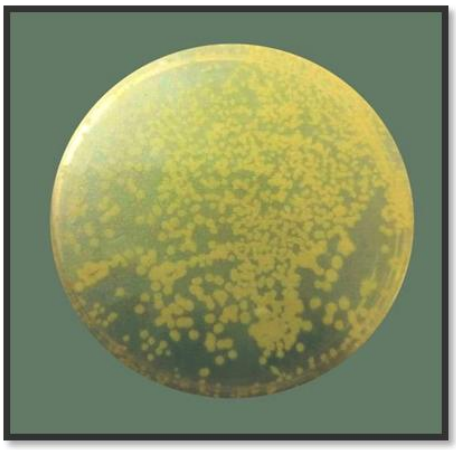


Fig 16: Ricinus communis

Confocal Microscopy:

After embedding the specimens in acrylic resin, each root was sectioned evenly to obtain the apical transverse sections, using a hard tissue microtome (Leica, Germany) (Fig.17, 18). The five experimental groups contained 5 sections/samples per group.

The fluorescent dyes Fluorescent Diacetate diluted in acetone (FDA) and propidium iodide(PI)(SIGMA,USA) diluted in distilled water were prepared to give a concentration of 4mg/mL of Fluorescein Diacetate and 1.4mg/mL of Propidium Iodide (Fig.19).The root sections were washed with Phosphate buffered saline twice to remove any debris present. The root sections were placed in microcentrifugal tubes and 400 µL of FDA was added per vial in dark and the sections were kept immersed in the solution for 10 minutes at room temperature. The roots were then removed from the vial and blotted dry and were immersed in PI solution for 2 minutes. It was then washed with saline to remove any excess stain and blotted dry.

All the sections were dried and were observed under a confocal laser scanning microscope (Zeiss, LSM 510 META,GERMANY) with 20X magnifications (Fig.20).The fluorescent images obtained were in terms of green and red pixels, corresponding to live and dead bacteria. AIM software was used to assess the viability of *E. faecalis* against the various intracanal medicaments used, by quantifying the bacteria individually as live and dead. (Fig.21)

Fig 17: Hard tissue microtome

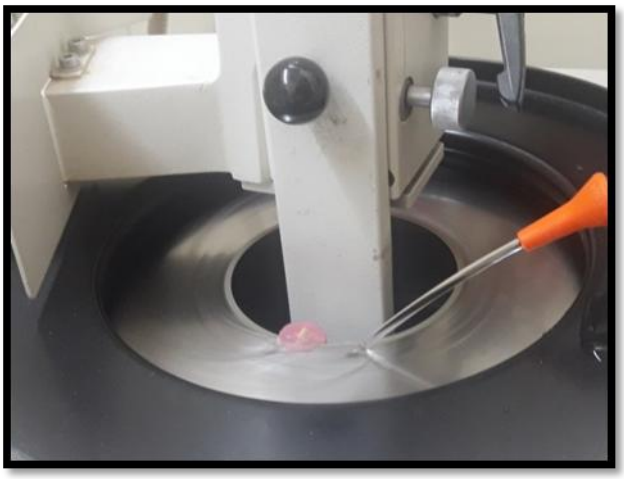


Fig 18: Sectioning of teeth

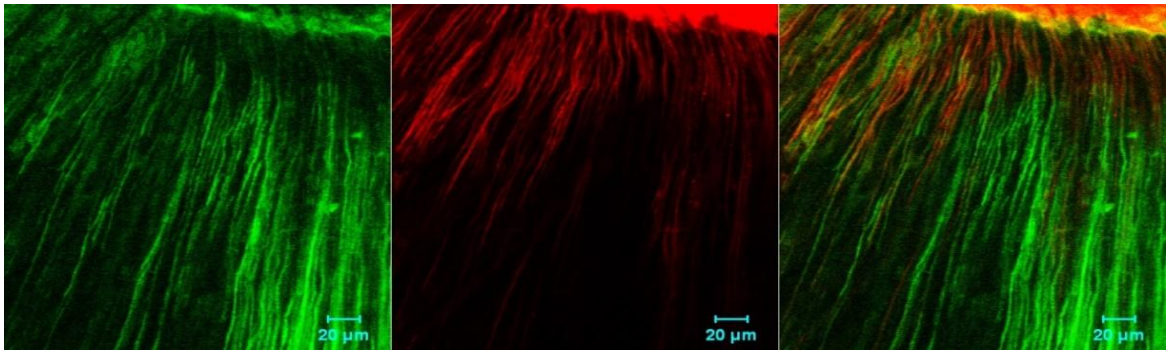
Fig 19: Fluorescent dyes FDA and PI

Fig 20: Confocal Laser Scanning Microscope

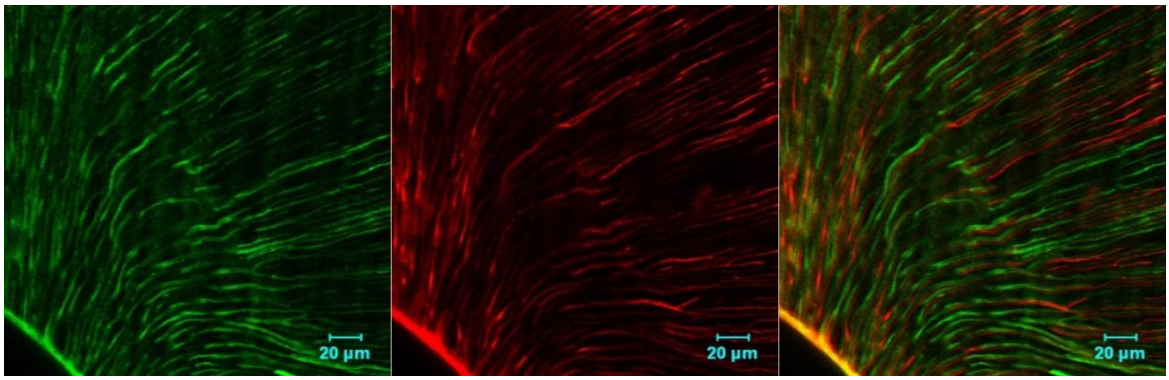
CONFOCAL LASER SCANNING MICROSCOPY IMAGES

FIG 21: Bacterial viability in the apical region of Tooth samples (20X)

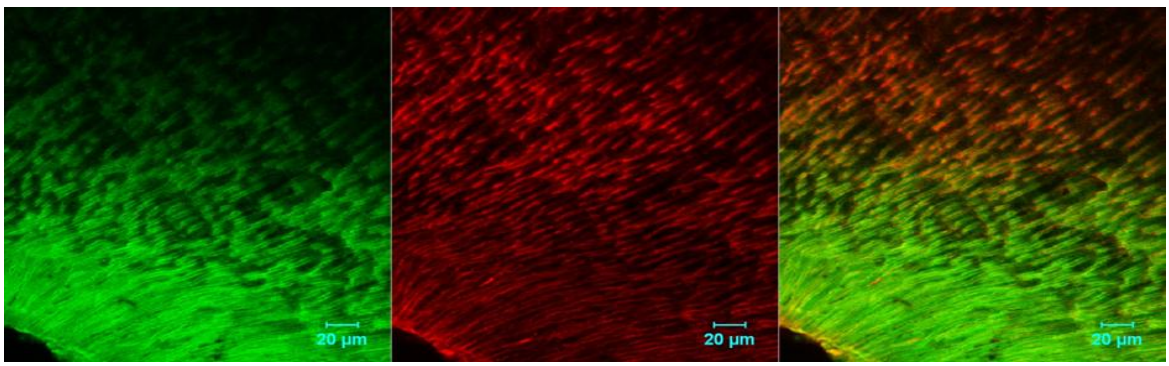
FDA **PI** **MERGE**



SALINE

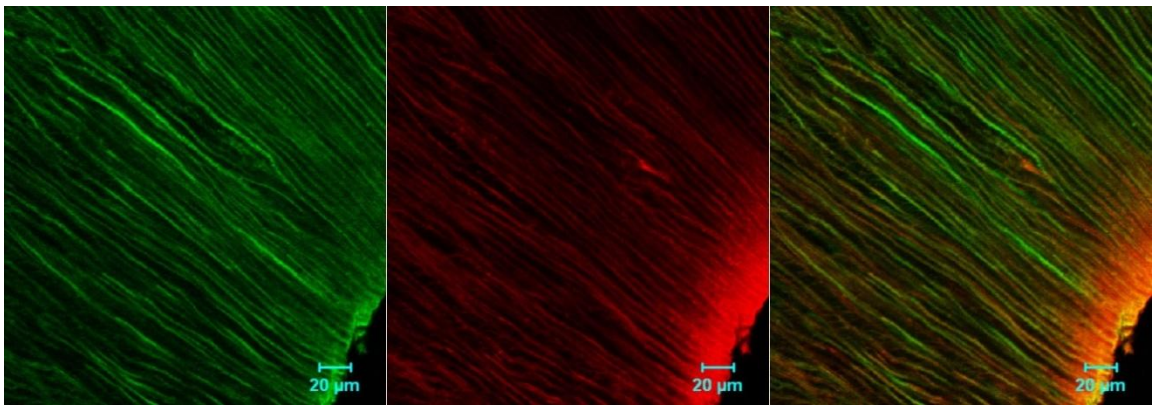


$Ca(OH)_2$

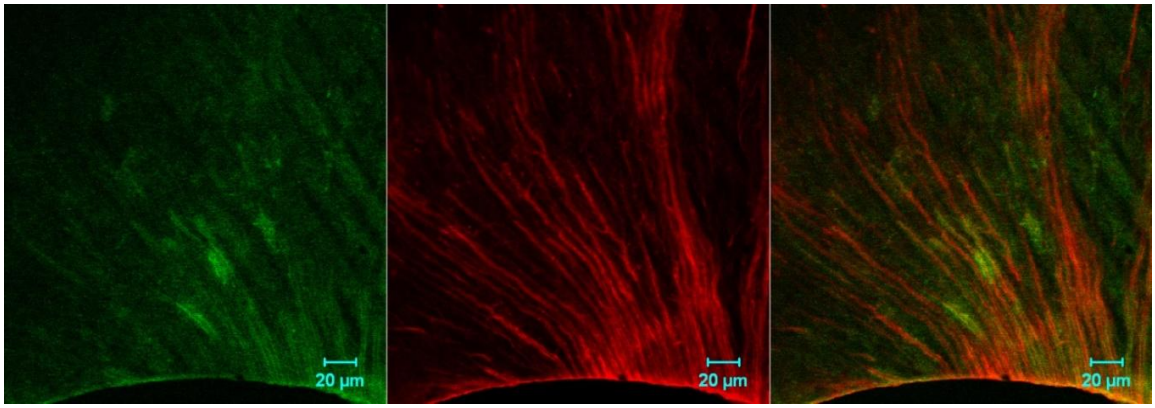


ALOEVERA

FDA **PI** **MERGE**



RICINUS COMMUNIS



LEMON

GREEN FLUORESCENCE INDICATE LIVE BACTERIA

RED FLUORESCENCE INDICATE DEAD BACTERIA

STATISTICAL ANALYSIS:

The statistical analysis was performed with the SPSS 17 software system (Chicago, USA). Descriptive statistics was performed using One Way Anova followed by Tukey HSD (Post Hoc) with levels of significance set at $P < 0.05$.

RESULTS

MASTER CHART

GREEN AND RED FLUORESCENCE (IN PIXELS)

GROUP	ROOT SAMPLES	APICAL	
		GREEN	RED
Saline	1	55401	13743
	2	48168	6168
	3	28257	1996
	4	68791	3435
	5	48631	1982
$Ca(OH_{2})$	6	4011	15335
	7	5138	7063
	8	7140	112746
	9	2025	55751
	10	5272	18322
ALOEVERA	11	6090	15221
	12	9912	45404
	13	3033	64172
	14	3293	27806
	15	30315	29947
RICINUS COMMUNIS	16	14125	6891
	17	41883	12501
	18	17538	15050
	19	68195	11895
	20	27512	15451
LEMON	21	8323	33643
	22	4666	37627
	23	37742	13112
	24	3599	28890
	25	3542	23174

GREEN : Corresponds to live bacteria

RED : Corresponds to dead bacteria

RESULTS

GREEN BACTERIA

TABLE 1: Difference in mean values in between the groups using ANOVA

	Groups	N	Mean	Std. Deviation	F value	P value
Green	**SALINE**	5	49849.60	14664.138	9.080	0.001
	CALCIUM HYDROXIDE	5	4717.20	1877.598		
	ALOEVERA	5	10528.60	11403.080		
	RICINUS COMMUNIS	5	38771.40	22977.222		
	LEMON	5	11574.40	14757.772		

TABLE 2: Difference in the mean values between the control (Saline) and test groups using post-hoc analysis

	Test Group	Comparison Group	Mean difference	P value
GREEN	SALINE	CALCIUM HYDROXIDE	45132.400*	0.001
		ALOEVERA	39321.000*	.004
		RICINUS COMMUNIS	11078.200	.760
		LEMON	38275.200*	.005

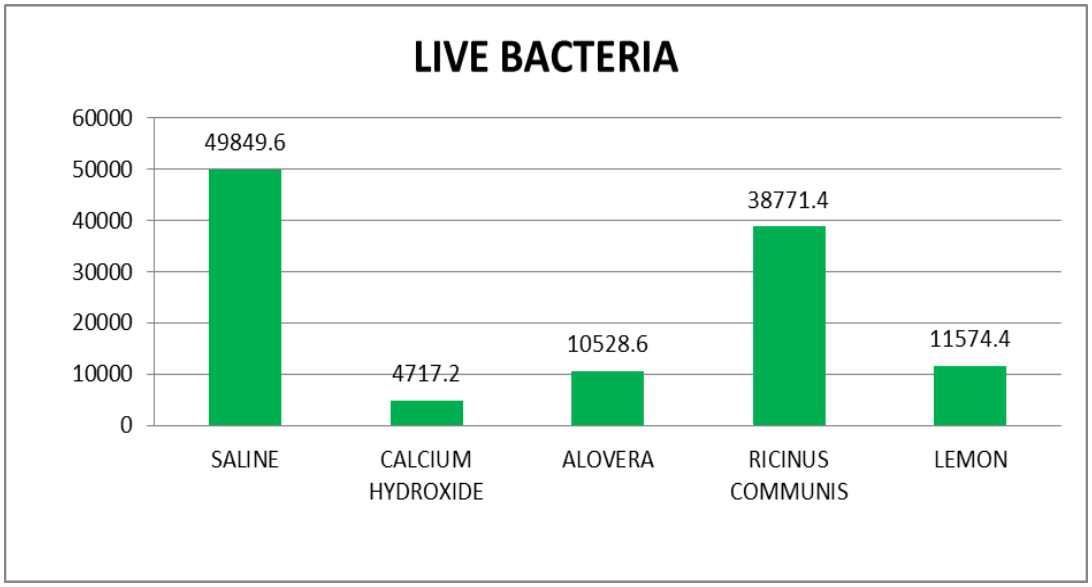
TABLE 3: MULTIPLE COMPARISONS – GREEN BACTERIA

Tukey HSD

(I) GROUPS	(J) GROUPS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Saline	$Ca(OH)_2$	45132.400*	9356.654	.001	17133.79	73131.01
	Aloevera	39321.000*	9356.654	.004	11322.39	67319.61
	Ricinus	11078.200	9356.654	.760	-16920.41	39076.81
	Lemon	38275.200*	9356.654	.005	10276.59	66273.81
$Ca(OH)_2$	Saline	-45132.400*	9356.654	.001	-73131.01	-17133.79
	Aloevera	-5811.400	9356.654	.970	-33810.01	22187.21
	Ricinus	-34054.200*	9356.654	.013	-62052.81	-6055.59
	Lemon	-6857.200	9356.654	.946	-34855.81	21141.41
Aloevera	Saline	-39321.000*	9356.654	.004	-67319.61	-11322.39
	$Ca(OH)_2$	5811.400	9356.654	.970	-22187.21	33810.01
	Ricinus	-28242.800*	9356.654	.047	-56241.41	-244.19
	Lemon	-1045.800	9356.654	1.000	-29044.41	26952.81
Ricinus Communis	Saline	-11078.200	9356.654	.760	-39076.81	16920.41
	$Ca(OH)_2$	34054.200*	9356.654	.013	6055.59	62052.81
	Aloevera	28242.800*	9356.654	.047	244.19	56241.41
	Lemon	27197.000	9356.654	.060	-801.61	55195.61
Lemon	Saline	-38275.200*	9356.654	.005	-66273.81	-10276.59
	$Ca(OH)_2$	6857.200	9356.654	.946	-21141.41	34855.81
	Aloevera	1045.800	9356.654	1.000	-26952.81	29044.41
	Ricinus	-27197.000	9356.654	.060	-55195.61	801.61

*. The mean difference is significant at the 0.05 level.

Graph 1: Bar diagram representing mean values of live bacteria between the groups.



In the above table, the F value 9.080 for the mean difference in the amount of live bacteria in the five groups is significant ($p < 0.05$).The mean amounts of live bacteria in the five groups (Saline, Calcium hydroxide, Aloevera, *Ricinus communis*, Lemon) were 49849.50, 4717.20, 10528.60, 38771.40, 11574.40, indicating that calcium hydroxide had less live bacteria compared to other groups. Further, post hoc analysis reveals that Calcium hydroxide is more effective when compared to the other medicaments followed by Aloevera, Lemon and *Ricinus communis.*

RED BACTERIA

TABLE 4: Difference in mean values in between the groups using ANOVA

	Groups	N	Mean	Std. Deviation	F value	P value
Red	**SALINE**	5	5464.80	4932.181	2.502	.075
	CALCIUM HYDROXIDE	5	41843.40	43835.567		
	ALOEVERA	5	36510.00	18817.170		
	RICINUS COMMUNIS	5	12357.60	3425.508		
	LEMON	5	27289.20	9588.161		

TABLE 5: Difference in the mean values between the control (Saline) and test groups using post-hoc analysis

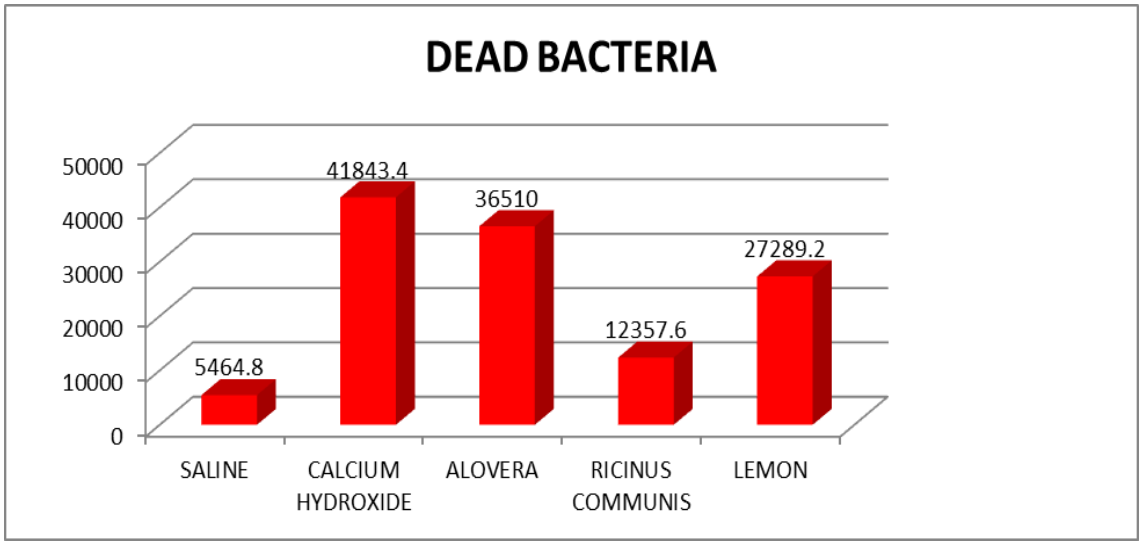
	Test Group	Comparison Group	Mean difference	P value
Red	SALINE	CALCIUM HYDROXIDE	-36378.600	.104
		ALOEVERA	-31045.200	.206
		RICINUS COMMUNIS	-6892.800	.987
		LEMON	-21824.400	.530

TABLE 6: MULTIPLE COMPARISONS (Tukey HSD) – RED BACTERIA

(I) GROUPS	(J) GROUPS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
SALINE	CALCIUM HYDROXIDE	-36378.600	13866.899	.104	-77873.56	5116.36
	ALOEVERA	-31045.200	13866.899	.206	-72540.16	10449.76
	RICINUS COMMUNIS	-6892.800	13866.899	.987	-48387.76	34602.16
	LEMON	-21824.400	13866.899	.530	-63319.36	19670.56
CALCIUM HYDROXIDE	SALINE	36378.600	13866.899	.104	-5116.36	77873.56
	ALOEVERA	5333.400	13866.899	.995	-36161.56	46828.36
	RICINUS COMMUNIS	29485.800	13866.899	.248	-12009.16	70980.76
	LEMON	14554.200	13866.899	.829	-26940.76	56049.16
ALOEVERA	SALINE	31045.200	13866.899	.206	-10449.76	72540.16
	CALCIUM HYDROXIDE	-5333.400	13866.899	.995	-46828.36	36161.56
	RICINUS COMMUNIS	24152.400	13866.899	.433	-17342.56	65647.36
	LEMON	9220.800	13866.899	.962	-32274.16	50715.76
RICINUS COMMUNIS	SALINE	6892.800	13866.899	.987	-34602.16	48387.76
	CALCIUM HYDROXIDE	-29485.800	13866.899	.248	-70980.76	12009.16
	ALOEVERA	-24152.400	13866.899	.433	-65647.36	17342.56
	LEMON	-14931.600	13866.899	.816	-56426.56	26563.36
LEMON	SALINE	21824.400	13866.899	.530	-19670.56	63319.36
	CALCIUM HYDROXIDE	-14554.200	13866.899	.829	-56049.16	26940.76
	ALOEVERA	-9220.800	13866.899	.962	-50715.76	32274.16
	RICINUS COMMUNIS	14931.600	13866.899	.816	-26563.36	56426.56

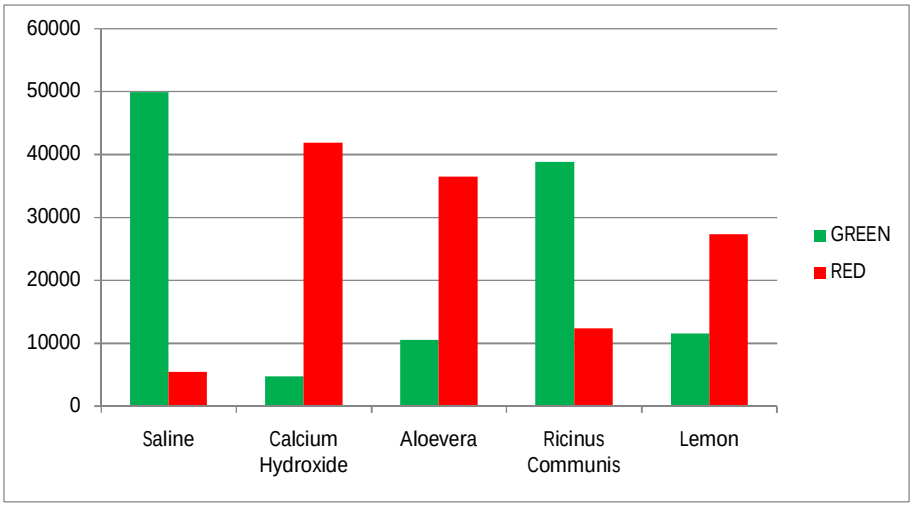
*. The mean difference is significant at the 0.05 level.

Graph 2: Bar diagram representing mean values of dead bacteria between the groups.



In the above table, the F value 2.502 for the mean difference in the amount of dead bacteria in the five groups is significant ($p < 0.05$). The mean amounts of dead bacteria in the five groups (Saline, Calcium hydroxide, Aloevera, *Ricinus communis*, Lemon) were 5464.80, 41843.40, 36510, 12357.6, and 27289.2 respectively, indicating that more dead bacteria were found in the calcium hydroxide group. Further, post hoc analysis reveals that calcium hydroxide is more effective when compared to the other medicaments followed by Aloevera, Lemon and *Ricinus communis*

Graph 3: Graphical representation of live and dead bacteria present in the five groups



CULTURE STUDY:

TABLE 7: Difference in mean values in colony count in between the groups using ANOVA

Groups	N	Mean	Std. Deviation	F	Sig.
SALINE	16	25.80	1.0481730	781.538	.000
CALCIUM HYDROXIDE	16	10.14	.9230159		
ALOEVERA	16	14.82	.8933085		
RICINUS COMMUNIS	16	21.73	.7274384		
LEMON	16	16.76	.7144928		

TABLE 8: Difference in the mean values in colony count in between the control (Saline) and test groups using post-hoc analysis

Test Group	Comparison Group	Mean difference	P value
SALINE	CALCIUM HYDROXIDE	15.6562500*	.001
	ALOEVERA	10.9750000*	.001
	RICINUS COMMUNIS	4.0625000*	.001
	LEMON	9.0375000*	.001

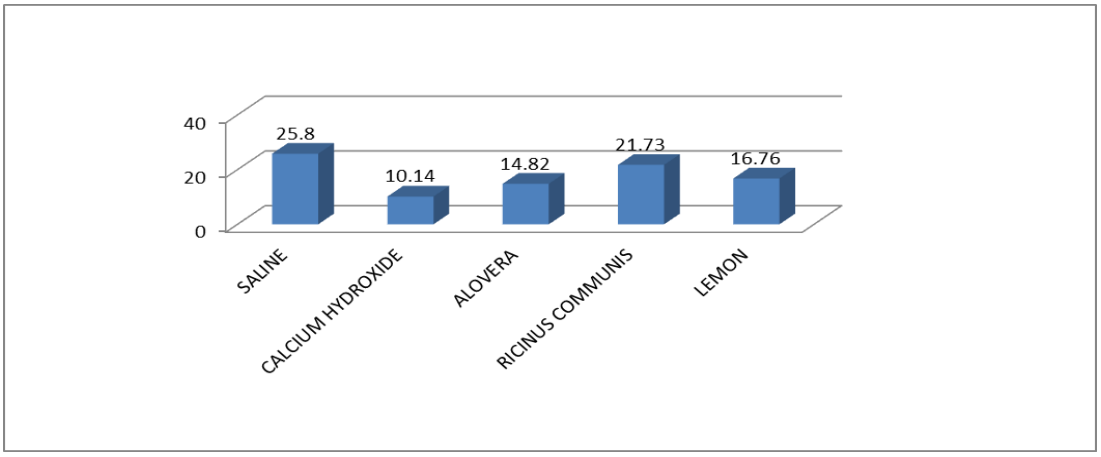
TABLE 9: Multiple Comparisons

Tukey HSD

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
SALINE	CALCIUM HYDROXIDE	15.6562500*	.3077455	.000	14.796024	16.516476
	ALOEVERA	10.9750000*	.3077455	.000	10.114774	11.835226
	RICINUS COMMUNIS	4.0625000*	.3077455	.000	3.202274	4.922726
	LEMON	9.0375000*	.3077455	.000	8.177274	9.897726
CALCIUM HYDROXIDE	SALINE	-15.6562500*	.3077455	.000	-16.516476	-14.796024
	ALOEVERA	-4.6812500*	.3077455	.000	-5.541476	-3.821024
	RICINUS COMMUNIS	-11.5937500*	.3077455	.000	-12.453976	-10.733524
	LEMON	-6.6187500*	.3077455	.000	-7.478976	-5.758524
ALOEVERA	SALINE	-10.9750000*	.3077455	.000	-11.835226	-10.114774
	CALCIUM HYDROXIDE	4.6812500*	.3077455	.000	3.821024	5.541476
	RICINUS COMMUNIS	-6.9125000*	.3077455	.000	-7.772726	-6.052274
	LEMON	-1.9375000*	.3077455	.000	-2.797726	-1.077274
RICINUS COMMUNIS	SALINE	-4.0625000*	.3077455	.000	-4.922726	-3.202274
	CALCIUM HYDROXIDE	11.5937500*	.3077455	.000	10.733524	12.453976
	ALOEVERA	6.9125000*	.3077455	.000	6.052274	7.772726
	LEMON	4.9750000*	.3077455	.000	4.114774	5.835226
LEMON	SALINE	-9.0375000*	.3077455	.000	-9.897726	-8.177274
	CALCIUM HYDROXIDE	6.6187500*	.3077455	.000	5.758524	7.478976
	ALOEVERA	1.9375000*	.3077455	.000	1.077274	2.797726
	RICINUS COMMUNIS	-4.9750000*	.3077455	.000	-5.835226	-4.114774

*. The mean difference is significant at the 0.05 level.

Graph 4: Bar diagram representing mean values of bacteria between the groups.



In the above table, the F value 781.538 for the mean difference in the amount of bacteria through colony counting in the five groups is significant ($p < 0.05$).The mean amounts of bacteria in the five groups (Saline, Calcium hydroxide, Aloevera, *Ricinus communis*, Lemon) were 25.8, 10.14, 14.82, 21.73, 16.76, indicating that calcium hydroxide had less bacteria compared to the other groups. Further, post hoc analysis reveals that Calcium hydroxide is more effective when compared to the other medicaments followed by Aloevera, Lemon and *Ricinus communis.*

DISCUSSION

DISCUSSION

Complete disinfection of the root canal space is an important prerequisite for achieving longterm success of nonsurgical endodontics [57].The use of a biocompatible intracanal medicaments possessing antimicrobial properties between appointments may reduce or eliminate bacteria in the root canal system and increase the success of root canal treatment [58].

The most commonly used intracanal medicament in Endodontics is Calcium hydroxide $Ca(OH)_2$. Since the introduction of calcium hydroxide by Herman in 1920, it has been used in endodontics as an intracanal medicament due to its high antimicrobial and anti-inflammatory properties [59]. It plays an important role in endodontics by its ability to induce hard tissue formation, exerting antibacterial action and interrupting the nutrient supply to remaining bacteria [60]. Its high pH (of about 11–12.5) has a destructive effect on bacterial cell membrane and protein structure. The antimicrobial efficacy of calcium hydroxide is dependent upon direct contact with bacteria and it decreases with time [61]. Moreover, calcium hydroxide is not considered very effective in eliminating bacteria from the dentinal tubules [61]. *Enterococcus faecalis* is small enough to proficiently invade and live within dentinal tubules[50]. **Gomes et al. (2003)** reported that *Enterococcus faecalis* present in the dentinal tubules are resistant to calcium hydroxide intracanal dressing over 10 days [47].

Nature has bestowed a very rich botanical wealth, and a large number of diverse types of plants grow in different parts of world. Antimicrobial agents of plant origin have enormous therapeutic potential. They are effective in the treatment for infectious diseases, and simultaneously they also mitigate many of the side effects

that are often associated with synthetic antimicrobials [62]. Phytomedicines have been used as anti-inflammatory, antibiotic, analgesic and sedative agents. In endodontics because of the cytotoxic reactions of the most of the commercial intracanal medicaments used and their inability to eliminate bacteria from dentinal tubules, trend of recent medicine attends to use biologic medication extracted from natural plants [63]. The main advantages of using herbal alternatives are easy availability, cost effectiveness, increased shelf life, low toxicity and lack of microbial resistance. Certain of these herbal derivatives has been tried in Endodontics as intracanal medicaments.

Aloevera belongs to the Liliaceae family. It is a plant species of the genus Aloe. The species was first described by **Carl Linnaeus** in **1753** [64]. It grows wild in tropical climates around the world and is cultivated for agricultural and medicinal uses. Aloevera is also used for decorative purposes and grows successfully indoors as a potted plant [65]. Aloe leaves contain clear gel and green part of the leaf that surrounds the gel is used to produce juice or dried substance. Cosmetic and some medicinal products are made from the mucilaginous tissue in the center of the aloevera leaf and are called as aloevera gel. Aloevera contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, anthraquinones, saponins, salicylic acids and amino acids. Total leaf extracts contain anthraquinones, which have antibacterial properties [66]. It provides 12 anthraquinones, which are phenolic compounds traditionally known as laxatives. Alloin and emodin act as analgesics, antibacterials and antivirals. It contains alloins and barbadoins as main chemical constituents. **Anuj Bhardwaj (2012)** has found aloevera gel has inhibitory effects on *S.pyogens* and *E.faecalis* because of anthraquinine [32].

Ricinus communis, the castorbean or castor-oil-plant, is a species of perennial flowering plant in the spurge family, Euphorbiaceae [67]. Although *Ricinus communis* is indigenous to the south eastern Mediterranean Basin, Eastern Africa and India, today it is widespread throughout tropical regions. Castor seed is the source of castor oil, which has a wide variety of uses. The constituents of *Ricinus communis* are ricinoleic acid , Oleic acid, linoleic acid, α-Linolenic acid, stearic acid, palmitic acid and dihydroxystearic acid. The seeds contain between 40% and 60% oil that is rich in triglycerides, mainly ricinolein [68]. Castor oil has many uses in medicine and other applications. The high percentage of ricinoleic acid residues in castor oil and its derivatives, inhibits many microbes, whether viral, bacterial or fungal [69] and can be used in Endodontics as a root canal irrigant as well as an intracanal medicament [11] . **Valera et al (2013)** found that the *Ricinus communis* extract was able to completely eliminate *C.albicans* and it was also able to significantly reduce the amount of *E. faecalis* [12]. **Garcia et al (2009)** observed that calcium hydroxide and *Ricinus communis* oil paste had good antimicrobial properties against microorganisms commonly found in endodontic infections [11].

The Lemon is a species of small evergreen tree in the flowering plant family Rutaceae, native to Asia. The chemical composition of lemon solution contains certain acidic substances called citric acid and carboxylic acid. The juice of the lemon is about 5% to 6% citric acid, with a pH of around 2.2, giving it a sour taste. Lemon solution has antimicrobial properties, making it excellent at reducing many types of bacteria, virus and fungi. The tree's ellipsoidal yellow fruit is used for culinary and non-culinary purposes throughout the world, primarily for its juice, which has both culinary and cleaning uses [70]. Lemon oil may be used in aromatherapy. Lemon oil aroma does not influence the human immune system, but

may contribute to relaxation [71]. **Sawsan T et al (2004)** found that fresh lemon solution was shown to have wide antibacterial efficiency against *Enterococcus faecalis* and hence can be used as an intracanal medicament [46].

Intracanal medication may be a valuable adjunct to chemomechanical preparation in the disinfection of the root canal system, reducing the endodontic microbiota and therefore favoring periapical tissue repair [58]. This study was conducted to compare and evaluate the antimicrobial effect of different intracanal medications including Calcium hydroxide, Aloevera, *Ricinus communis* and Lemon against *Enterococcus faecalis*.

Enterococcus faecalis is a Gram-positive cocci and a facultative anaerobe that occur singly, in pairs or in short chains [72]. It has been found to be one of the most persistent microorganisms with a prevalence of 24 to 77% in root filled teeth with periradicular lesions. It has a potential to adhere to both collagen and hydroxyapatite of dentin in the presence of human serum and also possesses certain virulence factors including lytic enzymes, cytolysin, aggregation substance, pheromones and lipoteichoic acid, which facilitates its survival in the root canal [73]. *E. faecalis* is known to colonize dentinal tubules, isthmus, rami, lateral and accessory canals. It remains to be the most frequently identified species in canals of root-filled teeth with periapical lesions [74]. Studies have shown *E.faecalis* to be viable inside the root canal dentin up to a period of 12 months [75]. Eradication of *E. faecalis* from the root canal remains a challenge, since it is resistant to a variety of antimicrobial agents [4]. Therefore, it is important to consider treatment regimens aimed at eliminating or preventing the infection of *Enterococcus faecalis* during all the phases of root canal treatment and hence *E.faecalis* was the microorganism of choice for the current study.

Colony Counting was chosen to evaluate the antibacterial efficacy of intracanal medicaments as this would signify the quantity of live residual bacteria present in the root canals. Microbiological root canal culturing is commonly used to assess the effectiveness of endodontic treatment measures. **Upadhyay et al (2015)** [20] tested the antimicrobial activity of dental materials and medicaments using the culture method. The advantage of this method is that it allows direct comparison of the materials against the organisms, indicating which material has the potential to eliminate bacteria in the local microenvironment of the root canal system. However, the disadvantage of this method is that the result not only depends on the toxicity of the material for the particular organism but is also influenced by the ability of the material to diffuse across the medium [76].

In a study by **S. D'Ercole et al (2008)** [77]**,** comparing molecular technique and culture technique, the authors concluded that molecular techniques cannot differentiate between viable and dead cultures. Culture methods have the advantage of being able to detect a wide variety of species; the characterization of all isolates may allow the identification of unexpected or new species. This technique enables us to search for all the microorganisms present in a non-specific way and it remains the most objective technique [77].

Bacterial viability assessment was done using a confocal laser scanning microscope which has advantages of visualization, differentiation of live and dead microorganisms and quantification individually, over the conventional methods. Scanning confocal microscopy includes the ability to adjust magnification electronically by varying the area scanned by the laser without having to change objectives. **Parmer et al (2011)**[78] assessed the capacity of confocal laser scanning

microscopy to differentially image and quantify viable and non-viable bacteria within mineralized tissue and showed it to be a convenient and reproducible approach for assessing viability of the bacteria and the extent of bacterial penetration into the dentinal tubules. **Weiger et al (2002)** [79] applied the FDA / PI stain to circumpulpal filings from an endodontic infection model to check the vitality status of bacteria and found that Confocal laser scanning microscope is helpful for characterization of live and dead bacteria in different physiological states.

Confocal laser scanning microscope necessitates the use of fluorescent binding agents to make visualization possible by the property of fluorescence. Such agents used in the current study are fluorescein diacetate (FDA) and propidium iodide (PI). The two stains differ in their spectral characteristics and their ability to penetrate bacterial cell membranes. The excitation/emission maxima for these dyes are about 480/500nm for FDA stain and 490/635nm for propidium iodide. When used, the FDA stain penetrates intact biological membranes, whereas PI penetrates only bacteria with compromised plasma membranes and reduces the FDA fluorescence on binding with the nucleic acid. Thus, with an appropriate staining, live bacteria with intact bacterial cell membranes stain fluorescent green whereas dead bacteria with damaged cell membranes accumulate propidium iodide in the cell body and stain fluorescent red [80]. **Zapata et al (2008)** [81] in their study explored the potential of confocal laser scanning microscopy (CLSM) to visualize live and dead *Enterococcus faecalis* in infected dentin. They found it to be useful in identification of viable and dead bacteria in infected dentin after staining with FDA and PI.

In this present study the best antibacterial activity through Colony Counting was exhibited by calcium hydroxide followed by Aloevera, lemon and Ricinus

communis. After the confocal laser scanning microscopic analysis, the values obtained for each medicament was tabulated. In our study, the results indicated that calcium hydroxide paste with a mean value of (Live/Dead 4717.20/41843.5) was efficacious against *Enterococcus faecalis* compared to Aloevera (10528.6 / 36510), Lemon (11574.4 / 27289.2) and *Ricinus communis* (38771.4 / 12357.6) at a level of significance of $p < 0.05$. Significant difference in reduction of live bacteria was found between the four groups. Also complete elimination of *E.faecalis* was not achieved with all the four intracanal medicaments used.

Studies by **Garcia et al (2009)[11]** comparing the antibacterial efficacy of *Ricinus communis*, propylene glycol, calcium hydroxide and their combination against *Enterococcus faecalis* and found that combination of *Ricinus communis* and calcium hydroxide was better than using plain *Ricinus communis*. In another study by **Sawsan et al (2004)[46]** who evaluated the antibacterial activity of freshly minced garlic extract , fresh lemon solution ,10% citric acid, 5.25% sodium hypochloride (NaOCl) and camphorated parachlorophenol (CPCP) against *Enterococcus faecalis* in which fresh lemon solution showed good antimicrobial effect against *Enterococcus faecalis* which is similar to the results obtained in this study.

The results were also in accordance with the study by **Abbaszadegan et al (2014) [82],** in which the antibacterial efficacy of Aloevera, Zataria multiflora plant oil and calcium hydroxide was tested against *Enterococcus faecalis* and it was found that calcium hydroxide possessed the best antimicrobial effect. In another study by **Kurian et al (2016)[83]** compared the antimicrobial efficacy of calcium hydroxide, extracts of mushroom and Aloevera leaves against *Enterococcus faecalis* and found that Aloevera showed good antimicrobial effect.

However, the results of our study vary from that of the study conducted by **Bhardwaj et al (2012)[32],** in which Aloevera showed better antibacterial efficacy compared to calcium hydroxide against *Enterococcus faecalis*. This may be because after placement of intracanal medicaments, the antimicrobial efficacy was checked only till 5 days in that study unlike the efficacy checked after 7 days in our study.

The findings of this study demonstrated that calcium hydroxide showed maximum antimicrobial efficacy. Among the herbal medicaments Aloevera and Lemon has shown good antimicrobial effect against *Enterococcus faecalis*.

The nature of the endodontic microflora is polymicrobial but single species biofilm model of *Enterococcus faecalis* was used in our study, which can be a limitation. Therefore, further studies have to be done using multispecies biofilm model. Though biofilm model with *E. faecalis* was used on extracted teeth, this does not entirely simulate the oral environmental conditions.

Further investigations regarding the efficacy of these intracanal medicaments in vitro and in vivo are needed for better understanding and for clinical application of the results of this in vitro study. The use of these medicaments incorporated into irrigants may be the future in days to come……..

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The current study evaluated the effectiveness of Calcium hydroxide, Aloevera, *Ricinus communis* and Lemon against *Enterococcus faecalis* in a tooth biofilm model.

Eighty extracted human roots were debrided and decoronated. The samples were chemomechanically prepared and were infected with *Enterococcus faecalis*, and incubated at 37°C for 21 days under aseptic conditions. The canals were re-inoculated with fresh bacterial samples at every 3 days interval to ensure viability of bacteria .The samples were divided into five groups with 16 samples under each medicament.

- Group I : Normal saline(control)
- Group II : Calcium hydroxide mixed with saline
- Group III : Aloevera
- Group IV : *Ricinus communis* (castor oil)
- Group V : Fresh lemon solution

The samples were incubated for a period of 7 days. Following incubation, the antibacterial efficacy of the intracanal medicaments against *Enterococcus faecalis* was evaluated by culture study (colony counting) and bacterial viability was assessed by confocal microscopy.

For assessing the bacterial viability, the roots were sectioned transversely at the apical region (five samples in each group) using a hard tissue microtome. The sections were washed thoroughly and stained with fluorescent DNA binding reagents (fluorescent diacetate and propidium iodide). The bacterial viability was examined by

Confocal laser scanning microscopy. Computer assisted determinants of fluorescence for live and dead bacteria were analysed and compared statistically.

The results obtained from colony counting and confocal microscopy revealed that complete elimination of *Enterococcus faecalis* was not achieved in any of the experimental groups. Calcium hydroxide was most effective in causing reduction of *E. faecalis* followed by Aloevera, Lemon and *Ricinus communis*.

Within the limitations of the current study, the medicament that is effective against *E.faecalis in vitro* may not be necessarily effective against the same microbe *in vivo* because root canal system contains multiple microorganisms. Further studies should be carried out in clinical conditions to better understand the efficacy and conclusively recommend herbal solutions as root canal medicaments.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Manzur A, González AM, Pozos A, Silva-Herzog D, Friedman S. Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: a randomized clinical trial. J Endod 2007; Feb 33(2):114-8.

2. Chong BS, Pitt Ford TR. The role of intracanal medication in root canal treatment. Int Endod J 1992 Mar; 25(2):97-106.

3. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod 2006 Feb; 32(2):93-98

4. Castilho AL, Saraceni CH, Díaz IE, Paciencia ML, Suffredini IB. New trends in dentistry: plant extracts against Enterococcus faecalis. The efficacy compared to chlorhexidine. Braz Oral Res 2013 Mar-Apr; 27(2):109-115.

5. Zoletti GO, Pereira EM, Schuenck RP, Teixeira LM, Siqueira JF, dos Santos KR. Characterization of virulence factors and clonal diversity of Enterococcus faecalis isolates from treated dental root canals. Res Microbiol 2011 Feb-Mar; 162(2):151-158.

6. Delany GM, Patterson SS, Miller CH, Newton CW. The effect of chlorhexidine gluconate irrigation on the root canal flora of freshly extracted necrotic teeth. Oral Surg Oral Med Oral Pathol 1982; 53:518-23.

51. Hancock H.H, Asgeir Sigurdsson, Martin Trope, Julian Moiseiwitsch, Chapel Hill. Bacteria isolated after unsuccessful endodontic treatment in a North American population. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;91:579-86

52. Haapasalo H.K, SireÂn E.K, Waltimo T.M.T, Erstavik D, Haapasalo M.P.P. Inactivation of local root canal medicaments by dentine: an in vitro study. Int Endod J 2000; 33: 126-131.

53. Josd F. Siqueira, Milton de Uzeda. Intracanal Medicaments: Evaluation of the Antibacterial Effects of Chlorhexidine, Metronidazole, and Calcium Hydroxide Associated with Three Vehicles. J Endod 1997; 23(3):167-169.

54. Georgopoulou M, Kontakiotis E, Nakou M. In vitro evaluation of the effectiveness of calcium hydroxide and paramonochlorophenol on anaerobic bacteria from the root canal. Endod Dent Traumatol 1993; 9: 249-253.

55. Sjogren U, Figdor D, Spangberg L, Sundqvist G. The antimicrobial effect of calcium hydroxide as a short-term intracanal dressing. Int Endod J 1991; 24:119-125.

56. Kamran E. Safavi, William E. Dowden, Joseph H. Introcaso, Kaare Langeland. A Comparison of Antimicrobial Effects of Calcium Hydroxide and Iodine-Potassium Iodide. J Endod 1985; 11(10): 454-456.

57. Wang CS, Arnold RR, Trope M. Clinical efficiency of 2% Chlorhexidine gel in reducing intracanal bacteria. J Endod 2007; 33:1283-9.

7. de Souza-Filho FJ, Soares Ade J, Vianna ME, Zaia AA, Ferraz CC, Gomes BP. Antimicrobial effect and pH of chlorhexidine gel and calcium hydroxide alone and associated with other materials. Braz Dent J 2008; 19:28-33.

8. Ellof JN. Which extractant should be used for the screening and isolation of antimicrobial components from plants. J Ethnopharmacol 1998; 60: 1-6.

9. Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria; potential application in prevention and treatment of oral diseases. (Online article) http:// ecam. Oxford journals. org/ cgi/ content/ full/ nep067.

10. Newall CA, Anderson LA, Phillipson JD. Herbal medicines. A guide for health-care professionals. London: The Pharmaceutical Press; 1996.

11. Lucas Da Fonseca Roberti Garcia, Gabriela Lemos De Almeida, Fernanda De C. P. Pires-De-Souza, Simonides Consani. Antimicrobial activity of a calcium hydroxide and Ricinus communis oil paste against microorganisms commonly found in endodontic infections. Rev Odonto Ciênc 2009; 24(4): 406-409.

12. Marcia Carneiro Valera, Lilian Eiko Maekawa, Luciane Dias de Oliveira, Antonio Olavo Cardoso Jorge, Érika Shygei, and Cláudio Antonio Talge Carvalho. In vitro antimicrobial activity of auxillary chemical substances and natural extracts on Candida albicans and Enterococcus faecalis in root canals. J Appl Oral Sci 2013; 21(2): 118–123.

13. Maya Verma, Ashwani Kumar. Antimicrobial activity of some folk medicinal plants used in rajasthan against selected pathogenic microorganisms. Int J Curr Pharm Res 2017; 9(3): 84-86.

14. Mohammed Mustafa .Antibacterial Efficacy of Neem (Azadirachta indica) Extract against Enterococcus faecalis : An in vitro Study. J Contemp Dent 2016;17(10):791-794

15. Alpa Gupta-Wadhwa, Jitesh Wadhwa , Jigyasa Duhan. Comparative evaluation of antimicrobial efficacy of three herbal irrigants in reducing intracanal E. faecalis populations: An in vitro study. J Clin Exp Dent 2016; 8(3): 230-5.

16. Pradeep Muttagadur Chandrappa, Akash Dupper, Pragya Tripathi, Ramakrishna Arroju, Preeti Sharma,Konthoujam Sulochana. Antimicrobial activity of herbal medicines (tulsi extract, neem extract) and chlorhexidine against *Enterococcus faecalis* in Endodontics: An *in vitro* study. J Int Soc Prev Community Dent 2015 Dec; 5(2): S89–S92.

17. Attia D.A, Farag A.M, Afifi I.K, Darrag A.M. Antimicrobial effect of different intracanal medications on various microorganisms. Tanta Dent J 2015; 12: 41-47.

18. Sonali Taneja, Pragya Kumar, Komal Malhotra, and Jaidev Dhillon. Antimicrobial effect of an oxazolidinone, lantibiotic and calcium hydroxide against *Enterococcus faecalis* biofilm: An *in vitro* study. Indian J Dent 2015 Oct-Dec; 6(4): 190–194.

19. Sanjay Madhavan, Muralidharan. Comparing the antibacterial efficacy of intracanal medicaments in combination with clove oil against Enterococcus faecalis. Asian J Pharm Clin Res 2015; 8(5): 136-138.

20. Kanchi Upadhyay, Mallikarjuna Kencheppa, Shilpi Gupta, Priyamvada Sharma, Soumik Roy, Puneet Gupta.Comparison of antibacterial efficacy of

combination of turmeric and calcium hydroxide with three intracanal medicaments against various endodontic bacteria: An in vitro study. J orofac res 2015; 5(4):113-117.

21. Murad C.F, Sassone L.M, Faver M, Hirata R, Figueiredo L, Feres M . Microbial Diversity in Persistent Root Canal Infections investigated by Checkerboard DNA-DNA Hybridization. J Endod 2014;1-8

22. Jaheer Shaik, Roopadevi Garlapati, Bolla Nagesh, Varri Sujana, Thumu Jayaprakash, Sudhakar Naidu. Comparative evaluation of antimicrobial efficacy of triple antibiotic paste and calcium hydroxide using chitosan as carrier against Candida albicans and Enterococcus faecalis: An *in vitro* study. J Conserv Dent 2014 Jul-Aug; 17(4): 335–339

23. Abbaszadegan A, Nabavizadeh M, Gholami A, Aleyasin Z.S, Dorostkar S, Saliminasab M, Ghasemi Y, Hemmateenejad B, Sharghi H. Positively charged imidazolium-based ionic liquid-protected silver nanoparticles: a promising disinfectant in root canal treatment. Int Endod J 2014;1-11

24. Jeison B. Carbajal Mejía. Antimicrobial effects of calcium hydroxide, chlorhexidine, and propolis on Enterococcus faecalis and Candida albicans. J Investig Clin Dent 2013; 4: 1–7

25. Gupta A, Duhan J, Tewari S, Sangwan P, Yadav A, Singh G, Juneja R , Saini H. Comparative evaluation of antimicrobial efficacy of Syzygium aromaticum, Ocimum sanctum and Cinnamomum zeylanicum plant extracts against Enterococcus faecalis: a preliminary study. Int Endod J 2013; 46: 775–783.

26. Kandaswamy Eswar, Nagendrababu Venkateshbabu, Kalaiselvam Rajeswari, and Deivanayagam Kandaswamy. Dentinal tubule disinfection with 2% chlorhexidine, garlic extract, and calcium hydroxide against *Enterococcus faecalis* by using real-time polymerase chain reaction: *In vitro* study. J Conserv Dent 2013 May-Jun; 16(3): 194–198

27. Wasudeo Namdeo Ghonmode, Omkar D Balsaraf, Varsha H Tambe, K P Saujanya, Ashishkumar K Patil, Deepak D Kakde. Comparison of the antibacterial efficiency of neem leaf extracts, grape seed extracts and 3% sodium hypochlorite against E. feacalis – An in vitro study. J Int Oral Health 2013 Dec; 5(6): 61–66.

28. Atila-Pektas B , Yurdakul P, Gulmez D , Gorduysus O. Antimicrobial effects of root canal medicaments against *Enterococcus faecalis* and *Streptococcus mutans*. Int Endod J 2013; 46: 413–418.

29. Jin-Kyung Lee,Seok Woo Chang,Hiran Perinpanayagam,Sang-Min Lim, Yoon-Jeong Park,Seung Hyun Han,Seung-Ho Baek,Qiang Zhu, Kwang-Shik Bae, Kee-Yeon Kum. Antibacterial Efficacy of a Human b-Defensin-3 Peptide on Multispecies Biofilms. J Endod 2013; 39(12):1625-1629.

30. Nilima Thosar, Silpi Basak, Rakesh N Bahadure, Monali Rajurkar. Antimicrobial efficacy of five essential oils against oral pathogens: An *in vitro* study. Eur J Dent 2013; 7(5):71-77.

31. Jaya Nagendra Krishna, Ramya Raghu1, Nagesh Bolla, Keerthi Muddanna. An in vitro comparative evaluation of the antibacterial efficacy of 10% metronidazole gel, 2% chlorhexidine gel, and a combination of calcium

hydroxide and 2% chlorhexidine gel against Enterococcus faecalis. J Orofac Sci 2012; 4(1):26-31.

32. Anuj Bhardwaj, Suma Ballal1, Natanasabapathy Velmurugan.Comparative evaluation of the antimicrobial activity of natural extracts of Morinda citrifolia,papain and aloevera gel(all in gel formulation),2% chlorhexidine gel and calcium hydroxide, against Enterococcus faecalis : An in vitro study. J Conserv Dent 2012; 15(3):293-297.

33. de Lucena J.M.V.M, Decker E.M, Walter C, Boeira L.S, Lost C, Weiger R.Antimicrobial effectiveness of intracanal medicaments on Enterococcus faecalis: chlorhexidine versus octenidine. Int Endod J 2013; 46: 53–61

34. Miranda R.G, Santos E.B, Souto R.M, Gusman H , Colombo A.P.V. Ex vivo antimicrobial efficacy of the EndoVac® system plus photodynamic therapy associated with calcium hydroxide against intracanal Enterococcus faecalis. Int Endod J 2013; 46: 499–505.

35. Baydaa H. Abd-Awn, Zainab A. Al-Dhaher, Raya R, Al-Dafaai.The effect of black seed oil extracts on mutans streptococci in comparison to chlorhexidine gluconate (in vitro). J Bagh Coll Dent 2012; 24(4):126-131.

36. Liu V, Dashper S, Parashos P, Liu S.W , Stanton D, Shen P, Chivatxaranukul P, Reynolds EC. Antibacterial efficacy of casein-derived peptides against Enterococcus faecalis. Aust Dent J 2012; 57: 1–5

37. Badr A.E, Omar N, Badria F.A. A laboratory evaluation of the antibacterial and cytotoxic effect of Liquorice when used as root canal medicament. Int Endod J 2011; 44: 51–58.

38. Lima R.K.P, Guerreiro-Tanomaru J.M, Faria-Junior N.B, Tanomaru-Filho M. Effectiveness of calcium hydroxide-based intracanal medicaments against Enterococcus faecalis. Int Endod J 2012; 45: 311–316.

39. Dakshita Joy Vaghela, Deivanayagam Kandaswamy, Nagendrababu Venkateshbabu, Narasimman Jamini, Arathi Ganesh. Disinfection of dentinal tubules with two different formulations of calcium hydroxide as compared to 2% chlorhexidine: As intracanal medicaments against *Enterococcus faecalis* and *Candida albicans*: An *in vitro* study. J Conserv Dent 2011 Apr-Jun; 14(2): 182–186.

40. Hemadri M, Sophia Thakur, Girija Sajjan. Nisin Vs Calcium Hydroxide – Antimicrobial Efficacy on Enterococcus Feacalis – An In-vitro Study. Int J Contemp Dent 2011; 2(3):55-61.

41. Manavalan Madhana Madhubala, Narasimhan Srinivasan, Shafie Ahamed . Comparative Evaluation of Propolis and Triantibiotic Mixture as an Intracanal Medicament against Enterococcus faecalis. J Endod 2011; 37(9):1287-1289.

42. Kandaswamy D, Venkateshbabu N, Gogulnath D, Kindo A.J. Dentinal tubule disinfection with 2% chlorhexidine gel, propolis, morinda citrifolia juice, 2% povidone iodine, and calcium hydroxide. Int Endod J 2010; 43: 419–423.

43. Ballal V, Kundabala M, Acharya S, Ballal M. Antimicrobial action of calcium hydroxide, chlorhexidine and their combination on endodontic pathogens. Aust Dent J 2007;52:(2):118-121

44. Jogikalmat Krithikadatta, Rajamani Indira, Alfred Leo Dorothykalyani. Disinfection of Dentinal Tubules with 2% Chlorhexidine, 2% Metronidazole,

Bioactive Glass when Compared with Calcium Hydroxide as Intracanal Medicaments. J Endod 2007; 33(12) 1473-1476.

45. Edgar Schäfer and Klaus Bössmann. Antimicrobial Efficacy of Chlorhexidine and Two Calcium Hydroxide Formulations against Enterococcus faecalis. J Endod 2005; 31(1): 53-56.

46. Sawsan T, Abu Zied, Somaia A. L. Eissa. Comparative Study on Antibacterial Activities of Two Natural Plants Versus Three different intracanal Medications. (Online-article) http:// www. kau. edu. sa/ Files/ 165/ Researches/ 19240_Comparative%20Study%20On.pdf

47. Gomes B.P.F.A, Souza S.F.C, Ferraz C.C.R, Teixeira F.B, Zaia A.A, Valdrighi L, Souza-Filho F.J. Effectiveness of 2% chlorhexidine gel and calcium hydroxide against Enterococcus faecalis in bovine root dentine in vitro. Int Endod J 2003; 36: 267-275.

48. Evans M, Davies J.K, Sundqvist G, Figdor D. Mechanisms involved in the resistance of Enterococcus faecalis to calcium hydroxide. Int Endod J 2002; 35: 221-228.

49. Carlos Estrela, Cyntia Rodrigues de Araujo Estrela. Two Methods to Evaluate the Antimicrobial Action of Calcium Hydroxide Paste. J Endod 2001; 27(12):720-723.

50. Love R.M. Enterococcus faecalis – a mechanism for its role in endodontic failure. Int Endod J 2001; 34: 399-405.

58. Bystro¨m A, Claesson R, Sundqvist G .The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol, and calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol 1985; 1: 170–5.

59. Kim D,Kim E. Antimicrobial effect of calcium hydroxide as an intracanal medicament in root canal treatment-a literature review. Part I: in vitro studies. Restor Dent Endod 2014 Nov; 39(4):241-252.

60. Hasselgren G, Olsson B, Cvek M. Effects of calcium hydroxide and sodium hypochlorite on the dissolution of necrotic porcine muscle tissue. J Endod 1998;14:125-127

61. Sundqvist G, Figdor D, Persson S, Sjogren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative retreatment. Oral Surg Oral Med Oral Path Oral Radiol Endod 1998;85:86-93

62. Rıos JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol 2005; 100: 80 –4.

63. Groppo FC, Bergamaschi CC, Cogo K, Montan M, Motta RHL, de Andrade ED. Use of Phytotherapy in Dentistry. Phytother Res.2008: 22: 993-998.

64. Linnaeus C. *Species plantarum, exhibentes plantas rite cognitas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas.* 1753 Vol. 2: 561–1200, [1–30, index]. Holmiae [Stockholm]: Impensis Laurentii Salvii.

65. *Perkins, Cyndi. "Is Aloe a Tropical Plant?". SFgate.com. February*2016

66. Newall CA, Anderson LA, Phillipson JD. Herbal medicines. A guide for health-care professionals. London: The Pharmaceutical Press; 1996.

67. Botanical Society of Britain and Ireland. Archived from the original on 2015-02-25.

68. Phillips, Roger, Rix, Martyn . *Annuals and Biennials*. London: Macmillan. 1999; 106.

69. Sabina E.P., Rasool M.K., Mathew L., Parameswari."Studies on the protective effect of *Ricinus communis* leaves extract on carbon tetrachloride hepatotoxicity in albino rats". *Pharmacologyonline* 2009: 905–916.

70. Julia F. Morto. "Lemon in Fruits of Warm Climates". Purdue University.1987; 160–168

71. Cooke B, Ernst E. *"Aromatherapy: A systematic review"* . British Journal of General Practice 2000*;* 50 (455): 493–6.

72. Distel JW, Hatton JF, Gillespie MJ. Biofilm formation in medicated root canals. J Endod 2002; 28(10):689-93.

73. Rocas IN, Siqueira JF, Santos KRN. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endod 2004; 30: 315-20.

74. Mistry KS, Sanghvi Z, Parmar G, Shah S. The antimicrobial activity of Azadirachta indica, Mimusops elengi, Tinospora cardifolia, Ocimum sanctum and 2% chlorhexidine gluconate on common endodontic pathogens: An in vitro study. Eur J Dent 2014; 8(2):172-7.

75. Sedgley CM, Lennan SL, Appelbe OK. Survival of Enterococcus faecalis in root canals ex vivo. Int Endod J 2005; 38: 735-742.

76. Neelakandan P, Sanjeev K,Subbarao CV.Duration-dependent susceptibility of endodontic pathogens to calcium hydroxide and chlorhexidine gel used as intracanal medicament: an in vitro evaluation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007 Oct; 104: e138-e141.

77. Simonetta D'Ercole, Giovanni Catamo, Domenico Tripodi, Raffaele Piccolomini. Comparison of culture methods and multiplex PCR for the detection of periodontopathogenic bacteria in biofilm associated with severe forms of periodontitis. NEW MICROBIOLOGICA 2008; 31: 383-391.

78. Parmar D, Hauman C.H.J, Leichter J.W, McNaughton A, Tompkins G.R .Bacterial localization and viability assessment in human ex vivo dentinal tubules by fluorescence confocal scanning laser scanning microscopy. Int Endod J 2011; 44: 644-651.

79. Weiger R, de Lucena J, Decker HE, Lost C. Vitality status of microorganisms in infected human root dentine. Int Endod J 2002; 35: 166–71.

80. Auty MAE, Gardiner GE, McBrearty SJ .Direct in situ viability assessment of bacteria in probiotic dairy products using viability staining in conjunction with confocal scanning laser microscopy. Applied and Environmental Microbiology 2001; 67: 420–5.

81. Zapata RO, Bramante CM, de Moraes IG. (2008) Confocal laser scanning microscopy is appropriate to detect viability of Enterococcus faecalis in infected dentin. J Endod 2008; 34: 1198–201.

82. Abbas Abbaszadegan, Safoora Sahebi, Ahmad Gholami,Alireza Delroba, Amin Kiani,Aida Iraji,Paul Vincent Abbott. Time-dependent antibacterial effects of *Aloe vera* and *Zataria multiflora* plant essential oils compared to calcium hydroxide in teeth infected with *Enterococcus faecalis*. J investig clin dent 2014; 7(1): 93-101.

83. Bijo Kurian, Swapna DV, Roopa R Nadig, Ranjini MA, Rashmi K, Subha Rani Bolar. Efficacy of calcium hydroxide, mushroom, and *Aloe vera* as an intracanal medicament against *Enterococcus faecalis*: An *in vitro* study. Endodontology 2016; 28(2): 137-42.