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

DISSERTATION

Submitted to the

BABU BANARASI DAS UNIVERSITY LUCKNOW, UTTAR PRADESH

In the partial fulfillment of the requirement for the degree

of

MASTER OF DENTAL SURGERY

In the subject of

CONSERVATIVE DENTISTRY & ENDODONTICS

Submitted by

DR. RICKU MATHEW REJI

Under the guidance of

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DEPARTMENT OF CONSERVATIVE DENTISTRY & ENDODONTICS

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES, LUCKNOW

Batch: 2020-2023 **Enrollment No.: 1200322006**

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I hereby declare that this dissertation entitled **"A Comparative Evaluation of the Antibacterial Efficacy of Various Root Canal Irrigants Against E. Fecalis: An *In-Vitro* Study"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. Vishesh Gupta**, Professor, Department of Conservative Dentistry & Endodontics, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

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LIST OF ABBREVIATIONS

ABBREVIATION	FULL FORM
E. fecalis	Enterococcus fecalis
NaOCl	Sodium Hypochlorite
CHX	Chlorhexidine
EDTA	Ethylene Diamine Tetra Acetic Acid
$Ca(OH)_2$	Calcium Hydroxide
AgNP	Silver Nanoparticles
CsNP	Chitosan Nanoparticles
W/v	Weight by Volume
%	Percentage
BHI	Brain Heart Infusion
mL	Millilitre
µL	Microlitre
CFU	Colony Forming Units
GIC	Glass Ionomer Cement

Introduction: The root canal system anatomy is extremely complex and diverse, it's not always possible to clean and shape it effectively. So, irrigation and disinfection of Root Canals plays a very important role in the success of a Root Canal Treatment. **Aim:** The aim of this study was to evaluate & compare antimicrobial efficacy of 3.8% Silver Diamine Fluoride, 5.25% Sodium Hypochlorite, 100mg/ml Vitex Negundo Linn , 0.2 % Nano Chitosan , 0.9% Normal Saline and 2% Chlorhexidine against Enterococcus fecalis as root canal irrigant. **Method:** Sixty single rooted human permanent teeth were taken and decoronated to standardize the canal length. After biomechanical preparation, teeth were inoculated with E.fecalis & randomly divided into 6 groups and the final irrigation was carried out with tested irrigants. Group A(*n* = 10): 0.9% Normal Saline(control); Group B(*n* = 10): 3.8% Silver Diamine Fluoride; Group C(*n* = 10): 5.25% Sodium hypochlorite; Group D(*n* = 10): 100mg/ml vitex negundo linn; Group E(*n*=10): 0.2% Nano Chitosan and Group F(n=10): 2% Chlorhexidine. The obtained constituent was cultured on agar plates & the number of CFUs (Colony forming units) per plate was determined using a digital colony counter, and statistically analysed using one-way ANOVA followed by post hoc Tukey test. **Results:** 5.25% Sodium Hypochlorite(Group C) was found to be most effective against E. fecalis followed by 2% Chlorhexidine (Group F), 0.2% Nano-chitosan(Group E), 100mg/ml Vitex negundo linn (Group D) and least effective for 3.8% Silver Diamine Fluoride (Group B). Group A (control group) - 0.9% normal saline had the lowest mean reduction in CFU count. **Conclusion:** 5.25% Sodium Hypochlorite showed maximum antimicrobial activity against E. fecalis.

Keywords: Enterococcus fecalis, Vitex negundo, Nano-chitosan, Chlorhexidine, Root Canal Irrigant, Root Canal Irrigation, Silver Diamine Fluoride.

Endodontic infection consists of a heterogeneous combination of microbial species.[1] Once the bacteria such as Streptococci, Veillonella parvula, Pepto streptococcus, Propioni bacterium, Lactobacilli, Eubacterium, Actinomyces, Bacteroides, Fusobacterium invade the pulpal tissue, the root canal becomes a "privileged sanctuary" for clusters of bacteria, their by products and degradation products of both pulpal tissue as well as bacterial microorganism.[2] In persistent infections, different microorganisms are associated with intraradicular and extraradicular infection.[3] The microenvironment of root canal favors the selection of few bacterial species like Enterococcus fecalis, Streptococcus anginosus and Fusobacterium nucleatum.[4] Enterococcus fecalis is a non-fastidious, gram positive facultative anaerobe that can proficiently invade dentinal tubules, survive during chemomechanical instrumentation and intracanal medication, adjust to altered nutrient supply and continue to remain viable inside the dentinal tubules.[4] The failure of root canal treatment has been attributed to these bacteria residing in the lateral canals, tubules of the dentin, ramifications and delta. These viable bacteria constantly act as a source of reinfection or continuous inflammation.[5]

Traditional use of mechanical instrumentation alone reduces the bacterial load from the canal by approximately 50%.[6] Thus, additional treatment modalities like endodontic irrigants are required to assist in microorganism elimination from the inaccessible areas.[7] Ideally an irrigant should possess qualities such as: powerful antibacterial activity, dissolution of organic tissue remnants, root canal cleansing, expulsion of dentinal debris from canals post instrumentation and the periradicular tissue should be free of any cytotoxicity from the contents of the irrigant.[8] Several types of endodontic chemical irrigating solutions have been used for disinfection of root canals. The important properties of root canal irrigants are maximum antibacterial action and pulp tissue dissolving properties with minimal tissue toxicity.[9] Most frequently used intracanal irrigant is sodium hypochlorite because of its high tissue dissolving property.[10] Various concentrations of Sodium hypochlorite have been used in dentistry but the most effective concentration recommended is 5.25% w/v.[10] Sodium hypochlorite with concentration 3% w/v is still the most regularly used concentration of sodium hypochlorite because it causes minimal tissue toxicity.[10] The major drawbacks of this irrigant includes primarily caustic effect on periapical tissues and moreover its potential to weaken dentin and reduce its flexural strength.[10]

Another widely used irrigating solution is Chlorhexidine with 2% w/v concentration which has the property of substantivity.[11] Dentin medicated with CHX acquires antibacterial substantivity. The absorption of positively charged ions released by CHX prevents bacterial colonization on the dentin surface, and the duration of this effect exceeds the period of medicament application.[12] The antibacterial efficacy of Chlorhexidine as a root canal irrigant is concentration dependent. Depending on its concentration, Chlorhexidine can have both bacteriostatic and bactericidal effects.[13] At high concentrations, it has bacteriocidal effects whereas at low concentrations, Chlorhexidine is bacteriostatic. Now-a-days its application in endodontics is diminishing as it causes unwanted discoloration of the tooth structure and its inability to dissolve the pulpal tissues.[14] Although CHX is useful as a final irrigant, its use as a main endodontic irrigant of the canal is not advised due to its inability to dissolve necrotic remnants.[15] Therefore, the demand for ideal root canal irrigants continues with the development of new materials and methods.[16]

Chitosan is one of the recently introduced endodontic irrigants.[17] Chitosan is the second most abundant polysaccharide after cellulose and it is widely distributed in nature.[18,19] Chitosan is a nontoxic cationic biopolymer obtained from deacetylation of chitin.[20] As a result of their polycationic/ polyanionic nature, nanoparticles exhibit higher antibacterial activity.[21] Its use in the field of endodontics was based on its broad-spectrum antimicrobial activity and considerable chelating effects.[22] This versatile polymer can be synthesized into nanoforms for various biomedical and pharmaceutical applications.[23] Chitosan nanoparticles have a significant antibacterial activity because of a higher surface area and charge density that enable them to react with the negative charge surface of bacterial cells, resulting in bacterial cell death.[24] The higher affinity of cationic chitosan antibacterial nanoparticles to bacterial cell surfaces and singlet oxygen release after photoactivation of photosensitiser provided a synergistic mechanism for photosensitiser functionalised chitosan nanoparticles to exert their antibacterial efficacy even in the presence of tissue inhibitors.[25] The fact that chitosan nanoparticles were accompanied by lower post-operative pain levels could be attributed to the biocompatibility of chitosan, being a natural polysaccharide with no reported cytotoxicity.[26] It was reported that Chitosan nanoparticles were significantly less cytotoxic than chitosan itself owing to the crosslinking.[27] These properties make Chitosan nanoparticles more tissue friendly than both sodium

hypochlorite and Chlorhexidine if it gets extruded.[28] Enterococcus fecalis has shown resistance against most of these medicaments, Thus, newer medicaments are required to eliminate the endodontic microflora more effectively.[29]

Silver has been around in dentistry since its inception and has shown good anti-bacterial properties and is used in several areas for disinfection and sterilization.[30] Silver Diamine Fluoride is an anticariogenic material with a high fluoride release capacity.[31] It has been proposed to be a very effective agent as an irrigant in endodontic therapy.[32]

Silver diamine fluoride is employed in dentistry due to its antimicrobial and anticariogenic effect.[33] The advantage of Silver diamine fluoride over 2% Chlorhexidine is that in addition to having similar anti-bacterial properties, the interaction of Silver diamine fluoride to teeth results in synergistic formation of fluorapatite.[34]

Various herbal extracts like Neem, Triphala, Aloe vera, Propolis, Green tea, Morinda citrifolia, Chamomile, Garlic extract etc. have antibacterial and medicinal effects, thus implicating their possibility to be used as an endodontic irrigant.[35]

Vitex negundo linn, a well-recognized plant in the field of Ayurveda, commonly known as Nirgundi.[36] It has many beneficial actions like antibacterial, antiinflammatory, analgesic, antifungal, anti-histaminic and antioxidant.[37] The antibacterial effect of vitex negundo is due to the presence of monoterpene compounds that cause membrane damaging effects.[38] Largest components of the Vitex negundo Linn. (Nirgundi) are glycosides, alkaloids and tannins which also contribute to antibacterial effects.[39] Aqueous and alcoholic extracts of Vitex negundo linn. have antibacterial effect against the bacteria that give positive and negative results in the gram stain test.[40] Nirgundi has been previously used as a mouthwash in treatment of periodontal diseases and in relieving tooth pain.[41]

Thus the purpose of this study is to evaluate the antibacterial efficacy of various recently introduced root canal irrigants against Enterococcus fecalis.

AIM

The aim of this in-vitro study is to evaluate & compare the antimicrobial efficacy of various root canal irrigants against Enterococcus fecalis.

OBJECTIVES

- To evaluate the antibacterial effect of Normal Saline root canal irrigant against Enterococcus fecalis.
- To evaluate the antibacterial effect of Silver Diamine Fluoride root canal irrigant against Enterococcus fecalis.
- To evaluate the antibacterial effect of Sodium Hypochlorite root canal irrigant against Enterococcus fecalis.
- To evaluate the antibacterial effect of Vitex negundo linn root canal irrigant against Enterococcus fecalis.
- To evaluate the antibacterial effect of Chitosan Nanoparticle root canal irrigant against Enterococcus fecalis
- To evaluate the antibacterial effect of Chlorhexidine root canal irrigant against Enterococcus fecalis.
- Inter-Comparison of each root canal irrigant group used in this in-vitro study.
- To conclude which is the best irrigant to provide highest antibacterial effect against E. fecalis in root canal system.

1. **Siqueira Jr JF, Machado AG, Silveira RM et al (1997)**[42] evaluated the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of Enterococcus fecalis from the root canal. Eighty freshly extracted human canine teeth were selected for this study. Conventional access preparations were made and the root canals were instrumented 1 mm beyond the apical foramen. Each root canal was inoculated with 10 mL of the E. fecalis suspension. After incubation, the contaminated root canals were divided into three groups according to the irrigation regimen used: Group 1: 20 root canals were manually irrigated with 2 mL of a 4.0% NaOCl solution; Group 2: 20 root canals were manually irrigated and ultrasonically activated with 2 mL of a 4.0% NaOCl solution; Group 3: 20 root canals were manually irrigated using a combination of 4.0% NaOCl and 3% H2O2. Contaminated canals irrigated with sterile saline solution served as the control. Paper points were used to sample bacteria from the root canals were transferred to tubes containing brain heart infusion (BHI) broth. The results showed that greatest reduction in bacterial count was in contaminated root canals that received manual irrigation with 4% NaOCl yielded positive cultures followed by root canals in which the 4% NaOCl solution was ultrasonicated. When NaOCl was used alternately with 3% hydrogen peroxide, the result was similar to the manual irrigation group. All specimens of the control group yielded positive cultures as E. fecalis was always recovered from all cultures.

2. **Sundqvist G., Figdor D., Persson S. et al. (1998)** [43] conducted a study to determine which microbial flora were present in teeth after failed root canal therapy and established the outcome of conservative re-treatment. Fifty-four root-filled teeth with persisting periapical lesions were selected for re-treatment. After removal of the root filling, canals were sampled by means of advanced microbiologic techniques. The result of the study showed that the microbial flora found was mainly single species of predominantly gram-positive organisms. The isolates most commonly recovered were bacteria of the species Enterococcus fecalis.
3. **Heling I, Chandler NP et al (1998)**[44] compared and evaluated the antimicrobial effect of irrigant combinations within dentinal tubules against Enterococcus fecalis. The irrigation solution regimes included saline for 10 min (control); 0.2% CHX for 10 min; 3% H2O2 for 10 min; mixture of 0.1% CHX and 1.5% H2O2 for 10 min;

mixture of 1.8% CHX and 3% H2O2 for 10 min; 0.2% CHX for 5 min then 3% H2O2 for 5 min; 1% NaOCI for 10 min; 17% EDTA for 10 min; 3% H2O2 for 5 min then 1% NaOCl for 5 min; 1% NaOCI for 5 min then 3% H2O2 for 5 min; and 17% EDTA for 5 min then 1% NaOCl for 5 min. Six standardized root specimens, which had been infected with Enterococcus fecalis were exposed to each solution. All the solutions proved to be more effective than saline in killing bacteria within the tubules. All the solutions killed more bacteria than EDTA, which was not significantly different from saline. There was no difference found between the hypochlorite and the chlorhexidine during the 10-min incubation, with both these solutions killing significantly more organisms close to the lumen. The hypochlorite solution proved to be superior to hydrogen peroxide close to the lumen but at deeper layers the difference was not significant. Alternating the two irrigating solutions did not affect their bactericidal effectiveness. When these solutions were combined they were no more efficient than 1% sodium hypochlorite solution, but significantly better than the 3% hydrogen peroxide alone. Both the two different chlorhexidine and peroxide concentrations used in combination were more effective than chlorhexidine alone. As anticipated, combining EDTA with hypochlorite (or chlorhexidine) was more effective than using EDTA alone.

4. **D'Arcangelo C, Varvara G, De Fazio P (1999)[45]** compared and evaluated action of different root canal irrigants on Enterococcus fecalis. The various irrigants used were 3% sodium hypochlorite, 0.2 % chlorhexidine, and 0.2 % cetrimide. 3 test tubes each for all irrigants were kept in contact with the bacterial species used for the experiment suspended in Brain Heart Infusion broth for 10, 20, or 30 min. Results showed that all irrigants had a bactericidal effect on Enterococcus fecalis. The greatest bactericidal effect was shown by 3% sodium hypochlorite followed by 0.2 % chlorhexidine, and then least bactericidal effect by 0.2 % cetrimide.

5. **Ayhan H, Sultan N, Cirak M et al (1999)[46]** studied and compared the antimicrobial effect of various endodontic irrigants against six selected microorganisms which included Staphylococcus aureus, Enterococcus fecalis, Streptococcus salivarius, Str. pyogenes, Escherichia coli and Candida albicans. The various endodontic irrigants used were 5.25% solution of NaOCl, 0.5% solution of NaOCl, 2.0% solution

chlorhexidine gluconate, Alcohol (21%), Cresophene (paramonochlorophenol, 30%; thymol, 5%; dexamethasone, 0.1%), Sterile physiological saline solution. Disc diffusion assay was used to evaluate the antibacterial activity of the endodontic irrigants by measuring the zones of inhibition. The tests were repeated five times for all strains. The results of the positive control (5.25% NaOCl) showed that it was effective against all test microorganisms with a substantial zone of inhibition. Saline was always ineffective. Decreased concentration of NaOCl significantly reduced its antimicrobial effect. Cresophene showed a significantly larger average zone of inhibition compared to the other experimental irrigants. Alcohol had smaller but not significantly different zones of inhibition than chlorhexidine.

6. **Love RM (2001)[47]** identified a possible mechanism that would explain how E. fecalis could survive and grow within dentinal tubules and reinfect an obturated root canal. Cells of Streptococcus gordonii, streptococcus mutans and Enterococcus fecalis were grown as colonies on Brain Heart Medium plates. In 100 teeth collected from numerous subjects, longitudinal root specimens with intact cementum from each root were selected at random from the pool of prepared roots and they were presoaked in Brain Heart Infusion medium containing bacterial cells for 2 days. The ability of the three bacterial species to invade dentine was assessed by dentine invasion and microtitre well experiments. From the results, it was postulated that a virulence factor of E. fecalis in failed endodontically treated teeth is related to the ability of E. fecalis cells to maintain the capability to invade dentinal tubules and adhere to collagen in the presence of human serum.

7. **Oliviera DP, Barbizam JVP, Trope M et al (2007)[48]** conducted a study to compare in vitro antimicrobial activity of 2% chlorhexidine gel against *Enterococcus fecalis* with sodium hypochlorite in 2 different concentrations (1.5% and 5.25%). Eighty human lower premolars with single root canals were prepared, autoclaved, and infected for 7 days with *E. fecalis* monocultures. The roots were then separated into 5 experimental groups according to the irrigant solution used during the standardized preparation. The results showed that 2% chlorhexidine gel and 5.25% sodium hypochlorite significantly reduced the *E. fecalis* Colony Forming Units in the posttreatment and final microbiological samples. The 1.5% sodium hypochlorite also

reduced the *E. fecalis* Colony Forming Units immediately after the root canal instrumentation.

8. **Hiraishi N, Yiu CKY, King NM et al (2010)[49]** investigated the use of 3.8% silver diamine fluoride as an antibacterial agent against *Enterococcus fecalis* biofilms and its ability to penetrate dentinal tubules by the formation of silver salts. They inferred that both NaOCl and $Ag(NH_3)_2F$ were effective against *E. fecalis* biofilms, with no significant difference in reduction of microorganisms for both exposure times. Silver deposits were present on 66.5% of the radicular dentin surfaces after 72-hour application of $Ag(NH_3)_2F$ as simulated interappointment dressings. Penetration of the silver deposits was observed at most 40 μm into dentinal tubules after smear layer removal.

9. **Retamozo B, Torabinejad M, Shabahang S et al (2010)[50]** conducted a study to determine the concentration of sodium hypochlorite and the irrigation time required to disinfect dentin cylinders infected with *Enterococcus fecalis.* The results showed that the most effective irrigation regimen was 5.25% at 40 minutes, whereas irrigation with 1.3% and 2.5% NaOCl for this same time interval was ineffective in removing *E. fecalis* from infected dentin cylinders.

10. **Balakrishnan A, Sam JE, Kumar A et al (2012)[51]** evaluated in their study the antibacterial efficacy of triphala, morinda citrifolia, aloe vera, vitex negundo and sodium hypochlorite against enterococcus fecalis. To study the antibacterial efficacy of the irrigants, the agar disk diffusion method was used. Sterile discs containing the herbal irrigants were placed onto the agar plate which contained the bacterial culture. The results showed that 5.25% sodium hypochlorite was the most efficient which was followed by triphala, morinda citrifolia, aloe vera and vitex negundo in decreasing order of antibacterial efficacy.

11. **Mathew VB, Madhusudhanan K, Sivakumar N et al (2012)[52]** studied and compared the anti-bacterial action of 3.8% silver diamine fluoride and 2% chlorhexidine gluconate against Enterococcus fecalis in root canals. Forty-four single-rooted teeth were decoronated, and the root section was enlarged with peeso-reamer (No: 3) to standardize length and diameter. The samples were then autoclaved and

divided into two study groups for irrigation. They inferred that 3.8% silver diamine fluoride (SDF) and 2% chlorhexidine showed a superior capacity to sterilize the root canals than control groups. The results of the study showed that SDF is as effective as 2% CHX in removing *E. fecalis* from infected root canals.

12. **Shrestha A, Kishen A et al (2014)**[53] assessed the antibacterial effect of chitosan nanoparticles with a photosensitiser against enterococcus fecalis. The following agents were tested for the inhibitory effects: 28 mg dentin powder; 10 mg fresh bovine pulp, frozen and powdered; 5 mg dentin-matrix; and 2% and 18% BSA and LPS (1 mg/mL). Extracted human third molars and bovine teeth from the slaughterhouse were obtained. The experiments were carried out in Colony forming unit counts with 3 samples per group each time. The results proved that the inherent antibacterial activity of polycationic chitosan nanoparticles and the singlet oxygen released after photoactivation of the photosensitiser synergistically provided chitosan particles the potential to achieve significant antibacterial efficacy even in the presence of tissue inhibitors within root canals.

13. **Bhalla V., Bhoiwala V., Rajkumar B. et al. (2015)**[54] in their study compared the antimicrobial efficacy amongst 2% Tea Tree Oil, 2% Chlorhexidine Digluconate, 3% Sodium Hypochlorite and the control (Distilled Water) using the Minimum Inhibitory Concentration(MIC) Test. The MIC was performed using 10-fold dilution in 96 U-Well Micro Test plates. It was concluded that Tea Tree Oil was the most effective in inhibiting E. fecalis, followed by sodium hypochlorite, and chlorhexidine digluconate was the least successful. Distilled water taken as control group showed no effect on the gram positive organisms.

14. **Del Carpio-Perochena A., Bramantel C.M., Duarte1 M.A.H. et al. (2015)**[55] investigated in their study the ability of bioactive Chitosan Nanoparticles (CNP's) to remove the smear layer and inhibit bacterial recolonization on dentin. One hundred bovine dentin sections were divided into five groups (n = 20 per group) according to the treatment. The irrigating solutions used were 2.5% sodium hypochlorite (NaOCl) for 20 min, 17% ethylenediaminetetraacetic acid (EDTA) for 3 min and 1.29 mg/mL Chitosan Nanoparticles for 3 min. The samples were irrigated with either distilled water (control), NaOCl, NaOCl-EDTA, NaOCl-EDTA-CNPs or NaOCl-CNPs. After

the treatment, half of the samples (n = 50) were used to assess the chelating effect of the solutions using portable scanning electronic microscopy, while the other half (n = 50) were infected intra-orally to examine the post-treatment bacterial biofilm forming capacity. The biovolume and cellular viability of the biofilms were analysed under confocal laser scanning microscopy. The results revealed that the smear layer was significantly reduced in all of the groups except the control and NaOCl groups. The CNPs treated samples were able to resist biofilm formation significantly better than other treatment groups and was concluded that CNPs could be used as a final irrigant during root canal treatment with the dual benefit of removing the smear layer and inhibiting bacterial recolonization on root dentin.

15. **Samiei M., Shahi S., Abdollahi A.A. et al. (2016)[56]** in their study used and compared 2% chlorhexidine (CHX) and 2.5% NaOCl for antibacterial activity against Enterococcus fecalis (E. fecalis) in the root canals of 60 maxillary central incisors. The root canals were irrigated either with 5 mL of 2% CHX or 2.5% NaOCl solutions, respectively. They concluded that the inhibition of bacterial growth in all the experimental groups was significantly superior to the control group. In addition, 2.5% NaOCl was significantly better than 2% CHX. effective in reducing the E. fecalis counts in comparison with the control group, but 2.5% NaOCl solution was the most effective protocol.

16. **Hayam YH, Zakeer S., Mahmoud N.F. (2017)[57]** evaluated the anti-bacterial activity of four selected solutions (Biopure MTAD, 2% Nano-Chitosan, 2% Chitosan, 2.5% Sodium hypochlorite) against Enterococcus fecalis (E. fecalis). The antimicrobial efficacy of four tested solutions was primarily determined using the agar disk-diffusion method (Mueller-Hinton agar plates). Furthermore, a total of 50 standardized single-canaled human teeth were collected and sterilized. Five teeth were cultured directly as negative control. Others were infected by a trypticase soy broth inoculated with E. fecalis. Infected roots incubated at 37ºC for 48 hours. Then five teeth cultured without treatment as positive control. While, the remaining were randomly divided into four groups (n=10) for being tested using the four selected solutions. Two or three sterile paper points were inserted into every root canal then transferred aseptically into corresponding Wassermann tubes that contain sterile Trypticase soy broth. All Wassermann tubes were incubated at 37ºC for 48 hours then

microbial growth of E. fecalis was verified by turbidity of the broth. Growth was further checked after 2 and 10 days then obtained data was statistically analysed. In their study they concluded that, Biopure MTAD significantly inhibited the bacteria growth and the 2% NanoChitosan, 2% chitosan and 2.5% sodium hypochlorite recorded mixed positive and negative results.

17. **Benbelaïd F., Khadira A., Bendahou M. et al. (2018)[58]** proposed an alternative irrigation solution based on Cinnamomum cassia essential oil was evaluated for antimicrobial activity and total eradication of E. fecalis and C. albicans biofilms. The most used irrigation solutions by dentists, namely 2% chlorhexidine digluconate and 3% sodium hypochlorite, were also evaluated for comparison. The obtained results revealed that Cinnamomum cassia essential oil has shown antimicrobial activity against the studied microbial species whatever their grouping in biofilms or planktonic. In the irrigation assay carried out in vitro, 1.25% C. cassia essential oil eradicated all E. fecalis and C. albicans viable cells protected in biofilms after 30 seconds of exposure, while 2% chlorhexidine digluconate and 2.5% sodium hypochlorite requires one and five minutes for the total elimination of studied pathogens, respectively.

18. **Bukhari S., Kim D., Liu Y. et al (2018)[59] -** tested a new disinfection technology using biomimetic iron oxide nanoparticles (IO-NPs) with peroxidase-like activity to enhance antibacterial activity on root canal surfaces and in dentinal tubules. The canal surfaces and dentinal tubules of single-rooted intact extracted teeth were infected by growing Enterococcus fecalis biofilms for 3 weeks. The samples were divided into 6 treatment groups: (1) phosphate-buffered saline (PBS) (negative control), (2) 3% hydrogen peroxide (H_2O_2) (test control), (3) IO-NPs (0.5 mg/mL) (test control), (4) IO-NPs (0.5 mg/mL) + 3% H_2O_2, (5) 3% sodium hypochlorite (positive control), and (6) 2% chlorhexidine (positive control). The nanoparticle irrigant used in the study was observed to be 9 times more effective in antibacterial activity against enterococcus fecalis than 3% sodium hypochlorite and 2% chlorhexidine. 3% sodium hypochlorite and 2% chlorhexidine were found to be significantly more effective than 3% hydrogen peroxide in all zones. The results revealed the potential to exploit nanocatalysts with enzyme-like activity as a potent alternative approach for the treatment of endodontic infections.

19. **Pillai BS, Madhubala MM, Velmurugan A et al(2018)[60]** evaluated the antibacterial efficacy of chlorhexidine, Mixture of Tetracycline, Acid and Detergent (MTAD) and Chitosan against Enterococcus fecalis when used as a root canal irrigant. The bacterial E. fecalis culture was grown overnight in Brain Heart Infusion (BHI) broth and inoculated in Mueller-Hinton agar plates. All four study irrigants were added to respective wells in agar plate (n=10) and incubated at 37°C for 24 hour. Diameter of bacterial inhibition zone around each well was recorded. They inferred that MTAD showed the highest antibacterial efficacy against Enterococcus fecalis followed by CHX and Chitosan.

20. **Patil P.H., Gulve M.N., Kolhe S.J. et al. (2018)[61]** evaluated and compared the smear layer removal efficacy of etidronic acid‐based irrigating solution with sodium hypochlorite (NaOCl) with surfactant, ethylenediaminetetraacetic acid (EDTA) with surfactant, BioPure MTAD and Chloroquick solution in the apical third of the root canal of forty human single‐rooted mandibular premolar teeth. The tested irrigants included Group I: normal saline (negative control); Group II: 5.25% sodium hypochlorite (NaOCl) with surfactant and 17% ethylenediaminetetraacetic acid (EDTA) with surfactant; Group III: freshly mixed BioPure MTAD; and Group IV: freshly mixed Chloroquick solution. The results showed that 5.25% NaOCl with surfactant followed by 17% EDTA with surfactant showed least smear layer scores. This was followed by MTAD and then Chloroquick.

21. **Al-Madi EM, Al -Jamie MA ,. Al-Owaid NM etal (2019)[62]** evaluated the antibacterial efficacy of 3.8% silver diamine fluoride (SDF) in comparison with 2% chlorhexidine (CHX) against Enterococcus fecalis biofilm. Extracted human teeth were used to make 70 dentin discs that were then inoculated with *E. fecalis* to generate a 3-week-old biofilm model. The discs were split into the experimental groups A and B (each $n = 20$) and control groups C and D (each $n = 15$). The discs in group A were irrigated with 3.8% SDF, while those in group B were irrigated with 2% CHX. The specimens in group C (positive control) were irrigated with 5.25% NaOCl, and those in group D (negative control) were irrigated with sterile saline. The results showed that the NaOCl group showed the greatest percentage of dead cells

(62.26%) among all groups. The SDF group showed a significantly higher percentage of dead cells (57.39%) than the 2% CHX and saline groups. In this study, it was concluded that SDF possessed higher antimicrobial activity than 2% CHX against E. fecalis biofilms.

22. **Kondreddi N, Venigalla BS, Singh TV et al (2020)[63]** compared the antibacterial activity of 0.2 % chitosan, 5.25% NaOCl, 2% chlorhexidine and alternating solutions of chitosan, sodium hypochlorite and chlorhexidine against Enterococcus fecalis. 60 extracted single-rooted teeth were selected and used for the study. After incubation with Enterococcus fecalis, samples were divided into six groups according to the solutions used for irrigation, that is, CHX, NaOCl, chitosan, alternating solution of chitosan and hypochlorite, alternating solution of chitosan and CHX, and saline. Antibacterial efficacy was assessed by obtaining the samples from root canal before and after the irrigation using paper points, culturing them on blood agar plates, and measuring the number of colony-forming units (CFUs) formed. The results revealed maximum antibacterial activity when chitosan was used alternatively with CHX and NaOCl. Independently, hypochlorite showed maximum antibacterial activity followed by CHX and chitosan which showed almost similar antibacterial activity.

23. **Rafid J. Al –Badr & Hussain F. Al-Huwaizi (2020)[64]** evaluated the antibacterial activity of chitosan-coated iron oxide nanoparticles (Chi-IONP) by agar direct diffusion method and determined the minimum inhibitory concentration and minimum bactericidal concentration (MIC/MBC) of this nanosolution against E.fecalis, S.mutans and Candida albicans the three oral microorganisms commonly isolated from endodontic infections. The results showed that Chi-IONP was a powerful antimicrobial agent against the tested microorganisms, and it was found that antifungal activity of Chi-IONP was higher than NaOCl 5.25% and had comparable antibacterial effect to NaOCl 5.25%.

24. **Kalita C, Raja D, Saikia A et al (2020)[65]** assessed the antibacterial efficacy of Azadirachta indica (Neem), Ocimum sanctum (Tulsi), and Vitex negundo (Pochotia) against oral microorganisms. Isolated microorganisms were Klebsiella oxytoca, Kochuria kristinae, Acinetobacter boumani, Sphingomonas paucimobilis, Pseudomonas fluorescens, Streptococcus gordonii, Enterococcus fecalis, and Bacillus

subtilis. Bacterial inoculums were poured and spread into Mueller Hinton plates. Plant extract was poured into prepared wells taking ciprofloxacillin as the positive control and dimethyl sulfoxide as the negative control.and evaluated for the zone of inhibition. Highest zone of inhibition of 23 mm observed in Enterococcus fecalis by Vitex negundo, Streptococcus gordonii of 21 mm by Azaradichta indica, Bacillus subtilis of 19 mm by both Azaradichta indica, and V. negundo, Klebsiella oxytoca and Sphingomonas paucimobilis showed zone of inhibition of 16 mm in Vitex negundo.

25. G**upta SS , Thosar N, Rathi N et al (2020)**[66] evaluated the antibacterial efficacy of Vitex negundo Linn. extract as root canal irrigant against Enterococcus fecalis and its penetration into root dentin. Forty single rooted premolars were randomly divided into 4 groups: 3% Sodium hypochlorite (NaOCl), 2% Chlorhexidine (CHX) , 100mg/ml Vitex negundo Linn. and saline as control all mixed with Rhodamine B dye. Test samples were analysed for bacterial count before and after irrigation using absorbent paper points and the colony forming units were recorded and measured. Sectioning of the samples was performed at three levels 3mm,6mm,9mm from apex and then these samples were analysed using confocal laser scanning microscopy for penetration depth of the irrigant within the dentinal tubules. They concluded that although 3% NaOCl was the most effective irrigant, all agents exerted acceptable antimicrobial activity against Enterococcus fecalis and penetration depth within tubules of dentin.

26. **Minavi B, Youssefi A, Quock R et al (2020)**[67] conducted a study and evaluated the antimicrobial substantivity effect of 3.8% SDF against other endodontic irrigants such as 2% CHX and 6.25% Sodium hypochlorite (NaOCl). They concluded that SDF possesses antimicrobial properties against the opportunistic pathogen E. fecalis. Moreover, the substantivity of 3.8% SDF was significantly greater than 6.25% NaOCl, but was comparable to 2% CHX.
27. **Abiding T, Susilo D, Gani BA et al (2022)**[68] conducted a study to evaluate the efficiency of 0.2% nanochitosan as root canal irrigant against E. fecalis. The experimental design was a randomized block design with a total number of 27 teeth divided equally into three treatment groups, namely, Group A: 0.2% high molecular nano-chitosan + PUI after remodeling treatment of root canals, Group B: 2.5% NaOCl with additional PUI after remodelling root canal treatment, and Group C: distilled water solution + PUI after remodeling root canal treatment.Following the treatments,

the growth of E. fecalis and the surface roughness of the tooth root canal were assessed.They concluded that the irrigation of root canal treatment with 0.2% high molecular nano-chitosan had significant antibacterial activities against *E. fecalis*.

28. **Sadek H, Hassan MYM, Elshishtawy HMM et al (2022)[69]** evaluated and compared the antibacterial efficacy of Chlorhexidine, nano-chitosan and their combination against enterococcus fecalis (E. fecalis) with and without ultrasonic activation. 110 extracted teeth were divided into 6 groups according to the antibacterial agent used; 1: Normal Saline (control group), 2: chitosan, 3: chitosan+2% chlorohexidine, 4: 2% chlorohexidine, 5: chitosan extra-strength, 6: chitosan extra-strength+ 2% chlorohexidine. Then each group was subdivided into two subgroups, with and without ultrasonic activation (n=10). Microbial samples were collected from all the root specimens and colony forming units were counted and transformed into log CFU. The control group showed the highest bacterial count while CHX with ultrasonic activation group showed the lowest bacterial count. Chlorohexidine, chitosan+chlorohexidine and chitosan extra-strength + chlorohexidine groups with ultrasonic activation showed significantly lower bacterial count than chitosan with ultrasonic activation group, chitosan extra-strength without ultrasonic activation group and the control group.

29. **Goel P, Galhotra V, Makkar S et al (2022)[70]** conducted an in-vitro study to compare the antibacterial efficacy of 0.2% Chitosan, 3% Sodium Hypochlorite, 2% Chlorhexidine against *Enterococcus fecalis.* The root canals of 72 extracted intact human single-rooted teeth with single canals were prepared, and *E. fecalis* was incubated in the root canals for 7 days. The teeth were then randomly divided into the following four experimental groups: group I: Saline, group II: 0.2% Chitosan, group III: 3% Sodium hypochlorite, and group IV: 2% Chlorhexidine. The effect of each irrigant was evaluated by counting the number of colony-forming units observed on inoculation. The results of the study showed that maximum number of colony-forming units were observed in the group I: Saline (106.83 CFU/mL), followed by group III: 3% Sodium hypochlorite (54.33), group II : 0.2% Chitosan (0.50 CFU/mL), and group IV: 2% Chlorhexidine (0 CFU/mL). Significant reductions were noted in *E. fecalis* colony counts in all groups ($p < 0.05$). The greatest reduction in colony count was noted in group IV:2% Chlorhexidine followed by group II: 0.2% Chitosan.

30. **Elkillany RZM, El-Ashry S, Sabet N et al (2022)[71]** compared the antibacterial efficacy of various nanoparticles against E. fecalis. Sixty-six human mandibular molars were contaminated with E. fecalis then treated with Calcium hydroxide, Calcium hydroxide nanoparticles (np), CHX, CHX loaded by silver nanoparticles (Ag-np) and CHX loaded by chitosan nanoparticles (CH-np). Six teeth were randomly assigned as a positive control group. Three of these teeth were used to obtain the average colony forming units (CFU) after three weeks incubation. The remainder sixty teeth were randomly divided into five groups, group A: Calcium hydroxide,group B: Calcium hydroxide nanoparticles, group C: Chlorhexidine ,group D: Chlorhexidine -Agnp and group E: Chlorhexidine -CHnp , each group comprising of twelve teeth. The results showed that Chlorhexidine \silver-np had the highest percentage reduction with a significant difference. Chlorhexidine and Calcium hydroxide had the lowest percentage reduction. The maximum mean value of bacterial percentage reduction was presented by Chlorhexidine -Agnp, Calcium hydroxide -np and Chlorhexidine -CHnp followed by Chlorhexidine then finally Calcium Hydroxide

This in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics, Babu Banarasi Das College of Dental Sciences, Lucknow in collaboration with R.S. Pathology Centre, Lucknow.

The following inclusion & exclusion criteria were set to select the teeth samples:

INCLUSION CRITERIA

1. Non carious, sound and intact human single rooted teeth.
2. Teeth with single canal (one orifice and one foramen) determined radiographically.

EXCLUSION CRITERIA

1. Teeth with any crack, caries or calcification.
2. Teeth with developmental anomaly.
3. Teeth with any restoration.
4. Endodontically treated teeth.

ARMAMENTARIUM USED

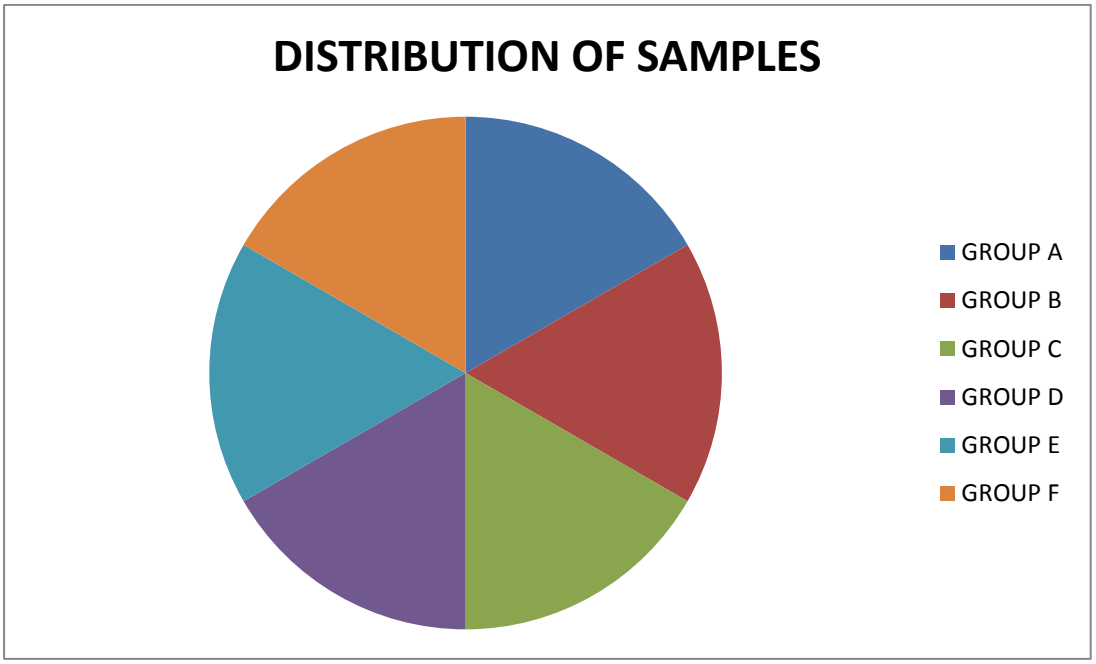
- Ultrasonic Scaler with tips(Coltene, Switzerland)
- Hand Scaler (periodontal) (GDC, India)
- Ruler (6") (Classmate, India)
- Divider (Classmate, India)
- Marker (Camlin, India)
- Straight Hand Piece (NSK , Japan)
- Micro Motor (Unicorn Denmart, India)
- Diamond Disc (Shofu, Japan)
- Autoclave (Confident, India)
- Glass ionomer Cement(Prevest, India)
- Curing Light(Woodpecker,China)
- Composite Restoration Instrument (GDC, India)
- Modelling Wax(Pyrex, India)
- Nail Varnish (Nykaa, India)
- PSP (Acteon)
- K-files (#6,8,10,15,20) (Dentsply, USA)
- Rotary HyFlex CM files(4% #20 & 6% #25) (Coltene, Switzerland)
- 17% Ethylenedieamine tetra acetic acid solution (EDTA) (Prevest Denpro, India)
- 3% Sodium Hypochlorite (NaOCl) (Pyrax, India)
- Normal Saline (0.9%w/v) (KRPL, India)
- Disposable syringe of 2ml with 24 gauge needle (Dispo Van, India)
- Endo block (Dentsply, USA)
- 3.8% Silver Diamine Fluoride (Ammdent,India)
- 5.25% Sodium Hypochlorite (Pyrax, India)
- 100mg/ml Vitex Negundo Linn (Navchetna Kendra,India)
- 0.2 % Nano Chitosan (Nano wings, India)
- 0.9% Normal Saline (KRPL, India)
- 2% Chlorhexidine (Prevest Denpro, India)
- Distilled Water (D.R Laboratories)

- Sterile Gloves (size 6.5) (Surgicare, India)
- Enterococcus fecalis (ATCC 29212)
- Absorbent Paper Points (Meta-Biomed, Republic of Korea)
- Sterile Tweezer (GDC, India)
- Eppendorf tubes containing 1 mL of sterile saline (Amanta, India)
- Blood agar plates (Accumix, India)
- Digital Colony Counter (ESICO, India)
- Vortex (MixMate Eppendorf AG, Germany)

DISTRIBUTION OF SAMPLES

GROUPS	NO. OF SAMPLES	TEST IRRIGANTS
GROUP A	10	0.9% Normal Saline(control).
GROUP B	10	3.8% Silver Diamine Fluoride
GROUP C	10	5.25% Sodium hypochlorite
GROUP D	10	100mg/ml vitex negundo linn
GROUP E	10	0.2% Nano Chitosan
GROUP F	10	2% Chlorhexidine

Table 1: **Distribution of Samples**



Graph 1. : Distribution of Samples

Methodology:

Test Organism

The microorganism used in this study was *Enterococcus fecalis* (ATCC 29212)

Test Irrigant

The irrigants tested were 3.8% Silver Diamine Fluoride, 3% Sodium Hypochlorite, 100mg/ml Vitex Negundo Linn , 0.2 % Nano Chitosan , 0.9% Normal Saline and 2% Chlorhexidine.

Material Profile:

SILVER DIAMINE FLUORIDE:

Silver antimicrobial medicaments have been used in the form of silver-containing topical ointments, silver ion-releasing zeolites, silver-containing catheters, and colloidal silver water-purifying systems to prevent bacterial infections and reduce the spread of infectious

diseases.[72] Silver diamine fluoride has been shown to arrest caries initiation and progression.[73] When esthetics is not the major concern, the application of 38% silver diamine fluoride to caries affected teeth is a proven method for arresting caries and a treatment alternative when restorations in primary teeth is not an option.[73] The caries inhibition/antibacterial mechanism of silver fluoride and $Ag(NH_3)_2F$ is attributed to the deposition of a ‘‘black crust’’ that forms a sclerotic protective coating of the underlying secondary dentin.[74] Despite its benefit in arresting caries, blackened tooth structure after the use of 38% $Ag(NH_3)_2F$ raises esthetic concerns particularly in the permanent dentition.[75] A 1:10 dilution of 38% SDF can be used as a potential root canal irrigant.[76]

SODIUM HYPOCHLORITE

Sodium hypochlorite is both an oxidizing and hydrolyzing agent.[77]It is bactericidal and proteolytic. Sodium hypochlorite solutions have been used as wound irrigants since at least 1915and as an endodontic irrigant as early as 1920.[78]Its use as an infant sanitizer is nearly universal. Irrigation of root canals with sodium hypochlorite solutions (in concentrations ranging from 1 percent to 5.25 percent) is now a widely accepted technique. As an endodontic irrigant, sodium hypo-chlorite solution is relatively cheap; is bactericidal and virucidal.[79,80] It dissolves proteins, has a low viscosity, and it has a reasonable shelf life. It is not without disadvantages, principally due to its toxicity– it damages all living tissues except keratinized epithelia.[81]Sodium hypochlorite is extremely corrosive to metals; is strongly alkaline, hypertonic and has a very unpleasant taste.[81] Observance of correct storage procedures is critical to obtaining the expected shelf life.[82] In endodontics most of these disadvantages can be obviated by confining the hypochlorite to the pulp chamber and root canal.[83]

CHLORHEXIDINE

Chlorhexidine is a cationic molecule, which can be used during treatment.[84] Its cationic structure provides a unique property named substantivity.[11] At low concentration (0.2%), low molecular weight substances, specifically potassium and phosphorous, will leak out of the cell. On the other hand, at higher concentration (2%), CHX is bactericidal as precipitation of the cytoplasmic contents occurs, which results in cell death.[14] The antimicrobial substantivity of CHX has been assessed in several periodontal and endodontic studies.[85] The positively charged ions released by CHX can adsorb into dentine and prevent microbial colonization on the dentine surface for some time beyond the actual the period of time of application of the medicament.[86] CHX is somewhat effective against bacterial biofilms also.[87]

CHITOSAN

Chitosan has been widely used in dentistry because of many beneficial properties, such as biocompatibility, biodegradability, bioadhesion, and nontoxic.[88] Chitosan is a polysaccharide, and it is attained from chitin, which is obtained from the seashell of crustaceans and shrimps, through the process called deacetylation.[20] In addition, in an acid environment, chitosan has the capability to chelate with various metal ions. Chitosan, in the form of nanoparticles, has been undertaken to optimize the effectiveness of chitosan as root canal irrigation because it has better absorption and penetration into dentinal tubules. Chitosan also has chelation properties as EDTA. Moreover, this connection is stipulated by the pH of the solution, ion involvement, and chitosan chemical structure.[89] Chitosan nanoparticle is hydrophilic; hence, it can maintain tight contact and can be adsorbed to the root canal dentin.[90] Chitosan has an enormous amount of hydroxyl and amino groups, which create chitosan becoming cationic, inducing ionic interactions with calcium dentin ions.[91]

VITEX NEGUNDO LINN

Vitex negundo (Pochotia) is considered as a tonic and also used as an anti-inflammatory, and astringent in toothache.[92] Vitex negundo linn, a well-recognized plant in the field of Ayurveda, commonly known as Nirgundi.[36] It has many beneficial actions like antibacterial, antiinflammatory, analgesic, antifungal, anti-histaminic and antioxidant.[37] The antibacterial effect of vitex negundo is due to the presence of monoterpene compounds that cause membrane damaging effects.[38] Largest components of the Vitex negundo Linn. (Nirgundi) are glycosides, alkaloids and tannins which also contribute to antibacterial effects.[39] Aqueous and alcoholic extracts of Vitex negundo linn. have antibacterial effect against the bacteria that give positive and negative results in the gram stain test.[40] Nirgundi has been previously used as a mouthwash in treatment of periodontal diseases and in relieving tooth pain.[41]

Sampling Method:

Human single rooted teeth extracted for periodontal reasons were obtained from private clinics in Lucknow. Collected samples were checked for inclusion and exclusion criteria and thereafter were considered for further use making sample size of sixty. Sixty samples were divided into total six groups (five groups based on irrigants and one group that acted as control group) each group comprising of ten teeth.

Sample Preparation:

Sixty single rooted human teeth selected were cleaned for any tissue remnants, plaque and calculus on the roots with hand and ultrasonic scalers. All the selected samples were marked with marker and then sectioned 14mm from the apex with a diamond disc using a low speed straight hand piece to standardize roots of all samples.

Root Canal Preparation:

Working length was determined using ISO #10K file by a digital radiograph through PSP. Root canal was prepared using HyFlex CM rotary file, by crown-down technique till size 25/ 6% taper file. During shaping, each canal was irrigated with 3% Sodium hypochlorite and final irrigation of 17% EDTA solution.

Innoculation and Irrigation of Specimens:

After root canal preparation, all the samples were autoclaved under steam at 121^{o} C, 15 lbs pressure for 15 minutes.

After sterilization, the samples root canal apices were sealed with light-cure GIC using instrument and light cure gun. Then the samples were coated with two coats of nail varnish and mounted on wax making wax block of dimensions 12mm x 12mm x 17mm.

Then the samples were inoculated with Enterococcus fecalis and thereafter the teeth were randomly divided into 5 experimental groups of 10 samples each & 1 control group of 10 samples. The following irrigation protocol was followed by all tested groups:

A sterile 2 mL syringe with 24 gauge needle was used to deliver 0.5 ml of irrigant into the canal for two minutes. All experimental teeth were then flushed with distilled water to prevent potential carry-over of the used irrigant as per irrigant to be tested.

Post Irrigation Microbial Sampling

An endodontic hand file was used in a circumferential filing motion to a level around 1 mm short of the root apex. A 6%, ISO size 25 paper point was then placed into the canal till the working length for 30 seconds each to soak up the canal contents. Paper points were then transferred to the eppendorf tubes containing 1 mL saline and were agitated in vortex mixer for 1 minute. Aliquots of 500 µl dilutions were cultured into Blood agar plates. For 48 hours, all plates were cultured at 37°C in a microaerophilic environment with 5% CO_2. After that, a Digital Colony Counter was used to determine the number of CFUs (Colony Forming Units) per plate.

Analysis of the sample

The number of colony-forming units (CFU) per millilitre of sample was calculated as per the following formula :

$$\text{Colony Forming Unit /ml} = \frac{\text{Number of colonies obtained X Dilution Factor}}{\text{Volume of sample inoculated}}$$

After obtaining the mean colony forming units of each group, statistical analysis was performed using SPSS software Version 19.0 (IBM Corporation, Chicago, USA).

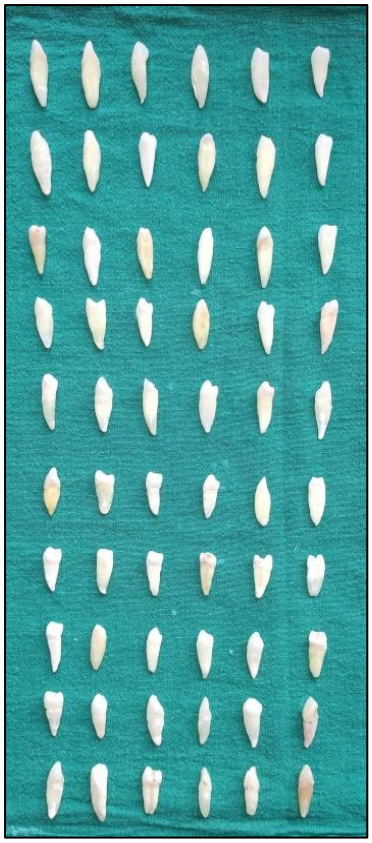


Fig.1- Samples (Human permanent single rooted teeth)

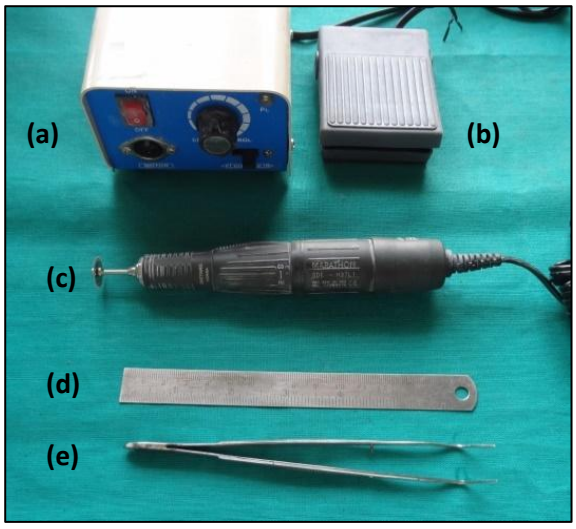


Fig.2- Armamentarium for Sample preparation

a. control box b. foot control c. straight handpiece with abrasive disc d. ruler e. forceps

Fig. 3 – Sixty Decoronated Samples

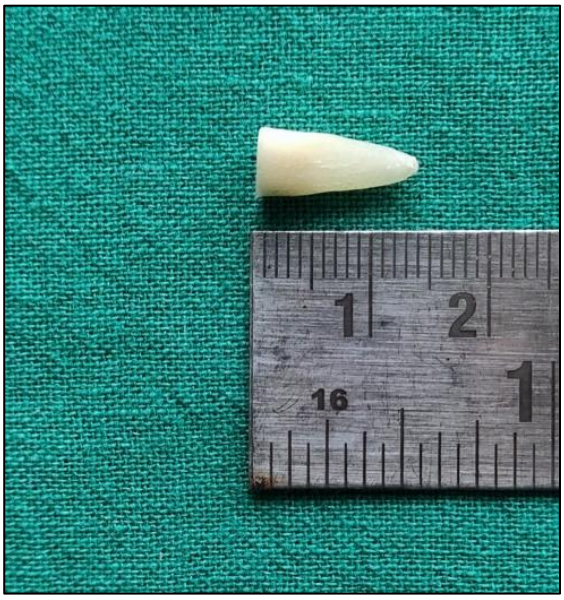


Fig. 4 – Standardisation of Length

COLOUR PLATE-I

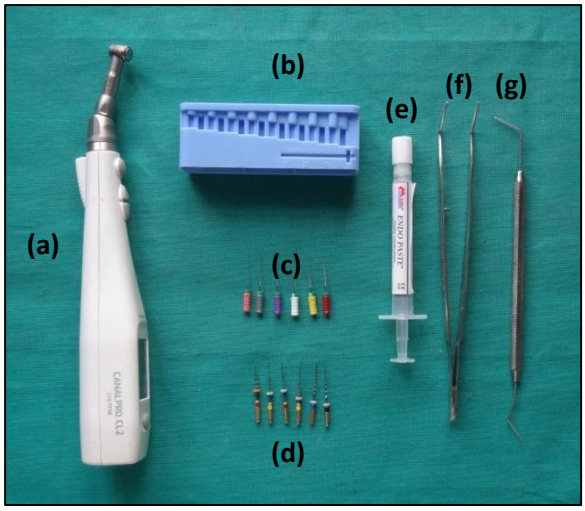


Fig.5- Armamentarium for Cleaning & Shaping

a. endomotor b.endoblock c.handfiles d. rotary files e. EDTA gel f.tweezer g. DG 16 explorer

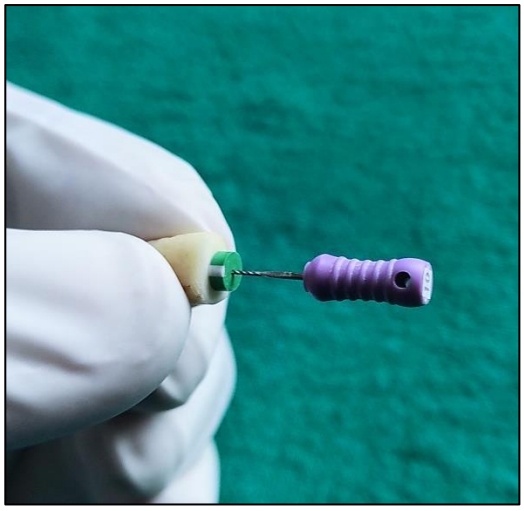


Fig.6- Working length determination

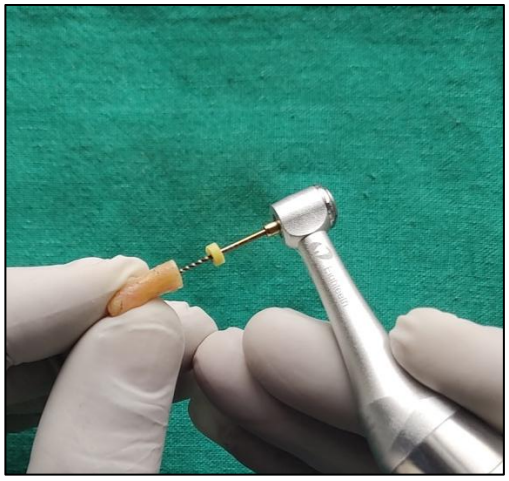


Fig.7- Cleaning and shaping using rotary files

COLOUR PLATE-II

Fig.8- Autoclave

Fig.9- Autoclaved samples stored normal saline

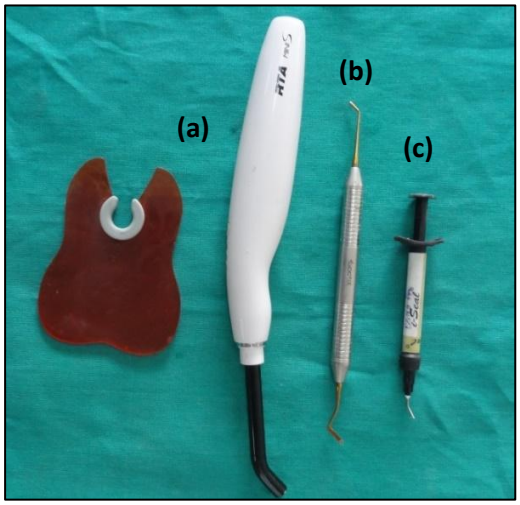


Fig.10- Armamentarium for sealing the apex

a.light curing unit b. composite instrument
c.lightcure GIC

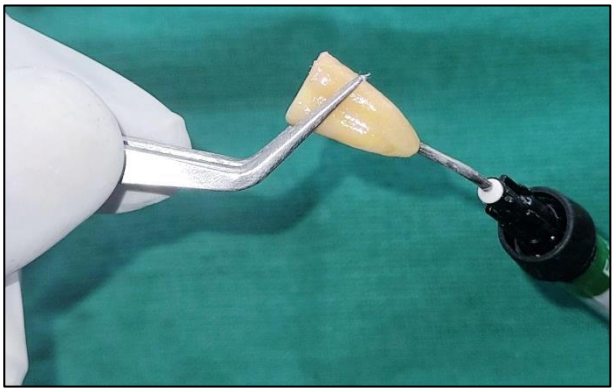


Fig.11 – sealing apex with lightcure GIC

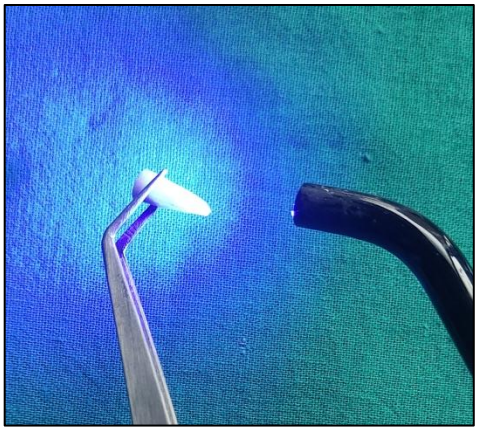


Fig.12- curing of GIC applied to sealapex

COLOUR PLATE-III

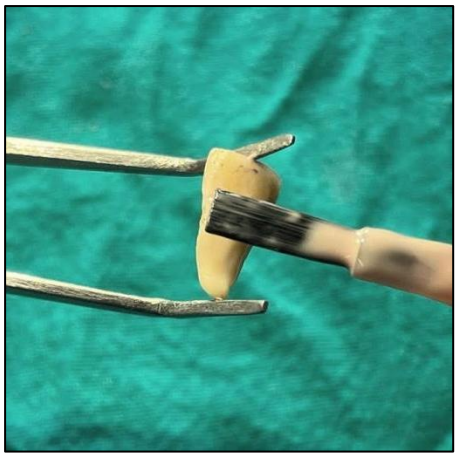


Fig.13 – Applying varnish on sample

Fig.14 – samples with two coats of nail varnish

Fig.15- Wax mounted sample

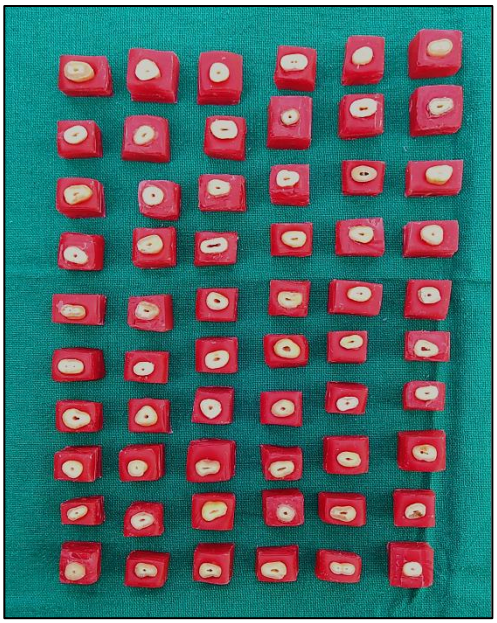


Fig.16– Sixty wax mounted samples

COLOUR PLATE-IV

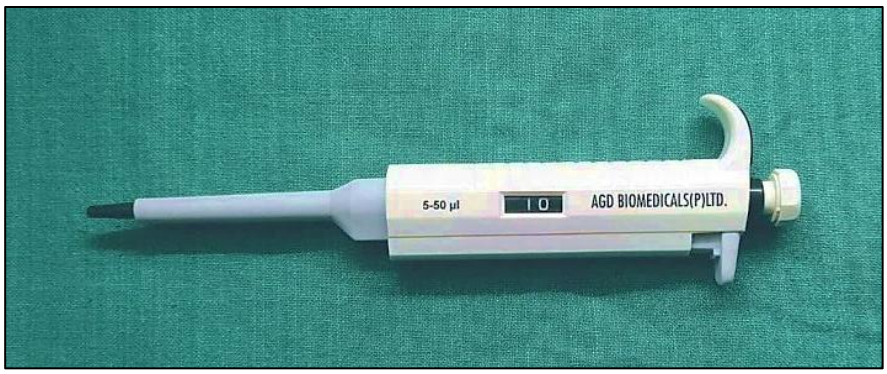


Fig.17- Enterococcus fecalis (ATCC 29212) Fig.18 - Micropipette used to carry E. faecalis

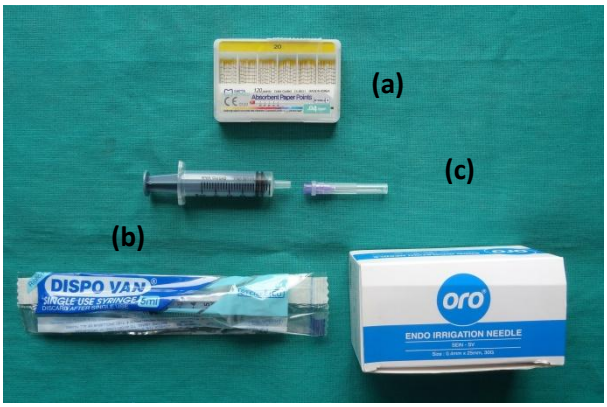


Fig.19-Armamentarium for Irrigation
a. paper points b. syringe c. irrigation needle

Fig.20- 17% EDTA Fig.21- 5.25% Sodium Hypochlorite Fig.22-Deionized water

COLOUR PLATE-V

Fig.23- 0.9% Normal Saline

Fig.24- 2% Chlorhexidine

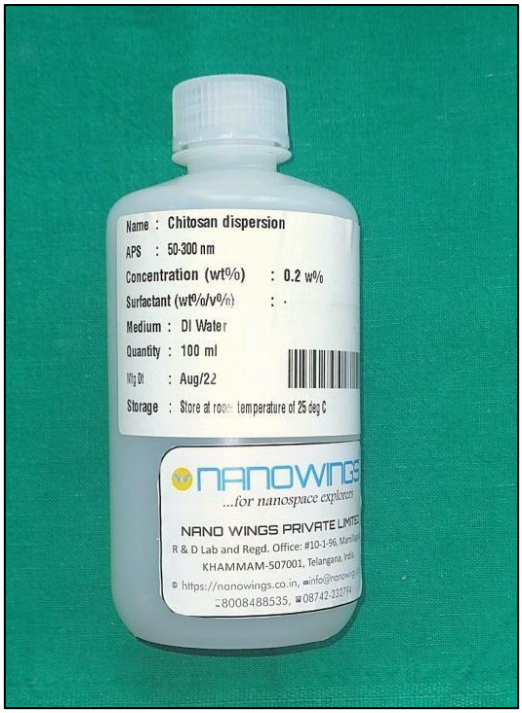


Fig.25- 0.2% Nanochitosan dispersion

Fig.26- 100 mg/ml Vitex negundo Linn.

COLOUR PLATE-VI

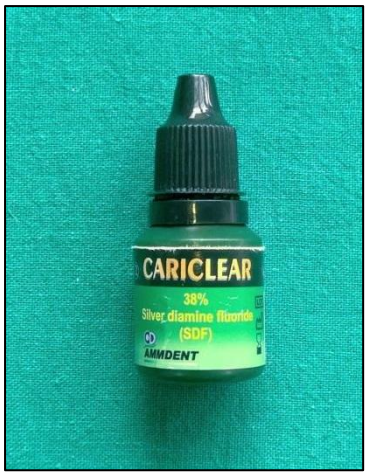


Fig.27-38% Silver Diamine Fluoride

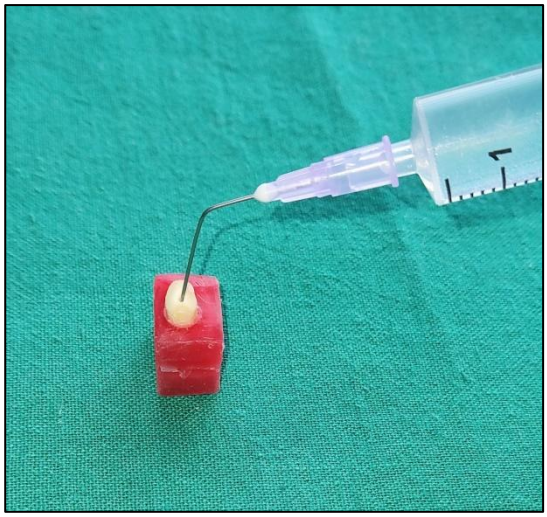


Fig.28 – Irrigation of test samples by respective agents

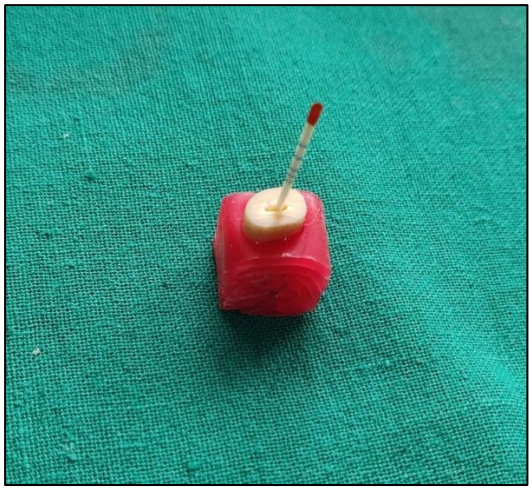


Fig.29-ISO 25# Paper point soaking canal content

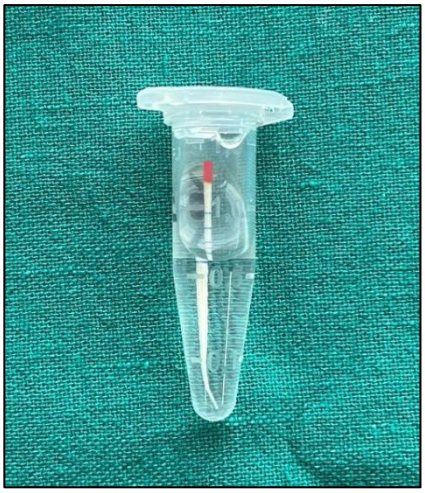


Fig 30- Eppendorf tube with soaked paper point

Fig.31- Inoculation loop

COLOUR PLATE-VII

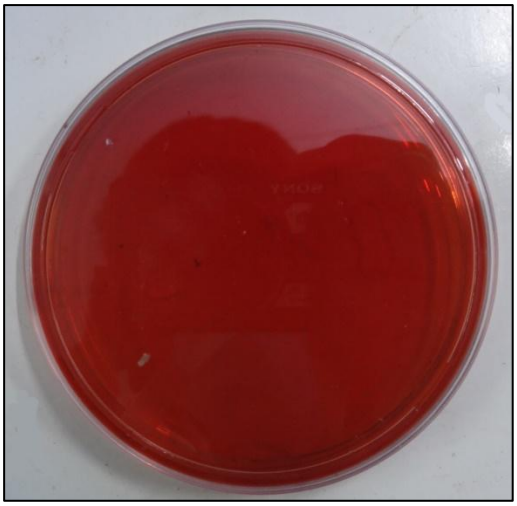


Fig.32- Culture of blood agar plate

Fig.33- Laminar flow chamber

Fig.34-Incubation Chamber

COLOUR PLATE-VIII

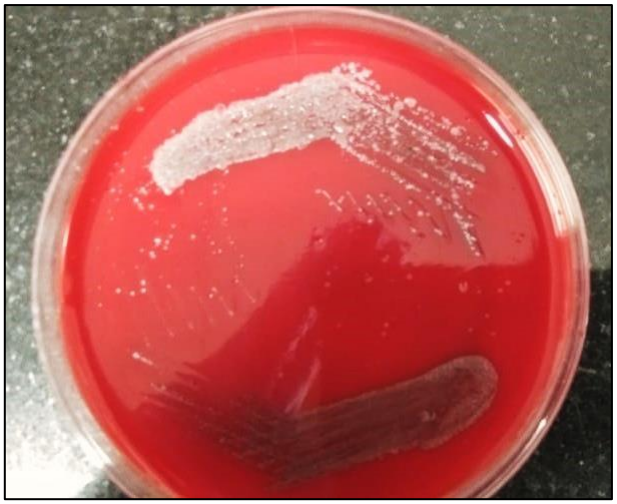


Fig.35-Enterococcus fecalis growth

Fig 36- Digital Colony Counte

COLOUR PLATE-IX

The results obtained were tabulated (Annexure 4). The descriptive statistics included mean and standard deviation. The Shapiro–Wilk test was used to investigate the distribution of the data and Levene's test to explore the homogeneity of the variables. The data were found to be homogeneous and normally distributed. The intergroup comparison for the difference of mean scores between independent groups was done using the One Way ANOVA followed by post hoc analysis. The intragroup comparison for the different time intervals was done using paired t test to find the difference between the individual time intervals. The level of the significance for the present study was fixed at 5%.

Software: SPSS (Statistical Package for Social Sciences) Version 19.0 (IBM Corporation, Chicago, USA).

Output Tables:

Groups	**N**	**Max.**	**Min.**	**Mean**	**S.D.**	**P value**	**F value**
Group A	10	17	9	12.10	2.46	0.01	25.534
Group B	10	26	19	21.80	2.34		
Group C	10	67	46	56.00	8.45		
Group D	10	27	19	23.40	3.09		
Group E	10	31	21	26.90	3.17		
Group F	10	37	26	31.20	3.55		

Table 2: Comparison of reduction in mean CFU counts among the groups

The mean CFU counts were compared among the six groups. The analysis done by one-way ANOVA showed statistically significant differences (**p<0.001**) in mean CFUs counts. The Group C – 5.25% Sodium Hypochlorite had the greatest mean reduction in CFU count of 56.00±8.45 followed by Group F – 2% Chlorhexidine (31.20±3.55), Group E – 0.2% Nanochitosan(26.90±3.17), Group D- 100mg/ml vitex negundo linn(23.40±3.09) and Group B - 3.8% Silver Diamine Fluoride (21.80±2.34). The Control Group A - 0.9% normal saline had the lowest mean reduction in CFU count of 12.10±2.46 (Graph 2).

	Mean Reduction	**Percentage Reduction**	**P value**	**Significance**
Group A vs Group B	12.10±2.46	11.95±2.97	**0.001**	**Significant**
	21.80±2.34	22.26±6.85		
Group A vs Group C	12.10±2.46	11.95±2.97	**0.001**	**Significant**
	56.00±8.45	56.64±19.15		
Group A vs Group D	12.10±2.46	11.95±2.97	**0.001**	**Significant**
	23.40±3.09	21.12±4.18		
Group A vs Group E	12.10±2.46	11.95±2.97	**0.001**	**Significant**
	26.90±3.17	24.45±7.05		
Group A vs Group F	12.10±2.46	11.95±2.97	**0.001**	**Significant**
	31.20±3.55	29.42±7.83		
Group B vs Group C	21.80±2.34	22.26±6.85	**0.001**	**Significant**
	56.00±8.45	56.64±19.15		
Group B vs Group D	21.80±2.34	22.26±6.85	0.791	Non-Significant
	23.40±3.09	21.12±4.18		
Group B vs Group E	21.80±2.34	22.26±6.85	0.813	Non-Significant
	26.90±3.17	24.45±7.05		

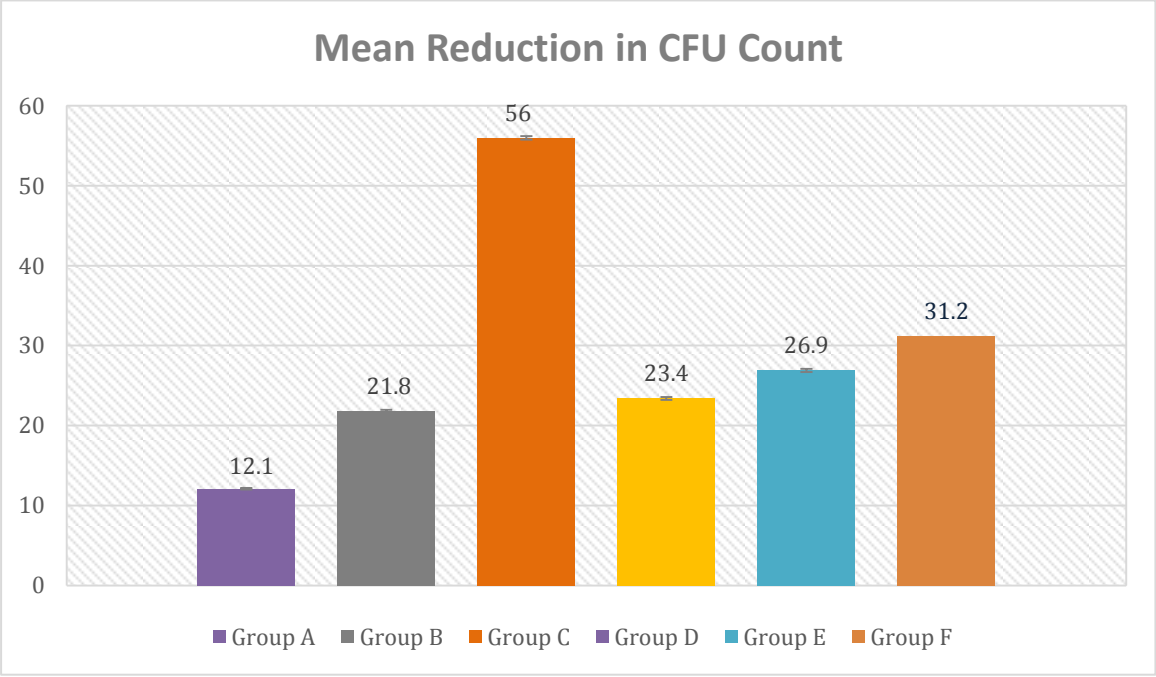
Group B vs Group F	21.80±2.34	22.26±6.85	0.101	Non-Significant
	31.20±3.55	29.42±7.83		
Group C vs Group D	56.00±8.45	56.64±19.15	**0.001**	**Significant**
	23.40±3.09	21.12±4.18		
Group C vs Group E	56.00±8.45	56.64±19.15	**0.001**	**Significant**
	26.90±3.17	24.45±7.05		
Group C vs Group F	56.00±8.45	56.64±19.15	**0.001**	**Significant**
	31.20±3.55	29.42±7.83		
Group D vs Group E	23.40±3.09	21.12±4.18	0.442	Non-Significant
	26.90±3.17	24.45±7.05		
Group D vs Group F	23.40±3.09	21.12±4.18	0.068	Non-Significant
	31.20±3.55	29.42±7.83		
Group E vs Group F	26.90±3.17	24.45±7.05	0.251	Non-Significant
	31.20±3.55	29.42±7.83		

Table 3: Post Hoc pair wise comparison of mean CFU count among the groups

The post hoc pair wise comparative analysis was done. In the Group I the mean E.Fecalis count at the pre irrigation level was 103.70±19.07 and at the post irrigation level was 91.60±18.44. The percentage reduction in bacterial count was 11.95±2.97 In the Group II, the mean E.Fecalis count at the pre irrigation level was 105.50±29.68 and at the post irrigation level was 83.70±30.24. The percentage

reduction in bacterial count was 22.26±6.85 In the Group III, the mean E.Fecalis count at the pre irrigation level was 106.30±27.43 and at the post irrigation level was 50.30±32.47. The percentage reduction in bacterial count was 56.64±19.15 In the Group IV, the mean E.Fecalis count at the pre irrigation level was 113.60±21.62 and at the post irrigation level was 90.20±20.81. The percentage reduction in bacterial count was 21.12±4.18 In the Group V, the mean E.Fecalis count at the pre irrigation level was 116.40±29.46 and at the post irrigation level was 89.50±28.68. The percentage reduction in bacterial count was 24.45±7.05 In the Group VI, the mean E.Fecalis count at the pre irrigation level was 109.60±16.98 and at the post irrigation level was 78.40±18.99. The percentage reduction in bacterial count was 29.42±7.83

The intergroup comparison was statistically significant between Group I and Group II, Group I and Group III, Group I and Group IV, Group I and Group V, Group I and Group VI. Group II and Group III, Group III and Group IV, Group III and Group V, Group III and Group VI.



Graph 2: Comparison of mean reduction in CFU counts among the groups

This in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics, Babu Banarasi Das College of Dental Sciences, Lucknow in collaboration with R.S. Pathology Centre, Lucknow.

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

In the present study, sixty single rooted human teeth were taken into consideration after accomplishing the inclusion and exclusion criteria. Single rooted teeth were chosen in this study as a standardisation and an in vitro closed-end canal model was used in this study by sealing the apex with GIC as this stimulated in vivo condition in the root canal more accurately.[93]

Enterococcus fecalis was chosen as a test organism for the present study as it is the most common bacteria associated with resistant or recurrent infections leading to endodontic treatment failure.[94] One of the most potent virulence factors of the Enteroccoccus group that enhance the adaptation and also the survival in various environments are collagen binding protein, Enterococcal surface proteins.[95] They can tolerate an extensive range of growth conditions, including temperatures ranging from10°C to 45°C.[96] It has been suggested in studies that Enterococcus fecalis can resist various intracanal treatment procedures.[97]

A major cause of endodontic failure lies in the inability to locate the canal; its debridement and obturation of the main as well as numerous lateral and accessory canals where endodontic instruments cannot reach during routine endodontic treatment due to which root canal disinfection is not accomplished.[94] To overcome this, root canal irrigants are being used to disinfect these areas where instrumentation is not possible.[99]

Irrigation has a critical role in endodontics.[95] During and after instrumentation, the irrigants facilitates the removal of microorganisms, tissue remnants and dentinal chips from root canals.[95] In addition to the debriding action, irrigation serves the purpose of facilitating instrumentation by lubricating the canals and by flushing out the dentinal debris.[96] Commonly used irrigants not just possess tissue dissolving and antibacterial

properties, but also have cytotoxic potential and may cause harm to the periapical tissues.[97] The most popular irrigants being used in dental practice are: Sodium Hypochlorite, Chlorhexidine, EDTA, Hydrogen Peroxide and Normal Saline; but none of them can be regarded as optimal or ideal.[100]

Sodium Hypochlorite (NaOCl) is one of the most popular irrigating solution.[101] Sodium Hypochlorite ionizes in water into Na^+ & hypochlorite (HCO^-) ions, establishing equilibrium with hypochlorous acid (HOCl). This hypochlorous acid is responsible for the antibacterial activity.[102] The antibacterial & tissue dissolving property of Sodium hypochlorite was supported by research conducted by Zehnder et al.[103] The action of sodium hypochlorite is dependent on its concentration, and so is its toxicity.[104] Previous studies have shown that the tissue-dissolving ability of sodium hypochlorite solution decreases if it is diluted.[105,106] Siqueira et al suggested that 4% NaOCl would display substantial efficacy in comparing the saline solution in disinfecting the Enterococcus fecalis contaminated root canal.[107] 5.25% NaOCl was more successful against Enterococcus fecalis than its lower concentrations.[108] Ercan et al. suggested that the microorganisms were greatly decreased using 5.25% NaOCl.[109] 5.25% NaOCl was used as an irrigant in this present study due to the greater antibacterial efficacy as obtained from evidence of previous research.[110] The shortcomings of NaOCl include unpleasant taste, toxicity & its inability to remove smear layer by itself.[110] The efficiency of Sodium Hypochlorite is counteracted by exudate from the lesioned periapical area, inflamed pulp tissue, dentin collagen, and microbial biomass.[110]

Another commonly used irrigant is Chlorhexidine, which is an antibacterial root canal irrigant. The antibacterial efficacy of chlorhexidine is due to the interaction of positive charge of the molecule and negatively charged phosphate groups on the microbial cell walls, thereby altering the cell's osmotic equilibrium.[111] This increases the permeability of the cell wall, which allows the CHX molecule to penetrate into the bacteria.[111] Chlorhexidine is available in various concentrations ranging from 0.2% to 2%.[111] At low concentration (0.2%), low molecular weight substances specifically potassium and phosphorous will leak out. On the other hand, at higher concentration (2%), CHX is bactericidal; precipitation of cytoplasmic contents occurs resulting in cell death.[14] Chlorhexidine's antibacterial activity is dependent on achieving an

optimal pH of 5.5-7.[112] White et al reported these effect of 2% Chlorhexidine persisted for 72 hours to 12 weeks and possesses substantivity because of its ability to bind to the hard tissue and maintain its antibacterial activityfor a long period of time.[113] These studies have assessed the antimicrobial efficacy of Chlorhexidine as a good irrigant. It is relatively non-toxic and possesses a higher substantivity rate than other commonly used root canal irrigants, therefore 2% Chlorhexidine was taken into consideration in this present study.[114]

In the pursuit of alternatives for a natural chemical; Chitosan emerged in the field of dentistry which is a natural polysaccharide that is the principle component of crustacean exoskeleton.[110] It is described as a nontoxic cationic biopolymer which is biocompatible, biodegradable and has the ability to improve dentin surface properties and elevates dentin resistance to collagenase degradation.[111] Moreover, chitosan possesses prolonged antibacterial activity against a broad range of microorganisms.[112,113] Furthermore; it significantly improves bond strength to dentin. Waltimo et al and Busscher et al found that the size of nanoparticles plays an important role in their antibacterial activity, with smaller particles showing higher antibacterial activity than the macro scaled ones.[115,116] Chitosan nanoparticles (CNPS) had a significant antibacterial activity because of a higher surface area and charge density (Positive charge) to react with the negative charge surface of bacterial cells, which lead to bacterial cell death.[111]

Antimicrobial silver compounds have also been advocated for endodontic use in an invitro study conducted by Padachey N et al where an antimicrobial silver compound was used as a root canal sealer.[117] Silver fluoride regimens were found to be effective in inhibiting the growth of cariogenic bacteria because of their antimicrobial effect and the deposition of silver compounds.[118]

Inorganic or chemically manufactured irrigants have numerous harmful effects and safety issues.[119] To overcome the harmful effects, herbal substitutes can be recommended. Vitex negundo Linn, a well-recognized plant in the field of Ayurveda, commonly known as Nirgundi has potential antibacterial properties.[36] Aqueous and alcoholic extracts of Vitex negundo Linn. have antibacterial effect against the bacteria that give positive and negative results in the gram stain test[40]. Singh et al. reported antibacterial activity of the oil extracted from the leaves of V. negundo against B.

subtilis in different concentrations.[120] The rich source of secondary metabolites in the plant such as tannin, flavonoids, terpenoids, and alkaloids is thought to be responsible for antibacterial properties.[121] Khan et al. inferred that methanolic extract of V. negundo is highly effective against Gram-positive organism.[122] In the study performed by Deogade et al, various concentrations of Vitex negundo Linn. like 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml had been used. Therefore 100mg/ml concentration of Vitex negundo Linn. was used in the present study.[123]

Study design of the present study was similar to that used by Nourzadeh et al, in which microbial reduction was assessed after chemomechanical debridement.[124] They took microbial samples from the root canals with the use of absorbent paper points before and after chemo-mechanical debridement.[124] Same methodology was followed in the present study to check the optimum growth of microbes. In the present study, blood agar was used for the cultivation of Enterococcus fecalis collected before and after irrigation. The present study used blood agar as the culture medium, as this medium is readily available and commonly used to culture E. fecalis.[125] Microbial count for Enterococcus fecalis in the form of colony forming units were recorded in the present study to assess the reduction in microbial growth pre and post irrigation.

In the present study, 5.25% Sodium Hypochlorite(Group C) was found to be most effective against E. fecalis followed by 2% Chlorhexidine (Group F), 0.2% Nano-chitosan(Group E), 100mg/ml Vitex negundo linn (Group D) and least effective for 3.8% Silver Diamine Fluoride (Group B). Group A (control group) - 0.9% normal saline had the lowest mean reduction in CFU count.

The mean CFU/ml for Enterococcus fecalis reduced from 106.30±27.43 to 50.30±32.47 after irrigation with Sodium Hypochlorite (Group C). The reason for sodium hypochlorite showing the highest antibacterial activity can be attributed to its ability to denature endotoxins and dissolve organic tissue and bacterial biofilms. Similar results were shown in the study conducted by Reyhani et al in which it was observed that even lowest concentration i.e., 2.5% NaOCl was able to denature endotoxins produced by bacteria and cause organic tissue dissolution.[126]

In Group F samples after irrigation with Chlorhexidine, it was found that the mean CFU/ml for Enterococcus fecalis before irrigation were 109.60±16.98 which reduced

to 78.40±18.99 post-irrigation. In the present study, mean CFU/ml for Enterococcus fecalis in Vitex negundo Linn. Group (Group D) before irrigation were 113.60±21.62 which reduced to 90.20±20.81 post irrigation. The higher antibacterial efficacy of chlorhexidine (Group F) compared to Vitex negundo Linn extract(Group D) could possibly be because of the ability of chlorhexidine to denature the bacterial cell wall and increase permeability whereas Vitex negundo Linn has antibacterial activity due to its secondary metabolites which cause cytoplasmic membrane degradation and not cell wall degradation as observed by Nagasarkar et al.[127] The outcome where sodium hypochlorite has more antibacterial efficacy compared to chlorhexidine can be validated due to presence of cationic bisbiguanides in Chlorhexidine which becomes inactivated on coming in contact with organic biofilm. In addition, it has limited penetration into matrix of bacterial biofilm.[128]

Similar results were obtained in the research done by Thosar SS et al who evaluated the antibacterial efficacy of sodium hypochlorite, chlorhexidine and vitex negundo linn. against Enterococcus fecalis and found out that sodium hypochlorite was most effective followed by chlorhexidine and then finally least effective by vitex negundo linn.[129] Studies done by Renisheya et al and Dubey and Padhy showed that ethanolic extracts of Vitex negundo Linn. had significant antibacterial activity against microorganisms like S. aureus and Enterococcus fecalis respectively.[130,131]

The CFU/ml of Enterococcus fecalis reduced from 116.40±29.46 to 89.50±28.68 after irrigation with 0.2% chitosan nanoparticles. Kishen et al. were the first in the field of Nanoparticles to evaluate the root canal disinfection by using Chitosan NPs.[132] Chitosan can be penetrated in the complexities of the root canal and dentinal tubules, thus eliminating microorganisms based on its concentration and time-dependent property even after 3 months.[132]

Nanochitosan particles(Group E) were observed to be having higher antibacterial efficacy than Vitex negundo Linn extract(Group D) and Silver Diamine Fluoride(Group B) which may be attributed to interaction between the positively charged CNP and the negatively charged bacterial cell membrane which impairs cellular exchange with medium; leading to leakage of the intracellular components and bacterial death.[133] Chitosan was also used in nanoparticles form which also may have attributed to the powerful antibacterial effect against E fecalis as this is well

documented in literature that nanoparticle size of a substance allows materials to acquire higher accuracy, efficiency and amplifies the antibacterial effect of many irrigants.[133,134]

Barreras *et al.* in their in-vitro study used Chitosan Nanoparticles and Chlorhexidine alone and in combinations to remove Enterococcus fecalis from the infected root canals and concluded that Chitosan Nanoparticle root canal irrigant alone was least effective when compared with chlorhexidine which is in accordance with the present study. [135] In contrast to the results of our study, the study done by Abu Al-timan where gel forms of chitosan nanoparticles and 5.25% sodium hypchlorite were evaluated against Enterococcus fecalis concluded that the chitosan nanoparticles were more effective in reducing Enterococcus fecalis with respect to sodium hypochlorite.[136]

The CFU/ml of Enterococcus fecalis reduced from 105.50±29.68 to 83.70±30.24 after irrigation with 3.8% Silver Diamine Fluoride (Group B) which makes SDF the least effective antibacterial irrigant in the present study. It is possible that an increase of the concentration might improve its efficiency. Contrast to the results observed in our study, the study conducted by Abrar E et al using showed 2% chlorhexidine was less effective than 3.8% silver diamine fluoride against Enterococcus fecalis.[137] In the study by Mathew et al , there was no difference between SDF and 2% chlorhexidine in antibacterial efficacy against Enterococcus fecalis.[138] On the other hand, in Al-Madi et al., 5.25% NaOCl had a higher antibacterial efficacy than either 3.8% SDF or 2% chlorhexidine.[62]

Although there are various root canal irrigants which are effective against Enterococcus fecalis, there has been always a necessity for newer alternatives to conventional irrigants which are more or less toxic to the periapical tissues. Herbal irrigants can be used as alternatives which has minimal cytotoxic effects when compared to conventional irrigants and considerable antibacterial efficacy.

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

In the present study, sixty single rooted human teeth were taken into consideration after accomplishing the inclusion and exclusion criteria. Single rooted teeth were chosen in this study as a standardisation and an in vitro closed-end canal model was used in this study by sealing the apex with GIC as this stimulated in vivo condition in the root canal more accurately.[93]

Enterococcus fecalis was chosen as a test organism for the present study as it is the most common bacteria associated with resistant or recurrent infections leading to endodontic treatment failure.[94] One of the most potent virulence factors of the Enteroccoccus group that enhance the adaptation and also the survival in various environments are collagen binding protein, Enterococcal surface proteins.[95] They can tolerate an extensive range of growth conditions, including temperatures ranging from10°C to 45°C.[96] It has been suggested in studies that Enterococcus fecalis can resist various intracanal treatment procedures.[97]

A major cause of endodontic failure lies in the inability to locate the canal; its debridement and obturation of the main as well as numerous lateral and accessory canals where endodontic instruments cannot reach during routine endodontic treatment due to which root canal disinfection is not accomplished.[94] To overcome this, root canal irrigants are being used to disinfect these areas where instrumentation is not possible.[99]

Irrigation has a critical role in endodontics.[95] During and after instrumentation, the irrigants facilitates the removal of microorganisms, tissue remnants and dentinal chips from root canals.[95] In addition to the debriding action, irrigation serves the purpose of facilitating instrumentation by lubricating the canals and by flushing out the dentinal debris.[96] Commonly used irrigants not just possess tissue dissolving and antibacterial

properties, but also have cytotoxic potential and may cause harm to the periapical tissues.[97] The most popular irrigants being used in dental practice are: Sodium Hypochlorite, Chlorhexidine, EDTA, Hydrogen Peroxide and Normal Saline; but none of them can be regarded as optimal or ideal.[100]

Sodium Hypochlorite (NaOCl) is one of the most popular irrigating solution.[101] Sodium Hypochlorite ionizes in water into Na^+ & hypochlorite (HCO^-) ions, establishing equilibrium with hypochlorous acid (HOCl). This hypochlorous acid is responsible for the antibacterial activity.[102] The antibacterial & tissue dissolving property of Sodium hypochlorite was supported by research conducted by Zehnder et al.[103] The action of sodium hypochlorite is dependent on its concentration, and so is its toxicity.[104] Previous studies have shown that the tissue-dissolving ability of sodium hypochlorite solution decreases if it is diluted.[105,106] Siqueira et al suggested that 4% NaOCl would display substantial efficacy in comparing the saline solution in disinfecting the Enterococcus fecalis contaminated root canal.[107] 5.25% NaOCl was more successful against Enterococcus fecalis than its lower concentrations.[108] Ercan et al. suggested that the microorganisms were greatly decreased using 5.25% NaOCl.[109] 5.25% NaOCl was used as an irrigant in this present study due to the greater antibacterial efficacy as obtained from evidence of previous research.[110] The shortcomings of NaOCl include unpleasant taste, toxicity & its inability to remove smear layer by itself.[110] The efficiency of Sodium Hypochlorite is counteracted by exudate from the lesioned periapical area, inflamed pulp tissue, dentin collagen, and microbial biomass.[110]

Another commonly used irrigant is Chlorhexidine, which is an antibacterial root canal irrigant. The antibacterial efficacy of chlorhexidine is due to the interaction of positive charge of the molecule and negatively charged phosphate groups on the microbial cell walls, thereby altering the cell's osmotic equilibrium.[111] This increases the permeability of the cell wall, which allows the CHX molecule to penetrate into the bacteria.[111] Chlorhexidine is available in various concentrations ranging from 0.2% to 2%.[111] At low concentration (0.2%), low molecular weight substances specifically potassium and phosphorous will leak out. On the other hand, at higher concentration (2%), CHX is bactericidal; precipitation of cytoplasmic contents occurs resulting in cell death.[14] Chlorhexidine's antibacterial activity is dependent on achieving an

optimal pH of 5.5-7.[112] White et al reported these effect of 2% Chlorhexidine persisted for 72 hours to 12 weeks and possesses substantivity because of its ability to bind to the hard tissue and maintain its antibacterial activityfor a long period of time.[113] These studies have assessed the antimicrobial efficacy of Chlorhexidine as a good irrigant. It is relatively non-toxic and possesses a higher substantivity rate than other commonly used root canal irrigants, therefore 2% Chlorhexidine was taken into consideration in this present study.[114]

In the pursuit of alternatives for a natural chemical; Chitosan emerged in the field of dentistry which is a natural polysaccharide that is the principle component of crustacean exoskeleton.[110] It is described as a nontoxic cationic biopolymer which is biocompatible, biodegradable and has the ability to improve dentin surface properties and elevates dentin resistance to collagenase degradation.[111] Moreover, chitosan possesses prolonged antibacterial activity against a broad range of microorganisms.[112,113] Furthermore; it significantly improves bond strength to dentin. Waltimo et al and Busscher et al found that the size of nanoparticles plays an important role in their antibacterial activity, with smaller particles showing higher antibacterial activity than the macro scaled ones.[115,116] Chitosan nanoparticles (CNPS) had a significant antibacterial activity because of a higher surface area and charge density (Positive charge) to react with the negative charge surface of bacterial cells, which lead to bacterial cell death.[111]

Antimicrobial silver compounds have also been advocated for endodontic use in an invitro study conducted by Padachey N et al where an antimicrobial silver compound was used as a root canal sealer.[117] Silver fluoride regimens were found to be effective in inhibiting the growth of cariogenic bacteria because of their antimicrobial effect and the deposition of silver compounds.[118]

Inorganic or chemically manufactured irrigants have numerous harmful effects and safety issues.[119] To overcome the harmful effects, herbal substitutes can be recommended. Vitex negundo Linn, a well-recognized plant in the field of Ayurveda, commonly known as Nirgundi has potential antibacterial properties.[36] Aqueous and alcoholic extracts of Vitex negundo Linn. have antibacterial effect against the bacteria that give positive and negative results in the gram stain test[40]. Singh et al. reported antibacterial activity of the oil extracted from the leaves of V. negundo against B.

subtilis in different concentrations.[120] The rich source of secondary metabolites in the plant such as tannin, flavonoids, terpenoids, and alkaloids is thought to be responsible for antibacterial properties.[121] Khan et al. inferred that methanolic extract of V. negundo is highly effective against Gram-positive organism.[122] In the study performed by Deogade et al, various concentrations of Vitex negundo Linn. like 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml and 100mg/ml had been used. Therefore 100mg/ml concentration of Vitex negundo Linn. was used in the present study.[123]

Study design of the present study was similar to that used by Nourzadeh et al, in which microbial reduction was assessed after chemomechanical debridement.[124] They took microbial samples from the root canals with the use of absorbent paper points before and after chemo-mechanical debridement.[124] Same methodology was followed in the present study to check the optimum growth of microbes. In the present study, blood agar was used for the cultivation of Enterococcus fecalis collected before and after irrigation. The present study used blood agar as the culture medium, as this medium is readily available and commonly used to culture E. fecalis.[125] Microbial count for Enterococcus fecalis in the form of colony forming units were recorded in the present study to assess the reduction in microbial growth pre and post irrigation.

In the present study, 5.25% Sodium Hypochlorite(Group C) was found to be most effective against E. fecalis followed by 2% Chlorhexidine (Group F), 0.2% Nano-chitosan(Group E), 100mg/ml Vitex negundo linn (Group D) and least effective for 3.8% Silver Diamine Fluoride (Group B). Group A (control group) - 0.9% normal saline had the lowest mean reduction in CFU count.

The mean CFU/ml for Enterococcus fecalis reduced from 106.30±27.43 to 50.30±32.47 after irrigation with Sodium Hypochlorite (Group C). The reason for sodium hypochlorite showing the highest antibacterial activity can be attributed to its ability to denature endotoxins and dissolve organic tissue and bacterial biofilms. Similar results were shown in the study conducted by Reyhani et al in which it was observed that even lowest concentration i.e., 2.5% NaOCl was able to denature endotoxins produced by bacteria and cause organic tissue dissolution.[126]

In Group F samples after irrigation with Chlorhexidine, it was found that the mean CFU/ml for Enterococcus fecalis before irrigation were 109.60±16.98 which reduced to

78.40±18.99 post-irrigation. In the present study, mean CFU/ml for Enterococcus fecalis in Vitex negundo Linn. Group (Group D) before irrigation were 113.60±21.62 which reduced to 90.20±20.81 post irrigation. The higher antibacterial efficacy of chlorhexidine (Group F) compared to Vitex negundo Linn extract(Group D) could possibly be because of the ability of chlorhexidine to denature the bacterial cell wall and increase permeability whereas Vitex negundo Linn has antibacterial activity due to its secondary metabolites which cause cytoplasmic membrane degradation and not cell wall degradation as observed by Nagasarkar et al.[127] The outcome where sodium hypochlorite has more antibacterial efficacy compared to chlorhexidine can be validated due to presence of cationic bisbiguanides in Chlorhexidine which becomes inactivated on coming in contact with organic biofilm. In addition, it has limited penetration into matrix of bacterial biofilm.[128]

Similar results were obtained in the research done by Thosar SS et al who evaluated the antibacterial efficacy of sodium hypochlorite, chlorhexidine and vitex negundo linn. against Enterococcus fecalis and found out that sodium hypochlorite was most effective followed by chlorhexidine and then finally least effective by vitex negundo linn.[129] Studies done by Renisheya et al and Dubey and Padhy showed that ethanolic extracts of Vitex negundo Linn. had significant antibacterial activity against microorganisms like S. aureus and Enterococcus fecalis respectively.[130,131]

The CFU/ml of Enterococcus fecalis reduced from 116.40±29.46 to 89.50±28.68 after irrigation with 0.2% chitosan nanoparticles. Kishen et al. were the first in the field of Nanoparticles to evaluate the root canal disinfection by using Chitosan NPs.[132] Chitosan can be penetrated in the complexities of the root canal and dentinal tubules, thus eliminating microorganisms based on its concentration and time-dependent property even after 3 months.[132]

Nanochitosan particles(Group E) were observed to be having higher antibacterial efficacy than Vitex negundo Linn extract(Group D) and Silver Diamine Fluoride(Group B) which may be attributed to interaction between the positively charged CNP and the negatively charged bacterial cell membrane which impairs cellular exchange with medium; leading to leakage of the intracellular components and bacterial death.[133] Chitosan was also used in nanoparticles form which also may have attributed to the powerful antibacterial effect against E fecalis as this is well

documented in literature that nanoparticle size of a substance allows materials to acquire higher accuracy, efficiency and amplifies the antibacterial effect of many irrigants.[133,134]

Barreras *et al.* in their in-vitro study used Chitosan Nanoparticles and Chlorhexidine alone and in combinations to remove Enterococcus fecalis from the infected root canals and concluded that Chitosan Nanoparticle root canal irrigant alone was least effective when compared with chlorhexidine which is in accordance with the present study. [135] In contrast to the results of our study, the study done by Abu Al-timan where gel forms of chitosan nanoparticles and 5.25% sodium hypchlorite were evaluated against Enterococcus fecalis concluded that the chitosan nanoparticles were more effective in reducing Enterococcus fecalis with respect to sodium hypochlorite.[136]

The CFU/ml of Enterococcus fecalis reduced from 105.50±29.68 to 83.70±30.24 after irrigation with 3.8% Silver Diamine Fluoride (Group B) which makes SDF the least effective antibacterial irrigant in the present study. It is possible that an increase of the concentration might improve its efficiency. Contrast to the results observed in our study, the study conducted by Abrar E et al using showed 2% chlorhexidine was less effective than 3.8% silver diamine fluoride against Enterococcus fecalis.[137] In the study by Mathew et al , there was no difference between SDF and 2% chlorhexidine in antibacterial efficacy against Enterococcus fecalis.[138] On the other hand, in Al-Madi et al., 5.25% NaOCl had a higher antibacterial efficacy than either 3.8% SDF or 2% chlorhexidine.[62]

Although there are various root canal irrigants which are effective against Enterococcus fecalis, there has been always a necessity for newer alternatives to conventional irrigants which are more or less toxic to the periapical tissues. Herbal irrigants can be used as alternatives which has minimal cytotoxic effects when compared to conventional irrigants and considerable antibacterial efficacy.

The present in-vitro study made a sincere effort to evaluate and compare the antimicrobial efficacy of 3.8% Silver Diamine Fluoride, 5.25% Sodium Hypochlorite, 100mg/ml Vitex Negundo Linn , 0.2 % Nano Chitosan , 0.9% Normal Saline and 2% Chlorhexidine against Enterococcus fecalis as root canal irrigants.

Within the limitations of this study the following conclusions were drawn:

1. All irrigants were effective against E. fecalis when compared to the control group i.e. 0.9% Normal Saline, thus can be used as an effective root canal irrigant.

2. 5.25% Sodium Hypochlorite was found to be most effective against E. fecalis against all the tested root canal irrigants.

3. 5.25% Sodium Hypochlorite showed maximum antimicrobial activity against E. fecalis when compared to 3.8% Silver Diamine Fluoride, 100mg/ml Vitex Negundo Linn , 0.2 % Nano Chitosan , 0.9% Normal Saline and 2% Chlorhexidine.

However, further in-vivo studies are required to evaluate which of the various recently introduced test irrigants are more appropriate as root canal irrigants, against E. fecalis and similar pathogens.

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BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES
(FACULTY OF BBD UNIVERSITY), LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **"A comparative evaluation of the Antibacterial Efficacy of Various Root Canal Irrigants against E Fecalis: An In-Vitro Study"** submitted by **Dr Ricku Mathew Reji** Post graduate student from the **Department of Conservative Dentistry and Endodontics** as part of MDS Curriculum for the academic year 2020-2023 with the accompanying proforma was reviewed by the Institutional Research Committee present on **11th October 2021** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

Prof. Vandana A Pant
Co-Chairperson

Prof. B. Rajkumar
Chairperson

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the IXth Institutional Ethics Sub-Committee

IEC Code: 33 **BBDCODS/04/2022**

Title of the Project: A comparative evaluation of the antibacterial efficacy of various root canal irrigants against E fecalis: An in-vitro study.

Principal Investigator: Dr Ricku Mathew Reji **Department:** Conservative Dentistry & Endodontics

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: New, MDS Research

Dear Dr Ricku Mathew Reji,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 07th April, 2022.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Rana Pratap Maurya Member	Reader, Department of Orthodontics, BBDCODS, Lucknow
4.	Dr. Akanksha Bhatt Member	Reader, Department of Conservative Dentistry & Endodontics, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.
The comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Lakshmi Bala

(Dr. Lakshmi Bala)
Member-Secretary
IEC

Member-Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

(Dr. Puneet Ahuja)
Principal
BBDCODS

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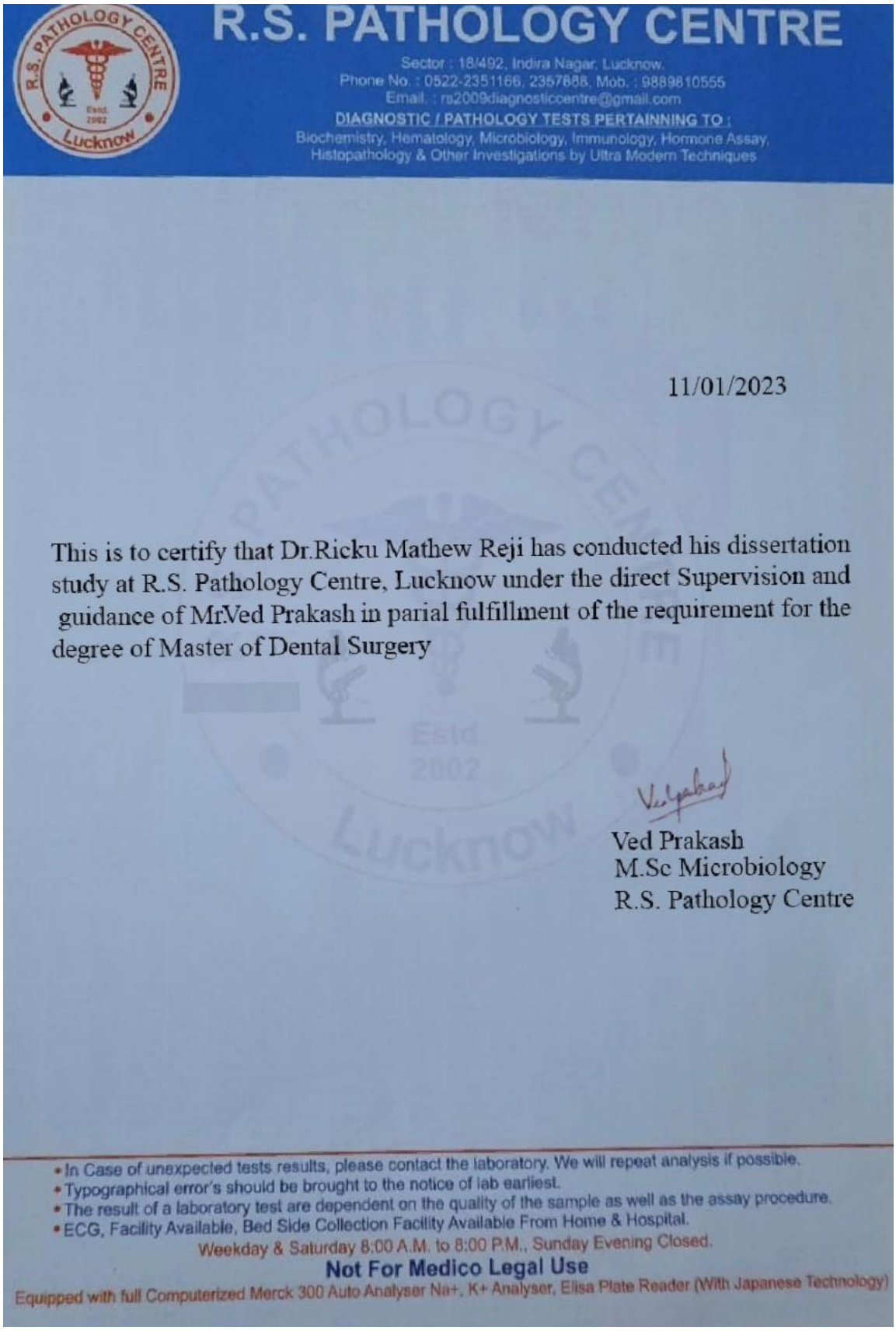

R.S. PATHOLOGY CENTRE

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11/01/2023

This is to certify that Dr.Ricku Mathew Reji has conducted his dissertation study at R.S. Pathology Centre, Lucknow under the direct Supervision and guidance of Mr.Ved Prakash in parial fulfillment of the requirement for the degree of Master of Dental Surgery

Ved Prakash
M.Sc Microbiology
R.S. Pathology Centre

- In Case of unexpected tests results, please contact the laboratory. We will repeat analysis if possible.
- Typographical error's should be brought to the notice of lab earliest.
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Reduction in E. fecalis colony forming units after irrigation with 6 different irrigants

Sample no.	Group A (NS) $X10^6$ CFU/ml	Group B (SDF) $X10^6$ CFU/ml	Group C (NaOCl) $X10^6$ CFU/ml	Group D (VNL) $X10^6$ CFU/ml	Group E (NC) $X10^6$ CFU/ml	Group F (CHX) $X10^6$ CFU/ml
1.	11	22	67	20	29	34
2.	12	21	68	21`	31	32
3.	13	22	56	23	30	29
4.	10	25	55	19	28	36
5.	9	19	46	26	23	37
6.	13	26	50	25	25	28
7.	15	20	49	24	21	29
8.	17	23	45	27	26	31
9.	11	19	62	21	27	30
10.	10	21	62	28	29	26

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INTRODUCTION In persistent endodontic infections, different microorganisms are associated with intraradicular and extraradicular infection.3 Depending on mechanical instrumentation alone brings about a reduction in the bacterial load from the canal by around 50%.6 So, additional treatment approaches like the use of root canal irrigants are necessary to achieve reduction of bacterial count in the root canal system.7 Because of its high ability to dissolve organic tissue, sodium hypochlorite is the most commonly used endodontic irrigant.10 Several different concentrations of sodium hypochlorite are used in endodontic treatment procedures but the concentration with maximum antibacterial efficacy is 5.25% w/v.10 Sodium hypochlorite has a few demerits which include toxic effects in the periapical region and denaturation of dentin collagen leading to loss of strength.10 Chlorhexidine is an endodontic irrigant which has the property of substantivity.11 The antibacterial efficacy of Chlorhexidine as an endodontic irrigant is dependent on its concentration. At different concentrations, Chlorhexidine can have bacteriostatic or bactericidal effects.13 At high concentrations, it has bacteriocidal effects whereas at low concentrations, Chlorhexidine is bacteriostatic.20 CHX is not advised to be used as a primary irrigant due to the lack of its ability to dissolve necrotic tissue from root canal space.21 Therefore,

68% **MATCHING BLOCK 1/7** W

the demand for ideal root canal irrigants continues with the development of new materials and methods.22

Vishesh Gupta