EVALUATION OF ANTIMICROBIAL EFFICACY OF CHEMICAL VERSUS HERBAL INTRACANAL MEDICAMENTS AGAINST ENTEROCOCCUS FAECALIS BIOFILM

Dr. Namita Tandon[1*], Dr. Abhishek Sharma[2], Dr. Jaspreet Kaur[3], Dr. Naman Grover[4], Dr. Tanisha Verma[5], Dr. Harmandeep Singh[6]

Abstract:
The main causative factors in the development of pulpal and periapical inflammation are bacteria, and the success of endodontic treatment depends on the eradication of microorganisms from the root canal system and prevention of reinfection

Objective: To evaluate the antimicrobial efficacy of chemical versus herbal intracanal medicaments against Enterococcus faecalis biofilm.

Method: Agar diffusion test was performed with Propolis (Group A), 2% Chlorhexidine (Group B), N-acetyl cysteine (Group C) and combination of 2%CHX+NAC (Group D) against planktonic cells. The diameter of zones bacterial inhibition were measured (mm) and recorded for each solution. For biofilm formation thirty freshly extracted teeth were vertically sectioned into two halves resulting in total of 60 samples.The samples were inoculated with bacterial suspension and incubated at 37°C for 2 weeks for biofilm formation. The samples were then divided into four experimental groups with 15 samples in each group. The samples were gently washed in saline and placed in culture wells containing the test solutions, i.e., Propolis, 2% CHX, NAC, a combination of 2% CHX and NAC in 1:1 ratio. Biofilm formed on root canal surface were removed using sterile scalpel and inoculated in 1ml of BHI broth, and mixed with the test medicament in equal proportions (1ml each). Spread plate method was performed in an interval of 2 hrs starting from 0- 24 hrs. to check the formation of E.faecalis colonies. The effect of medicament on the biofilm were expressed in terms of percent viability.

Results: In agar diffusion test, Propolis showed minimum zone of inhibition, 2% CHX and NAC had almost equal zones of inhibition whereas maximum inhibition was shown by a combination of NAC and 2% CHX suggesting a synergistic action. Intergroup comparison showed highly significant results between all the groups ($P < 0.001$) except in Group B (2% CHX) and Group C (NAC) where the results were statistically insignificant (P value= 0.11). In Biofilm Inhibitory Analysis, no viable bacteria was found in all the four groups, except in Group A (Propolis) with negligible (0.8%) viable bacteria at 24 hrs. Statistical analysis was not done for the cultures obtained from the biofilm as there was no viable bacteria left in all the test groups

Conclusion: The current prospective study showed that herbal intracanal medicaments are less effective as compared to chemical medicaments and among chemicals NAC has almost equal antimicrobial property as 2% CHX whereas their combination showed a synergistic action.

Keyword: N-acetyl cysteine, 2% Chlorhexidine, Propolis, Herbal/ Chemical Intracanal Medicaments, E.faecalis, Biofilm.

[1*]MDS, Senior resident, Oral health sciences centre, PGIMER Chandigarh.
[2]MDS, Senior lecturer, Dpt. Of prosthodontics crown bridge and implantology, Himachal institute of dental sciences paonta sahib.
[3]MDS Periodontics.
[4]BDS, Dental surgeon, Punjab.
[5]BDS, Dental surgeon, Punjab.
[6]MDS 1st year student, Himachal dental college, Sundernagar

***Corresponding Author:** Dr. Namita Tandon
*MDS, Senior resident, Oral health sciences centre, PGIMER Chandigarh.

DOI: 10.48047/ecb/2023.12.si5a.0516

INTRODUCTION

Microorganisms and their by-products are considered to be the major cause of pulp and periradicular pathosis. Hence, a major objective in root canal treatment is to disinfect the entire root canal system, which requires that all contents of the root canal system be eliminated as possible sources of infection. This goal may be accomplished using mechanical instrumentation and chemical irrigation, in conjunction with medication of the root canal system between treatment sessions. Antimicrobial agents are recommended for intracanal antisepsis, to prevent the growth of microorganisms between appointments.

Traditionally calcium hydroxide has been the choice as an intracanal medicament, because of its wide spectrum of action against many endodontic pathogens. One of the identifying features of E.faecalis is that it withstands the high alkalinity and so resistant to calcium hydroxide dressing.

CHX is a positively charged hydrophobic and lipophilic molecule that interacts with phospholipids and lipopolysaccharides on the cell membrane of bacteria and then enters the cell through some type of active or passive transport mechanism **(Athanassiadis et al. 2007)**. Its efficacy is because of the interaction of the positive charge of the molecule and the negatively charged phosphate groups on microbial cell walls **(Gomes et al. 2003a,b)** thereby altering the cells' osmotic equilibrium. This increases the permeability of the cell wall, which allows the CHX molecule to penetrate into the bacteria. 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%)..

N-acetyl cysteine is a derivative of the amino acid L-cysteine and is widely used in medical treatment via inhalation and oral and intravenous routes of chronic bronchitis and acetaminophen overdose **(El-Feky et al. 2009; Silveira et al. 2013)**, and has an excellent safety profile **(Kao et al. 2003)**. **Quah et al. (2012)** found antibacterial property of N-acetyl cysteine (NAC) is unaffected by the presence of dentin which can be considered as an advantage of NAC over CHX. NAC exerts anti-inflammatory activity through its ability to inhibit the expression and release of various proinflammatory cytokines. It is a thiol-containing antioxidant and a mucolytic agent. It is a non-antibiotic drug with antibacterial properties which decreases biofilm formation by a variety of bacteria. Recently, it was shown that NAC inhibits the growth of E. faecalis and eradicates biofilms consisting of it.

Even though E.faecalis makes up a small proportion of the flora in untreated canals, it is a persistent organism in root filled teeth in which root canal therapy had failed. As E.faecalis can secrete proteases such as serine, protease, gelatinase, and collagen binding protein, it can easily bind to dentin firmly, and thus it can live in dentinal tubules and endure prolonged times of starvation. E.faecalis biofilms are several folds more resistant to phagocytosis, antibodies, or antimicrobils than planktonic bacteria. NAC effectively reduces extracellular polysaccharide (EPS) production, disrupts mature biofilms, and therefore decreases the adhesion potential of the bacteria on surfaces.

Due to potential side effects, safety concerns, and ineffectiveness of conventional endodontic medicaments, we are now looking for herbal products. Herbal products have been used, since ancient times in dental and medical practice, and the trend is growing now due to their high antimicrobial activity, excellent biocompatibility, anti-inflammatory, and antioxidant properties. Among the herbal products Propolis, a resinous product of honey bee is a good antimicrobial and anti-inflammatory agent. It has been used since the Greek and Egyptian civilization because of its healing qualities. It is composed of resin (55%), essential oils, and waxes (30%) mixed with bee glue (bee salivary secreation), pollen (5%), amino acids, minerals, ethanol, vitamins and highly active bioflavonoids (10%). The active components are flavonoids and cinnamic acid, caffeic acid phenethyl ester that act as anti-inflammatory agents. Scientific research has revealed its antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, anti-tumor and immunomodulating properties. Current research involving propolis in dentistry spans many fields and highlights its antimicrobial and anti-inflammatory activities, particularly in cariology, oral surgery, pathology, periodontics and endodontics.

A large number of studies have been performed that have assessed the antimicrobial efficacy of 2% of CHX and propolis as an intracanal medicament against E.faecalis separately. However very few in-vitro studies have been documented in the literature that have compared

the antimicrobial efficacy of propolis, 2% CHX, N-acetyl cysteine and combination of 2% CHX and NAC as an intracanal medicament against E.faecalis.
Therefore, the purpose of this study was aimed to evaluate the antimicrobial activity of Propolis, 2% CHX and NAC alone and their combination against E.faecalis isolates and biofilms.

AIM AND OBJECTIVES

1. Aim of this prospective study was to evaluate the antimicrobial efficacy of chemical versus herbal intracanal medicaments against Enterococcus faecalis biofilm.
2. To evaluate the effectiveness of intacanal medicaments against E.faecalis biofilms, as their is Increased awareness of the role of biofilms in infections of the human body where a bacterial biofilm remain a major challenge
3. To conclude from the comparative evaluation of the four type of medicaments used, which one has got more bacterial reduction potential.

MATERIAL AND METHODOLOGY

The present in vitro study was conducted to evaluate and compare the antimicrobial efficacy of herbal intracanal medicament i.e Propolis with chemical medicaments, N-acetyl cystiene and 2% Chlorhexidine, alone and their combination. Susceptibility of both planktonic and biofilm form was checked through agar well diffusion test and biofilm inhibitory analysis respectively.

MICROBIOLOGICAL PROCEDURE

The microbiological study was carried out in Department of Conservative Dentistry and Endodontics in Government Dental College and Hospital, Amritsar in collaboration with Department of Microbiology, Government Medical College, Amritsar.

Determination of zones of inhibition through agar diffusion test

The microrganism used in the study was E.faecalis (ATCC 29212) strains. The E.faecalis was stored on trypticase soya agar/ soyabeen casein digest agar slants at 2-8°C with regular subculturing period of 15 days and the glycerol stock of the culture was maintained at -80°C.

The pathogenic bacteria was grown in Trypticase soy broth for 4 hours at 37°C, thereby Mc Farland standarized for its turbidity. Qualitative assay was performed using agar well diffusion test. Fifteen petri dishes containing BHI agar enriched with defibrinated sheep blood was seeded with E.faecalis isolates. In each petri dish, four wells measuring 6mm in diameter were cut using sterile cork borer
The experimental group for agar diffusion test will be as follows-

Group A- 11% ethanolic extracts of Propolis
The dried Propolis was procured from Jonty Api Agro Services, Ludhiana. The crude propolis was then coarsly powdered. 55 g of this powder was subjected to extraction using 500 ml of ethanol and incubated at 37ºC for 24 hours to get 11% of propolis extract. The crude extract was then filtered and concentrated under vaccum using rotary evaporator (IKA, Works INC, North America). The dried propolis extract was then packed and stored in a refrigerator to avoid contamination.

Group B- 2% CHX (Neelkanth Healthcare Pvt. Ltd, Jodhpur, Rajasthan)

Group C- N-acetyl cysteine (Samarth Life Sciences Pvt. Ltd. Baddi, Himachal Pradesh, India)
NAC solution at a concentration of 200mg/ml was freshly prepared by dissolving 0.2 g in 1ml of sterile distilled water accordind to **Quah et al. (2012)**.

Group D- Combination of 2% CHX and NAC (1:1)
The above mentioned four test solutions (100µl each) were delivered in the prepared wells through a micropipette. The petri dishes were incubated in upright position for 24hrs at 37°c. At the end of incubation period the diameter of the zones of the bacterial inhibition was measured with a Digital Vernier Caliper

Biofilm Inhibitory Analysis

For biofilm formation thirty intact, unrestored non carious mature human single rooted freshly extracted teeth will be collected from The Department of Oral and Maxillofacial Surgery of Punjab Government Dental College and Hospital, Amritsar. Teeth were cleaned of debris and soft tissue remnants and were stored in normal saline solution at room temperature and used within three months after extraction The teeth will be radiographed buccolingualy and mesiodistally to ensure that each tooth contains one canal.

Preparation of teeth

The tooth crown was decoronated at the cementoenamel junction, perpendicular to its long

axis by using a slow speed diamond rotary disc mounted on mandrel (SS White, Lakewood, New Jersey) in a straight handpiece (NSK, Tokyo, Japan) at 30,000 rpm Patency of each canal was determined with # 10 K-file (Sybron Endo, CA, United States). The canal length was obtained by using K-file (Sybron Endo, CA, United States) until the tip of the file was visible at the apical foramen and the working length was established by subtracting 1mm from the canal length.

Instrumentation of all teeth was performed with a crown-down technique using the Protaper rotary files (Dentsply Maillefer, Ballaigues, Switzerland) upto F3 (30/0.09) . The root canal were prepared to the working length with ProTaper instruments according to manufacturer's instructions. All the instruments were used in a torque controlled cordless endomotor (Endomate TC2, NSK, Japan) at a consistent rotation of 300 rpm.
Shapers (S1, S2 and SX) were used in brushing motion.

To achieve 0.03mm apical diameter. The finishers F1-F3 were used with in-and-out action.

In between the root canal irrigation was performed using 1ml of 17% ethylene diamine tetraacetic acid (Avuprep, Dental Avenue, India) and 2ml of 5.25% of sodium hypochlorite (Dentpro, India). All the canals were finally rinsed with 5ml of distilled water.

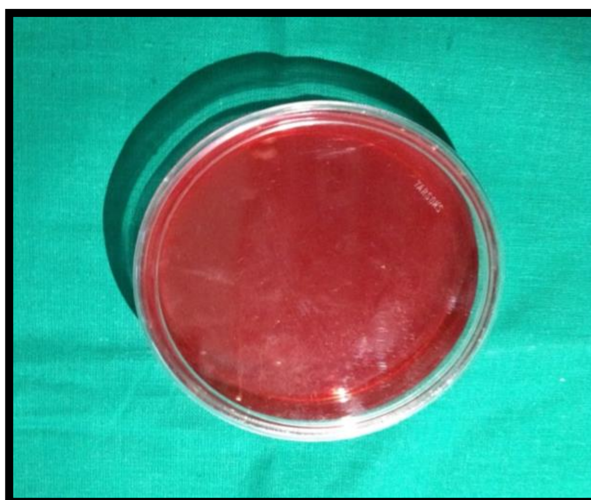
Teeth will be vertically sectioned into two halves to obtain sixty samples. The concave tooth surface will be minimally grounded to achieve flat surface to enable placement in the tissue culture wells, exposing the root canal surface to E.faecalis to form a biofilm

The sectioned tooth samples were divided into 4 experimental groups i.e Group A (Propolis), Group B (2% CHX), Group C (NAC) and Group D (2%CHX+NAC) containing 15 (fifteen) teeth each.

Samples in each group were placed in tissue culture wells where every well was inoculated with 2ml of bacterial suspension. Thereafter incubated at 37°C for 2 weeks. After formation of the biofilm which was otherwise monitered everyday was gently washed using saline and placed in culture wells containing the four test solutions i.e. Propolis, 2% CHX, N-acetyl Cysteine, a combination of 2% CHX and NAC in 1:1 ratio. After 7 days the tooth samples were washed with saline solutions for 5 minutes.

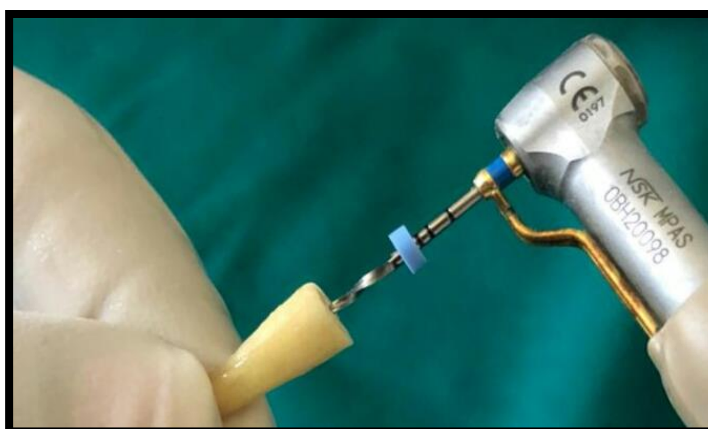
Quantitative Assay
The quantitative effect of the test medicaments were assayed by viable cell count method where the biofilm formed on root canal surface was removed using sterile scalpel and inoculated in 1ml of BHI broth . The inoculum was mixed together with the test medicament in equal proportions (1ml each). Spread plate method (using 10µl of above mixture) was performed in an interval of 2hrs starting from 0-24 hrs. The plates were incubated at 37°C for 24hrs. The number of colonies were counted and compared to that of control where the test medicament was replaced with autoclave distilled water.). The effect of medicament on the formed biofilm were expressed in terms of percent viability.

The obtained data were subjected to statistical analysis using one way anova with post- hoc scheffe's test.

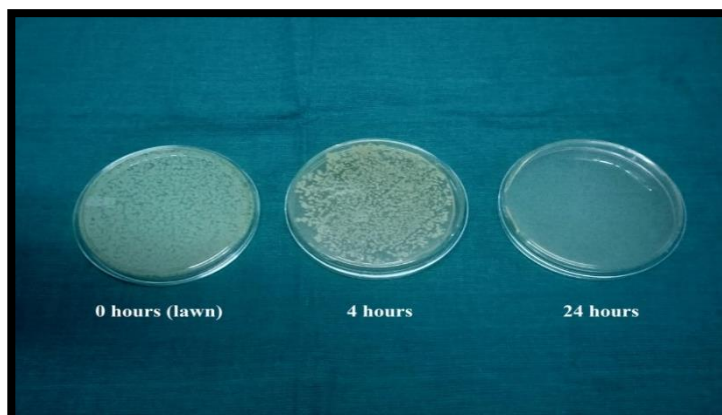


Petridish containing BHI agar enriched with defibrinated sheep blood

Inoculation of Enterococcus faecalis



Biomechanical preparation using Pro Taper using F3



Culture plates showing number of colonies at different time interval in propolis (Group-A)

(A) Culture plates showing number of colonies at different time interval in chlorhexidine (Group-B)

(A) Culture plates showing number of colonies at different time interval in CHX+NAC (Group-D)

OBSERVATIONS

Table I: The Values Of Inhibition Zones For Different Intracanal Medicaments Tested Against Enterococcus Faecalis

SERIAL NO.	Group A (PROPOLIS)	Group B (CHX)	Group C (NAC)	Group D (NAC + CHX)
1	13 mm	15 mm	15 mm	18 mm
2	12 mm	15 mm	14 mm	19 mm
3	11 mm	16 mm	14 mm	18 mm
4	12 mm	17 mm	15 mm	17 mm
5	13 mm	15 mm	13 mm	20 mm
6	13 mm	14 mm	12 mm	16 mm
7	11 mm	16 mm	16 mm	17 mm
8	10 mm	15 mm	15 mm	17 mm
9	12 mm	14 mm	14 mm	19 mm
10	11 mm	17 mm	15 mm	16 mm
11	13 mm	13 mm	13 mm	20 mm
12	10 mm	15 mm	14 mm	18 mm
13	12 mm	16 mm	15 mm	19 mm
14	11 mm	14 mm	12 mm	17 mm
15	13 mm	15 mm	14 mm	18 mm
MEAN	11.86 mm	15.133 mm	14.06 mm	17.93 mm

CHX- Chlorhexidine
NAC- N-acetyl Cysteine

TABLE II: Mean And Standard Deviation Ofinhibition Zones Of Tested Intracanal Medicaments

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group A: (PROPOLIS)	15	11.80	1.08	0.27	11.20	12.39	10.00	13.00
Group B: (CHX)	15	15.13	1.12	0.29	14.51	15.75	13.00	17.00
Group C: (NAC)	15	14.06	1.16	0.30	13.42	14.71	12.00	16.00
Group D: (NAC+CHX)	15	17.93	1.27	0.33	17.22	18.64	16.00	20.00
Total	60	14.73	2.49	0.32	14.08	15.37	10.00	20.00

One Way ANOVA Test: $F = 71.654$; $p < 0.001$; Highly significant
CHX- Chlorhexidine
NAC- N-acetyl Cysteine

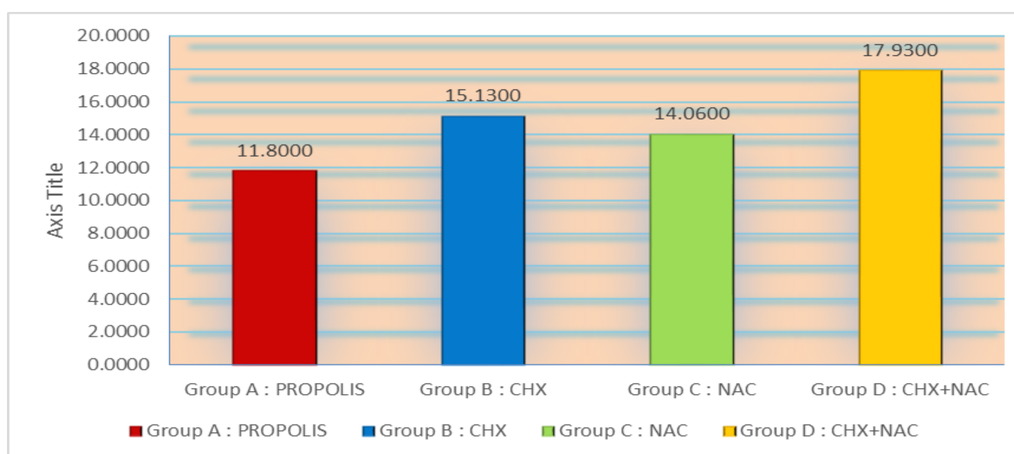


Figure I: Graphical representation of mean zone of inhibition between group A, B, C and D.

TABLE III: Comparison Between Group A And Group B Using One Way Anova With Post-Hoc Scheffe Test

	Mean	SD	Mean Difference	p value
Group A (PROPOLIS)	11.80	1.08	3.33	<0.001**
Group B (CHX)	15.13	1.12		

****p<0.001; Highly significant**

As per table V, statistical analysis by one way Anova with post-hoc Scheffe's test, p-value showed statistically highly significant difference (p<0.001) between group A (Propolis) and group B (CHX).

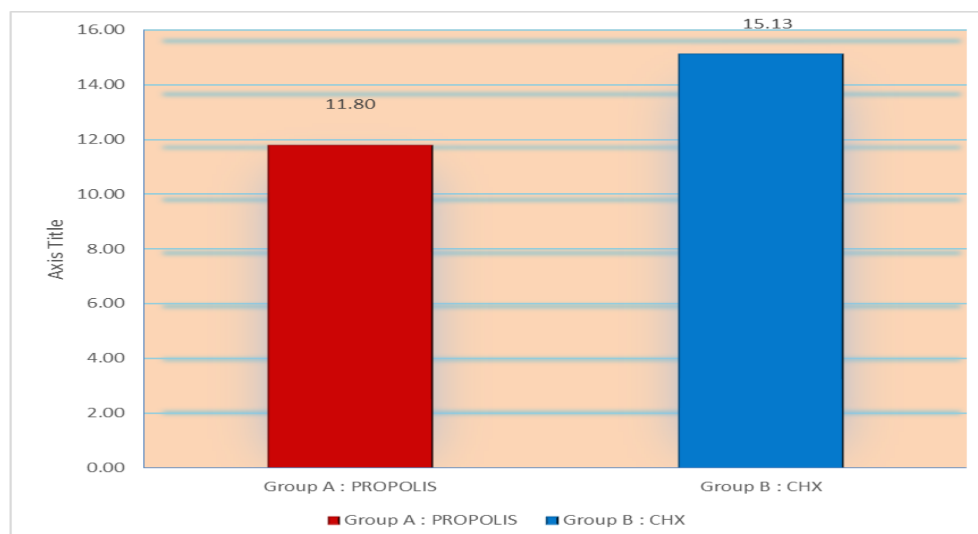


Figure II: Graphical representation of mean zones of inhibition between group A and B

TABLE IV: Comparison Between Group A And Group C Using One Way Anova With Post-Hoc Scheffe Test

	Mean	SD	Mean Difference	p value
Group A (PROPOLIS)	11.80	1.08	2.267	<0.001**
Group C (NAC)	14.06	1.16		

****p<0.001; Highly significant**

As per table VI, statistical analysis by one way Anova with post-hoc Scheffe's test, p-value showed statistically highly significant difference (p<0.001) between group A (Propolis) and group C (NAC).

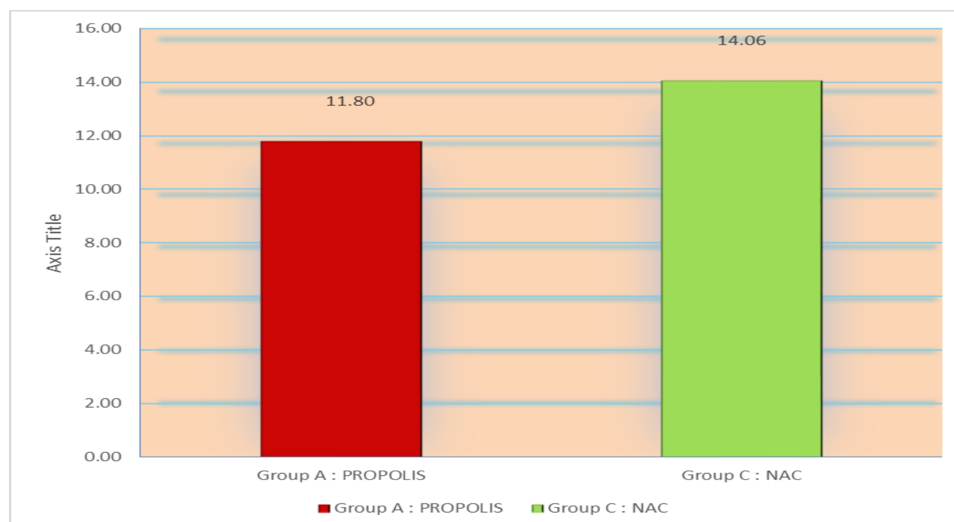


Figure III: Graphical representation of mean zones of inhibition between group A and C

TABLE V: Comparison Between Group A And Group D Using One Way Anova With Post-Hoc Scheffe Test

	Mean	SD	Mean Difference	p value
Group A (PROPOLIS)	11.80	1.08	6.133	<0.001**
Group D (CHX+NAC)	17.93	1.28		

$^{**}p<0.001$; Highly significant

As per table VII, statistical analysis by one way Anova with post-hoc Scheffe's test, p-value showed statistically highly significant difference ($p<0.001$) between group A (Propolis) and group D (CHX+NAC).

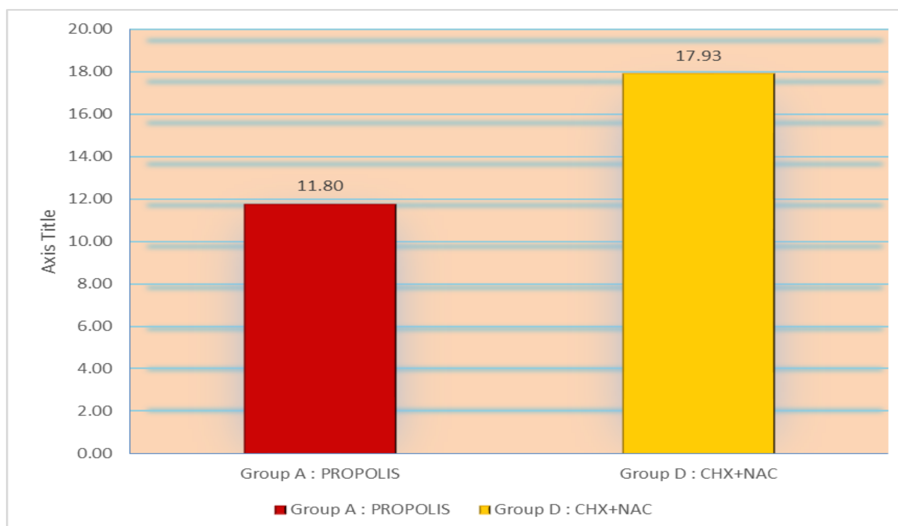


Figure IV: Graphical representation of mean zones of inhibition between group A and D

TABLE VI: Comparison Between Group B And Group C Using One Way Anova With Post-Hoc Scheffe Test

	Mean	SD	Mean Difference	p value
Group B (CHX)	15.13	1.12	1.067	0.111; NS
Group C (NAC)	14.06	1.16		

NS: $p>0.05$; Not significant

As per table VIII, statistical analysis by one way Anova with post-hoc Scheffe's test, p-value showed statistically insignificant difference ($p>0.05$) between group B (CHX) and group C (NAC).

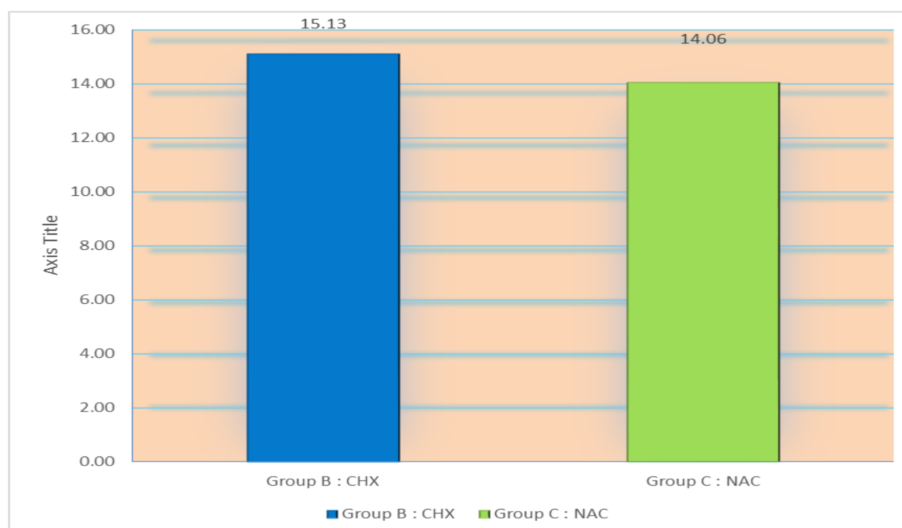


Figure V: Graphical representation of mean zones of inhibition between group B and C

TABLE VII: Comparison Between Group B And Group D Using One Way Anova With Post-Hoc Scheffe Test

	Mean	SD	Mean Difference	p value
Group B (CHX)	15.13	1.13	2.800	<0.001**
Group D (CHX + NAC)	17.93	1.28		

****p<0.001; Highly significant**

As per table IX, statistical analysis by one way Anova with post-hoc Scheffe's test, p-value showed statistically highly significant difference (p<0.001) between group B (CHX) and group D (CHX+NAC).

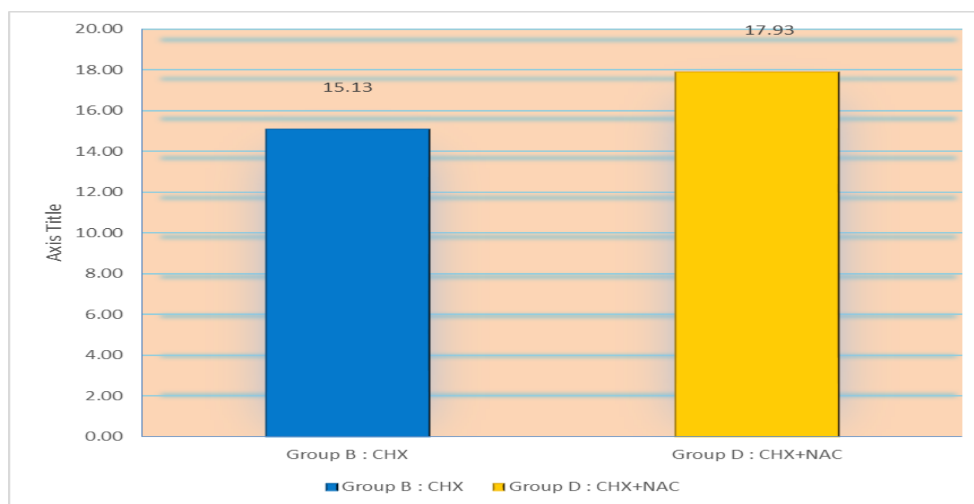


Figure VI: Graphical representation of mean zones of inhibition between group B and D

TABLE VIII: Comparison Between Group C And Group D Using One Way Anova With Post-Hoc Scheffe Test

	Mean	SD	Mean Difference	p value
Group C (NAC)	14.06	1.16	3.867	<0.001**
Group D (CHX + NAC)	17.93	1.28		

****p<0.001; Highly significant**

As per table X, statistical analysis by one way Anova with post-hoc Scheffe's test, p-value showed statistically highly significant difference (p<0.001) between group C (NAC) and group D (CHX+NAC).

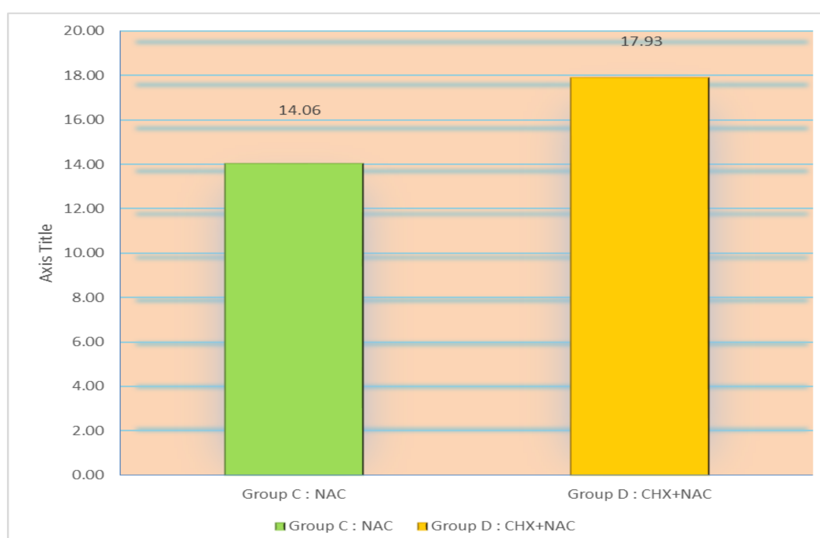


Figure VIII: Graphical representation of mean zones of inhibition between group C and D

TABLE IX: Inter Group Comparison For Zone Of Inhibition Using Post-Hoc Scheffe Test

Comparison	Mean Difference	P value	Significance
Group A vs B	3.33	<0.001	Highly significant
Group A vs C	2.267	<0.001	Highly significant
Group A vs D	6.133	<0.001	Highly significant
Group B vs C	1.067	0.111	Not significant
Group B vs D	2.800	<0.001	Highly significant
Group C vs D	3.867	<0.001	Highly significant

As per table IV, the intergroup comparison using post-hoc scheffe's test, the p value showed statistically significant difference between group A vs B, group A vs C, group A vs D, group B vs C, group C vs D and insignificant difference between group B vs C.

TABLE X: Percent Viability (%) Reduction In Number Of Colonies For Different Intracanal Medicaments At Different Time Intervals

TIME (hrs.)	GROUP A (PROPOLIS)	GROUP B (CHX)	GROUP C (NAC)	GROUP D (NAC + CHX)
0	100	100	100	100
2	72.4	57.6	64.4	43
4	54.2	18	35.6	14
6	40.4	14	21.4	6.2
8	26.8	3.2	15.2	1.4
10	29.6	1.2	7.4	0
12	12.4	0	0.8	0
24	0.8	0	0	0

CHX- Chlorhexidine
NAC- N-acetyl Cysteine

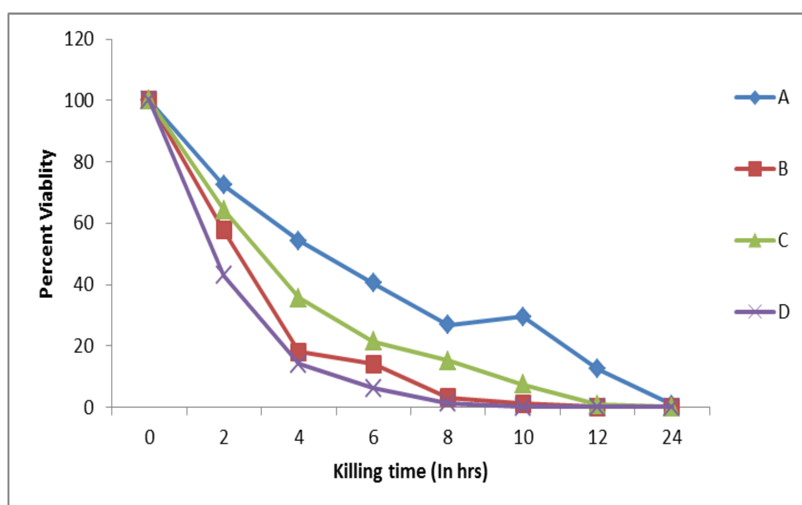


Figure VIII: Evaluation of efficacy of the different medicaments by viable cell count method. The test medicaments are projected on the x-axis while the percent viability is projected on the y-axis.

A – Propolis
B- Chlorhexidine (CHX)
C- N-acetyl cysteine (NAC)
D- (NAC+CHX)

DISCUSSION

The main causative factors in the development of pulpal and periapical inflammation are bacteria, and the success of endodontic treatment depends on the eradication of microorganisms from the root canal system and prevention of reinfection. Bacteria in the root canal are present either as free-floating planktonic single cells or biofilms, which are communities of microorganisms embedded in a self-produced polymeric matrix adherent to each other and/or to surfaces or interfaces (**Ricucci and Siqueira 2010; Jiang et al. 2011**).

Although endodontic treatment removes the majority of bacteria, it is difficult to completely eradicate them from the root canal system, and it is particularly difficult to remove sessile aggregates of multispecies bacterial biofilms formed on the root canal surfaces **(Ricucci and Siqueira 2010; Lee et al. 2013a).** Biofilm can be defined as a sessile, multi-cellular, microbial community characterized by cells that are firmly attached to a surface and enmeshed in a self-produced matrix of extra-cellular polymeric substance (EPS). EPS in biofilm are mostly composed of polysaccharides, but include other molecules such as proteins, extracellular DNA, and lipids (**Jachlewski et al. 2015**).

E.Faecalis a gram positive and facultative anaerobe, is one of the most prevalent bacterial strains in the root canal with endodontic failure, and is the most commonly isolated species from canals of teeth requiring postendodontic treatment. Enterococci possess a number of virulence factors that permit adherence to host cells and extracellular matrix, facilitate tissue invasion, effect immunomodulation and cause toxin-mediated damage. These factors include: (1) aggregation substance (AS), (2) enterococcal surface proteins such as esp, (3) gelatinase, (4) a cytolysin toxin, (5) extracellular superoxide production, (6) capsular polysaccharides and (7) antibiotic resistance determinant. E. faecalis can survive and grow at an alkaline environment (i.e., pH 9.6) and thus requires a higher pH to be inhibited. It has been shown that E. faecalis can be effectively killed or suppressed at a pH of 11.5 or higher.

In the present study E. faecalis was choosen as a bacterial marker due to the following reasons (1). Its resistance to many intracanal disinfectants is well documented **(da Silva et al. 2018)** (2). due to its strong association of it with failed root canal (3). extremly high recovery rates (approximately 70%) had been reported from persistent root canal infection **(Peciuliene et al. 2000)** (4) the organism is capable of surviving even under unusual stresses.(5) most commonly encountered in recalcitrant endodontic infections. (6) ability of E. faecalis to tolerate starvation, extremes of pH, salt concentration, biofilm formation, dentin tubular invasion, and emergence of antibiotic resistant strains.

Although the majority of bacteria are removed by mechanical and chemical methods during endodontic treatment, it is difficult to eradicate them completely from the root canal system. Hence, the use of intracanal medicaments to disrupt biofilms and thereby eradicate residual bacterial infections within root canals has been advocated to enhance the success of root canal treatment.

Calcium hydroxide and chlorhexidine have been widely used for this purpose due to their excellent biological and antimicrobial activities (**Lee et al. 2013b**). However, previous studies have demonstrated that the antimicrobial efficacy of calcium hydroxide varies depending on its location in the root canal **(Siqueira and Lopes 1999)** and is compromised by the buffering effect of dentin, The fact that chlorhexidine is inactivated by physiological salts and has limited ability to penetrate the deep layers of biofilms also limits its use as an intracanal medicament **(Portenier et al. 2002; Chavez de Paz et al. 2010).** It was reported that N-Acetyl cysteine inhibits the growth of E. faecalis and eradicates biofilms consisting of it (**Quah et al. 2012**).

Herbal products have been used, since ancient times in dental and medical practice, due to their high antimicrobial activity, excellent biocompatibility, anti-inflammatory, and antioxidant properties (**Podar et al. 2015**).

Therefore, this study was aimed to evaluate the antimicrobial efficacy of Chemical versus Herbal intracanal medicaments against Enterococcus faecalis and biofilms consisting of it. Susceptibility of both planktonic and biofilm form was checked through agar well diffusion test and biofilm inhibitory analysis, respectively.

Agar Diffusion Test

Initially, a qualitative assay was performed using agar well diffusion test to determine the efficacy of Propolis (Group A), 2% Chlorhexidine (Group B), N-acetyl cysteine (Group C) and combination of 2% Chlorhexidine and N-acetyl cysteine (Group D) against Enterococcus Faecalis.

In Group A, Propolis was used as intracanal medicament to check the antimicrobial activity against E. faecalis. It ranges from hard to brittle a freezing temperature to soft pliable at 20-30°C to sticky and gummy at temperature <45°. It becomes liquid at temperatures 60-70°C.

The antibacterial effect of propolis is bactericidal by inhibiting their mobility. Propolis kills the fungi and also the viruses while the growth of the

latter is also inhibited. It is effective against gram-positive rods in addition to Mycobacterium tuberculosis, with re-stricted activity against gram-negative bacilli **(Khurshid et al. 2017** and fungicidal properties better than fluconazole.

Propolis had significant antimicrobial activity against E. faecalis and suggested propolis to be used in endodontics (**Jahromi, Toubayani and Rezaei 2012). Saxena et al. (2011)** suggested that propolis has strong antimicrobial action against *E. faecalis* next to NaOCl

Recent studies have reported that propolis is more effective against resistant microrganisms and is biocompatible.

Current studies involving propolis had used different microbiological methods, such as agar dilution, agar well diffusion, agar disc diffusion techniques, as well as several concentrations of the propolis extract, which in general ranged from 11% to 30% weight to volume. Consequently, in Group A, we selected 11% of ethanolic extracts of Propolis as an intracanal medicament.

The ethanolic extract of propolis (EEP) shows high efficacy against the strains of bacteroides and Pepto-streptococcus but exhibits less efficiency against the strains of Clostridium, Eubacterium and Archnia. Ethanol, as the solvent of propolis, does not influence propolis antimicrobial effect.

Propolis extracts limit plaque formation on the tooth surface, which indirectly reduces dental caries.

The most common and reported side effect of propolis is allergy to the resinous wax-cum material. Allergic reactions may be seen as contact cheilitis, contact stomatitis, perioral eczema, labial edema, oral pain, peeling of lips, and dyspnea (**Malhotra and Gupta 2014**).

Chlorhexidine has been used as an intracanal medicament in Group B. CHX has been used in endodontics and proposed as both an irrigant and an intracanal medicament.

Its efficacy is because of the interaction of the positive charge of the molecule and the negatively charged phosphate groups on microbial cell walls **(Gomes et al. 2003a,b)**, thereby altering the cells' osmotic equilibrium. The most common oral preparation, CHX gluconate, is water soluble and at physiologic pH, it readily dissociates and releases the positively charged CHX component **(Greenstein et al. 1986)**. 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 **(Gomes et al. 2003a)**.

CHX has a broad-spectrum antimicrobial activity, targeting both gram-positive and gram-negative microbes.

At low concentrations of 0.005–0.01%, a stable monolayer of CHX is adsorbed and formed on the tooth surface, which might change the physical and chemical properties of the surface and may prevent or reduce bacterial colonization. At higher concentrations (> 0.02%), a multilayer of CHX is formed on the surface, providing a reservoir of chlorhexidine, and this multilayer can rapidly release excess CHX into the environment as the concentration of the CHX in the surrounding environment decreases. The antibacterial substantivity of three concentrations of CHX solution (4%, 2%, and 0.2%) after 5 min of application has been evaluated. The results have revealed a direct relationship between the concentration of CHX and its substantivity.

.

Only 2% CHX-containing medications were able to thoroughly eliminate most of both the 1-day and 3-day E. faecalis Biofilms.

In search of ideal root canal medicaments revealed another candidate, N-acetylcysteine (NAC). NAC was used as an intracanal medicament in Group C to evaluate its antimicrobial activity against E. faecalis. NAC a derivative of the amino acid L cysteine, is a potent thiol-containing antioxidant and mucolytic agent that disrupts disulfide bonds in mucus and reduces the viscosity of secretions **(El-Feky et al. 2009; Zhao and Liu 2010)**. It is widely used in medical treatment via inhalation and oral and intravenous routes of chronic bronchitis and acetaminophen overdose **(El-Feky et al. 2009; Silveira et al. 2013)**, and has an excellent safety profile **(Kao et al. 2003)**.

The exact mechanisms responsible for the antimicrobial and antibiofilm activities of NAC are still speculative. These speculations include (1) inhibition of cysteine utilization in bacteria, (2) reaction between the thiol group of NAC and bacterial cell proteins, (3) reduction of bacterial extracellular polymeric substances that are

responsible for bacterial adhesion and pathogenicity, and (4) disturbance of intracellular redox equilibrium with potential indirect effects on cell metabolism and intracellular signal transduction Pathways.

The biofilm disrupting activity of NAC is significantly higher than that of saturated calcium hydroxide or 2% chlorhexidine, the antimicrobial efficacy of NAC was not reduced by the presence of dentin powder for up to 14 days.

In addition NAC possesses anti-inflammatory effect on lipopolysaccharide -induced inflammatory responses and analgesic property for relieving postendodontic pain .

As a consequence, we have used combination of 2% Chlorhexidine and N-acetyl Cysteine in Group D to obtain the synergestic benefits of both the medicaments.

The techniques usually employed to assess antimicrobial efficacy include Broth Dilution, Agar Disc Diffusion, Agar Disc Dilution, Spiral Gradient Test, E-Test and Automated Antimicrobial Testing Systems. Each of these techniques has their own inherent advantages and disadvantages **(Aravind et al. 2006)**.

Broth Dilution method includes both Microdilution and Macrodilution. The advantage of this method is that it evaluates both quantitatively and qualitatively, whereas it is disadvantageous as chemical property of the material being tested can be altered and it is time consuming as well, especially when the clinician wants to evaluate the susceptibility of an organism to a number of antibiotics.

The Spiral Gradient Test, which is based on the agar dilution derivations, has the ability to test many organisms at a given time. Although, it is time saving, it is rather technique sensitive and needs special kits currently unavailable in India.

The E-Test, based on the agar diffusion derivations, offers the convenience of the disc diffusion procedure. It has the ability to generate minimum inhibitory concentration data, but is also technique sensitive like the previous test and needs special kits currently unavailable in India.

The Automated antimicrobial testing systems (also known as Vitek and Walkaway system), eliminates the need for overnight incubation and hence time saving. The limitations in these systems are that the chance of misinterpretation is high due to the turbid metric method of analysis and these are very expensive and currently unavailable in India.

In our study, Kirby-Bauer method (Agar Disc Diffusion method) was chosen instead of the Agar Dilution method. The agar well diffusion method or well plate method or the agar diffusion method was used in this study as it is the most commonly used method of antimicrobial activity determination especially of newer substances like plant extracts, new drug formulations, etc.

Fifteen petri dishes containing Brain Heart Infusion agar enriched with defibrinated sheep blood was seeded with E.faecalis isolates. BHI agar is a nutritious buffered culture medium that contains infusions of brain heart tissue along with peptones to supply proteins and other nutrients necessary to support the growth of microorganisms. It was used for cultivation of Enterococcus faecalis employed in the preparation of inoculate that were further used for antimicrobial activity testing.

The pathognic bacteria was grown in Triptone Soya Broth for 4 hours at 37°C, thereby Mc Farland standarized for its turbidity. The activated culture was swabbed onto BHI media supplemented with defibrinated blood (100µl). In each petri dish, four wells measuring 6mm in diameter were cut using sterile cork borer. The test medicaments (100µl) of each were delivered in the prepared wells through a micropipette. The petri dishes were incubated in upright position for 24hrs at 37°C. The antimicrobial activity of each extract was expressed in terms of diameter of zone of inhibition (in mm) produced by each extract at the end of incubation period and the diameter of the zones of the bacterial inhibition was measured in mm with a Digital Vernier Caliper.

The results of present study revealed that the mean values of inhibition zone for tested intracanal medicaments in Group A (Propolis), Group B (Chlorhexidine), Group C (NAC) and Group D (CHX+NAC) was 11.86mm, 15.13mm, 14.06mm and 17.93mm respectively **(Table I).**

The observations above showed maximum inhibition zone in Group D followed by Group B, Group C and Group A. The maximum zone of inhibition shown by combination of CHX and NAC may be attributed to synergestic effects of

antimicrobial activity of Chlorhexidine with NAC, an agent of proven mucolytic activity and antioxidant agent. In addition CHX has a wide range of antimicrobial activity. The minimum inhibition zone in Propolis may be due to difference in the medical preparation of propolis and its heterogeneous chemical composition, which may differ from area to area and from season to season, affecting its antimicrobial efficacy.

The results of the present study are in concurrence with **Palaniswamy et al. (2016)** who evaluted the effectiveness of N-acety cysteine, 2% CHX and their combination as intracanal medicaments on Enterococcus faecalis and found that 2%CHX and NAC showed almost equal zones of inhibition whereas their combination showed a maximum zone of inhibition suggesting a synergestic action.

An intergroup comparison for the zones of inhibition was done using *post-hoc* Scheffe's test, which revealed a statistically highly significant difference between Group A (Propolis) vs B (Chlorhexidine), Group A (Propolis) vs C (N-acetyl Cysteine), Group A (Propolis) vs D (CHX+NAC), Group B (CHX) vs D (CHX+NAC), Group C (NAC) vs D (CHX+NAC) (p value<0.001), whereas a statistically insignificant difference (P value = 0.11) was observed between Group B (CHX) and Group C (NAC)

The results of current study are in aggrement with **Mattigatti et al. (2012)** who found statistically significant difference between 2% CHX and Propolis when used as an intracanal medicament against E. faecalis. Also, the result of this study is in support with the study by **Kandaswamy et al. (2010)** where 2% chlorhexidine performed better than Propolis.

The results of this study are in consistent with those reported by **Ridhalaksani et al. (2017)** who compared the antibacterial potential of NAC and 2% CHX on isolates of E.faecalis biofilms and found that there was no statistically significant difference among the materials.

Biofilm Inhibitory Analysis

The antibacterial studies on agar plates are very common tests, but the microbial inhibition zone depends on the test substance's solubility and ability to diffuse through agar, so it may not express its full effective potential **(Mathew et al. 2015).** Therefore, the information obtained from agar diffusion studies does not reliably reflect their in vitro or in vivo antimicrobial activity. The results obtained from this test must be interpreted with caution, as this assay may not demonstrate the full clinical potential of the material being tested and does not distinguish microbiostatic and microbicidal properties of dental materials neither does it provide any information about the microorganisms viability after the test **(Mattigatti et al. 2012).**

For biofilm formation thirty intact, unrestored non carious mature human single rooted freshly extracted teeth were collected from The Department of Oral and Maxillofacial Surgery of Punjab Government Dental College and Hospital, Amritsar. Teeth were cleaned of debris and soft tissue remnants and were stored in normal saline solution at room temperature and used within three months after extraction. The teeth will be radiographed buccolingualy and mesiodistally to ensure that each tooth contains one canal.

All the samples were decoronated using a diamond disc in a low speed straight handpiece. Then working length for each root was recorded using a size 10 K-file. The root canals were instrumented using crown down technique with rotary instruments because it has better removal of bulk microorganisms at the coronal third to prevent accidental debris pushing into the apical part, enhanced volumes of irrigants can be placed into the canal more quickly than with other methods of instrumentation, such as the classic "Step Back" technique, reduces the hydrostatic pressure that can occur in the canal, gives better access to the apical part of the root canal, minimize loss of working length, better tactile sense is possible because restrictive dentin is removed coronally, less iatrogenic errors and more rapid instrumentation. Apical enlargement during canal cleaning and shaping procedures has the potential to eliminate more bacteria from the root canal system **(Vijaykumar 2010)**. So in the present study apical preparations were done up to F3 Protaper in all the specimens.

Sodium hypochlorite (NaOCl) in concentrations from 0.5% to 6% is the most commonly recommended irrigating solution**. Giardino et al. (2007) and Carson et al. (2005)** found that 5.25% NaOCl showed the highest antimicrobial activity. Therefore, 5.25% of sodium hypochlorite was used as an irrigating solution in the study. Moreover, smear layer was removed with 17% EDTA followed by irrigation with distilled water

to avoid the prolonged effect of EDTA and Sodium Hyochlorite solutions.

All the teeth will be vertically sectioned into two halves to obtain sixty samples. The concave tooth surface will be minimally grounded to achieve flat surface to enable placement in the tissue culture wells, exposing the root canal surface to E.faecalis to form a biofilm. Dentin was selected because it represents the primary substratum for bacterial adhesion and biofilm formation **(Palaniswamy et al. 2016)**. There are numerous advantages of using an in- vitro biofilm model, which include ease of modification if necessary, control of variables, low cost and ease of replication. They are also very useful in answering some initial but fundamental questions, providing preliminary data, which is essential for future confirmation by in vivo testing.

The sectioned tooth samples were divided into 4 experimental groups. Samples in each group were placed in tissue culture wells where every well was inoculated with 2ml of bacterial suspension. Tissue culture wells are flat-bottomed, cylindrical containers made of polystyrene. These well plates can be used to form static biofilms that are similar to biofilms formed in the root canal, which is a closed environment, not a dynamic system. The biofilms formed on well plates also share the same characteristics as mature biofilms, which are tolerant to antibiotics and resistant to the immune system (**Ridhalaksani et al. 2017**).

Bacterial adhesion has been suggested to occur in 2 main phases. Phase 1 is a physicochemical process which occurs within seconds to minutes, whereas phase 2 is considered as a biological cellular-molecular process of biofilm maturation, occurring in a time frame of few hours to few days **(Chai et al. 2013)**. Thus, in the present study dentin samples were inoculated with bacteria for the maximum period 2 weeks at 37°C to allow maturation of the biofilm structure on dentin.

After formation of the biofilm which was otherwise monitered everyday was gently washed using saline to remove the unattached bacteria and placed in culture wells containing the four test solutions ie. Propolis, 2% CHX, N-acetyl Cysteine and their combination. After 7 days the tooth samples were washed with saline solutions for 5 minutes.

Biofilm formed on root canal surface was removed using sterile scalpel and inoculated in 1ml of BHI broth. The inoculum was mixed together with the test medicament in equal proportions (1ml each). Spread plate method (using 10µl of above mixture) was performed in an interval of 2hrs starting from 0-24 hrs. This method is used to obtain isolated colonies and the plates were incubated at 37°C for 24hrs. The number of colonies were counted and compared to that of control where the test medicament was replaced with autoclave distilled water. The quantitative effect of the test medicaments were assayed by viable cell count method and expressed in terms of percent viability.

The results of the present study indicated that in Group A (Propolis) negligible percentage of (0.8%) colonies were found to be viable even at 24 hrs. While in Group B (2% Chlorhexidine) took 12hrs respectively for effective killing, and in Group C (N-Acetyl cysteine) took 24hrs for the same, while in Group D (CHX+NAC) complete killing was observed in 10 hrs of duration. **(Table X)**

From the results above it is evident that the combination of CHX and NAC correlated well with the results obtained from agar well diffusion assay where it showed maximum potential with a percent viability of 1.4% at 8hrs and complete inhibition thereafter.The probable reason for this may be the biofilm disruption action of NAC is due to its effect on exopolysaccharide (EPS) production, which is one of the major components in the biofilm. This can occur through several ways. First, the disulfide bonds of the bacterial enzyme involved in EPS production can be disrupted by the sulfhydryl group of NAC, or there can be an excretion through the thiol-disulfide exchange. Second, as NAC is antioxidant, it can exert an indirect effect on bacterial cell metabolism as well as on EPS production. The association of an antimicrobial substance CHX with an agent of proven mucolytic activity or a potential EPS disruptor such as NAC should enhance antimicrobial efficacy against biofilms.

Both CHX and NAC used in Group B and Group C showed complete inhibition at 12 hrs and 24 hrs respectively. However, Propolis was not effective enough to completly inhibit E. faecalis even after 24 hrs of treatment.

The data could not be subjected to statistical analysis as their is no viable bacteria left in all the

four test groups except the control i.e. autoclave distilled water.

The results obtained by viable cell count, were in line with the result obtained from agar well diffusion test.

From the disscusion above it may be infered that all tested intracanal medicaments showed antibacterial activity against planktonic and biofilm form of Enterococcus Faecalis. NAC has almost equal antimicrobial efficacy as 2% CHX and their combination shows synergestic effects. This clearly indicates that the combination of NAC and CHX will inhibit the growth of E.faecalis fastidiously as compared to CHX, NAC used alone as well as the herbal medicament Propolis. Chemical medicaments were more effective as compared to Propolis which is a herbal medicament. Here we also acknowledge the efficacy of Propolis as compared to the synthetic medicaments in terms of bioavailability and biosafety. Further exploration of combinational studies as depicted above might encompass better efficacy and lesser concentration emplyoed of medicaments and herbal resources as well. Hence combination of medicament (Chlorhexidine and NAC) may insight to better treatment practises.

However, before any definite conclusion can be drawn, clinical evaluation with larger number of samples and more extensive research with a definitive data distribution should be done to evaluate the antimicrobial potential of these intracanal medicaments in future.

SUMMARY & CONCLUSION

- ❖ Most of Earlier literature about endodontic disinfection has focused on killing of planktonic microorganisms which can be easily eliminated by a variety of different methods. Increased awareness of the role of biofilms in infections of the human body where a bacterial biofilm remain a major challenge has caused a shift in study design, so that biofilms rather than planktonic microorganisms are now used in studies of the efficacy of various disinfecting methods and agents.
- ❖ The present study was undertaken to evaluate the antimicrobial efficacy of herbal and chemical intracanal medicaments against planktonic and biofilm forms of Enterococcus faecalis. Among the herbal propolis was used and Chlorhexidine and N-acetyl cysteine alone and their combination was used as a chemical medicaments.
- ❖ In this in-vitro study initially a qualitative assay was performed using agar well diffusion test. Fifteen petri dishes containing Brain Heart Infusion agar enriched with defibrinated sheep blood was seeded with E.faecalis isolates.
- ❖ In each petri dish, four wells measuring 6mm in diameter were cut using sterile cork borer. The test medicaments (100µl) of each were delivered in the prepared wells and the petri dishes were incubated in upright position for 24hrs at 37°C. At the end of incubation period the diameter of zones of the bacterial inhibition was measured in mm with a Digital Vernier Caliper.
- ❖ The results of present study revealed that the mean value of inhibition zone of tested intracanal medicaments in Group A (Propolis), Group B (Chlorhexidine), Group C (NAC) and Group D (CHX+NAC) was 11.86mm, 15.13mm, 14.06mm and 17.93mm respectively **(Table I)**. The observations showed maximum inhibition zone in Group D followed by Group B, Group C and Group A.
- ❖ The data when subjected to statistical analysis using one way Anova and Post-hoc Scheffe's Test revealed a statistically highly significant difference between group A (Propolis) vs B (Chlorhexidine), group A (Propolis) vs C (N-acetyl Cysteine), group A (Propolis) vs D (CHX+NAC), group B (CHX) vs D (CHX+NAC), group C (NAC) vs D (CHX+NAC) (p value<0.001). Whereas a statistically insignificant difference (P value = 0.11) was observed between Group B (CHX) and Group C (NAC)
- ❖ After analyzing the efficacy of the test medicaments on the planktonic form of E.faecalis, its effect on the preformed biofilms was assesed through viable cell count method and percent viability. For biofilm formation thirty intact, unrestored non carious mature human single rooted freshly extracted teeth were collected.
- ❖ All the samples were decoronated using a diamond disc in a low speed straight handpiece. Then working length for each root was recorded and the root canals were instrumented using crown down technique with rotary instruments and apical preparations were done up to F3 Protaper. The root canals were irrigated using 5.25% of sodium hypochlorite and 17% EDTA, and finally rinsed with distilled water.

- All the teeth will be vertically sectioned into two halves to obtain sixty samples and. The sectioned tooth samples were divided into 4 experimental groups. Samples in each group were placed in tissue culture wells where every well was inoculated with 2ml of bacterial suspension.
- After formation of the biofilm which was otherwise monitered everyday was gently washed using saline and placed in culture wells containing the four test solutions ie. Propolis (Group A), 2% CHX (Group B), N-acetyl Cysteine (Group C) and their combination (Group D).
- Biofilm formed on root canal surface was removed using sterile scalpel and inoculated in 1ml of BHI broth.
- The inoculum was mixed together with the test medicament in equal proportions (1ml each). Spread plate method (using 10µl of above mixture) was performed in an interval of 2hrs starting from 0-24 hrs.
- The number of colonies were counted and compared to that of control where the test medicament was replaced with autoclave distilled water. The quantitative effect of the test medicaments were assayed by viable cell count method and expressed in terms of percent viability.
- The results of the present study demonstrated that in Group A (Propolis) negligible percentage of (0.8%) colonies were found to be viable even at 24 hrs. While in Group B (2% Chlorhexidine) took 12hrs respectively for effective killing, and in Group C (N-acetyl Cysteine) took 24hrs for the same, while in Group D (CHX+NAC) complete killing was observed in 10 hrs of duration
- The data could not be subjected to statistical analysis as their is no viable bacteria left in all the four test groups except the control i.e. autoclave distilled water. The results obtained were biologically significant but statistically insignificant.
- Under the condition of present study, it may be concluded that among all the tested medicaments Propolis was least effective as compared to the Chemical medicaments . This may be due to lack of standardized protocol for extraction of various herbal agents tested against various microorganisms, as well as the solvents & the concentration of the products used. Hence standardized protocol for extraction of herbal agents to make use of their benefits in Endodontics as root canal disinfectants is strongly recommended.
- Among the Chemical medicaments NAC has almost equal antimicrobial property as 2% CHX which opens new avenues to be used as newer medicament. The dyadic combination of 2% CHX and NAC exhibits a synergestic effect against plantonic and biofilm form of E.faecalis biofilms, may be recommended as an intacanal medicament.
- However, before any definite conclusion can be drawn, clinical evaluation with larger number of samples and more extensive research with a definitive data distribution should be done to evaluate the antimicrobial potential of these intracanal medicaments in future.

BIBLIOGRAPHY

1. Abdulrab, S, Halboub, E, Barngkgei, I & Al-Hebshi, N 2017, 'N-Acetylcysteine as a Candidate Therapeutic for Recurrent Aphthous and Aphthous-Like Ulcers', *Dental Hypotheses*, vol. 8, pp. 17-22.
2. Abul, N, Karthick, K, Mathew, S & Deepa, NT 2017, 'Herbal extracts in endodontics', *Journal of Indian Academy of Dental Specialist Researchers*, vol. 4, pp. 23-27.
3. Afzal, A, Gopal, VR, Pillai, R, Jacob, AS, U-Nu, S & Shan, S 2013, 'Antimicrobial activity of various irrigants against E. faecalis biofilm An in vitro study', *Journal of Interdisciplinary Dentistry*, vol. 3, no. 2, pp. 103-108.
4. Agrawal, A & Gpv, S 2016, 'Herbal Root Canal Irrigants - A Review', *Annals and Essences of Dentistry,* vol. 8, no. 3, pp. 7c-10c.
5. Akca, AE, Akca, G, Topçu, FT, Macit, E, Pikdöken, L & Özgen, I[a] 2016, 'The Comparative Evaluation of the Antimicrobial Effect of Propolis with Chlorhexidine against Oral Pathogens: An In Vitro Study', *BioMed Research International*, pp. 1–8.
6. Alharbi, A, Aliuddin, S, Alharbi, TA, Alharbi, S, Alanzi, AO & Alharbi, AA 2017, 'Herbal Endodontic Irrigants', *International Journal of Preventive and Clinical Dental Research,* vol. 4, no.4, pp. 311–314.
7. Al-Qathami, H & Al-Madi, E 2003, 'Comparison of sodium hypochlorite, propolis and saline as root canal irrigantsA pilot study', *Saudi Dental J*, Vol. 5, pp. 100-102.
8. Al-Shaher, A, Wallace, J, Agarwal, S, Bretz, WA & Baugh, D 2004, 'Effect of propolis on human fibroblasts from the pulp and

periodontal ligament', *J Endod,* vol. 30, no. 5, pp. 359-361.

9. Amaral, EP, Conceição, EL, Costa, DL, Rocha, MS, Marinho, JM, Cordeiro-Santos, M, D'Império-Lima, MR, Barbosa, T, Sher, A & Andrade, BB 2016, 'N-acetyl-cysteine exhibits potent anti-mycobacterial activity in addition to its known anti-oxidative functions', *BMC Microbiology*, vol. 16.
10. Amaral, EP, Conceição, EL, Costa, DL, Rocha, MS, Marinho, JM, Cordeiro-Santos, M, D'Império-Lima, MR, Barbosa, T, Sher, A & Andrade BB 2016, 'N-acetyl-cysteine exhibits potent anti-mycobacterial activity in addition to its known anti-oxidative functions', *BMC Microbiology*, vol. 16, pp. 1-11.
11. Aravind, Gopikrishna V, Kandaswamy D, Jeyavel & RK 2006, 'Comparitive evaluation of the antimicrobial efficacay of five endodontic root canal sealers against Enterococcus Faecalis and Candida Albicans', *J Cons Dent*, vol. 9, pp. 2-12.
12. Aslam, S & Darouiche, RO 2011, 'Role of Antibiofilm-Antimicrobial Agents in Controlling Device-Related Infections', *The International Journal of Artificial Organs*, Vol. 34, pp. 752–758.
13. Aslam, S, Trautner, BW, Ramanathan, V & Darouiche, RO 2007, 'Combination of Tigecycline and N-Acetylcysteine Reduces Biofilm-Embedded Bacteria on Vascular Catheters', *Antimicrobial Agents and Chemotherapy*, vol. 51, no. 4, pp. 1556–1558.
14. Athanassiadis, B, Abbott, PV & Walsh, LJ 2007, 'The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics', *Australian Dental Journal, vol.* 52, pp. 64–82.
15. Attia, DA, Farag, AM, Afifi, IK & Darrag, AM 2015, 'Antimicrobial effect of different intracanal medications on various microorganisms', *Tanta Dental Journal*, Vol. 12, pp. 41–47.
16. Baranwal, R, Duggi, V, Avinash, A, Dubey, A, Pagaria, S & Munot, H 2017, 'A Smart Supplement for an Intracanal Medicament', *International journal of Clinical Pediatric Dentistry*, vol. 10, no. 4, pp. 324-329.
17. Bechgaard, E, Ploug, E & Hjorth, N 1985 'Contact sensitivity to chlorhexidine', *Contact Dermatitis*, vol. 13, pp. 53–5.
18. Bohora, A, Hegde, V & Kokate, S 2010 'Comparison of the antibacterial efficiency of neem leaf extract and 2% sodium hypochlorite against E. faecalis, C. albicans and mixed culture - An in vitro study', Endodontology, vol. 22, no. 1, pp. 8-12.
19. Brailo, V, Boras, VV, Alajbeg, I & Juras, V 2006, 'Delayed contact sensitivity on the lips and oral mucosa due to propolis-case report', *Med Oral Patol Oral Cir Bucal,* vol. 11, no. 4, pp. 303-304.
20. Bui, TB, Baumgartner, JC & Mitchell, JC 2008, 'Evaluation of the Interaction between Sodium Hypochlorite and Chlorhexidine Gluconate and its Effect on Root Dentin', *Journal of Endodontics*, vol. 34, no. 2, pp. 181–185.
21. Calnan, CD 1962, 'Contact dermatitis from drugs', *Journal of the Royal Society of Medicine'*, vol. 55, pp. 39–42.
22. Carbajal Mejía, JB 2013, 'Antimicrobial effects of calcium hydroxide, chlorhexidine, and propolis on Enterococcus faecalis and Candida albicans', *Journal of Investigative and Clinical Dentistry,* vol. 4, pp. 1-7.
23. Carbajal Mejía, JB 2013, 'Antimicrobial effects of calcium hydroxide, chlorhexidine, and propolis on Enterococcus faecalis and Candida albicans', *Journal of Investigative and Clinical Dentistry,* vol. 4, pp. 1-7.
24. Carrilho, MR, Carvalho, RM & de Goes, MF 2007a, 'Chlorhexidine preserves dentine bond in vitro', *Journal of Dental Research,* vol. 86, pp. 90–4.
25. Carson, KR, Goodell, GG & McClanahan, SB 2005, 'Comparison of the antimicrobial activity of six irrigation primary endodontic pathogens', *J Endod*, vol. 31, pp. 471-3.
26. Chai WL, Hamimah H & Abdullah M 2013, 'Evaluation of Antimicrobial Efficacy of Antibiotics and Calcium Hydroxide against Enterococcus faecalis Biofilm in Dentine', *Sains Malaysiana,* Vol. 42, no.1, pp. 73–80.
27. Chávez de Paz, LE, Bergenholtz, G, Svensäter, G 2010, 'The effects of antimicrobials on endodontic biofilm bacteria', *J Endod,* vol. 36, no. 1, pp. 70-7.
28. Chávez De Paz, LE., Dahlén, G, Molander, A, Möller, A, & Bergenholtz, G 2003, 'Bacteria recovered from teeth with apical periodontitis after antimicrobial endodontic treatment', *Int Endod J,* vol. 36. pp. 500-508.
29. Choi, YS, Kim, C, Moon, JH & Lee, JY 2018, 'Removal and killing of multispecies endodontic biofilms by N-acetylcysteine', *brazilian journal of microbiology,* Vol. 49, pp. 184–188.

30. Clegg, MS, Vertucci, FJ, Walker, C, Belanger, M & Britto, LR 2006, 'The effect of exposure to irrigant solutions on apical dentine biofilms in vitro', *Journal of Endodontics,* vol. 32, pp. 434–7.
31. Costa, F, Sousa, D.M, Parreira, P, Lamghari, M, Gomes, P & Martins, M.C.L 2017, 'N-acetylcysteine-functionalized coating avoids bacterial adhesion and biofilm formation', *Scientific Reports,* vol. 7, pp. 1-13.
32. da Silva, TM, Alves, FR, Lutterbach, MTS, Paiva, MM & Ferreira, DdeC, 2018, 'Comparison of antibacterial activity of alexidine alone or as a final irrigant with sodium hypochlorite and chlorhexidine', *Journal of Endodontics*, vol. 34, no. 2, pp. 181-185.
33. Daga, P, Asrani, H, Farista, S & Mishra, P 2017, 'Comparative Evaluation of Antimicrobial Efficacy of Neem, Miswak, Propolis, and Sodium Hypochlorite against Enterococcus faecalis using EndoVac', *International Journal of Prosthodontics & Restorative Dentistry,* Vol. 7, no. 2, pp. 60–65.
34. Darrag, AM 2013, 'Antimicrobial efficacy of endodontic irrigation solutions against planktonic microorganisms and dual-species biofilm', *Tanta Dental Journal,* Vol. 10, pp. 129–137.
35. de Brito Costa, EMM, de Araújo Evangelista, AP, de Medeiros, ACD, Dametto, FR & de Carvalho, RA 2012, 'In vitro evaluation of the root canal cleaning ability of plant extracts and their antimicrobial action', *Brazilian Oral Research*, vol. 26, no. 3, pp. 215-221.
36. de Paz, LC 2007, 'Redefining the Persistent Infection in Root Canals: Possible Role of Biofilm Communities', *Journal of Endodontics,* Vol. 33, no. 6, pp. 652–662.
37. De, Rezende, GPDSR, Pimenta, FC & Costa, LRDRSD 2006, 'Antimicrobial activity of two brazilian commercial propolis extracts', *Brazilian Journal of Oral Sciences,* vol. 5, no. 16, pp. 967-970.
38. del Prado, G, Ruiz, V, Naves, P, Rodríguez-Cerrato, V, Soriano, F & del Carmen Ponte, M 2010, 'Biofilm formation by Streptococcus pneumoniae strains and effects of human serum albumin, ibuprofen, N-acetyl-l-cysteine, amoxicillin, erythromycin, and levofloxacin', *Diagnostic Microbiology and Infectious Disease*, vol. 67, pp. 311–318.
39. Delgado, RJR, Gasparoto, TH, Sipert, CR, Pinheiro, CR, de Moraes, IG, Garcia, RB, Duarte, MAH, Bramante, CM, Torres, SA, Garlet, GP, Campanelli, AP & Bernardineli, N 2013, 'Antimicrobial activity of calcium hydroxide and chlorhexidine on intratubular Candida albicans', Int J Oral Sci, vol. 5, no. 1, pp. 32-36.
40. Dinicola, S, Grazia, SD, Carlomagno, G & Pintucci, JP 2014, 'N-acetylcysteine as powerful molecule to destroy bacterial biofilms. A systematic review', *European Review for Medical and Pharmacological Sciences,* Vol. 18, pp. 2942-2948.
41. Domenech, M & García, E 2016, 'N -Acetyl-L-cysteine and Cysteamine: New Strategies Against Mixed Biofilms of Non-encapsulated Streptococcus pneumoniae and Non-typeable Haemophilus influenzae', *Antimicrobial Agents and Chemotherapy*, Vol. 61, no. 2.
42. Dukes, MN 1992, 'Meyler's Side Effects of Drugs: An Encyclopedia of Adverse Reactions and Interactions', *Amsterdam: Elsevier,* pp. 576–9.
43. Ehsani, M, Moghadamnia, A, Zahedpasha, S, Maliji, G, Haghanifar, S, Mir, SM, & Kani, NM 2012. The role of prophylactic ibuprofen and N-acetylcysteine on the level of cytokines in periapical exudates and the post-treatment pain. *Daru.* 20, 30.
44. El-Aziz, AAA, El-Banna, T, Sonbol, FI, Abo-Kamar, A & Seif-Eldin, DW 2012, 'Evaluation of the combination of N-acetylcysteine and or sodium salicylate with ciprofloxacin on bacterial adhesion and biofilm formation on urinary catheters', *The International Arabic Journal of Antimicrobial Agents*, vol. 2, pp. 1-12.
45. El-FEKY, MA, El-REHEWY, MS, Hassan, MA, Abolella, HA, El-BAKY, RMA & Gad, GF 2009, 'Effect of Ciprofloxacin and N-acetylcysteine on Bacterial Adherence and Biofilm Formation on Ureteral Stent Surfaces', *Polish Journal of Microbiology*, Vol.58, no. 3, pp. 261-267.
46. Ercan, E, Dalli, M, Dulgergil, CT 2006, 'In vitro assesement of the efficetiveness of chlorhexidine gel & Calcium hydroxide paste with chlorhexidine against Entero coccus faecalis & Candida Albicans; Oral Survery, Oral Pathology, Oral Radiology & Endodontics', Vol. 102, pp. 27-31.
47. Ercan, E, Dalli, M, Türksel Dülgergil, Ç & Yaman, F 2007, 'Effect of Intracanal Medication with Calcium Hydroxide and 1% Chlorhexidine in Endodontic Retreatment Cases with Periapical Lesions: An In Vivo

Study', *Journal of the Formosan Medical Association*, vol. 106, no. 3, pp. 217–224.

48. Ercan, N, Erdemir, E, Ozkan, S & Hendek, M 2015, 'The comparative effect of propolis in two different vehicles; mouthwash and chewing-gum on plaque accumulation and gingival inflammation', *European Journal of Dentistry*, vol. 9, no. 2, pp. 272-276.
49. Estrela, C, Silva, JA, Alencar, AHGD, Leles, CR & Decurcio, DA 2008, 'Efficacy of sodium hypochlorite and chlorhexidine against Enterococcus faecalis', *Journal of applied oral sciences,* vol. 16, no. 6, pp. 364-368.
50. Estrela, C, Sydney, GB, Figueiredo, JAP & Estrela CR de A 2009, 'Antibacterial efficacy of intracanal medicaments on bacterial biofilm: a critical review', *Journal of Applied Oral Science*, Vol. 17, no.1, pp. 1–7.
51. Evans, M, Davies, JK, Sundqvist, G, & Figdor, D 2002, 'Mechanisms involved in the resistance of Enterococcus faecalis to calcium hydroxide', *Int Endod J, vol.* 35, pp. 221–228.
52. Ferris, RA, McCue, PM, Borlee, GI, Loncar, KD, Hennet, ML & Borlee, BR 2016, 'In Vitro Efficacy of Nonantibiotic Treatments on Biofilm Disruption of Gram-Negative Pathogens and an In Vivo Model of Infectious Endometritis Utilizing Isolates from the Equine Uterus', *Journal of Clinical Microbiology,* Vol. 54, no. 3, pp. 631–639.
53. Gendron, R, Grenier, D, Sorsa, T & Mayrand, D 1999, 'Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine', *Clinical and Diagnostic Laboratory Immunology,* vol. 6, pp. 437–9.
54. Giardino, L, Ambu, E, Savoldi, E, Rimondini, R, Cassanelli, C & Debbia, EA 2007, 'Comparative evaluation of antimicrobial efficacy of sodium hypochlorite, MTAD, and Tetraclean against Enterococcus faecalis biofilm', *J Endod*, vol. 33, no. 7, pp. 852-5.
55. Gomes, BP, Sato, E, Ferraz, CC, Teixeira, FB, Zaia, AA & Souza- Filho, FJ 2003b, 'Evaluation of time required for recontamination of coronally sealed canals medicated with calcium hydroxide and chlorhexidine', *International Endodontic Journal*, vol. 36, pp. 604–9.
56. Gomes, BP, Souza, SF, Ferraz, CC, Teixeira, FB, Zaia, AA, Valdrighi, L, & Souza-Filho, FJ 2003, 'Effectiveness of 2% chlorhexidine gel and calcium hydroxide against Enterococcus faecalis in bovine root dentine in vitro*', Int Endod J,* vol. 36, pp. 267–275.
57. Gomes, F, Leite, B, Teixeira, P, Azeredo, J & Oliveira, R 2012, 'Farnesol in combination with N-acetylcysteine against Staphylococcus epidermidis planktonic and biofilm cells', *Brazilian Journal of Microbiology,* Vol. 43, pp. 235–242.
58. Goncalves, RD, Domingues, FHF, Onoda, HK, Guerisolp, DMZ, Pereira, KFS & Yoshinarp, GH 2012, 'Analysis of smear layer removal by different irrigants', *Rev Gaucha Odontol, Porto Alegre,* vol. 60, no. 2, pp. 157-61.
59. Greenstein, G, Berman, C & Jaffin, R 1986, 'Chlorhexidine: an adjunct to periodontal therapy', *Journal of Periodontology,* vol. 57, pp. 370–6.
60. Haapasalo, M, Qian, W, Porteiner, I, & Waltimo, T 2007, 'Effects of dentin on the antimicrobial properties of endodontic medicaments', *J Endod*, vol. 33, pp. 917-925.
61. Hamidi, MR, Mahmoudi, E, Moghadamnia, AA & Zahedpasha, S 2012, 'Effect of Calcium Hydroxide and Chlorhexidine Medicaments on the Apical Seal', *Iranian Endodontic Journal*, vol. 7, no. 1, pp. 15-19.
62. Hebling, J, Pashley, DH, Tja¨derhane, L & Tay, FR 2005, 'Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo', *Journal of Dental Research*, vol. 84, pp. 741–6.
63. Ibrahim, N, Zakaria AJ, Ismail, Z & Mohammmad, KS 2016, 'Antibacterial and Phenolic Content of propolis produced by two Malaysian Stingless Bees, Heterotrigona itama and Geniotrigona thoracica', *International Journal of Pharmacognosy and Phytochemical Research*, vol. 8, pp. 156-161.
64. Jachlewski, S, Jachlewski, WD, Linne, U, Bräsen, C, Wingender, J, & Siebers, B 2015, 'Isolation of extracellular polymeric substances from biofilms of the thermoacidophilic archaeon Sulfolobus acidocaldarius', *Frontiers in Bioengineering and Biotechnology,* vol. 27, pp. 123.
65. jahromi, MZ, Mirzakoucheki- Boroujeni, P, Daneshpour, S & Saei, MR 2015, 'Effect of triple Antibiotic paste and 2% chlorhexidine gel on shear bond strength of composite resin to dentin; an in vitro study', *Caspian Journal Of Dental Research*, vol. 4, pp. 8-9.
66. Jahromi, MZ, Toubayani, H & Rezaei, M 2012, 'Propolis: A New Alternative for Root Canal Disinfection', *Iranian Endodontic Journal,* Vol. 7, no. 3, pp. 127-133.

67. Jain, P & Ranjan, M 2014, 'Role of herbs in intracanal medicaments', *Int J Pharm Bio Sci*, vol. 5, no. 3, pp. 126-131.
68. Jain, P & Ranjan, M 2014, 'Role of herbs in root canal irrigation-A review', *IOSR Journal of Pharmacy and Biological Sciences*, vol. 9, no.2, pp. 06–10.
69. Jaiswal, N, Sinha, DJ, Singh, UP, Singh, K, Jandial, UA & Goel, S 2017, 'Evaluation of antibacterial efficacy of Chitosan, Chlorhexidine, Propolis and Sodium hypochlorite on Enterococcus faecalis biofilm : An in vitro study', *Journal of Clinical and Experimental Dentistry*, Vol. 9, no. 9, pp. E1066- 1074.
70. Jhajharia, K, Parolia, A, Shetty, KV & Mehta, LK 2015, 'Biofilm in endodontics: A review', *Journal of International Society of Preventive and Community Dentistry,* Vol no. 5, no. 1, pp. 1-12.
71. Jiang, LM, Hoogenkamp, MA, van der Sluis, LWM, Wesselink, PR, Crielaard, W, & Deng, DM 2011, 'Resazurin metabolism assay for root canal disinfectant evaluation on dual-species biofilms', *Journal of Endodontics*, vol. 37, pp. 31-35.
72. Jyothi, KN & Gopal, A, 2016, 'Comparison of Antimicrobial Efficacy of 0.3% Propolis, 10% Neem, 10% Triphala and 5% Sodium hypochlorite on Candida albicans and E. faecalis Biofilm formed on Root Dentin: An in–Vitro Study', *Research & Reviews: Journal of Dental Sciences*, Vol no. 4, no. 3, pp. 90-94.
73. Kala, DM & AR, DS 2017, 'Comparision of Healing of Periapical Lesion with Different Intracanal Medicament Using Mineral Trioxide Aggregate as Apical Barrier', *IOSR Journal of Dental and Medical Sciences*, vol. 16, no. 5, pp. 17–22.
74. Kandaswamy, D, Venkateshbabu, N, Gogulnath, D & Kindo, AJ 2010, 'Dentinal tubule disinfection with 2% chlorhexidine gel, propolis, morinda citrifolia juice, 2% povidone iodine, and calcium hydroxide', *Int Endod J,* vol. 43, no. 5, pp. 419-23.
75. Kao, LW, Kirk, MA, Furbee, RB, Mehta, NH, Skinner, JR, & Brizendine, EJ 2003, 'What is the rate of adverse events after oral *N*-acetylcysteine administered by the intravenous route to patients with suspected acetaminophen poisoning?', *Ann Emerg Med,* vol. 42, pp. 741–750.
76. Kashi, TSJ, Kermanshahi, RK, Erfan, M, Dastjerdi, EV, Rezaaei, Y & Tabatabae, FS 2011, 'Evaluating the in-vitro Antibacterial effects of Iranian Propolis on Oral Microorganisms', *Iranian Journal of Pharmaceutical Research*, vol. 10, no. 2, pp. 363-368.
77. Kayaoglu, G1, Ömürlü, H, Akca, G, Gürel, M, Gençay, Ö, Sorkun, K & Salih, B 2011, 'Antibacterial activity of Propolis versus conventional endodontic disinfectants against Enterococcus faecalis in infected dentinal tubules', *J Endod*, vol. 37, no. 3, pp. 376-81.
78. Khademi, AA, Mohammadi, Z & Havaee, A 2006, 'Evaluation of the antibacterial substantivity of several intra-canal agents', *Australian Endodontic Journal*, vol. 32, pp. 112–5.
79. Khurshid, Z, Naseem, M & Zohaib, S 2017, 'Propolis: A natural biomaterial for dental and oral healthcare, Journal of Dental Research', Dental Clinics, *Dental Prospects*, vol. 11, no. 4, pp. 265-274.
80. Kim Seow, W 1990, 'The effects of dyadic combinations of endodontic medicaments on microbial growth inhibition', *The American Academy of Pediatric Dentistry*, vol. 12, no. 5, pp. 292-297.
81. Kirmusaoðlu, S, Yurdugül, S & Koçoðlu, ME, 2012, 'The effect of N-acetylcysteine on growth and biofilm formation in Staphylococcus epidermidis strains', *Turkish Journal Of Medical Sciences*, Vol. 42. no. 4, pp.689-694.
82. Komorowski, R, Grad, H, Wu, XY & Friedman, S 2000, 'Antimicrobial substantivity of chlorhexidine-treated bovine root dentine', *Journal of Endodontics*, vol. 26, pp. 315–7.
83. Krautheim, AB, German, THM & Bircher, AJ 2004, 'Chlorhexidine anaphylaxis: case report and review of the literature', *Contact Dermatitis,* vol. 50, pp. 113–6.
84. Kumar, VM, Kumar, PR, Khanna, R & Agarwal, J 2014, 'The antimicrobial effectiveness of 25% propolis extract in root canal irrigation of primary teeth', *Journal of Indian Society of Pedodontics and Preventive Dentistry,* vol. 32, no. 2, pp. 120-124.
85. Kumar, N, Ahmad, KKM, Dang, R & Husain, A 2008, 'Antioxidant and antimicrobial activity of propolis from Tamil Nadu zone', *Journal of Medicinal Plants Research,* Vol. 2, no.12, pp. 361-364.
86. Kuþtarcý, A, Oktay, EA, Kýlýç, A, Özan, U & Altunbaþ, D 2011, 'Evaluation of antimicrobial efficacy of sodium hypochlorite, propolis, octenidine

dihydrochloride and chlorhexidine on microorganisms', *Cumhuriyet Dental Journal*, vol. 14, no. 3, pp. 183-190.

87. Lee, JK, Chang, SW, Perinpanayagam, H, Lim, SM, Park, YJ, Han, SH, Baek, SH, Zhu, Q, Bae, KS, & Kum, KY 2013a, 'Antibacterial efficacy of a human β-defensin-3 peptide on multispecies biofilms', *Journal of Endodontics,* vol. 39, pp. 1625–1629.
88. Lee, JK, Park, YJ, Kum, KY, Han, SH, Chang, SW, Kaufman, B, Jiang, J, Zhu, Q, Safavi, K, & Spångberg, L 2013b, 'Antimicrobial efficacy of a human β-defensin-3 peptide using an *Enterococcus faecalis* dentine infection model', *International Endodontic Journal,* vol. 46, pp. 406–412.
89. Leite, B, Gomes, F, Melo, P, Souza, C, Teixeira, P, Oliveira, R & Pizzolitto, E 2013, 'N-acetylcysteine and vancomycin alone and in combination against staphylococci biofilm', *Brazilian Journal of Biomedical Engineering*, Vol. 29, no. 2, pp. 184–192.
90. Lima, KC, Fava, LR & Siqueira, JFJr 2001, 'Susceptibilities of Enterococcus faecalis biofilms to some antimicrobial medications', *Journal of Endodontics*, vol. 27, pp. 616–9.
91. Lin, S, Zuckerman, O, Weiss, EI & Fuss, Z 2003a, 'Antibacterial efficacy of a new chlorhexidine slow-releasing device to disinfect dentinal tubules', *Journal of Endodontics,* vol. 29, pp. 416–8.
92. M, H, Thakur S & Sajjan, G 2011, 'Nisin vs Calcium Hydroxide- Antimicrobial efficacy on Enterococcus Faecalis- An In vitro study', *International Journal of Contemporary Dentistry*, Vol. 2, (3), pp.55-61.
93. Madhubala, MM, Srinivasan, N & Ahamed, S 2011 'Comparative Evaluation of Propolis and Triantibiotic Mixture as an Intracanal Medicament against Enterococcus faecalis', *Journal of Endodontics,* Vol no. 37, no. 9, pp.1287–1289.
94. Mahendra, M, Agrawal, N, Munaga, S & Tyagi, S 2016, 'Antimicrobial activity of different biological extracts as intracanal medicament against Enterococcus faecalis: An in vitro study', *Endodontology,* Vol. 28, no. 2, pp. 166-170.
95. Makkar, S, Aggarwal, A, Pasricha, S & Kapur, I 2015, 'Comparative evaluation of octenidine hydrochloride and chlorhexidine as antibacterial root canal irrigant', *Indian Journal of Oral Sciences*, Vol. 6, no.1, pp. 183-190.
96. Makkar, S, Aggarwal, A, Pasricha, S & Kapur, I 2015, 'Comparative evaluation of octenidine hydrochloride and chlorhexidine as antibacterial root canal irrigant', *Indian Journal of Oral Sciences*, Vol. 6, no.1, pp. 183-190.
97. Malhotra, S & Gupta, VK 2014, 'Use of propolis in pediatric dentistry', *Journal of Dental and Allied Sciences*, Vol. 3, pp. 93-98.
98. Marchese, A, Bozzolasco, M, Gualco, L, Debbia, EA, Schito, GC & Schito AM 2003, 'Effect of fosfomycin alone and in combination with N-acetylcysteine on E. coli biofilms', *International Journal of Antimicrobial Agents*, vol. 22, pp. 95–100.
99. Mathew, J, Pathrose, S, Kottoor, J, Karaththodiyil, R, Alani, M & Mathew, J 2015, 'Evaluation of an indigenously prepared herbal extract (endopam) as an antimicrobial endodontic irrigant: An ex vivo study', *Journal of International Oral Health*, vol. 7, no.6, pp. 88-91.
100. Mattigatti, S, Jain, D, Ratnakar, P, Moturi, S, Varma, S & Rairam, S 2012, 'Antimicrobial Effect of Conventional Root Canal Medicaments vs Propolis against Enterococcus faecalis, Staphylococcus aureus and Candida albicans', *The Journal of Contemporary Dental Practice*, vol. 13, no. 3, pp. 305–309.
101. Mehmandoust, P, Farhadmollashahi, N & Ghasemi, A 2016, 'Antibacterial efficacy of lavandula officinalis extract, sodium hypochlorite and chlorhexidine gluconate solutions as root canal irrigations: A comparative analysis', *Caspian J Dent Res,* vol. 5, pp. 14-20.
102. Mittal, R, Singla, MG, Garg, A, Gupta, S & Dahiya, V 2012, 'Comparative evaluation of the antimicrobial efficacy of MTAD, oxytetracycline, sodium hypochlorite and chlorhexidine against Enterococcus faecalis: An ex-vivo study', *Saudi Endodontic Journal*, vol. 2, no. 2, pp. 70-74.
103. Mohammadi, Z & Abbott, PV 2009, 'The properties and applications of chlorhexidine in endodontics', *International Endodontic Journal*, vol. 42, pp. 288–302.
104. Moon, JH, Choi, YS, Lee, HW, Heo, JS, Chang, SW, & Lee, JY 2016, 'Antibacterial effects of N-acetylcysteine against endodontic pathogens', *Journal of Microbiology,* Vol. 54, no. 4, pp. 322–329.
105. Moon, JH, Jang, EY, Shim, KS & Lee, JY 2015, 'In vitro effects of N-acetyl cysteine alone and in combination with antibiotics on

Prevotella intermedia', *Journal of Microbiology*, vol. 53, no. 5, pp. 321–329.

106. Mozayeni, MA, Haeri, A, Dianat, O & Jafari, AR 2014, 'Antimicrobial Effects of Four Intracanal Medicaments on Enterococcus Faecalis: An in Vitro Study', *Iranian Endodontic Journal*, vol. 9, no. 3, pp. 195-198.
107. Nalawade, TM, Bhat, KG & Sogi, S 2016, 'Antimicrobial Activity of Endodontic Medicaments and Vehicles using Agar Well Diffusion Method on Facultative and Obligate Anaerobes', *Int J Clin Pediatr Dent*, vol. 9, no. 4, pp. 335-341.
108. Nara, A, Dhanu, Chandra, P, Anandakrishna, L & Dhananjaya 2010, 'Comparative Evaluation of Antimicrobial Efficacy of MTAD, 3% NaOCl and Propolis Against E Faecalis', *International Journal of Clinical Pediatric Dentistry*, vol. 3, no. 1, pp. 21–25.
109. Neelakantan, P, Romero, M, Vera, J, Daood, U, Khan, A, Yan, A & Cheung, G 2017, 'Biofilms in Endodontics—Current Status and Future Directions', *International Journal of Molecular Sciences,* Vol. 18, no. 8, pp. 1-21.
110. Neha, S, Jyoti, L, Manoj, C & Ajay, S 2014, 'Chlorhexidine Gluconate – A Promising Endodontic Irrigant: A Review', *IOSR Journal of Dental and Medical Sciences*, vol. 13, no.1, pp. 40–46.
111. Nomura, M, Okano, M & Okada, N 1989, 'Four cases with anaphylaxis induced by chlorhexidine', *Archives of Dermatology*, vol. 125, pp. 50–2.
112. Ohtoshi, T, Yamauchi, N & Tadokoro, K 1986, 'Ig E antibody-mediated shock reaction caused by topical application of chlorhexidine', *Clinical Allergy,* vol. 16, pp. 155–61.
113. Olofsson, AC, Hermansson, M & Elwing, H 2003, 'N-Acetyl-L-Cysteine Affects Growth, Extracellular Polysaccharide Production, and Bacterial Biofilm Formation on Solid Surfaces', *Applied and Environmental Microbiology*, vol. 69, no. 8, pp. 4814–4822.
114. Oncag, O, Hosgor, M, Hilmioglu, S, Zekioglu, O, Eronat, C & Burhanoglu, D 2003, 'Comparison of antibacterial and toxic effects of various root canal irrigants', *International Endodontic Journal*, vol. 36, pp. 423–32.
115. Ong, TH, Chitra, E, Ramamurthy, S, Siddalingam, RP, Yuen, KH, Ambu, SP & Davamani, F 2017, 'Chitosan-propolis nanoparticle formulation demonstrates anti-bacterial activity against Enterococcus faecalis biofilms', *PLOS ONE*, vol. 12, no. 3, pp. 1/22- 22/22.
116. Osmundsen, PE 1982 'Contact dermatitis to chlorhexidine', *Contact Dermatitis*, vol. 8, pp. 81–3.
117. Palaniswamy, U, Lakkam, SR, Arya, S & Aravelli, S 2016, 'Effectiveness of N-acetyl cysteine, 2% chlorhexidine and their combination as intracanal medicaments on Enterococcus faecalis biofilm', *Journal of Conservative Dentistry,* vol. 19, pp. 17-20.
118. Pamplona-Zamenhan, LC, Pamplona, BC, da Silva C, Marcucci, MC & Mimica, LMJ 2012, 'Evaluation of the In Vitro Antimicrobial Activity of Ethanol extract of Brazilian Classified Propolis on Strains of Staphylococccus aureus'*, Brazilian Journal of Microbiology*, vol. 42, pp. 259-1264.
119. Peciuliene, V, Balciuniene, I, Eriksen, HM & Haapasalo, M, 2000, 'Isolation of Enterococcus faecalis in Previously Root-Filled Canals in a Lithuanian Population', *Journal of Endodontics,* Vol. 26, no. 10, pp. 593-595.
120. Pei, Y, Liu, H, Yang, Yi, Yang, Y, Jiao, Y, Tay, FR & Chen, J 2018, 'Biological Activities and Potential Oral Applications of N-Acetylcysteine: Progress and Prospects', *Oxidative Medicine and Cellular Longevity,* pp. 1–14.
121. Perez-Giraldo, C, Rodríguez-Benito, A, Morán, FJ, Hurtado, C, Blanco MT & Gómez-García AC 1997, 'Influence of N-acetylcysteine on the formation of biofilm by Staphylococcus epidermidis', *Journal of Antimicrobial Chemotherapy,* vol. 39, pp. 643–646.
122. Pimenta, HC, Violante, IMP, Musis, CR de, Borges, ÁH & Aranha, AMF 2015, 'In vitro effectiveness of Brazilian brown propolis against Enterococcus faecalis', *Brazilian Oral Research*, vol. 29, no.1, pp. 1–6.
123. Plutzer, B, Zilm, P, Ratnayake, J & Cathro, P 2018, 'Comparative efficacy of endodontic medicaments and sodium hypochlorite against Enterococcus faecalis biofilms', *Australian Dental Journal,* vol. 63, pp. 208–216.
124. Podar, R, Kulkarni, GP, Dadu, SS, Singh, S & Singh, SH 2015, 'In vivo antimicrobial efficacy of 6% Morinda citrifolia, Azadirachta indica, and 3% sodium hypochlorite as root canal irrigants', *European Journal of Dentistry,* Vol. 9, pp. 529-34.

125. Portenier, I, Haapasalo, H, Orstavik, D, Yamauchi, M, & Haapasalo, M 2002, 'Inactivation of the antibacterial activity of iodine potassium iodide and chlorhexidine digluconate against Enterococcus faecalis by dentin, dentin matrix, type-I collagen, and heat-killed microbial whole cells', *J Endod, vol.* 28, pp. 634–637
126. Portenier, I, Haapasalo, H, Rye, A, Waltimo, T, Orstavik, D & Haapasalo, M 2001, 'Inactivation of root canal medicaments by dentine, hydroxylapatite and bovine serum albumin', *International Endodontic Journal*, vol. 34, pp. 184–8.
127. Portenier, I, Waltimo, TMT & Haapasalo, M 2003, 'Enterococcus faecalis- the root canal survivor and "star" in post-treatment disease', *Endodontic Topics*, vol. 6, pp. 135–159.
128. Quah, SY, Wu, S, Lui, JN, Sum, CP & Tan, KS 2012, 'N-Acetylcysteine Inhibits Growth and Eradicates Biofilm of Enterococcus faecalis', *Journal of Endodontics,* Vol. 38, no. 1, pp. 81–85.
129. Ramani, N & Mathew, S 2012, 'Comparative evaluation of antimicrobial efficacy of chlorhexidine digluconate and propolis when used as intracanal medicament: ex vivo study', *J Int Oral Health*, vol. 13, pp. 223-228.
130. Ramos, IF, Biz, MT, Paulino, N, Scremin, A, Della Bona, A & Barletta, FB 2012, 'Histopathological analysis of corticosteroid-antibiotic preparation and propolis paste formulation as intracanal medication after pulpectomy', An in vivo study, *J Appl Oral Sci*, vol. 20, pp. 50-6.
131. Rezende, GPDSRD, Costa, LRDRSD, Pimenta, FC & Baroni, DA 2008, 'In vitro Antimicrobial Activity of Endodontic Pastes with Propolis Extracts and Calcium Hydroxide: A Preliminary Study', *Brazilian Dental Journal*, vol. 19, no. 4, pp. 301-305.
132. Ricucci, D & Siqueira, JFJr 2010, 'Biofilms and apical periodontitis periodontitis: study of prevalence and association with clinical and histopathologic findings', *Journal of Endodontics,* vol. 36, pp. 1277–1288.
133. Ridhalaksani, R, Nazar, K, Djauharie, NK, Meidyawati, R & Npa, DA 2017, 'The antibacterial potential of N- acetyl cysteine as an endodontic irrigant on the clinical isolates of the Enterococcus Faecalis Biofilm', *International Journal of Applied Pharmaceutics,* Vol. 9, no. 2, pp. 17-19.
134. Rôças, IN & Siqueira, JF 2011, 'Comparison of the In Vivo Antimicrobial Effectiveness of Sodium Hypochlorite and Chlorhexidine Used as Root Canal Irrigants: A Molecular Microbiology Study', Journal of Endodontics, vol. 37, no. 2, pp. 143–150.
135. Rouhani, A, Erfanzadeh, M, Jafarzadeh, H & Najafi, E 2017, 'Comparison of Residual Triple Antibiotic Paste, Propolis and Calcium Hydroxide on Root Canal Walls in Natural Open Apex Teeth: An In Vitro Study', *Iranian Endodontic Journal*, vol. 13, no. 1, pp. 25-29.
136. Saxena, P, Pant, VA, Wadhwani, KK, Kashyap, MP, Gupta, SK & Pant, AB 2011, 'Potential of the propolis as storage medium to preserve the viability of cultured human periodontal ligament cells: an in vitro study', *dental traumatology*, vol. 27, no. 2, pp. 102-108.
137. Shahani, MN & Reddy, SVV 2011, 'Comparison of antimicrobial substantivity of root canal irrigants in instrumented root canals up to 72h: An in vitro study', *Journal of Indian Society of pedodontics and preventive dentistry,* vol. 29, no. 1, pp. 28-33.
138. Shen, Y, Stojicic, S, Qian, W, Olsen, I & Haapasalo, M 2010, 'The Synergistic Antimicrobial Effect by Mechanical Agitation and Two Chlorhexidine Preparations on Biofilm Bacteria', *Journal of Endodontics*, vol. 36, no. 1, pp. 100–104.
139. Shrivastava, R, Rai, VK, Kumar, A, Sinha, S, Tripathi, P, Gupta, K & Sabharwal, S 2015, 'An in vitro Comparison of Endodontic Medicaments Propolis and Calcium Hydroxide alone and in Combination with Ciprofloxacin and Moxifloxacin against Enterococcus Faecalis', *The Journal of Contemporary Dental Practice*, vol. 16, no. 5, pp. 394–399.
140. Shruthi, E & Suma, BS 2012, 'Health from the hive: potential uses of propolis in general health', *Int J Clin Med*, vol. 3, no. 3, pp. 159-162.
141. Silva, FB, Almeida, JM & Sousa, SM 2004, 'Natural medicaments in endodontics — a comparative study of the anti-inflammatory action', *Braz Oral Res,* Vol. 18, pp. 174-9.
142. Silveira, LF, Baca, P, Arias-Moliz, MT, Rodríguez-Archilla, A, & Ferrer-Luque, CM 2013, 'Antimicrobial activity of alexidine alone and associated with N-acetylcysteine against *Enterococcus faecalis* biofilm', *Int. J. Oral Sci, vol.* 5, pp. 146–149.
143. Siqueira, JFJr & Lopes, HP 1999, 'Mechanisms of antimicrobial activity of calcium hydroxide: a critical review',

International Endodontic Journal, vol. 32, pp. 361–369.

144. Siqueria, ABS, Gomes, BS, Cambium, I, Maia, R, Abreu, S, Souza-Motta CM,de Queiroz, LA & Porto, ALF 2009, 'Trichophyton species susceptibility to green and red propolis from Brazil', *Letters in applied Microbiology*, vol. 48, pp. 90-96.
145. Snellman, E & Rantanen, T 1999, 'Severe anaphylaxis after a chlorhexidine bath', *Journal of the American Academy of Dermatology,* vol. 40, pp. 771–2.
146. Stewart, PS & William Costerton, J 2001, 'Antibiotic resistance of bacteria in biofilms', *The Lancet*, vol. 358, pp. 135–138.
147. Tatnall, FM, Leigh, IM & Gibson, JR 1990, 'Comparative study of antiseptic toxicity on basal keratinocytes, transformed human keratinocytes and fibroblasts', *Skin Pharmacology,* vol. 3, pp. 157–63.
148. Tervit, C, Paquette, L, Torneck, CD, Basrani, B & Friedman, S 2009, 'Proportion of Healed Teeth With Apical Periodontitis Medicated With Two Percent Chlorhexidine Gluconate Liquid: A Case-Series Study', Journal of Endodontics, vol. 35, no. 9, pp. 1182–1185.
149. Tirali, RE, Bodur, H & Ece, G 2012, 'In vitro antimicrobial activity of Sodium hypochlorite, Chlorhexidine gluconate and Octenidine Dihydrochloride in elimination of microorganisms within dentinal tubules of primary and permanent teeth', *Med Oral Patol Oral Cir Bucal*, vol. 17, no. 3 pp. 517-22.
150. Torricelli, R & Wuthrich, B 1996, 'Life-threatening anaphylactic shock due to skin application of chlorhexidine', *Clinical and Experimental Allergy,* vol. 26, pp. 112–3.
151. Ulusoy, AT, Kalyoncuoglu, E, Reis, A & Cehreli, ZC 2016, 'Antibacterial effect of N-acetylcysteine and taurolidine on planktonic and biofilm forms of Enterococcus faecalis', *Dental Traumatology,* vol. 32, pp. 212-218.
152. Üstün, Y, Arslan, S & Aslan, T, 2013, 'Effects of calcium hydroxide and propolis intracanal medicaments on bond strength of resin-based endodontic sealer as assessed by push-out test', *Dental Materials Journal*, vol. 32, no. 6, pp. 913–919.
153. Vasconcelos, WA , Braga, NMA, Chitarra VR, Santos VR, Andrade AL & Domingues RZ 2014, 'Bioactive Glass-Green and Red Propolis Association Antimicrobial Activity Against Oral Pathogen Bacteria', *Natural Products and Chemical Research*, vol. 2, no.6, pp. 1-5.
154. Vasudeva, A, Sinha, DJ, Tyagi, SP, Singh, NN, Garg, P & Upadhyayd D 2017, 'Disinfection of dentinal tubules with 2% Chlorhexidine gel, Calcium hydroxide and herbal intracanal medicaments against Enterococcus faecalis: An in-vitro study', *Singapore Dental Journal*, vol. 38, pp. 39-44.
155. Victorino, FR, Bramante, CM, Watanabe, E, Ito, IY, Franco, SL & Hidalgo, MM 2009, 'Antibacterial activity of propolis-based toothpastes for endodontic treatment', *Brazilian Journal of Pharmaceutical Sciences*, vol. 45, no. 4, pp. 795–800.
156. Victorino, FR, Franco, SL, Svidzinski, TIE, Avila-campos, MJ, Cuman, RKN, Hidalgo, MM & Bersani-Amado, CA 2007 'Pharmacological evaluation of propolis solutions for endodontic use', *Pharm Biol*, vol. 45, pp. 721-727.
157. Vijaykumar, S, GunaShekhar, M & Himagiri, S 2010, 'In vitro effectiveness of different endodontic irrigants on the reduction of Enterococcus faecalis in root canals', *Journal of Clinical Experimental Dentistry*, vol. 2, no. 4, pp. 169-72.
158. Viuda-Martos, M, Ruiz-Navajas, Y, Fernández-López, J & Pérez-Alvarez, JA 2008, 'Functional properties of honey, propolis, and royal jelly', *J food Sci*, vol. 73, no. 9, pp. 117-24.
159. Waldner-Tomic, N, Vanni, R, Belibasakis, G, Thurnheer, T, Attin, T & Schmidlin, P 2014, 'The in Vitro Antimicrobial Efficacy of Propolis against Four Oral Pathogens: A Review', *Dentistry Journal*, vol. 2, pp. 85–97.
160. Waltimo, TM, Ørstavik, D, Siren, EK & Haapasalo, MP 1999, 'In vitro susceptibility of Candida albicans to four disinfectants and their combinations' *International Endodontic Journal*, vol. 32, pp. 421–9.
161. Zhao, T & Liu, Y 2010, '*N*-acetylcysteine inhibit biofilms produced by Pseudomonas aeruginosa', *BMC Microbiol*, vol. 10, pp. 140.
162. Zirwas, MJ & Otto, S 2010, 'Toothpaste Allergy Diagnosis and Management' *The Journal of Clinical and aesthetic dermatology*, vol. 3, no. 5, pp. 42-47.