BHARATH INSTITUTE OF HIGHER EDUCATION AND RESEARCH
(Declared as Deemed-to-be University under section 3 of UGC Act 1956)
#173, Agharam Road Selaiyur Chennai 600 073 Tamilnadu, INDIA

ANTIBACTERIAL EFFICACY OF TEA TREE OIL IN COMBINATION WITH VARIOUS SURFACTANTS AGAINST ENTEROCOCCUS FAECALIS: AN IN VITRO STUDY

BY

Dr. NIVASHINI G S V

Dissertation submitted to the

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MASTER OF DENTAL SURGERY

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CONSERVATIVE DENTISTRY AND ENDODONTICS

SREE BALAJI DENTAL COLLEGE & HOSPITAL

CHENNAI – 600 100

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DECLARATION BY THE CANDIDATE

I hereby declare that this Thesis titled **"ANTIBACTERIAL EFFICACY OF TEA TREE OIL IN COMBINATION WITH VARIOUS SURFACTANTS AGAINST ENTEROCOCCUS FAECALIS AN IN VITRO STUDY"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. A. VENKATESH, M.D.S.,** Professor, Department of Conservative dentistry and Endodontics, Sree Balaji Dental College and Hospital, Chennai.

Signature of the Candidate

Dr. NIVASHINI G S V

Date: 20-04-23

Place: Chennai

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EVALUATION REPORT OF THE RESEARCH PROPOSAL

Name, Designation & Dept of the Principal Investigator: Dr. Nivashini G.S.V, PG student, Conservative Dentistry & Endodontics.
Name, Designation & Dept of the Co- Investigator: Dr. A.Venkatesh, Professor, Conservative Dentistry & Endodontics.

Title of the proposed research: Antibacterial efficacy of various surfactants with Tea Tree oil against Enterococcus faecalis - An *In Vitro* study.

IRB - Approval Number: SBDCH - IRB/ 22-06/06.

The Institutional Review Board had reviewed your application with the necessary documents to conduct the study entitled "Antibacterial efficacy of various surfactants with Tea Tree oil aginst Enterococcus faecalis - An *In Vitro* study." on 7th July 2022.

The following documents were reviewed:

i. Research proposal submitted by the Principal Investigator.
ii. Patient Information Sheet and Informed Consent Form in English and/or vernacular language.
iii. Questionnaire / clinical trial protocol.
iv. Advertisements/ information brochure to be used for recruiting the patients for the purpose of your research/study.
v. Insurance Policy / Compensation for participation and for serious adverse events occurring during the study participation.
vi. Investigator's Agreement with the Sponsor(s).
vii. Principal Investigator's and Co-Investigators- *Curriculum Vitae*.
viii. Investigator(s) - self-declaration form

The following members of the Institutional Review Board were present at the IRB meeting at Lakshmi Ammal auditorium, SBDCH on 7th July 2022 at 10.00 a.m.

Dr. S. Jimson, Convener.
Dr. Badri Thiruvenkatachari, Chairman.
Dr. K. Padmavathy, Member secretary.

Dr. S. Raghavendra Jayesh, clinician- Prosthodontics, Dr. A. Ponnudurai, clinician-Pedodontics, Dr. A. Radhakrishnan, clinician-General Medicine, Dr. C. Sumathi Jones- Scientific Member -Pharmacology, Dr. Vijay Ebenezer, clinician- Oral surgery, Dr. N. Aravinda Babu- clinician-Oral pathologist, Dr. V. Prakash, clinician-Endodontics, Dr. T. Sarumathi, clinician-Oral Medicine & Radiology, Dr. Anitha Balaji, clinician-Periodontics, Dr. M. Anita, clinician-Public Health dentistry.

The Institutional Review Board approves the study to be conducted in the form presented by the PI.

The Institutional Review Board hereby advices that the PI / Co-I(s) / researcher, should get approval from the Institute's Ethics Committee prior to commencement of the study and should maintain "confidentiality" *vis-a-vis* subjects.

The Institutional Review Board expects to be informed about the half-yearly progress of the study, changes in the research/study protocol (if any) and to submit a copy of the final report of the research study.

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Dr. NIVASHINI GSV

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INTRODUCTION

Microbial infection in the root canal system causes an inflammatory response in the periapical tissues. The goal of root canal therapy is to retain healthy periapical tissues or to promote their healing, in teeth that can be restored and have adequate periodontal support [1]. The number of microorganisms, their virulence, and host reactions all have an impact on the severity of periapical inflammation and symptoms. However, certain microbial species are able to penetrate the dentine tubules to variable depths. Microbial biofilm growth is commonly detected on root canal walls. Root canal sample for microbiological diagnosis is advised in cases of persistent and complex infections or when there is a possibility of systemic transmission of infection [2]. In primary infections, anaerobic gram-negative rods are often identified microbes. In post-treatment condition, facultatively anaerobic gram-positive cocci and rods such Streptococcus, Enterococcus, Peptostreptococcus, and Actinomyces species predominate in the microflora. The removal of microbial species from the infected root canal system requires performing instrumentation, disinfection, and interappointment medicament under strictly controlled aseptic circumstances [3]. In the past, treatment-resistant infections have frequently been associated with Enterococcus faecalis and Candida albicans [4].

Enterococci can appear alone, in pairs, or in short chains. They are facultative anaerobes, meaning they can grow either with or without oxygen [5,6]. The human intestinal lumen is the habitat to several Enterococcus species, which have 105–108 colony-forming units (cfu) per gram of faeces and, in most cases, do no harm to their hosts. In smaller numbers they are also found in the oral cavity and the female genital tracts of humans [7]. Carbohydrates, glycerol, lactate, malate, citrate, arginine, agmatine, and numerous keto acids are among the various energy sources that they catabolize. Extremely abrasive

conditions, such as salt concentrations and a pH of 9.6, do not kill enterococci. They are resistant to ethanol, azide, heavy metals, detergents, bile salts, and desiccation. They proliferate between 10 and 45 degrees Celsius and sustain 60 degrees Celsius for 30 minutes. There are now 23 species of enterococci and based on how they interact with mannitol, sorbose, and arginine, these are separated into five groups. E. faecalis is a member of the same family as E. faecium E. Casseliflavus, E.mundtii, and E.gallinarum. These five species hydrolyze arginine and produce acid in mannitol broth, but they are unable to do so in sorbose broth [5]. Except for a few atypical forms, E.faecalis is the only member of the group to use pyruvate and to an extent tolerate tellurite. It is also arabinose negative. More recently, molecular methods that can quickly and precisely identify the Enterococcus species have been created. For taxonomic purposes, methods such as DNA-DNA hybridization, 16S rRNA gene sequencing, whole-cell protein (WCP) analysis, and gas liquid chromatography of fatty acids have been utilised. The majority of these techniques are based on nucleic acids and use PCR amplification tests, followed by electrophoretic examination of the PCR products, probing, sequencing, or a combination of the two [8]. In addition to lytic enzymes, cytolysin, aggregation material, pheromones, and lipoteichoic acid, E. faecalis also possesses other virulence factors. It has been demonstrated that it can attach to host cells and produce proteins that enable it to outcompete other bacterial cells, and modify host reactions [7]. Because E. faecalis has the ability to inhibit lymphocyte activity, endodontic failure may result. The virulence factors that E. faecalis possesses are just one aspect of its diversity [9]. It can also pass along similar virulence features to other species, which helps it survive and spread disease. It is capable of withstanding protracted hunger until a sufficient nutritional supply is made available. When serum becomes available, hungry cells can recover by utilising it as a food supply, which comes from the periodontal ligament and alveolar bone [10]. E.faecalis adhere to collagen I type [11]. E. faecalis has been

demonstrated to withstand calcium hydroxide intracanal dressings for more than ten days [12,13]. E. faecalis can create a biofilm that protects it from being destroyed. By allowing bacteria to develop resistance to phagocytosis, antibodies, and antimicrobials 1000 times greater than that of nonbiofilm generating organisms [14]. Numerous studies have been conducted in an effort to identify an efficient a means of eliminating or preventing E. faecalis from accessing the area of a root canal. It is possible for E.faecalis to enter the root canal system throughout therapy, in-between appointments, or even following the course of treatment is over [6]. Consequently, it's crucial to take into account treatment plans intended to get rid of or stop E.faecalis infection. 3% sodium hypochlorite which have a proteolytic action, has the power to eliminate E. faecalis from the root canal system, if used appropriately following the proper irrigation protocol. All forms of E. faecalis can be effectively eradicated with sodium hypochlorite, even when it is in the form of biofilm [15]. However, on the other hand, when extruded into the periapical region, exerts cytotoxicity and neurotoxicity. It has drawbacks, mostly due to its toxicity, which causes harm to all living tissues except keratinized epithelia [16]. Sodium hypochlorite is very corrosive to metals, is highly alkaline, hypertonic, and has a foul odour. Specific storage techniques must be followed in order to achieve the specified shelf life. The majority of these drawbacks may be avoided in endodontics by restricting the hypochlorite to the pulp chamber and root canal. Rubber dams and precise watering procedures are essential [16]. There are various other root canal irrigants that are tested and marketed for root canal treatment in the present scenario. However, research focuses on the efficacy of herbal extracts as root canal irrigants considering its biocompatibility and ongoing rise in the antibiotic resistant strains and the negative effects of synthetic drugs.

Tea tree (Melaleuca alternifolia), grows in Australia, mostly in the western regions, close to streams and rivers. One of the first uses of tea tree oil in medical practice was wound disinfection and treatment of wounds already infected with bacteria [17,18]. Currently, tea tree oil is mostly used topically in dermatology and gynaecology. However, completely new applications have appeared, for instance as inhalations to treat the upper respiratory tract and orally in the therapy of infections of the urinary tract. TTO's dissociation in test media is restricted by the fact that it is only weakly soluble in water. Various tactics have been employed to combat this issue, surfactants are being added to broth and agar test media [18]. Endpoint determination in susceptibility tests can be challenging when TTO is dispersed in liquid media because the suspension it provides is often turbid. Various bacteria have been investigated invitro with TTO. From 1940 through the 1980s, there are just a few reports of TTO's antibacterial activity in the literature. The initial study was published by Atkinson and Brice (1955), [18] who used both agar and broth dilution assays to test the antibacterial activity of plants in the Myrtaceae family. The oil also possesses a plethora of characteristics that make it appropriate for use in dentistry. TTO's mechanism of action has now partially been identified. On the basis of its hydrocarbon structure and attendant lipophilicity, assumptions concerning its mode of action were made that it selectively disrupts the cell membrane and stop its the vital functions. In the event that none of the key antibacterial components of TTO, terpinen-4-ol and -terpineol, nor 1,8-cineole, were found to trigger autolysis in S. aureus cells, but all were found to leak 260 nm-absorbing material and make cells sensitive to sodium chloride [19]. Interestingly, 1,8-cineole, a chemical compound, had the strongest impacts. Frequently regarded as having quite weak antibacterial properties. This suggests that even though cineole might not be one of the main antibacterial elements in TTO, it might permeabilize bacterial membranes and make it easier for other, more potent elements to enter. There hasn't been much research

on how TTO components affect cell shape [20]. Using electron microscopy, researchers examined Mesosomes and other lesions, including those found after TTO therapy, were visible in S.aureus cells [21]. The loss of viability, suppression of glucose-dependent respiration, and induction of lysis observed after TTO treatment all happen more frequently in exponentially growing organisms than in stationary ones. The larger degree of morphological alterations observed in these cells by researchers also demonstrated the heightened sensitivity of actively growing cells. Using electron microscopy various bacteria exhibit different levels of susceptibility, which suggests that there may be additional targets at play [22]. Despite having great qualities, the physical properties of TTO provide several challenges for its use as an irrigant. Its lipophilicity causes miscibility issues with aqueous bases. To overcome this problem, Surface modifiers are therefore added to irrigation solutions to lessen surface tension and contact angle in order to obtain deeper penetration into dentinal tubules and canal anatomical complexity.

A surfactant (or detergent) is a component that is added to a dentifrice to provide cleaning and antibacterial benefits via surface action, based on hydrophilic and hydrophobic qualities. Surfactants are broadly categorized into four broad categories: anionic, cationic, amphoteric and non-ionic [23].

Anionic surfactants when dissolved in water, produce a negatively charged surfactant ion which is generally harmless. A saturated/unsaturated C12-C18 aliphatic group makes up the straight chain. The presence of double bonds in the surfactant determines its potential for water solubility. One such anionic surfactant is the sodium salt of lauryl alcohol is known as SLS (1-dodecanol). Sulfuric acid monododecyl ester sodium salt is the term coined to it. The most common concentrations range from 0.5 to 2%. SLS inhibits a number of microorganisms. The mechanism of action of SLS antimicrobial effect is due to its adsorption and penetration through the porous cell wall, followed by

interactions with cell membrane components, lipids, and proteins. The penetration of SLS into the membrane induces a surge in bacterial cell permeability, which can lead to intracellular component leakage and cell lysis [24].

Cationic surfactants are excellent emulsifiers. Surfactants have also been discovered to be effective bactericides, and some are used as topical antiseptics. They're very beneficial in hand sanitizers because of their germicidal qualities. Surfactants that are cationic are drawn to negatively charged sites. Cationic surfactants in solution, the head is positively charged. These surfactants have also been found to be good bactericides and some find use as topical antiseptics. Their germicidal properties make them especially useful in hand sanitizers [25]. Cationic surfactants are attracted to negatively- charged sites. Cetrimide is a cationic quaternary ammonium surfactant that has been shown to be efficient against Gram-positive and Gram-negative bacteria, as well as antifungal properties. It's used topically and is completely nontoxic at concentrations of up to 2%. CT lowers the surface tension of liquids since it is made up of amphipathic molecules. This property might enable it penetrate into difficult-to-reach places like the inner lumen of dentinal tubules [25].

Nonionic surfactants of the non-dissociable kind, such as alcohol, phenol, ether, ester, or amide, do not ionise in aqueous solution due to their hydrophilic group. Due to the lack of an electrical charge, these surfactants are resistant to water hardness deactivation. The inclusion of a polyethylene glycol chain, generated through polycondensation of ethylene oxide, makes a high fraction of these nonionic surfactants hydrophilic. Surfactants that aren't ionic are perhaps the most commonly employed in medication delivery. Polyol esters, polyoxyethylene esters, poloxamers, and pluronics are examples of nonionic surfactants. Polyethylene glycol is a common component of polyoxyethylene esters (PEGs).

Tween 80 (polysorbate 80, polyoxyethylene sorbitan monooleate) is a nonionic surfactant commonly used in cosmetics, medicines, and food items as an emulsifier. The US Food and Drug Administration has approved it for usage in up to 1% of certain foods. Tween 80 has got attention in recent years after research showed that putting 1% Tween 80 in drinking water causes inflammation and increased adiposity in rats. Tween80 was previously reported to decrease bacterial adhesion and limit biofilm formation in Pseudomonas sp. AKS2 and several isolates of Pseudomonas aeruginosa, Escherichiacoli, S. aureus, Staphylococcus aureus, Staphylococcus epidermidis, and more. This study aimed to evaluated the antibacterial efficacy of tea tree oil in combination with cationic, anionic and non-ionic surfactants against enterococcus faecalis dentinal biofilm [26].

In complex root canal anatomies, the antibacterial efficacy of oil based irrigants are lesser compared to other irrigants, which may be because of its decreased wettability so surface modifiers are added [27]. In the past, various studies reported the efficacy of various irrigants with or without surface active agents but never intercompared the efficacy of all the types of surfactants (anionic, cationic and non-ionic) in the same study. Hence, we aimed to estimate the minimum inhibitory and bactericidal concentrations of all the surfactants individually and also in combination with tea tree oil and to analyse the antibacterial efficacy of various surfactants with tea tree oil against E. faecalis.

AIM AND OBJECTIVES

AIM

The aim of the present study is to estimate the minimum inhibitory and bactericidal concentrations of all the surfactants individually and also in combination with tea tree oil and to analyze the antibacterial efficacy of various surfactants with tea tree oil against E. faecalis.

OBJECTIVES

1. To evaluate the antibacterial efficacy of cetrimide, sodium dodecyl sulphate, tween 80 and its combination with tea tree oil and sodium hypochlorite on *E. faecalis* (ATCC 29212) by agar well diffusion assay.
2. To determine the Minimum Inhibitory Concentration (MIC) by broth microdilution assay.
3. To confirm the MIC by Minimum Bactericidal Concentration (MBC).
4. To determine the colony forming units of E. faecalis (ATCC 29212) exposed to experimental irrigants.

REVIEW OF LITERATURE

P N R Nair (2004) provided a comprehensive overview on the Pathogenesis of apical periodontitis and the causes of endodontic failures where the apical periodontitis is discussed in detailed. The author included apical periodontitis is a sequel to endodontic infection and manifests itself as the host defense response to microbial challenge emanating from the root canal system. It is viewed as a dynamic encounter between microbial factors and host defenses at the interface between infected radicular pulp and periodontal ligament that results in local inflammation, resorption of hard tissues, destruction of other periapical tissues, and eventual formation of various histopathological categories of apical periodontitis, commonly referred to as periapical lesions. The treatment of apical periodontitis, as a disease of root canal infection, consists of eradicating microbes or substantially reducing the microbial load from the root canal and preventing re-infection by orthograde root filling. The treatment has a remarkably high degree of success. Nevertheless, endodontic treatment can fail. Most failures occur when treatment procedures, mostly of a technical nature, have not reached a satisfactory standard for the control and elimination of infection. Even when the highest standards and the most careful procedures are followed, failures still occur. This is because there are root canal regions that cannot be cleaned and obturated with existing equipments, materials, and techniques, and thus, infection can persist. In very rare cases, there are also factors located within the inflamed periapical tissue that can interfere with post-treatment healing of the lesion. The data on the biological causes of endodontic failures are recent and scattered in various journals.

Göran Sundqvist et al (2004) in his review life as an endodontic pathogen describes the type of microbial flora in the untreated root canal and the root-filled canal with

persistent infection. Recent contributions of molecular methods of microbial identification are outlined along with a discussion of advantages and limitations of these methods. Ecological and environmental factors are the prime reasons for differences in the microbial flora in these distinct habitats. Shared phenotypic traits and an ability to respond to the modified environment select for the species that establish a persistent root canal infection.

José F Siqueira Jr et al (2022) in his review Present status and future directions: Microbiology of endodontic infections describes the microbiologic aspects of endodontic infections and provides perspectives for future research and directions in the field. The author states that Apical periodontitis has a microbial aetiology and is one of the most common inflammatory diseases that affect humans. Fungi, archaea and viruses have been found in association with apical periodontitis, but bacteria are by far the most prevalent and dominant microorganisms in endodontic infections. Bacterial infection of the root canal system only occurs when the pulp is necrotic or was removed for previous treatment. In some specific cases, including acute and chronic abscesses, the bacterial infection may reach the periradicular tissues. Intracanal bacteria are usually observed as sessile multispecies communities (biofilms) attached to the dentinal root canal walls. Infection in the main root canal lumen can spread to other areas of the root canal system. Although more than 500 bacterial species have been detected in endodontic infections, a selected group of 20 to 30 species are most frequently detected and may be considered as the core microbiome. There is a high interindividual variability in the endodontic microbiome in terms of species composition and relative abundance. Obligate anaerobic species are more abundant in the intraradicular bacterial communities of teeth with primary apical periodontitis, while both anaerobes and facultatives dominate the communities in post-treatment apical periodontitis. Bacterial interactions play an essential role in determining the overall virulence of the community, which has been

regarded as the unit of pathogenicity of apical periodontitis.

Domenico Ricucci et al (2010) conducted a study that evaluated the prevalence of bacterial biofilms in untreated and treated root canals of teeth evincing apical periodontitis. The associations of biofilms with clinical conditions, radiographic size, and the histopathologic type of apical periodontitis were also investigated. It resulted that Bacteria were found in all but one specimen. Overall, intraradicular biofilm arrangements were observed in the apical segment of 77% of the root canals (untreated canals: 80%; treated canals: 74%). Biofilms were also seen covering the walls of ramifications and isthmuses. Bacterial biofilms were visualized in 62% and 82% of the root canals of teeth with small and large radiographic lesions, respectively. All canals with very large lesions harbored intraradicular biofilms. Biofilms were significantly associated with epithelialized lesions (cysts and epithelialized granulomas or abscesses) ($p < 0.001$). The overall prevalence of biofilms in cysts, abscesses, and granulomas was 95%, 83%, and 69.5%, respectively. No correlation was found between biofilms and clinical symptoms or sinus tract presence ($p > 0.05$). Extraradicular biofilms were observed in only 6% of the cases. The author concludes that the overall findings are consistent with acceptable criteria to include apical periodontitis in the set of biofilm-induced diseases. Biofilm morphologic structure varied from case to case and no unique pattern for endodontic infections was identified. Biofilms are more likely to be present in association with longstanding pathologic processes, including large lesions and cysts.

Preeti N. Malani et al (2002) A book Enterococcal Disease, Epidemiology, and Treatment which acts as a guide to understanding the vanguard of antibiotic-resistant bacteria. - Offers a comprehensive primary text on enterococci and covers the pathogenesis of infection, molecular biology, and antibiotic resistance.

Isabela N Rôças et al (2004) A study conducted on Association of Enterococcus faecalis with different forms of periradicular diseases. This molecular study was undertaken to investigate the prevalence of E. faecalis in endodontic infections and to determine whether this species is associated with particular forms of periradicular diseases. E. faecalis was significantly more associated with asymptomatic cases than with symptomatic ones. E. faecalis was detected in 20 of 30 cases of persistent endodontic infections associated with root-filled teeth. When comparing the frequencies of this species in 30 cases of persistent infections with 50 cases of primary infections, statistical analysis demonstrated that E. faecalis was strongly associated with persistent infections. The average odds of detecting E. faecalis in cases of persistent infections associated with treatment failure were 9.1. The results of this study indicated that E. faecalis is significantly more associated with asymptomatic cases of primary endodontic infections than with symptomatic ones.

Stefanie Koch et al (2004) in his review on Enterococcal infections: host response, therapeutic, and prophylactic possibilities. The author states that the emergence of resistance against multiple antibiotics and the increasing frequency with which Enterococcus faecalis and Enterococcus faecium are isolated from hospitalized patients underscore the necessity for a better understanding of the virulence mechanisms of this pathogen and the development of alternatives to current antibiotic treatments. The genetic plasticity of enterococci and their ability to rapidly acquire and/or develop resistance against many clinically important antibiotics and to transfer these resistance determinants to other more pathogenic microorganisms makes the search for alternative treatment and preventive options even more important. A capsular polysaccharide antigen has recently been characterized that is the target of opsonic antibodies. A limited number of clinically relevant serotypes exist, and the development of an enterococcal vaccine based on capsular polysaccharides may improve our ability to prevent and treat

these infections. Additional enterococcal surface antigens, including ABC transporter proteins and other virulence factors, such as aggregation substance (AS), may also be useful targets for therapeutic antibodies.

Richard R. Facklam et al (2002) in his book History, Taxonomy, Biochemical Characteristics, and Antibiotic Susceptibility Testing of Enterococci. The author states that the enterococci are a complex, diverse, and important group of bacteria in terms of their interaction with humans. This chapter presents a variety of techniques and procedures that can be used for isolation and identification of these important microorganisms. Early documentation of microorganisms that are now included in the genus *Enterococcus* is mainly related to the "streptococci of fecal origin". Enterococci are gram-positive cocci that occur singly, in pairs, or as short chains. Most enterococci, apart from *Enterococcus cecorum, Enterococcus columbae, Enterococcus pollens*, and *Enterococcus saccharolyticus*, hydrolyze pyrrolidonyl-β- naphthylamide (PYR); all strains produce leucine aminopeptidase. The tables depicting the phenotypic characteristics of the *Enterococcus* species in the chapter are based on correlations between the whole-cell protein (WCP) profiles and the phenotypic tests and, on some occasions, in conjunction with DNA-DNA reassociation experiments. Techniques for isolation of enterococci from inanimate hospital surfaces have been developed. For several years, controversies and confusion surrounded antimicrobial susceptibility testing of enterococcal isolates, particularly with regard to the reliability of phenotypic methods for detection of high-level resistance (HLR) to aminoglycosides and resistance to vancomycin. Some detailing of the specific standard recommendations for detecting HLR to aminoglycosides, as well as resistance to β-lactams and vancomycin, is presented in the chapter as they are considered very helpful for routine detection of these important resistance markers in enterococci. Molecular methods can rapidly detect specific antimicrobial-drug resistance genes and have substantially contributed to the

understanding of the spread and genetics of acquired enterococcal resistance.

T S Hubble et al (2003) in his study Influence of Enterococcus faecalis proteases and the collagen-binding protein, Ace, on adhesion to dentin. where tested the hypothesis that the E. faecalis proteases, serine protease and gelatinase, and the collagen-binding protein (Ace) contribute to adhesion to the root canal. Statistical analysis revealed that adherence of OG1RF was significantly greater than the mutant strains ($P < 0.001$), while significant differences were not detected between the protease mutants. The data indicate that serine protease and Ace aid E. faecalis binding to dentin, while the role of gelatinase is uncertain.

D Figdor et al (2003) in his aticle titled Starvation survival, growth and recovery of Enterococcus faecalis in human serum summarized the ability of Enterococcus faecalis to survive starvation for long periods in the obturated root canal is likely to be an important factor in the pathogenesis and maintenance of a persistent infection after endodontic treatment. The response of E. faecalis to starvation survival in water and glucose-, phosphate- or amino acid-limited chemically defined medium was studied, along with the capacity for growth and recovery of starved cells of E. faecalis in pooled human serum. Author concludes by stating E. faecalis is capable of withstanding prolonged periods of starvation in a minimal metabolic state provided that there is a high cell density at the onset of starvation. Starved cells were capable of recovery upon addition of human serum.

R M Love (2001) in his study Enterococcus faecalis--a mechanism for its role in endodontic failure, aimed to identify a possible mechanism that would explain how E. faecalis could survive and grow within dentinal tubules and reinfect an obturated root canal. It resulted that all three species remained viable over the period of the experiment when grown in human serum. Cells of all three bacteria were able to invade dentine and

bind to immobilized collagen. Both of these properties were inhibited by the presence of collagen in the cell solution. Human serum inhibited dentine invasion and collagen adhesion by S. gordonii DL1 and S. mutans NG8, whilst dentine invasion by E. faecalis JH2-2 was reduced in the presence of serum, but not inhibited, and binding to collagen was enhanced. It is postulated that a virulence factor of E. faecalis in failed endodontically treated teeth may be related to the ability of E. faecalis cells to maintain the capability to invade dentinal tubules and adhere to collagen in the presence of human serum.

Gamal M. EL-Sherbiny et al (1990) studied the Antimicrobial Susceptibility of Bacteria Detected from the Root Canal Infection (Before and After) Root-Filled Teeth. the study was to investigate the bacterial species from root canal infection (before and after) filled teeth and evaluate susceptibility to antibiotics, antimicrobial agents and plant extracted from ginger (*Zingiber officinale*). Thirty-three adult patients with symptoms of root canal infection was receiving at the outpatient clinic, Faculty of Dental Medicine, Al-Azhar University, Cairo, Egypt, 2014, diagnosed by a physician participated in the study. Forty- five bacterial samples were taken from root canals infection, 33 before and 12 after filled teeth. A total 126 bacterial isolates were isolated and identify from this samples. The predominant bacterial isolates were found *Enterococcus faecalis* 15%, followed by *Streptococcus mutans* 9.5%, *Streptococcus acidominimus* 8.7% and *Porphyromonas gingivalis* 7.93%. The bacterial isolates highly sensitive to amoxicillin-clavulanic acid, vancomycin and erythromycin. About 36.50 % of the isolates were resistant to tetracycline. More than 50 % of all isolates were resistant to metronidazole. The sensitivity of bacterial isolates to antimicrobial agents revealed that sensitive 87.30% to potassium iodide 2.0 %, 73.01 % to calcium hydroxide 2 %, 69 % to chlorxidine 1% and 54% to sodium hypochlorite 0.5%. The in vitro antibacterial activity of *Zingiber officinale* was studied against bacterial isolates. The aqueous and ethanolic

extracts of *Zingiber officinale* exhibited antibacterial activity against all bacterial isolates with MIC ranged from 0.5 to 1.3 mg/ml and 0.3 to 1.0 mg/ml respectively. In conclusions, amoxicillin-clavulanic acid and vancomycin most potent antibiotics and potassium iodide 2% most antimicrobial agents against bacterial isolates. *Zingiber officinale* extract has potential antimicrobial action against bacterial isolates.

M Haapasalo et al (1987) conducted a study on In vitro infection and disinfection of dentinal tubules. Cylindrical dentin specimens, 4 mm high with a diameter of 6 mm and a canal 2.3 mm wide, were prepared from freshly extracted bovine incisors. The cementum was removed from all dentin blocks. The tubules were opened by four-minute treatments with 17% EDTA and 5.25% NaOCl before being infected with Enterococcus faecalis ATCC 29212 in yeast extract-glucose broth. Bacteria rapidly invaded the tubules. After three weeks of incubation, a heavy infection was found 400 micron from the canal lumen, and the front of the infection reached 1000 micron in some blocks. Camphorated paramonochlorophenol (CMCP) and a calcium hydroxide compound, Calasept, were tested for their disinfecting efficacy toward E. faecalis-infected dentin. Liquid CMCP rapidly and completely disinfected the dentinal tubules, whereas CMCP in gaseous form disinfected tubules less rapidly. Calasept failed to eliminate, even superficially, E. faecalis in the tubules. The method used in bacteriological sampling allowed for sequential removal of 100-micron-thick zones of dentin from the central canal toward the periphery. Control specimens were uniformly infected and yielded growth in bur samples up to some 500 microns from the surface. The model proved quite sensitive and seems suitable for in vitro testing of root canal medicaments.

John W Distel et al (2002) in his clinical trial Biofilm formation in medicated root canals The hypothesis that Enterococcus faecalis resists common intracanal medications

by forming biofilms was tested. E. faecalis colonization of 46 extracted, medicated roots was observed with scanning electron microscopy (SEM) and scanning confocal laser microscopy. SEM detected colonization of root canals medicated with calcium hydroxide points and the positive control within 2 days. SEM detected biofilms in canals medicated with calcium hydroxide paste in an average of 77 days. Scanning confocal laser microscopy analysis of two calcium hydroxide paste medicated roots showed viable colonies forming in a root canal infected for 86 days, whereas in a canal infected for 160 days, a mushroom-shape typical of a biofilm was observed. Analysis by sodium dodecyl sulfate polyacrylamide gel electrophoresis showed no differences between the protein profiles of bacteria in free-floating (planktonic) and inoculum cultures. Analysis of biofilm bacteria was inconclusive. These observations support potential E. faecalis biofilm formation in vivo in medicated root canals.

J. Siqueira et al (1997) in his study the effectiveness of 4.0% sodium hypochlorite (NaOCl) used with three irrigation methods in the elimination of Enterococcus faecalis from the root canal was tested in vitro. Root canals contaminated with E. faecalis were treated as follows: (i) irrigation with 2 mL of NaOCl solution and agitation with hand files; (ii) irrigation with 2 mL of NaOCl solution and ultrasonic agitation; (iii) irrigation with NaOCl alternated with hydrogen peroxide. Contaminated canals irrigated with sterile saline solution served as the control. Paper points used to sample bacteria from the root canals were transferred to tubes containing 5 mL of brain heart infusion (BHI) broth. Tubes were incubated and the appearance of broth turbidity was indicative of bacteria remaining in the root canal. There were no statistically significant differences between the experimental groups. However, NaOCl applied by the three methods tested, was significantly more effective than the saline solution (control group) in disinfecting the root canal.

R M Clarkson et al (1998) reviewed about sodium hypochlorite. It has been used as an endodontic irrigant for more than 70 years, and is now one of the most common solutions for this purpose. The chemical properties and production of commercial sodium hypochlorite are reviewed. Domestic bleaches and an infant sanitizer are compared from the point of view of cost and ease of use--Milton being recommended where a 1% solution is required. The cost of syringes and needles for endodontic irrigation is many times greater than the hypochlorite they contain, and total annual practice costs for hypochlorite are low. Brief guidelines for clinical use, storage, handling and disposal are included.

C F Carson et al (2006) reviewed the antimicrobial and other medicinal properties of Melaleuca alternifolia (Tea Tree) oil. Complementary and alternative medicines such as tea tree (melaleuca) oil have become increasingly popular in recent decades. This essential oil has been used for almost 100 years in Australia but is now available worldwide both as neat oil and as an active component in an array of products. The primary uses of tea tree oil have historically capitalized on the antiseptic and anti-inflammatory actions of the oil. This review summarizes recent developments in our understanding of the antimicrobial and anti-inflammatory activities of the oil and its components, as well as clinical efficacy. Specific mechanisms of antimicrobial and anti-inflammatory action are reviewed, and the toxicity of the oil is briefly discussed.

Nancy Atkinson et al (1946) in his preliminary survey on the antibacterial substance provided by the flowering plants. Fresh leaves, stems and flowers, and where possible seeds and roots were thoroughly ground up separately with a little water. The extracts thus produced were tested for antibacterial activity by the cylinder-plate method devised by Heatley (1944). In most cases, a mixture of all the extracts for any one plant was also tested. The tests were made against Staph. aureus and Bacttyphosum. This method of

testing is convenient as it does not require the preparation of a sterile extract. Some extracts may possibly prove negative by this method and positive by some other method. It resulted that One of the most active of all the Myriaceae tested was Regelis grandifora. Other geners of which only one or two species were tested and proved inactive were Backea, Eugenia, Kremass, Beaufortia, Hypocalymns, Sysaphae, Actinodium and Synorpia.

J Sikkema et al (1995) reviewed the Mechanisms of membrane toxicity of hydrocarbons. Most importantly, lipophilic hydrocarbons accumulate in the membrane lipid bilayer, affecting the structural and functional properties of these membranes. As a result of accumulated hydrocarbon molecules, the membrane loses its integrity, and an increase in permeability to protons and ions has been observed in several instances. Consequently, dissipation of the proton motive force and impairment of intracellular pH homeostasis occur. In addition to the effects of lipophilic compounds on the lipid part of the membrane, proteins embedded in the membrane are affected. The effects on the membrane-embedded proteins probably result to a large extent from changes in the lipid environment; however, direct effects of lipophilic compounds on membrane proteins have also been observed. Finally, the effectiveness of changes in membrane lipid composition, modification of outer membrane lipopolysaccharide, altered cell wall constituents, and active excretion systems in reducing the membrane concentrations of lipophilic compounds is discussed. Also, the adaptations (e.g., increase in lipid ordering, change in lipid/protein ratio) that compensate for the changes in membrane structure are treated.

S D Cox et al (2000) discussed the mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil). The essential oil of Melaleuca alternifolia (tea tree) exhibits broad-spectrum antimicrobial activity. Its mode of action against the Gram-

negative bacterium Escherichia coli AG100, the Gram-positive bacterium Staphylococcus aureus NCTC 8325, and the yeast Candida albicans has been investigated using a range of methods. We report that exposing these organisms to minimum inhibitory and minimum bactericidal/fungicidal concentrations of tea tree oil inhibited respiration and increased the permeability of bacterial cytoplasmic and yeast plasma membranes as indicated by uptake of propidium iodide. In the case of E. coli and Staph. aureus, tea tree oil also caused potassium ion leakage. Differences in the susceptibility of the test organisms to tea tree oil were also observed and these are interpreted in terms of variations in the rate of monoterpene penetration through cell wall and cell membrane structures. The ability of tea tree oil to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control is the most likely source of its lethal action at minimum inhibitory levels.

Christine F Carson et al (2002) described the Mechanism of action of Melaleuca alternifolia (tea tree) oil on Staphylococcus aureus determined by time-kill, lysis, leakage, and salt tolerance assays and electron microscopy. The essential oil of Melaleuca alternifolia (tea tree) has broad-spectrum antimicrobial activity. The mechanisms of action of tea tree oil and three of its components, 1,8-cineole, terpinen-4-ol, and alpha-terpineol, against Staphylococcus aureus ATCC 9144 were investigated. Treatment with these agents at their MICs and two times their MICs, particularly treatment with terpinen-4-ol and alpha-terpineol, reduced the viability of S. aureus. None of the agents caused lysis, as determined by measurement of the optical density at 620 nm, although cells became disproportionately sensitive to subsequent autolysis. Loss of 260-nm-absorbing material occurred after treatment with concentrations equivalent to the MIC, particularly after treatment with 1,8-cineole and alpha-terpineol. S. aureus organisms treated with tea tree oil or its components at the MIC or two times the MIC showed a significant loss of tolerance to NaCl. When the agents were tested at one-half

the MIC, only 1,8-cineole significantly reduced the tolerance of S. aureus to NaCl. Electron microscopy of terpinen-4-ol-treated cells showed the formation of mesosomes and the loss of cytoplasmic contents. The predisposition to lysis, the loss of 260-nm-absorbing material, the loss of tolerance to NaCl, and the altered morphology seen by electron microscopy all suggest that tea tree oil and its components compromise the cytoplasmic membrane.

Nathan E. Baker (2004) The study investigated the ability of endodontic irrigants and medicaments to eliminate Enterococcus faecalis from infected dentinal tubules, and whether their antimicrobial action was enhanced by surfactant. It resulted that the addition of surfactant did not enhance the antibacterial action of any medicament. When used as a 24-hour medicament, Ca(OH)2 consistently failed to eliminate E faecalis, whereas both Betadine Scrub and IKI rendered 90% of samples sterile. IKI was the only agent shown to consistently eliminate E faecalis in a 15-minute time frame. The author concluded that under the in vitro conditions of this study, IKI was able to eliminate E faecalis from bovine root dentin when used with a 15-minute contact time.

Ahmed S et al (2017) conducted a study on the Evaluation of Effect of Irrigants with or without Surfactant on Root Canal Transportation by Cone Beam Computed Tomography-An In vitro Study. It aimed to evaluate the influence of addition of surfactants to Sodium Hypochlorite (NaOCl) and Ethylenediaminetetraacetic Acid (EDTA) on transportation of root canal. Irrigation regimens followed: G1 (n=10)-irrigation with saline solution(control); G2 (n=10)-irrigation with 2.5% NaOCl; G3 (n=10)-irrigation with 2.5% NaOCl added with surfactant; G4 (n=10)-irrigation with 17% EDTA; G5 (n=10)-irrigation with 17% EDTA added with surfactant. The mean transportation values were higher in G5. Transportation in G3 and G5 was not significantly different compared to G2 and G4 respectively ($p<0.05$). they concluded that

instrumentation using irrigating solutions added with surfactant like 1% cetrimide maintained the canal curvature well.

T. J. Paul et al (2019) sodium lauryl sulphate (SLS) is a synthetic product that is broadly utilised in toothpaste. Recently, a systematic review reported on SLS based dentifrices and their influence on recurrent aphthous stomatitis. The results also mentioned that SLS-free dentifrices showed significant reduction on number, duration, episodes and pain among recurrent aphthous ulceration (Sutton's disease) patients. There is a need for the search of natural and innovative substances that can fill the role provided by SLS in toothpastes, with less or no potential for harm. Plant-derived saponin may prove beneficial as it is likely to be relatively harmless when taken orally, and toxicity is minimised during ingestion by low absorption and hydrolysis. Being a natural source, it is biodegradable and less likely to bioaccumulate and cause toxicity and disease. Currently, there are no studies that show the occurrence of recurrent aphthous ulcers from the use of ackee-derived saponin.

Mehmet Burak Guneser et al (2016) aimed to was to compare the antimicrobial effect of sodium hypochlorite (NaOCl), 2% chlorhexidine (CHX), a CHX/cetrimide solution (CHX+CTR), octenidine hydrochloride (OCT) and Salvia officinalis plant extract against Enterococcus faecalis. Seventy decoronated single-rooted human teeth were infected and divided into 6 test (n=10) and 2 control groups (n=5) (negative, sterile samples and positive, infected samples). Following irrigants were then applied to test groups: 2.5% NaOCl, 5.25% NaOCl, CHX, CHX+CTR, S. officinalis extract and OCT. The dentin chips were obtained from inner root canal walls and analyzed by counting the number of colony forming units (CFU). The 2.5% NaOCl, 5.25% NaOCl, CHX and OCT groups presented no bacterial growth (CFU=0). S. officinalis and CHX+CTR groups reduced the number of E. faecalis cells but could not eliminate all. OCT may

have potential as an endodontic irrigant in treatment of infected root canals.

Gannu P.Kumar et al (2011) reported an overview of Nonionic surfactant vesicular systems for effective drug delivery. Vesicular systems are a novel means of drug delivery that can enhance bioavailability of encapsulated drug and provide therapeutic activity in a controlled manner for a prolonged period of time. Liposomes were the first such system but they suffer from a number of drawbacks including high cost and lack of stability at various pHs. Niosomes are a nonionic surfactant vesicular system, which can be easily and reliably made in the laboratory. Many factors affect noisome formation such as the method of manufacture, nature of surfactant and encapsulated drug, temperature at which the lipids are hydrated and the critical packing parameter. This review describes all aspects of niosomes including their different compositions, the various methods of preparation, the effect of changing manufacturing parameters, methods of characterization, factors that affect their stability, their use by various routes of administration, their therapeutic applications and the most important patents. The review also provides detailed information of the various types of niosomes that provide effective drug delivery.

Nielsen CK et al (2016) reported the Effects of Tween 80 on Growth and Biofilm Formation in Laboratory Media. Tween 80 is a widely used non-ionic emulsifier that is added to cosmetics, pharmaceuticals, and foods. Because of its widespread use we need to understand how it affects bacteria on our skin, in our gut, and in food products. The aim of this study is to investigate how Tween 80 affects the growth and antimicrobial susceptibility of *Staphylococcus aureus*, *Listeria monocytogenes*, and *Pseudomonas fluorescens*, which are common causes of spoilage and foodborne illnesses. Addition of 0.1% Tween 80 to laboratory growth media increased the growth rate of planktonic *S. aureus* batch cultures, and it also increased the total biomass when *S. aureus* was grown

as biofilms. In contrast, Tween 80 had no effect on batch cultures of *L. monocytogenes*, it slowed the growth rate of *P. fluorescens*, and it led to formation of less biofilm by both *L. monocytogenes* and *P. fluorescens*. Furthermore, Tween 80 lowered the antibacterial efficacy of two hydrophobic antimicrobials: rifampicin and the essential oil isoeugenol. Our findings underline the importance of documenting indirect effects of emulsifiers when studying the efficacy of hydrophobic antimicrobials that are dispersed in solution by emulsification, or when antimicrobials are applied in food matrixes that include emulsifiers. Furthermore, the species-specific effects on microbial growth suggests that Tween 80 in cosmetics and food products could affect the composition of skin and gut microbiota, and the effect of emulsifiers on the human microbiome should therefore be explored to uncover potential health effects.

Isabelle Portenier et al (2006) aimed to investigate the antibacterial activity of MTAD and chlorhexidine towards two strains of Enterococcus faecalis and the inhibitory effects of dentine and bovine serum albumin on the antibacterial activity. Survival of bacteria exposed to the medicaments in the presence or absence of inhibitors was monitored in an in vitro model. Full concentration (100%) MTAD and 0.2% chlorhexidine rapidly killed both strains. Combining chlorhexidine with cetrimide further reduced the time required for killing. The presence of dentine or BSA caused a marked delay in killing by both medicaments. The two E. faecalis strains tested showed minor differences in their susceptibility to the disinfectants.

Nimmy Sabu et al (2022) aimed to compare the antibacterial efficacy of 2% chlorhexidine (CHX), 0.2% cetrimide (CTR), and 0.2% CHX + 0.2% CTR against anaerobic bacteria and to test the influence of CTR added to CHX on its antibacterial action. When the mean values of Group I (2% CHX) were compared with Group II

(0.2% CTR), the data were statistically not significant (P = 0.2341), whereas Group I showed a significant difference when compared with Group III (0. 2% CHX + 0.2% CTR) (P = 0.0107). When the mean values of Group II and Group III were compared, the data were found to be statistically not significant (P = 0.0805). The efficacy of 2% CHX was found to be slightly superior when compared with that of 0.2% CTR, but the difference was not statistically significant. However, a significant difference was found between 2% CHX and combination irrigants.

Arathi Ganesh (2015) conducted a study to evaluate the sensitivity of *Enterococcus faecalis* biofilms treated with DNase enzyme and their susceptibility to 2% chlorhexidine used alone or in conjunction with a detergent in a dentin disinfection model and examine under confocal laser scanning microscopy (CLSM). Semi cylindrical shaped dentin specimens were infected with *E. faecalis* and incubated for 24 hours. Following incubation, the infected dentin specimens were exposed for 3 minutes to the four disinfecting solutions and grouped accordingly. {Group I- Sterile saline, Group II- 2% Chlorhexidine (CHX), Group III– Dnase1 Enzyme + 2% CHX, Group IV- DNase1 Enzyme + 2% CHX & Tween 80. Bacterial viability was then assessed by staining the specimens and examining under CLSM to analyse the proportion of dead and live bacteria within the dentinal tubules. The Groups II, III and IV showed statistically significant ($p<0.05$) percentage of dead bacteria compared to the control (Group I). However there was no significant difference in the killing effectiveness within the experimental groups (II-IV) at ($p<0.05$). The author concludes that EPS degrading enzyme (DNase I) disrupts the biofilm and increases the susceptibility of *E.faecalis* when exposed to 2% Chlorhexidine and the use of a surfactant with this combination significantly contributes to improving the antibacterial efficacy.

F Daschner (1977) Minimal bacteriostatic and minimal bactericidal concentrations of rolitetracycline, minocycline and doxycycline on 20 different E. coli serotypes and 16 staphylococcus aureus strains have been compared in bouillon and serum. In addition growth curves of all strains in bouillon and serum without and with antibiotics in concentrations corresponding to the minimal inhibitory concentration of each strain have been followed for 24 hours. In E. coli minimal bactericidal concentrations of all 3 tetracyclines in bouillon on average were only twice as high as the minimal bacteriostatic concentrations of the drugs, tested. Minimal bacteriostatic and bactericidal concentrations in serum were significantly lower than in bouillon. 27 out of 20 E. coli strains were inhibited by less than 0.012 microgram/ml. In staphylococci minimal bactericidal concentrations were substantially higher than the minimal bacteriostatic concentrations (on average at least four times higher). In E. coli addition of serum increased the antimicrobial activity of all tetracyclines tested. Growth curves in bouillon tended to show some bactericidal shape at 3-, 7- and 24-hours following addition of the tetracyclines at minimal bacteriostatic concentrations. Bactericidal activity of tetracyclines at the MIC was substantially improved by addition of serum. This was not true for staphylococci in which neither minimal bactericidal and bacteriostatic concentrations nor growth curves could be significantly improved by addition of serum.

L H Field (1980) A tube dilution test to evaluate the effectiveness of antibiotics against Bordetella pertussis is described. Five B. pertussis strains, including a well-characterized research strain and four fresh clinical isolates, were tested with several antibiotics. Erythromycin showed the highest in-vitro activity of the antibiotics tested. A concentration of 0.12 microgram/ml was bacteriostatic for all strains, while 2 microgram/ml was bactericidal. Minimal inhibitory and minimal bactericidal concentrations for ampicillin by tube tests were found to be higher than values

previously reported for agar plate tests.

T R Kurup (1991) Antibacterial activities of methyl-p-hydroxybenzoate, phenoxyethanol and chlorocresol against Pseudomonas aeruginosa were evaluated in the presence of varying concentrations of Tween 80. Below cmc level, the bactericidal activities increased with decrease in the surface tension values of Tween 80 solutions and with interfacial tension values of Tween 80 solution/liquid paraffin systems. Linear relationships were found to exist between the concentrations of each preservative required to reduce the microbial population by a factor 10(3) within 48 hours and the values of surface tension and interfacial tension respectively. Reduction in surface tension and interfacial tension would have increased the adsorption and uptake of preservatives by bacterial cells thereby killing the cells at a faster rate. Concentrations of Tween 80 above cmc also enhanced the antibacterial activities of these preservatives. This was attributed to the increase in the permeability of bacterial membranes to preservatives.

Mengting Duan et al (2022) Silver ions (Ag^+) have been proved to be a strong bactericide but with high cytotoxicity and discoloration property. Triton X-100 (TX-100) and Ag^+ were co-used for the first time as a clinical intracanal medication to obtain both enhanced antibacterial effect and low cytotoxicity. The synergistic antibacterial effect of TX-100 + Ag^+ was tested on both planktonic and biofilm-resident E. faecalis on dentine. And the cytotoxicity was tested on MC3T3-E1 cells. Results confirmed the antibacterial activity against both planktonic and biofilm-resident E. faecalis was dramatically improved after TX-100 incorporation. TX-100 and Ag^+ mixture demonstrated a similar inhibitory effect as the 2% chlorhexidine (CHX), while the cytotoxicity was much lower than 2% CHX ($p < 0.05$). In conclusion, TX-100 + Ag^+ mixture might be developed into

a new effective intracanal medication as the 2% CHX.

Bohora A et al (2022) aimed to (a) to evaluate the antibacterial efficacy of probiotics against common endodontic pathogens, i.e. *E. faecalis* and *C. albicans*, and (b) to evaluate the potential use of probiotic therapy as an additive in endodontic treatment procedures. Probiotic groups showed inhibitory activity against *E. faecalis* by the agar cup method, whereas there was no effect on *C. albicans*. In the biofilm stage, both the test groups had an antibacterial effect on pathogenic organisms. Author suggests that probiotic organisms of the species *Lactobacillus* and *Bifidobacterium* are effective for preventing the growth of *E. faecalis* and *C. albicansin vitro*. Because probiotics are available in varied compositions and concentrations, further evaluation for their role in treating endodontic infection is suggested and warranted. In addition, the study suggested that poloxamer 407 could be utilized as an ideal delivery vehicle for probiotics for use as a potential endodontic intracanal medicament.

B. A. NEWTON (1960) in his article summarized the mechanism of the bactericidal action of surface active compounds. The author described the evidence for the disorganization of cell permeability barriers, indirect disorganization through the dissolution of the cell wall, direct disorganization of the protoplast membrane.

C. Estrela et al (2012) The aim of this preliminary study was to verify the antibacterial potential of cetylpyridinium chloride (CPC) in root canals infected by Enterococcus faecalis. The results showed the presence of E. faecalis after root canal sanitization. The number of bacteria decreased after the use of CPC. In the agar diffusion test, CPC induced large microbial inhibition zones, similar to 2% chlorhexidine and large than 2.5% NaOCl. In conclusion, cetylpyridinium chloride showed antibacterial potential in endodontic infection with E. faecalis.

J Piret et al (2000) The efficacy of sodium lauryl sulfate (SLS), a sulfated anionic chaotropic surfactant, and dextran sulfate (DS), a polysulfated carbohydrate, against herpes simplex virus (HSV) and human immunodeficiency virus (HIV) infections was evaluated in cultured cells and in different murine models of HSV infection. Results showed that both SLS and DS were potent inhibitors of the infectivities of various HSV-1 and HSV-2 strains. Pretreatment of HIV-1 (strain NL4-3) with SLS also reduced its infectivity to 1G5 cells. DS prevented the binding of HSV to cell surface receptors and therefore its entry into cells. Pretreatment of HSV-1 (strain F) with 50 microM SLS resulted in a complete loss of virus infectivity to Vero cells. However, viruses were able to enter into cells and to produce in the nuclei capsid shells devoid of a DNA core. The amount of the glycoprotein D gene produced in these cells remained unchanged compared to controls, suggesting that SLS could interfere with the maturation of the virus. At a higher SLS concentration (100 microM), HSV was highly damaged by SLS pretreatment and only a few viral particles could enter into cells to produce abnormal capsids. Although DS was a more potent inhibitor of HSV infectivity in vitro, it was unable to provide any protection in murine models of HSV infection. However, SLS conferred a complete protection of animals infected cutaneously with pretreated viruses. In addition, skin pretreatment of mice with a polymer formulation containing SLS completely prevented the development of cutaneous lesions. More interestingly, intravaginal pretreatment of mice with SLS in a buffered solution also completely protected against lethal HSV-2 infection. Taken together, our results suggest that SLS could thus represent a candidate of choice as a microbicide to prevent the sexual transmission of HIV, HSV, and possibly other pathogens that cause sexually transmitted diseases.

Jacob M ten Cate (2006) Dental plaque has the properties of a biofilm, similar to other biofilms found in the body and the environment. Modern molecular biological

techniques have identified about 1000 different bacterial species in the dental biofilm, twice as many as can be cultured. Oral biofilms are very heterogeneous in structure. Dense mushroom-like structures originate from the enamel surface, interspersed with bacteria-free channels used as diffusion pathways. It has been found that many therapeutic agents bind to the biofilm EPS matrix before they even reach the bacteria, and are thereby inactivated. Taken together, these fundings highlight why the study of bacteria in the oral cavity is now taken on by studying the biofilms rather than individual species**.**

Sundqvist G (1992) reviews that ecology of root canal flora. The author elaborates that the root canal represents a special environment in which selective pressures result in the establishment of a restricted group of the oral flora. Population shifts occur over time with obligate anaerobes ultimately dominating the bacterial mix. Bacterial interrelationships and the nutritional supply are key factors in determining the outcome of the infection. Endodontic treatment, apart from directly eliminating bacteria, can completely disrupt the delicate ecology and deprive persisting bacteria of their nutritional source.

Ashraf F Fouad et al (2005) aimed to identify Enterococcus spp in nonhealing endodontic cases using PCR amplification and molecular sequencing, and to determine if the prevalence of enterococci is increased in diabetic patients. Specimens from 40 cases undergoing retreatment were incubated in prereduced thioglycollate broth at 37 degrees C. Extracted DNA had PCR amplification using primers that target the tuf gene of 14 Enterococcus spp. PCR products were directly sequenced and identified phylogenetically. In conclusion, E faecalis was the only enterococcal species detected, with an overall prevalence of 22%.

J C Baumgartner et al (2004) The polymerase chain reaction (PCR) is an innovative

nucleic acid-based assay that has the highest sensitivity of any microbiological technique for the detection of bacteria. The purpose of this study was to use PCR to detect the presence of specific species of bacteria in samples collected from two geographical locations. Microbial samples from abscesses of endodontic origin were collected from patients in Portland, Oregon, and Rio de Janeiro, Brazil. PCRs with species-specific oligonucleotide primers for the 16S ribosomal RNA gene were used for detection of the bacteria after DNA extraction from each clinical sample. Statistical analysis revealed that there was a significant difference in detection of the bacteria between the two geographical locations for Prevotella intermedia, Prevotella nigrescens, Prevotella tannerae, Fusobacterium nucleatum, and Porphyromonas gingivalis, but not for Porphyromonas endodontalis, Fusobacterium necrophorum, and Enterococcus faecalis. These results suggest that differences in bacteria detected or cultured in studies can be associated with geographical location.

Vineet Agrawal et al (2016) This article aims to provide a comprehensive review of various herbal endodontic irrigants evaluated for their effectiveness in the disinfection of a root canal system. The main purpose of a root canal treatment is to eliminate the bacteria and their products from the pulp space. Chemomechanical preparation (chemical—refers to irrigating solutions, i.e., either synthetic chemicals or herbal solutions and mechanical—refers to instrumentation of a root canals with endodontic files) of a root canal system plays a major role in obtaining the rationale of root canal treatment. Various synthetic chemicals known as endodontic irrigants play a major role in disinfection, but also have undesirable properties like allergic potential, toxicity, unacceptable taste, etc. Today there is a major change in trend towards the use of natural herbal medicines as a part of dental treatment due to its easy availability, less toxicity, and cost effectiveness.

M M Tong et al (1992) One hundred and four patients completed a randomized, double-blind trial to evaluate the efficacy of 10% w/w tea tree oil cream compared with 1% tolnaftate and placebo creams in the treatment of tinea pedis. Significantly more tolnaftate-treated patients (85%) than tea tree oil (30%) and placebo-treated patients (21%) showed conversion to negative culture at the end of therapy ($p < 0.001$); there was no statistically significant difference between tea tree oil and placebo groups. All three groups demonstrated improvement in clinical condition based on the four clinical parameters of scaling, inflammation, itching and burning. The tea tree oil group (24/37) and the tolnaftate group (19/33) showed significant improvement in clinical condition when compared to the placebo group (14/34; $p = 0.022$ and $p = 0.018$ respectively). Tea tree oil cream (10% w/w) appears to reduce the symptomatology of tinea pedis as effectively as tolnaftate 1% but is no more effective than placebo in achieving a mycological cure.

G B Mahady (2005) This review focuses on the medicinal plants and bacteria for which there is significant published in vitro, in vivo and clinical data available. Infectious diseases are a significant cause of morbidity and mortality worldwide, accounting for approximately 50% of all deaths in tropical countries and as much as 20% of deaths in the Americas. Despite the significant progress made in microbiology and the control of microorganisms, sporadic incidents of epidemics due to drug resistant microorganisms and hitherto unknown disease-causing microbes pose an enormous threat to public health. These negative health trends call for a global initiative for the development of new strategies for the prevention and treatment of infectious disease. For over 100 years chemical compounds isolated from medicinal plants have served as the models for many clinically proven drugs, and are now being re-assessed as antimicrobial agents. The reasons for this renaissance include a reduction in the new antibacterial drugs in the pharmaceutical pipeline, an increase in antimicrobial resistance, and the need of

treatments for new emerging pathogens. Literally thousands of plant species have been tested against hundreds of bacterial strains in vitro and many medicinal plants are active against a wide range of gram positive and gram negative bacteria. However, very few of these medicinal plant extracts have been tested in animal or human studies to determine safety and efficacy.

Karen W Martin et al (2004) Traditional medicine has made use of many different plant extracts for treatment of fungal infections and some of these have been tested for in vitro antifungal activity. This systematic review evaluates antifungal herbal preparations that have been tested in controlled clinical trials. Four electronic databases were searched for controlled clinical trials of antifungal herbal medicines. Data were extracted in a standardized manner by two independent reviewers and are reviewed narratively. Seven clinical trials met our inclusion criteria. Tea tree oil preparations were tested in four randomized clinical trials and some positive outcomes were attributed to the intervention in all trials. Solanum species (two trials) and oil of bitter orange preparations (one trial) were compared with conventional treatments. In all cases encouraging results were reported. There are few controlled clinical trials of herbal antifungal medicines. The most thoroughly clinically tested is tea tree oil, which holds some promise. All herbal remedies require further investigation in rigorous clinical trials.

Miao Li et al (2016) Tea tree oil (TTO) is a natural essential oil with strong antimicrobial efficacy and little drug resistance. However, the biomedical applications of TTO are limited due to its hydrophobicity and formulation problems. Here, we prepared an inhalable TTO nanoemulsion (nanoTTO) for local therapies of bacterial and fungal pneumonia. The optimal formulation of nanoTTOs consisted of TTO/Cremophor EL/water with a mean size of 12.5nm. The nanoTTOs showed strong in vitro

antimicrobial activities on Escherichia coli, Acinetobacter baumannii, Klebsiella pneumoniae, Staphylococcus aureus and Candida albicans. After inhalation to the lung, the nanoTTOs had higher anti-fungal effect than fluconazole on the fungal pneumonia rat models with reduced lung injury, highly microbial clearance, blocking of leukocyte recruitment, and decrease of pro-inflammatory mediators. In the case of rat bacterial pneumonia, the nanoTTOs showed slightly lower therapeutic efficacy than penicillin though at a much lower dose. Taken together, our results show that the inhalable nanoTTOs are promising nanomedicines for local therapies of fungal and bacterial pneumonia with no obvious adverse events.

Linda Halcón et al (2004) Antibiotic-resistant bacteria continue to be a major health concern worldwide. In particular, Staphylococcus aureus, both methicillin-resistant and -sensitive, are of concern in their ability to cause difficult skin and underlying tissue infections. Melaleuca alternifolia oil (tea tree oil), an essential oil, has demonstrated promising efficacy in treating these infections. Tea tree oil has been used for centuries as a botanical medicine, and has only in recent decades surfaced in the scientific literature as a promising adjunctive wound treatment. Tea tree oil is antimicrobial, anti-inflammatory, and has demonstrated ability to activate monocytes. There are few apparent side effects to using tea tree oil topically in low concentrations, with contact dermatitis being the most common. Tea tree oil has been effective as an adjunctive therapy in treating osteomyelitis and infected chronic wounds in case studies and small clinical trials. There is a need for larger clinical trials to further examine efficacy of tea tree oil as an adjunctive wound therapy, as well as improved guidelines for developing plant-based medicines.

Yuetian Zhang et al (2018) In the study, the minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC), and action mechanism of terpinen-4-ol

against S. agalactiae was investigated to evaluate antibacterial activity of terpinen-4-ol. Results showed the MIC and MBC of terpinen-4-ol were 98 and 196 μg/mL, respectively. Time-kill curves displayed that the antibacterial activity of terpinen-4-ol was in a concentration-dependent manner. Transmission electron micrographs showed that the cell membrane and wall of S. agalactiae were damaged, and plasmolysis and chromatins were inconspicuous. Release of Ca^{2+} and Mg^{2+} proved that terpinen-4-ol could increase cell membrane permeability. And the release of lactate dehydrogenase (LDH) suggested that cell wall was destroyed. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and 4',6-diamidino-2-phenylindole (DAPI) staining results showed that terpinen-4-ol could affect the synthesis of protein and DNA. These results suggested that terpinen-4-ol might be used as candidate for treating S. agalactiae infection.

N S Radulović et al (2013) Widespread antibiotic resistance, the emergence of new pathogens in addition to the resurgence of old ones, and the lack of effective new therapeutics exacerbate the problems. Thus, the need to discover and develop new antimicrobial agents is critical to improve mankind's future health. Plant secondary metabolites (PSMs) offer particular promise in this sense. Plant Kingdom could be considered a rich source of the most diverse structures (e.g. there are more than 12,000 known alkaloids, more than 8,000 phenolic compounds and over 25,000 different terpenoids), many of which were proven to possess strong antimicrobial properties (e.g. thymol, eurabienol, etc.). Having all of the mentioned advantages of PSMs as potential antimicrobials in mind, a major question arises: why is it that there are still no commercially available or commonly used antibiotics of plant origin? This review tries to give a critical answer to this question by considering potential mechanisms of antimicrobial action of PSMs (inhibition of cell wall or protein synthesis, inducing leakage from the cells by tampering with the function of the membranes, interfering with

intermediary metabolisms or DNA/RNA synthesis/function), as well as their physical and chemical properties (e.g. hydrophilicity/lipophilicity, chemical stability). To address the possible synergistic/antagonistic effects between PSMs and with standard antibiotics, special attention has been given to the antimicrobial activity of PSM-mixtures (e.g. essential oils, plant extracts). Moreover, possible ways of overcoming some of PSMs molecular limitations in respect to their usage as potential antibiotics were also discussed.

E. Bordini et al (2018) This study evaluated the antibacterial activity of terpinen-4-ol against Streptococcus mutans and Lactobacillus acidophilus and its influence on gbpA (S. mutans) and slpA (L. acidophilus) gene expression. As measured by XTT assay, the concentrations of terpinen-4-ol that effectively inhibited the biofilm were 0.24% and 0.95% for S. mutans and L. acidophilus, respectively. Confocal microscopy revealed the presence of a biofilm attached to the enamel and dentin block surfaces with significant terpinen-4-ol effects against these microorganisms. The expression of the gbpA and slpA genes involved in adherence and biofilm formation was investigated using RT-PCR. Expression of these genes decreased after 15 min with 0.24% and 0.95% terpinen-4-ol in S. mutans and L. acidophilus, respectively. These findings demonstrate the antimicrobial activity of terpinen-4-ol and its ability to modulate the expression of gbpA and slpA genes, emphasizing the therapeutic capacity of terpinen-4-ol as an alternative to inhibit adherence in biofilm.

Philip D Marsh (2006) The predominant species from diseased sites are different from those found in healthy sites, although the putative pathogens can often be detected in low numbers at normal sites. In dental caries, there is a shift toward community dominance by acidogenic and acid-tolerating species such as mutans streptococci and lactobacilli, although other species with relevant traits may be involved. Strategies to control caries

could include inhibition of biofilm development (e.g. prevention of attachment of cariogenic bacteria, manipulation of cell signaling mechanisms, delivery of effective antimicrobials, etc.), or enhancement of the host defenses. Additionally, these more conventional approaches could be augmented by interference with the factors that enable the cariogenic bacteria to escape from the normal homeostatic mechanisms that restrict their growth in plaque and out compete the organisms associated with health. Evidence suggests that regular conditions of low pH in plaque select for mutans streptococci and lactobacilli.

Stuart G Dashper et al (2013) aimed to characterise the biofilm disruptive component of CMP and compare its activity with the known antimicrobial agents chlorhexidine and zinc ions. CLSM analysis showed that S. mutans produced robust, structured biofilms with an average thickness of 7.37μm and a biovolume of 3.88μm(3)/μm(2) substratum after 16h of incubation in the flow cell system. A single application of 10mg/mL CMP that contained 2.4mg/mL KCG significantly reduced total biofilm biovolume and average biofilm thickness by 53% and 61%, respectively. This was statistically the same as a 2.4mg/mL KCG treatment that reduced the total biovolume and average thickness by 59% and 69%, respectively, suggesting the KCG was the biofilm disruptive component of CMP. Chlorhexidine treatment (0.1%) caused similar effects in the flow cell model. KCG-Zn caused significantly more disruption of the biofilms than either KCG or ZnCl2 treatment alone. In a static biofilm model chlorhexidine was shown to work by disrupting bacterial membrane integrity whilst KCG-Zn had no effect on membrane integrity.

Gaurav Patri (2017) study aimed to compare the efficacy of herbal antibacterial agents (Tea Tree Oil (TTO) and *Aloe vera*) with commercially available 2% chlorhexidine (CHX) as cavity disinfectant for use in minimally invasive dentistry. The results of

study showed that there was a statistically significant reduction in TVC when compared between pre and post excavation in all the groups ($p<0.05$) and post- excavation and post-disinfection in all the test groups ($p<0.05$) (except control group). Post-disinfection, 2% chlorhexidine showed highest reduction in TVC followed by 1% tea tree oil and aloe vera gel.

Guerreiro MYR (2020) aimed to To evaluate the effect of the addition of surfac-tants to sodium hypochlorite (NaOCl) on the removal of accumulated hard tissue debris (AHTD), before and after final irrigation with 17% EDTA, from mesial canals of mandibular molars through microcomputed tomographic (micro-CT) analysis. Results state that none of the irrigant solutions tested was able to completely eliminate hard tissue debris from mesial canals of mandibular molars. There were no significant differences in the percentage of AHTD amongst the different irrigation solutions ($P > 0.05$). Final irrigation with 17% EDTA significantly reduced the percentage of AHTD ($P < 0.05$), without differences amongst the groups ($P > 0.05$). The study concludes that addition of surfactants to NaOCl did not affect the removal of AHTD from mesial canals of extracted mandibular molars. Final irrigation with 17% EDTA significantly improved AHTD removal.

Manikandan Ravinanthanan (2018) The aim of this study was to evaluate the efficacy of irrigants and identify a cost-effective regimen to eradicate VRE. The study results that about 2.5% and 5% CHX were significant over mixture of tetracycline, acid and detergent (MTAD) ($P < 0.05$). 5% CHX could achieve 100% elimination while 2.5% CHX and 5% IKI had 99.90%. 2% CHX and 2.5% IKI had 99% effective kill percentage. All concentrations of NaOCl were ineffective (90%) as compared to MTAD (95%). CTR (0.5%, 1% and 2%) and SDS (2%) were significant ($P < 0.05$) over MTAD. Combination surfactant regimens of 2% CHX +0.5% CTR and 2% CHX +1% SDS

achieved 99.90% eradication potential and were significant ($P < 0.05$) over MTAD. It concludes that Surfactant regimens were highly effective and superior to MTAD. CTR and SDS by their organic solvent property enhanced the antibacterial action of CHX.

Luciano Giardino (2006) The aim of this study is to compare the surface tension of four common endodontic irrigants: Moltendo EDTA 17%, Cetrexidin, Smear Clear, Sodium hypochlorite 5.25%, with the surface tension of MTAD and Tetraclean. Freshly produced MilliQ water was used as a reference. All measurements were performed following the Wilhelmy plate technique, using a Cahn DCA-322 Dynamic Contact Angle Analyzer at the temperature of 22 degrees C. MilliQ water, sodium hypochlorite 5.25%, and EDTA 17% had the highest surface tension, whereas those of Cetrexedin and Tetraclean has shown the lowest surface tension value. Both new irrigants, MTAD and Tetraclean, are capable of removing the smear layer. Thanks to their low surface tension, increasing the intimate contact of irrigant solutions with the dentinal walls, they may permit deeper penetration.

Sinha DJ et al (2015) aimed to evaluate and compare the antibacterial efficacy of *Melaleuca alternifolia* (tea tree oil), Curcumalonga (turmeric), 2% chlorhexidine (CHX), and 5% sodium hypochlorite (NaOCl) against *Enterococcus faecalis.* Maximum antibacterial efficacy was exhibited by 2% CHX, followed by 5% NaOCl and C. longa with no statistically significant difference between them. It was followed by M. alternifolia (Tea tree oil). Ethanol and saline showed the least antibacterial action. Author concludes by saying *C. longa* and *M. alternifolia* can be used as an alternative root canal irrigant, although long-term in vivo studies are warranted.

F Taşman (2000) The aim of this study was to evaluate the surface tension values of established and potential endodontic irrigants to which a surface active agent had not been added. Additionally, Cetredixine, a surfactant-containing 0.2% chlorhexidine

gluconate solution, was included in the measurements. Surface tension measurements were performed using the ring method on a DuNouy tensiometer at a standard room temperature. Ringer's solution, saline solution, and distilled water had the highest surface tension values, whereas those of NaOCl (2.5% and 5%) and 17% EDTA were relatively low. Two anesthetic solutions, Ultracaine and Citanest, demonstrated values similar to NaOCl and EDTA, although a statistically significant difference was found between all solutions tested. Cetredixin displayed the lowest surface tension. A low surface tension agent should penetrate tubules better.

Gerald E. McDonnell (2020) book titled Antisepsis, Disinfection, and Sterilization, is a detailed and accessible presentation of the current methods of microbial control. Each major category, such as physical disinfection methods, is given a chapter, in which theory, spectrum of activity, advantages, disadvantages, and modes of action of the methods are thoroughly and clearly presented. Sufficient background on the life cycles and general anatomy of microorganisms is provided so that the reader who is new to microbiology will better appreciate how physical and chemical biocides work their magic on microbes.

Abani K Bhuyan (2010) To understand the mechanism of ionic detergent-induced protein denaturation, this study examines the action of sodium dodecyl sulfate on ferrocytochrome c conformation under neutral and strongly alkaline conditions. Equilibrium and stopped-flow kinetic results consistently suggest that tertiary structure unfolding in the submicellar and chain expansion in the micellar range of SDS concentrations are the two major and discrete events in the perturbation of protein structure. The nature of interaction between the detergent and the protein is predominantly hydrophobic in the submicellar and exclusively hydrophobic at micellar levels of SDS concentration. The observation that SDS also interacts with a highly

denatured and negatively charged form of ferrocytochrome c suggests that the interaction is independent of structure, conformation, and ionization state of the protein. The expansion of the protein chain at micellar concentration of SDS is driven by coulombic repulsion between the protein-bound micelles, and the micelles and anionic amino acid side chains.

E.Banin(2006) Biofilms consist of groups of bacteria attached to surfaces and encased in a hydrated polymeric matrix. Bacteria in biofilms are more resistant to the immune system and to antibiotics than their free-living planktonic counterparts. Thus, biofilm-related infections are persistent and often show recurrent symptoms. The metal chelator EDTA is known to have activity against biofilms of gram-positive bacteria such as Staphylococcus aureus. EDTA can also kill planktonic cells of Proteobacteria like Pseudomonas aeruginosa. In this study we demonstrate that EDTA is a potent P. aeruginosa biofilm disrupter. In Tris buffer, EDTA treatment of P. aeruginosa biofilms results in 1,000-fold greater killing than treatment with the P. aeruginosa antibiotic gentamicin. Furthermore, a combination of EDTA and gentamicin results in complete killing of biofilm cells. P. aeruginosa biofilms can form structured mushroom-like entities when grown under flow on a glass surface. Time lapse confocal scanning laser microscopy shows that EDTA causes a dispersal of P. aeruginosa cells from biofilms and killing of biofilm cells within the mushroom-like structures. An examination of the influence of several divalent cations on the antibiofilm activity of EDTA indicates that magnesium, calcium, and iron protect P. aeruginosa biofilms against EDTA treatment. Our results are consistent with a mechanism whereby EDTA causes detachment and killing of biofilm cells.

MATERIALS AND METHODS

Armamentarium

- Eighty freshly extracted single rooted teeth
- Straight hand piece (NSK Japan)
- Micromotor (Marathon M3) with diamond disc and round bur (MANI, INC)
- K –files no 10, 15 and 20 (MANI, INC)
- 2.5 ml syringe (Dispovan), (Figure 1)
- Protaper gold rotary files (Dentsply Maillefer)
- Normal Saline (IPSOI. healthcare Pvt. Ltd), (Figure 1)
- RC prep (Prime dental, India), (Figure 1)
- Endodontic motor (Changzhou sifary medical technology co.,Ltd) , (Figure 1)
- Ultrasonic unit (Changzhou sifary medical technology co.,Ltd) (Figure 1)
- Mueller Hinton agar (HiMedia laboratories Pvt Ltd, India)
- *E. faecalis* (ATCC 29212)
- Cetrimide 96% (Loba chemie Pvt.Ltd.) (Figure 2)
- Sodium lauryl sulphate / sodium dodecyl sulphate powder 85% (Loba chemie Pvt.Ltd.) (Figure 3)
- Tween 80 (Loba chemie Pvt.Ltd.) (Figure 4)
- Tea tree oil diluted in ethanol – irrigating solution (Figure 5) (Afro Meal; Vijay impex)

For Preparation of Test Solution:

1. Sterile uricols

2. Weighing paper

3. Distilled water

4. Electronic balance

5. Sterile disposable syringe-Membrane filters (0.22μm, Sartorius)

For Agar Well Diffusion and Minimal Inhibitory

Concentration:

1. Mueller Hinton Broth (Figure 6)

2. Mueller Hinton Agar (HiMedia laboratories Pvt Ltd, India) (Figure 9)

3. Petridishes

4. Test tubes

5. Microtitre plate

6. Micro pipettes

7. Micro pipette tips

8. Cork borer

For Biofilm Formation and *E. faecalis* Sampling:

1. Mueller Hinton broth (Figure 6)

2. Mueller Hinton agar (HiMedia laboratories Pvt Ltd, India), (Figure 9)

3. Petridishes

4. Test tubes

5. Micro pipettes

6. Eppendorf tubes

7. Inoculation loops

8. Sterile saline

Standard Bacterial Culture Used in the Study:

Stock culture of *E. faecalis* (ATCC 29212) that had been maintained in the Department of Microbiology, Sree Balaji Dental College and Hospital, BIHER, Chennai was used for the study.

Revival of Microbial Cultures:

The culture was revived on MacConkey Agar (HiMedia Laboratories Pvt Ltd, Mumbai, India) supplemented with 0.5% NaoCl (Nice chemicals (P) LTD). After overnight incubation, the growth obtained on the agar plates was checked for purity by Gram's staining.

For Testing Antibacterial Efficacy of Irrigating solutions on *E. Faecalis* Planktonic Cells:

Agar well diffusion and Broth microdilution assay were performed to assess the efficacy of surfactants-Cetrimide, Sodium lauryl sulphate and Tween 80 individually and in combination with Tea tree oil.

Inoculum Preparation:

Isolated colonies (5-6) of *E. Faecalis* from MacConkey agar plate cultures were suspended in sterile Muller Hinton Broth (MHB) (HiMedia Laboratories Pvt Ltd, Mumbai, India) and the cell densities were adjusted to match 0.5 McFarland standard (1.5×10^8 cfu/mL) as recommended by CLSI

(Clinical and Laboratory Standards Institute) guidelines for any antibacterial assay by diffusion technique.

Agar Well Diffusion Technique:

Agar well diffusion assay was performed as it is a convenient method for screening the antibacterial activity of endodontic irrigant solutions against bacterial strains. Cetrimide, Sodium lauryl sulphate and Tween 80 individually and in combination with 2% tea tree oil were tested for potential anti-enterococcal activity against *E. faecalis* (standard strain, ATCC 29212). The test irrigants were dispensed into the respectively labelled wells of MHA plate which had been seeded (lawn culture) with *E. faecalis* ATCC 29212.

Agar Well Diffusion Protocol:

The broth culture of *E. faecalis* ATCC 29212 matched with 0.5 McFarland standards was inoculated onto Mueller Hinton Agar plates using sterile swabs to obtain a lawn culture. Four wells of diameter of 8mm were punched aseptically with a sterile cork borer. The prepared test solutions (Tea tree oil (2%) (Group 7), SDS (2%) (Group 2), Cetrimide (2%) (Group 1), Tween 80 (0.2%) (Group 3), Cetrimide + Tea tree oil (Group 4), SDS + Tea tree oil (Group 5), Tween 80 + Tea tree oil (Group 6)) were placed into the respectively labelled wells. The agar plates were incubated at 37°C for 24 hours. The diameter of zone of inhibition was measured using a

graduated scale.

Test Groups for Micro Broth Dilution Assay:

The test solutions used for microbroth dilution assay were Groups 1, 2, 3, 4, 5, 6 and 7.

Preparation of Test Solutions:

The test solutions were divided into seven groups. They are:

Group 1: Cetrimide

Group 2: Sodium lauryl sulphate

Group 3: Tween 80

Group 4: Cetrimide + Tea tree oil

Group 5: Sodium lauryl sulphate + Tea tree oil

Group 6: Tween 80 + Tea tree oil

Group 7: Tea tree oil (in ethanol)

The test solutions were prepared in sterile injectable water and were filter sterilized using sterile membrane filters.

Micro Broth Dilution Assay (Figure 8):

Broth dilution assay involves challenging the organism of interest with antimicrobial agents in a broth environment. Broth microdilution denotes the performance of the broth dilution test in a microtiter plate. In this procedure a series of doubling dilution of the test solutions (potential antibacterial agents) was prepared in the broth medium. The lowest antimicrobial concentration that completely inhibits visible bacterial growth

was recorded as the minimal inhibitory concentration (MIC). From the prepared test solutions doubling dilutions were done to obtain a concentration gradient. The assay was performed in duplicate for each test solution.

Micro Broth Dilution Protocol:

The test solutions were added to the respectively labelled wells - A1 & B1: Cetrimide (4%), C1 & D1: Sodium lauryl sulphate (4%), E1 & F1: Tween 80 (4%), G1 & H1: Cetrimide (4%), + Tea tree oil (4%), I1 & J1: Sodium lauryl sulphate (4%), + Tea tree oil (4%), K1 & L1: Tween 80 (4%) + Tea tree oil (4%), M1 & N1: Tea tree oil (4%). Doubling dilution was performed from well A1 through A11, well B1 through B11, well C1 through C11, well D1 through D11, well E1 through E11, well F1 through F11, well G1 through G11, well H1 through H11, well I1 through I11, well J1 through J11, well K1 through K11, well L1 through L11, well M1 through M11, and well N1 through N11. Wells A12, B12, C12, D12, E12, F12, G12, H12, I12, J12, K12, L12, M12, N12 served as culture controls (without the test solution/irrigant). To all the wells, 10µl of *E. faecalis* ATCC 29212 suspension was added and incubated at 37°C for overnight. The MIC was determined by performing minimum bactericidal concentration (MBC).

Minimum Bactericidal Concentration:

Minimum Bactericidal Concentration was performed by inoculating 5µl of

broth culture from all the wells onto respectively labelled MHA plates. The MBC was regarded as the lowest concentration in the series of dilution which inhibited the growth of *E. faecalis*.

Tooth Sample Preparation:

- Eighty freshly extracted intact non carious, single rooted human teeth with fully formed apices were chosen for the study. The teeth were cleaned of superficial debris, calculus, tissue tags and stored in normal saline (IPSOI. healthcare Pvt. Ltd) . The teeth were then radiographed to confirm the presence of single canal. The tooth specimens were sectioned below the cementoenamel junction (CEJ) with a diamond disc to obtain a standard tooth length of 15mm. This enabled us to avoid cervical interference, easy exploration and to achieve better cleaning and shaping of the canal. A size 10 K file was used for scouting and establishing patency till the tip of the file was seen at the apical foramen from which 1 mm was subtracted in order to establish the working length. All the samples were instrumented using Protaper gold rotary system (Dentsply Maillefer) in a crown down technique using Eighteenth E-xtreme endomotor system (Changzhou sifary medical technology co.,Ltd). The apical third of each canal was enlarged to a size 30 with 9% taper (F3) to allow adequate flushing action of saline solution. During instrumentation, 0.85% saline solution (IPSOI. healthcare Pvt. Ltd) was used for flushing of root canals. The apices of teeth were sealed with two layers of nail varnish.

Sterilization of Tooth Samples:

The prepared tooth samples were placed in clean glass test tubes (5 teeth/test tube) containing 5 ml of Mueller Hinton Broth (HiMedia Laboratories Pvt Ltd, India) and autoclaved at 121°C at 15lbs pressure for 20 mins (Figure 10). The efficacy of sterilization was assessed by including a biological indicator, *Geobacillus stearothermophilus* MTCC 1518. After sterilization, the tubes were also placed in an incubator at 37°C for 48 hrs and observed for turbidity to double check the sterility of the samples.

Biofilm Formation (Figure 8):

The sterile MHB in 17 test tubes (5 teeth/test tube) were inoculated with 10µl of *E. faecalis* ATCC 29212 cultured overnight at 37°C in Mueller Hinton Broth (MHB, HiMedia laboratories Pvt Ltd, India) adjusted to an optical density of 1.5 X 10^8 cells/mL with 0.5 McFarland standard. To avoid nutrient depletion and accumulation of toxic end products sterile culture medium (Mueller Hinton broth) was replaced every alternate day. The culture purity was checked by inoculating a loopful of culture media onto Mueller Hinton agar (HiMedia laboratories Pvt Ltd, India) and by Gram staining. At the end of 4^{th} week the incubated samples were randomly assigned to one of the five groups.

Antibiofilm Activity of the Test Irrigants:

Twenty samples for each test group were used for quantitative assay. The teeth were randomly divided into four groups for the above-mentioned

irrigating solutions with 20 specimens in each group. Sterile paper points per tooth were used at working length to obtain pre-treatment sample (PRE-A) the canals from all 4 groups (Figure 12). After pre-treatment paper point sampling, the tooth specimens (with *E. faecalis* dentinal biofilm) were prepared with 30/09 (F3) and irrigated with 5 mL of the respective irrigant (6X MIC of the planktonic cells, Group 1: Cetrimide (0.024%) + Tea tree oil (6 %), Group 2: Tween 80 (24 %) + Tea tree oil (6%), Group 3: Sodium lauryl sulphate (0.18%) + Tea tree oil (6%), Group 4: Tea tree oil (6%)). All irrigants were delivered at a flow rate of 0.04 mL/s using a side-vented 30-gauge needle (Diadent). Irrigants were activated using passive ultrasonic irrigation technique for 3 minutes) (Figure 11). Post irrigation sample (POST-B) was taken following saline wash. Post treatment with the respective test irrigants, samples from each tooth were taken using sterile paper points. Following which the paper points from each tooth sample were placed individually in 1.5-mL microtubes containing 1 mL of sterile Phosphate Buffered Saline (PBS) (HiMedia Laboratories Pvt Ltd, Mumbai, India). The microfuge tubes were vortexed to suspend the bacteria (*E. faecalis*) adherent to the paper points. Spread plate technique was adopted to determine the viable count of *E. faecalis.* Briefly, 10 microlitres from each microfuge tube was plated on MHA plates and the plates were incubated at 37^0 C for 24 hours, after which the colonies were counted by using a colony counter. The number of colony forming units (CFU) was

calculated and subjected to statistical analysis.

Flow chart- METHODOLOGY:

PREPARATION OF TOOTH SAMPLES

SINGLE ROOTED TOOTH WERE COLLECTED (n=80)

↓

DECORONATED TO A LENGTH OF 15mm

↓

WORKING LENGTH ESTABLISHED USING 15K FILE

↓

APICAL ENLARGEMENT WAS DONE UNTIL F3

↓

IRRIGATED WITH 3% NaOCl (total vol 10 mL) FOR 5 MINS , FOLLOWED BY 17% EDTA (3 mL) FOR 1 MIN

↓

IRRIGATED WITH 10% SODIUM THIOSULPHATE (TO REDUCE THE CARRY OVER EFFECT)

↓

TOOTH SAMPLES WERE RINSED WITH DISTILLED WATER.

PREPARED TOOTH SAMPLES WERE STORED IN STERILE SALINE.

STERILZATION OF THE PREPARED TOOTH SAMPLES

PREPARED TOOTH SAMPLES WERE AUTOCLAVED THRICE (121°C, 20 MINS @ 15 LB PRESSURE)

↓

STERILIZED TOOTH SAMPLES WERE TRANSFERRED TO TEST TUBES CONTAINING STERILE MHB BROTH

↓

STERILITY WAS PERFORMED BY INCUBATING THE TOOTH SAMPLES AT 37°C FOR 48 HRS.

E. faecalis DENTINAL BIOFILM FORMATION

THE TOOTH SAMPLES WERE INNOCULATED WITH *E. faecalis* ATCC 29212

INCUBATE IT FOR 4 WEEKS AT 37°C

↓

THE ARTIFICALLY INFECTED TOOTH SAMPLES WERE INCUBATED AT 37°C FOR 4 WEEKS TO FORM *E. faecalis* DENTINAL BIOFILM

ANTI-BIOFILM ASSAY

AFTER 4 WEEKS OF INCUBATION, THE TOOTH SAMPLES WERE GENTLY RINSED WITH STERILE SALINE TO REMOVE THE PLANKTONIC CELLS.

↓

THE ARTIFICALLY INFECTED TOOTH SAMPLES WERE RANDOMLY DIVIDED INTO 4 GROUPS

↓

GROUP 1	GROUP 2	GROUP 3	GROUP 4
6% TEA TREE OIL + 0.024% CETRIMIDE	6% TEA TREE OIL + 24% TWEEN 80	6% TEA TREE OIL + 0.18% SODIUM LAURYL SULPHATE	6% TEA TREE OIL

↓

PRE TREATMENT SAMPLE (SAMPLE A) WERE COLLECTED FROM EACH TOOTH USING ABSORBENT PAPER POINTS (60 SECS)

↓

↓

PREPARED ROOT CANALS WERE IRRIGATED WITH THE RESPECTIVE TEST SOLUTIONS (2 mL/teeth)

↓

AGITATED WITH ULTRASONICS FOR 3 MINUTES

↓

POST TREATMENT SAMPLE (SAMPLE B) WERE COLLECTED FROM EACH TOOTH USING ABSORBENT PAPER POINTS (60 SECS)

↓

THE PAPER POINTS (WITH THE ADSORBED *E. faecalis* ATCC 29212) WERE TRANSFERRED 1.0 mL OF PBS IN RESPECTIVELY LABELLED MICROFUGE TUBES.

↓

SPREAD PLATE TECHNIQUE WAS PERFORMED ON MHA PLATES

↓

FIGURE 1: ENDODONTIC MOTOR AND ULTRASONIC UNIT

FIGURE 2: CETRIMIDE

FIGURE 3: SODIUM DODECYL SULPHATE / SODIUM LAURYL SULPHATE

FIGURE 4: TWEEN 80

FIGURE 5: TEA TREE OIL DILUTED IN ETHANOL

FIGURE 6: MUELLER HINTON BROTH MED

FIGURE 7: SECTIONED TOOTH SAMPLE OBTAINED AFTER AUTOCLAVING

FIGURE 8: TOOTH SAMPLES INCUBATED FOR *E. faecalis* BIOFILM FORMATION KEPT IN ORBITAL SHAKER

FIGURE 9: MUELLER HINTON BROTH MEDIUM

FIGURE 10: ELECTRONIC COLONY COUNTER

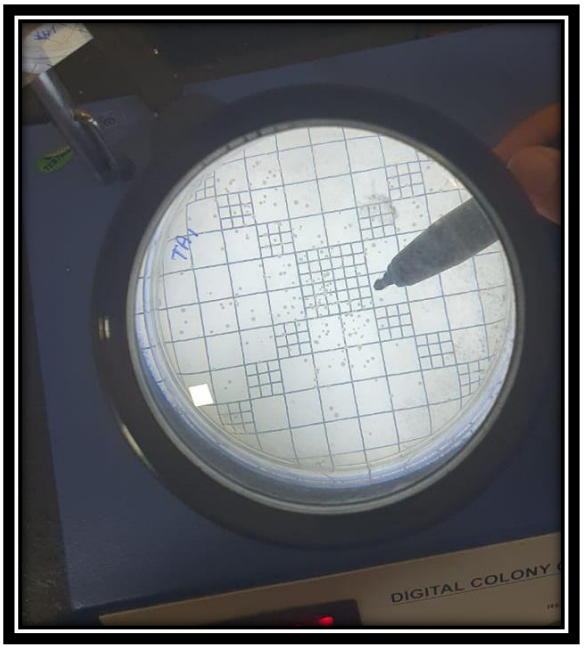


FIGURE 11: CLEANING AND SHAPING OF TOOTH SAMPLES

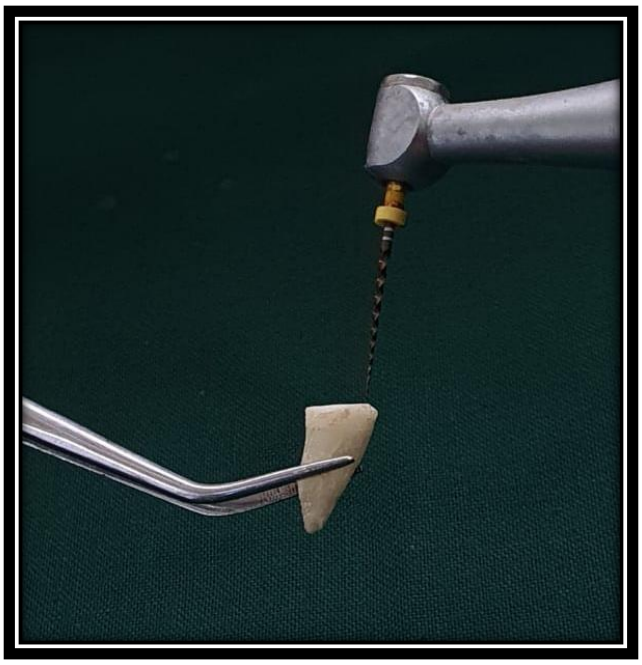
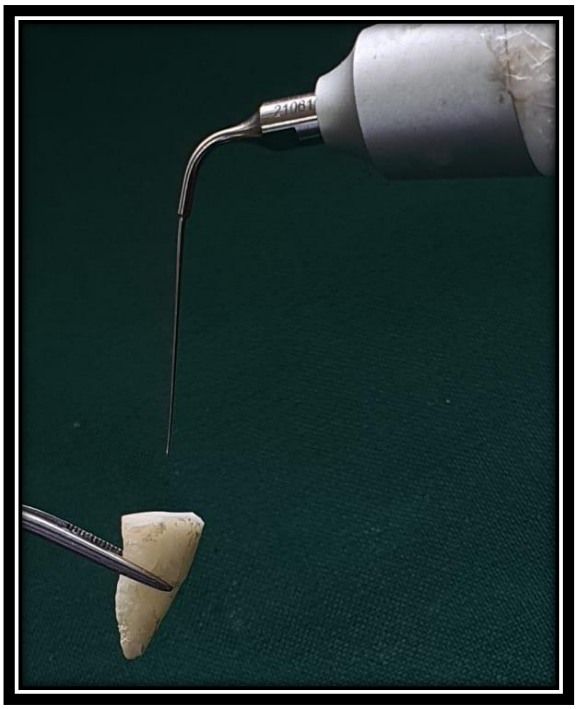


FIGURE 12: SAMPLE COLLECTION USING PAPER POINT



RESULTS

Agar well diffusion assay:

The agar well diffusion assay was performed in triplicates in order to confirm the reproducibility of the results. The Mean diameter of the zone of inhibition of *E. faecalis* with Tea Tree oil, test surfactants i.e Cetrimide, SDS, Tween 80 and combination of Tea Tree oil with the surfactants are depicted in Table 1. Statistical analysis by one way ANOVA revealed that significant difference was observed between the study groups. Post Hoc Test: Tukey HSD reveled that Tween 80 and Tea Tree oil + Tween 80 showed the least inhibition. The surfactants, 2% Cetrimide and 2% SDS showed a wider zone of inhibition compared the surfactants combined with tea Tree oil (2% Tea Tree oil + 2% Cetrimide and 2% Tea Tree oil + 2% SDS) (Table 2, Figure 1).

Table 1: Mean diameter of the zone of inhibition

Group	Mean	SD	One way ANOVA	
			F-Value	*p*-Value
2% Tea Tree oil	10.67	.58	533.452	.000
Ethanol	11.00	.00		

2% Cetrimide	33.67	2.08		
2% SDS	33.67	.58		
0.2% Tween	8.00	.00		
2% Tea Tree oil + 2% Cetrimide	26.67	.58		
2% Tea Tree oil + 2% SDS	28.67	.58		
2% Tea Tree oil + 0.2% Tween 80	8.67	.58		

Figure 13: Mean diameter of the zone of inhibition

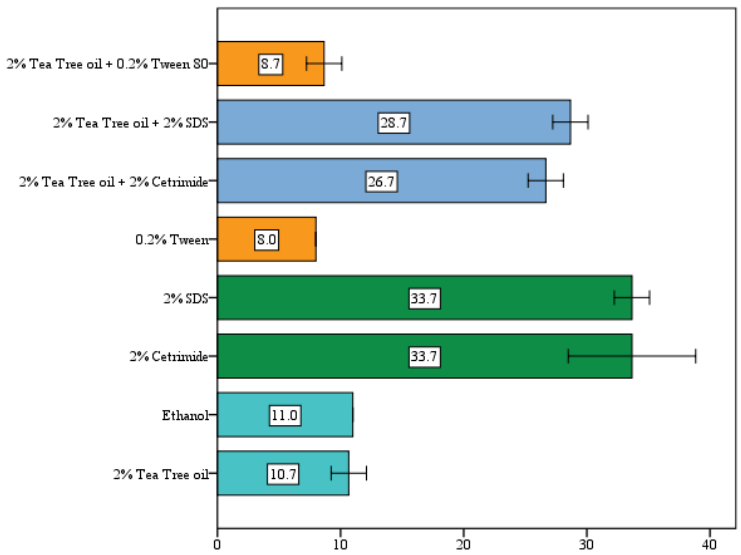


Table 2: Post Hoc Test: Tukey HSD – Homogeneous Subsets

Mean Diameter of zone of inhibition: Homogeneous Subsets					
		Subset for alpha = 0.05			
Group	N	1	2	3	4
0.2% Tween	3	8.00			
2% Tea Tree oil + 0.2% Tween 80	3	8.67			
2% Tea Tree oil	3		10.67		
Ethanol	3		11.00		
2% Tea Tree oil + 2% Cetrimide	3			26.67	
2% Tea Tree oil + 2% SDS	3			28.67	
2% Cetrimide	3				33.67
2% SDS	3				33.67
Sig.		.976	.068	.155	1.000

Pair-wise comparison of the mean values of the diameter of zone of inhibition between the groups are depicted in Table 3. Significant difference was observed between groups. Nevertheless, no statistical difference was observed between tea

Tree Oil *Vs* Ethanol, Cetrimide *Vs* SDS, and Tween 80 *Vs* Tea Tree Oil + Tween 80 (P value 0.317, 0.653 and 0.114 respectively) (Table 3).

Table 3: Pair-wise comparison of the mean values of the diameter of zone of inhibition between the groups.

Between groups	Test Statistic	Asymp. Sig		Between groups	Test Statistic	Asymp. Sig
TT *Vs* ET	-1.000	.317				
TT *Vs* CE	-1.993	.046		CE *Vs* TTCE	-1.993	.046
TT *Vs* SD	-2.023	.043		SD *Vs* TTSD	-2.023	.043
TT *Vs* TW	-2.121	.034		TW *Vs* TTTW	-1.581	.114
TT *Vs* TTCE	-2.023	.043		TTCE – TTSD	-2.023	.043
TT *Vs* TTSD	-2.023	.043		TTCE - TTTW	-2.023	.043
TT *Vs*	-2.023	.043		TTSD -	-2.023	.043

TTTW				TTTW		
CE *Vs* SD	-.449	.653				
CE *Vs* TW	-2.087	.037				
SD *Vs* TW	-2.121	.034				

Minimum Inhibitory concentration of the test irrigants against planktonic *E. faecalis*:

The MIC of the test irrigants (Tea Tree oil, Tea Tree oil with the different surfactants viz., Cetrimide, SDS and Tween 80) below (Table 4).

Table 4: Minimum Inhibitory concentration of the test irrigants against planktonic *E. faecalis*:

Group	MIC
Tea Tree oil	1%

Tween 80	4%
Cetrimide	< 0.004%
SDS	0.03%
Tea Tree oil + Tween 80	1% + 4%
Tea Tree oil + Cetrimide	1% + < 0.004%
Tea Tree oil + SDS	1% + 0.03%

Antibiofilm activity of Tea Tree oil and surfactants:

The mean *cfu /mL* of *E. faecalis* in the different groups both before and after treatment with the test irrigants (Tea Tree oil, Tea Tree oil with the different surfactants viz., Cetrimide, SDS and Tween 80) are depicted in table 5 (Figure 2). There was a significant difference between pre and post test values in all the four groups. The Post test values were significantly lower compared to pre test values.

Table 5: Pairwise comparison of the mean *cfu /mL* of *E. faecalis* in the different test groups.

Group	N	Pre Test			Post Test			Paired Samples t-test	
		Mean	SD	SEM	Mean	SD	SEM	*t*-Value	*p*-value
Tea Tree Oil	18	18294.444	20840.810	4912.226	405.556	647.595	152.640	3.610	.002
Tea Tree Oil + Cetrimide	18	33777.778	18901.340	4455.089	716.667	2597.793	612.306	7.317	.000
Tea Tree Oil + SDS	18	38350.000	44886.540	10579.859	333.333	860.916	202.920	3.580	.002
Tea Tree Oil + Tween 80	18	47216.667	29517.557	6957.355	866.667	1712.927	403.741	6.643	.000

Comparison of the effect of the test irrigants based on the mean difference in the cfu /mL is depicted in Figure 2. The percentage difference between pre and post tests do not differ significantly among the four groups compared, p-value > 0.05 (ANOVA) (Table 6).

- TT vs TT + Cetrimide
- TT vs TT + SDS
- TT vs TT + Tween 80
- TT+ Cetrimide vs TT +SDS
- TT + Cetrimide vs TT + Tween 80
- TT + SDS vs TT + Tween 80

Figure 14: Mean *difference in the cfu /mL* of *E. faecalis* between the in the different groups.

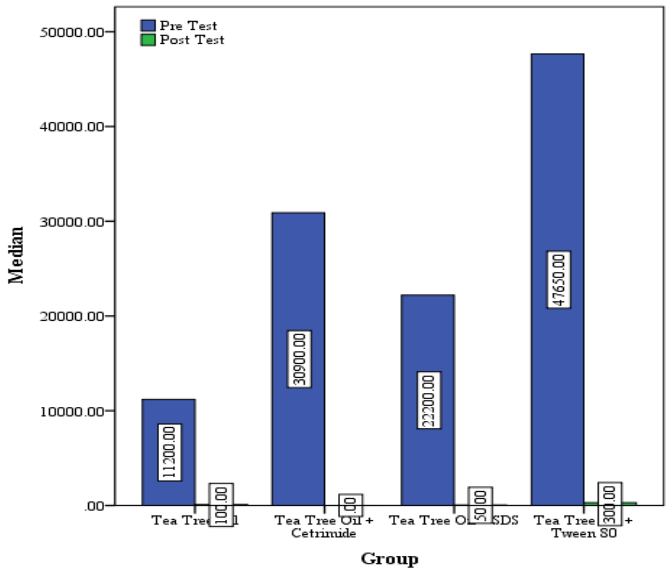


Table 6: Percentage mean difference between Pre and Post Tests.

	N	Mean	SD		One way ANOVA	
	Statistic	Statistic	Statistic	Std. Error	*F*-Value	*p*-Value
TT Diff (%)	18	95.391	7.428	1.751	.521	.669
TTCE Diff (%)	18	97.659	8.319	1.961		
TTSD Diff (%)	18	97.752	5.748	1.355		
TTTW Diff (%)	18	97.573	4.627	1.091		

Pair-wise comparison of the mean values between the groups indicate that the percentage difference between pre and post tests do not differ in all the six comparisons made (Table 7).

Table 7: Independent Samples t-test: Pair wise Comparisons

	Tea Tree Oil			Tea Tree Oil + Cetrimide			Independent Samples t-test	
N	Mean	SD	SEM	Mean	SD	SEM	*t*-Value	*p*-Value
18	95.391	7.428	1.751	97.659	8.319	1.961	-.863	.394
	Tea Tree Oil			Tea Tree Oil + SDS				
18	95.391	7.428	1.751	97.752	5.748	1.355	-1.066	.294
	Tea Tree Oil			Tea Tree Oil + Tween 80				
18	95.391	7.428	1.751	97.573	4.627	1.091	-1.058	.298
	Tea Tree Oil + Cetrimide			Tea Tree Oil + SDS				
18	97.659	8.319	1.961	97.752	5.748	1.355	-.039	.969
	Tea Tree Oil + Cetrimide			Tea Tree Oil + Tween 80				
18	97.659	8.319	1.961	97.573	4.627	1.091	.038	.970
	Tea Tree Oil + SDS			Tea Tree Oil + Tween 80				
18	97.752	5.748	1.355	97.573	4.627	1.091	.103	.919

ZONE OF INHIBITION OF TEST IRRIGANTS

FIGURE 15: Tea tree oil & Ethanol

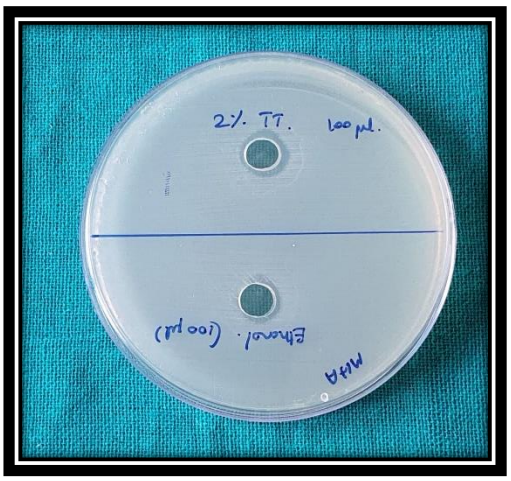
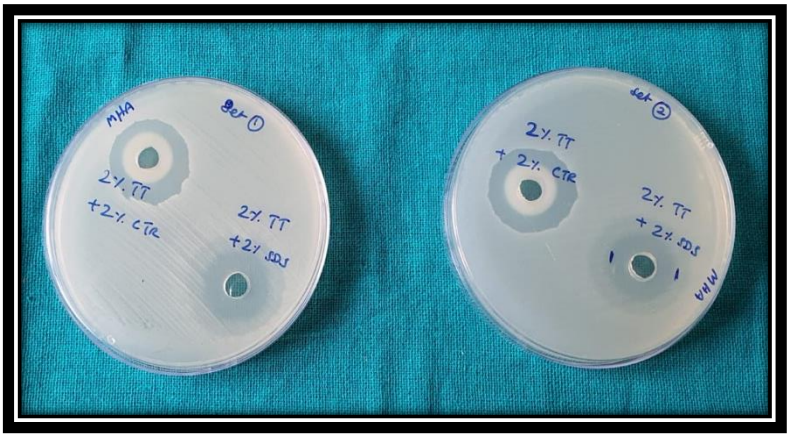


FIGURE 16: Tween 80 & Tween 80 + Tea tree oil

FIGURE 17: Tea tree oil , SDS & Tween 80

FIGURE 18: Cetrimide + Tea tree oil & SDS + Tea tree oil



URE 19: Tween 80

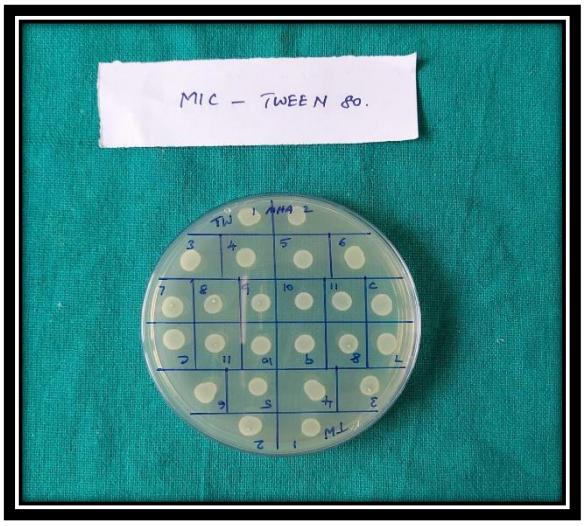


FIGURE 20: SDS

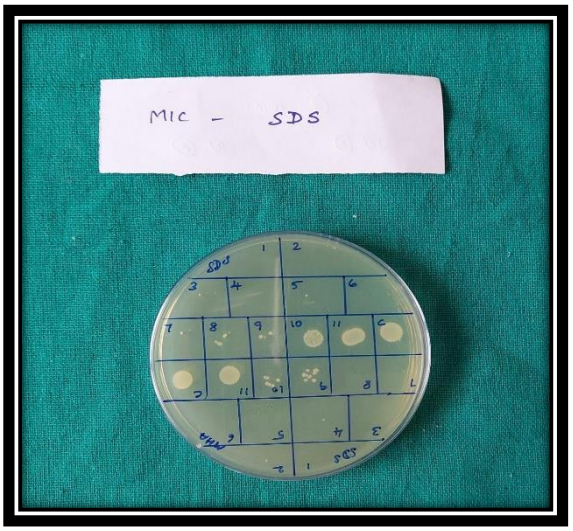


FIGURE 21: CETRIMIDE + TEA TREE OIL

FIGURE 22: TWEEN 80 + TEA TREE OIL

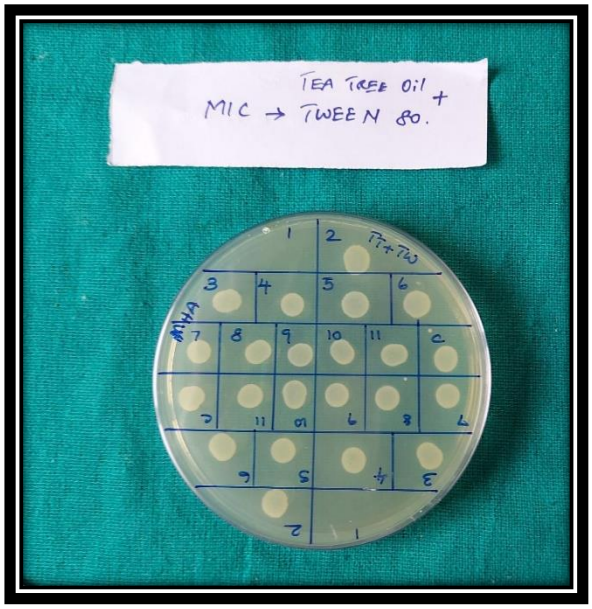


FIGURE 23: TEA TREE OIL

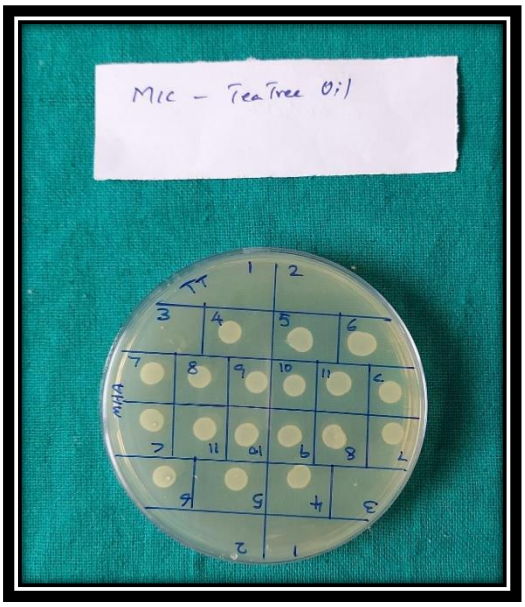


FIGURE 24: CETRIMIDE

ANTIBIOFILM ACTIVITY

COLONY FORMING UNITS OF IRRIGATION SOLUTIONS

FIGURE 25: TEA TREE OIL

FIGURE 26: TEA TREE OIL + CETRIMIDE

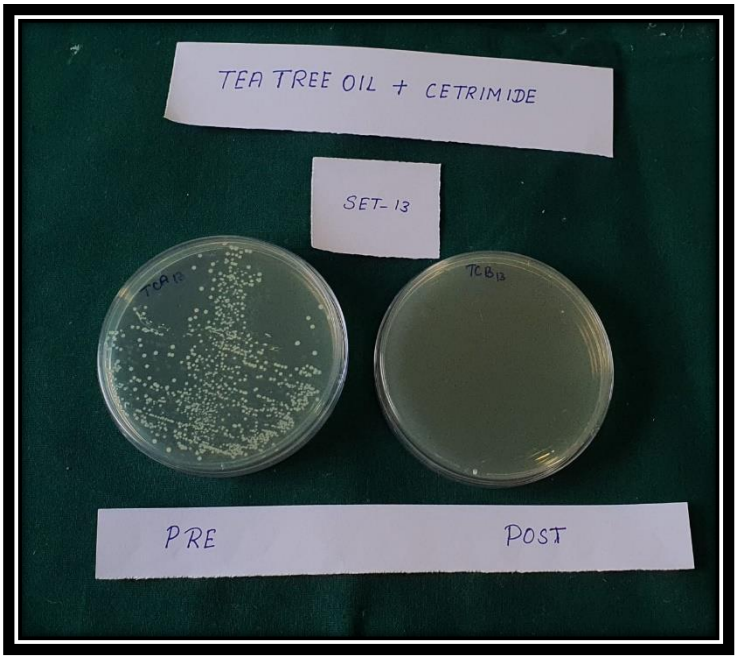
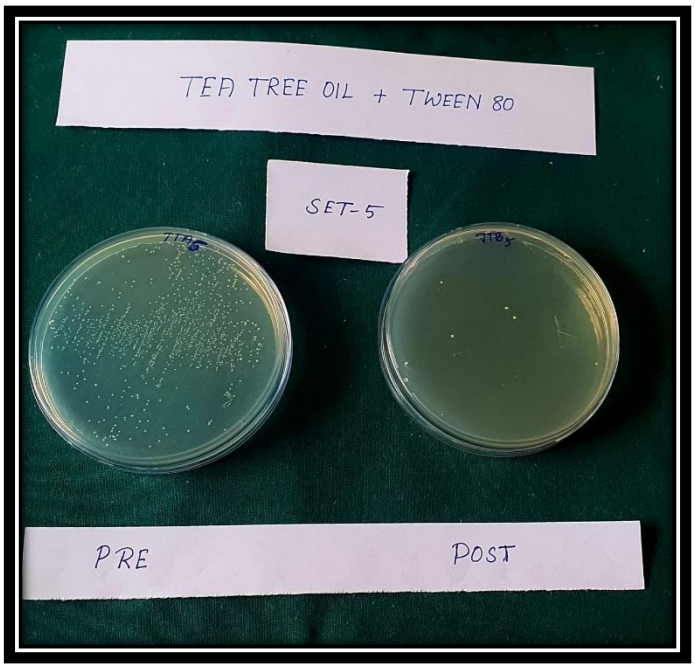


FIGURE 27: TEA TREE OIL + SDS

FIGURE 28: TEA TREE OIL + TWEEN 80



DISCUSSION

The study aimed to estimate the minimum inhibitory and bactericidal concentrations of all the surfactants individually and also in combination with tea tree oil and to analyse the antibacterial efficacy of various surfactants with tea tree oil against E. faecalis.

In the first part of the study, minimum inhibitory concentration was estimated for seven groups which includes Tea tree oil (Group 7), Cetrimide (Group 1), SDS (Group 2), Tween 80(Group 3), Tea tree oil + Cetrimide (Group 4), Tea tree oil + SDS (Group 5), Tea tree oil + Tween 80 (Group 6). Out of all the seven groups cetrimide individually (Group 1) and in combination with tea tree oil (Group 4) showed the lowest minimum inhibitory concentration followed by SDS (Group 2) and its combination groups. Previous studies have used cetrimide in various concentrations such as 0.1% [28], 0.2% [29] and 0.02% [30]. However, we used 0.024% of Cetrimide based on the minimum inhibitory concentration assay conducted as a part of the current study (Table:1) (Figure: 13). Studies conducted using cetrimide as surfactant showed rapid eradication of E. faecalis even at lower concentrations when used in combination with irrigants such as chlorhexidine. Both chlorhexidine and cetrimide target the organisms' cell wall and are synergistic to each other [31,32]. Until recently no study has shown the antibacterial efficacy of tea tree oil in combination with cetrimide or surfactants of various types. Tween 80(Group 3) on the other

hand, showed the highest minimum inhibitory concentration in the current study following Tea tree oil (Group 7). Tween 80 which is well known as a part of MTAD, a universal irrigant, appears to have rather weak antibacterial properties on its own whereas it is known to enhance the antibacterial effects of other compounds [33]. To ascertain the MIC of test irrigants in the current study against planktonic cells of E. faecalis ATCC 29212, a microbroth dilution assay was performed. The majority of research done to assess the antimicrobial efficiency of various irrigants have either been done on monocultures of planktonic cultures or biofilms of E. faecalis [34,35].

In order to assess the minimum bactericidal concentration, agar well diffusion assay was carried out and the zone of inhibition was estimated. We investigated the possible anti-enterococcal effects of cetrimide, SDS, and Tween 80 alone and in combination with 2% tea tree oil (standard strain, ATCC 29212). In the current study, Tea tree oil which was used across the groups was diluted with ethanol, in order to titre its concentration, a separate group containing Tea tree oil diluted with ethanol was taken as a positive control and ethanol as a negative control. The zone of inhibition was almost similar in 2% cetrimide (Group 1) and 2% SDS (Group 2) individually and also the Tea tree oil + 2% cetrimide (Group 4), Tea tree oil + 2% SDS (Group 5) groups exhibited similar zones of inhibition. Pair-wise comparison of the mean values of the diameter of zone

of inhibition between the groups are depicted in Table 3. Significant difference was observed between groups. Nevertheless, no statistical difference was observed between tea Tree Oil *Vs* Ethanol, Cetrimide *Vs* SDS, and Tween 80 *Vs* Tea Tree Oil + Tween 80 (P value 0.317, 0.653 and 0.114 respectively) (Table 3). It is stated that even at lower concentrations, CTR and SDS exhibited significant antibacterial action [36]. The CTR molecule's cationic environment promotes bonding with anionic compounds at the bacterial surface and has the ability to alter the integrity of the cytoplasmic membrane. Protein denaturation and cell death occur as a result of the cytoplasmic membrane enzymes becoming inactive. CTR has been utilised as an endodontic irrigant and is noncytotoxic [37]. A possible explanation for SDS's antimicrobial effect is that it is an anionic alkyl sulphate with low surface tension, the ability to solubilize proteins, an increase in LPS disaggregation, and the ability to inhibit bacterial coaggregation [38].

In the second part of the study, antimicrobial efficacy of various types of surfactants such as Cationic, anionic and non-ionic surfactants were tested against E. faecalis (ATCC 29212) 4-week-old biofilm in combination with tea tree oil.

Enterococcus faecalis is one of the most resistant species in the oral cavity and is a potential reason why root canal treatments fail. "Decreased oxygen tension and other environmental factors that are different from

those at the surface are exposed to cells that are further deep in the biofilm. This promotes altering phenotypes in terms of growth rate and gene transcription, which may facilitate survival and virulence [39]. Furthermore, the potential of E. faecalis cells to invade dentinal tubules and its ability to adhere to collagen in the presence of human serum has been hypothesised to be a virulence factor of E. faecalis in failed endodontically treated teeth [40]. The MIC & MBC for the present study used planktonic bacterial cells whereas E. Faecalis biofilm is used in order to assess the antibacterial efficacy. Since E.Faecalis in the form of biofilm is more resistant and will result in clinically relevant findings, the minimum inhibitory concentration was multiplied by six.

Most investigations in the literature assessed the antibacterial effectiveness of various drugs have been conducted on monocultures of E. faecalis biofilm or planktonic cultures [34,35,41,42]. However, it is widely known that initial endodontic infections are multi-microbial in nature, whereas E. faecalis has been documented to reside as a single organism or coexist with a number of other taxa in teeth that have had root canal therapy. Since the E. faecalis bacterium is crucial to the development of bacterial biofilms in the root canal environment, E. faecalis biofilms are regarded as a suitable model for evaluating novel antimicrobial therapies.

The results of the present study show that there was a significant difference between pre and post-test values, p-value < 0.05 (ANOVA) in all

the four groups. The Post-test values are significantly lower compared to pre-test values. (Table :5) (Figure: 14). Following proper irrigation protocol, ultrasonic activation for 3 minutes (Changzhou sifary medical technology co.,Ltd) (Figure 1) and the antibacterial efficacy of tea tree oil and with the combined effect of surfactants led to a significant difference between the pre and post-test values. In the current study, all of the test groups contain tea tree oil as a main irrigant which naturally possess antibacterial properties. Natural products have been used in dentistry and medicine for thousands of years, and they are now becoming increasingly popular because of their strong antibacterial, biocompatible, anti-inflammatory [43], and antioxidant characteristics. TTO, which is made from Melaleuca alternifolia leaves, has a long history of usage in traditional Indonesian medicine for treating toothaches and microbiological infections [44,45,46]. Due to its diverse spectrum of ingredients and antibacterial actions, the majority of current research highlights TTO as being natural, biodegradable, and having no microbial resistance [47]. Furthermore, based on the LD50 of TTO, Halcon et al. observed that severe responses would be exceedingly rare even in the absence of ingestion [48]. TTO has been demonstrated in several investigations to be able to rapidly destroy bacterial cell structure, severely limit DNA and protein synthesis, and disrupt the enzymatic activity. [49,50,51,52]. Even though there are literatures demonstrating the antibacterial activity of tea tree oil, there are no existing

studies that evaluated the efficacy of various types of surfactants in combination with a single herbal irrigant. Therefore, in the present study, we used tea tree oil to explore its antibacterial activity in combination with various types of surfactants.

The root canal system creates an environment that is conducive to the growth and colonisation of numerous microorganisms, as well as the formation of biofilms that may be resistant to the penetration and disruption of antibacterial agents. TTO's ability to resist bacteria mostly relies on its ability to penetrate and eliminate biofilms [53]. Due to the above reasons, in the current study initially 2% tea tree oil was used during agar well diffusion assay, later during minimal inhibitory concentration assay resulted to be 1% for the planktonic bacteria which is similar to the study conducted by Patri G et al [54].

There are many studies that focused on the antibacterial efficacy of various irrigants, herbal extracts, intracanal medicaments, sealers, etc. A very few assessed the effect of irrigants in combination with or without a single surfactant [28,29,30]. But the current study mainly focuses on a novel approach that assesses the effect of various types of surfactants in combination with a single irrigant (Tea tree oil), which is not reported so far in the literature.

The inter-group comparison did not reveal a significant percentage difference between pre and post-tests, p-value > 0.05 (Table 6). A study

concluded that the clearance of AHTD (accumulated hard tissue debris) from removed mandibular molar mesial canals was unaffected by adding surfactants to NaOCl. Using 17% EDTA as a final irrigation greatly enhanced AHTD elimination, which is in agreement with the current study [55]. In contrast, studies evaluated the effect of surfactants and concluded that Surfactant regimens were far more efficient than MTAD. By virtue of being organic solvents, CTR and SDS improved CHX's antimicrobial effects. They could allow for deeper penetration because of their low surface tension, which increases the close contact of irrigant solutions with the dentinal walls [56,57].

However, there was marginal increase in antibacterial efficacy between the group containing surfactants, p-value = 0.669 (ANOVA) (Group 1,2 &3) when compared to the group containing tea tree oil alone (Group 4) . This is because of the capability of the surfactant to increase the wettability of the irrigant and the mechanism by with it lyses the bacterial cells. Wettability of the irrigant to the dentinal wall is an important factor that has major impact on its efficacy that is strictly proportional to its surface tension. The surface tension is defined as “the force between molecules that produces a tendency for the surface area of a liquid to decrease” [58]. The ability of the solution to penetrate the main and lateral canals as well as the dentinal tubules is determined by its wettability. An irrigant solution may be able to boost its protein solvent capacity and enable

better antibacterial activity in uninstrumented parts of the root canal system by increasing its wettability [59].

Even though not statistically significant, of all the four groups in the current study, the antibacterial efficacy was highest in Tea Tree Oil + Cetrimide (Group 1) followed by Tea Tree Oil, Tea Tree Oil + SDS (Group 3) and Tea Tree Oil + Tween 80 (Group 2) respectively.

Positively charged ion in cationic surfactants integrates into the lipid membrane of the bacterial cell and disorganizes it in addition to increasing wettability. The negatively charged cell surface attracts cationic surfactants, which then bind to the cell wall through the hydrophobic head, react with the lipids and proteins that make up the cytoplasmic membrane, and then penetrate the cell to interact with intracellular components. Finally, this causes damage to the bacteria's outer coats, causing intracellular components to leak out [60]. A study Comparing CCP (Co-amoxiclav/Citric Acid/Polysorbate-80) to 2% CHX demonstrated greater antibacterial activity. In comparison to 2% CHX alone or CCP, combinations of 2% CHX + 0.2% CTR demonstrated superior antibacterial activity. Therefore, the current investigation also showed that the combination had a synergistic effect. of 0.2% CTR + 2% CHX [61].

when SDS monomers are present in quantities above the threshold micellar concentration, they bind to proteins primarily through hydrophobic contacts, which leads to the unfolding of the tertiary structure. The hallmark

of SDS action on proteins is that the micelles nucleate on the hydrophobic regions of the protein chain, causing it to expand [62]. However, it could not out-number the antibacterial efficacy of the other surfactants.

The least out of the four groups in the present study is the non-ionic surfactant, Tween 80 can prevent P. aeruginosa and S. aureus from forming biofilms. For S. aureus, 0.01% Tween 80 inhibited 8 out of the 12 clinical isolates that were examined. The high levels of lipase activity present in all the isolates that were not suppressed might cause Tween 80 to be cleaved at its ester link, releasing oleic acid and polyethylene sorbitan [63]. Lipase activity of the E. faecalis ATCC 29212 strain that is used in the current study has not been evaluated.

The null hypothesis for cfu/mL between test groups is accepted as there was no significant difference between the groups with or without surfactants.

LIMITATIONS

The effect of sodium hypochlorite being the “gold standard” endodontic irrigant has not been evaluated in the current study. The upcoming studies could include it as a control group in order to compare the efficacy of surfactants, tea Tree Oil and its combination.

The quantity of colony-forming units of cultivable bacteria and the quantitative analysis of the dentin infection may both be estimated using microbiological sample techniques. However, the spatial distribution of

microorganisms inside the dentin IGs was not well revealed by this approach. The study did not examine the chemical interaction between surfactants and tea tree oil. A number of investigations, including stability, biocompatibility, and potential chemical reactions, should be carried out before its clinical recommendations are made, taking into account that this study represents a preliminary evaluation of alternative endodontic irrigant for use in endodontic infections.

CONCLUSION

The following were the conclusions for the current study,

- The minimum inhibitory concentration of < 0.004% cetrimide with or without 1% tea tree oil against E faecalis was statistically highest among the compared groups.
- The minimum bactericidal concentration of 2% cetrimide and 2% SDS exhibited similar zones of inhibition and were highest among the compared groups.
- All groups containing surfactant showed almost similar (not statistically significant) but improved antibacterial efficacy post treatment compared to the pre-treatment values.

Within limitations of the study, surfactants provided a boost to the antibacterial efficacy of 6% tea tree oil. Addition of a cationic surfactant like 0.024% cetrimide to tea tree oil can further improve its antibacterial effects ().

SUMMARY

AIM

The aim of the present study is to estimate the minimum inhibitory and bactericidal concentrations of all the surfactants individually and also in combination with tea tree oil and to analyze the antibacterial efficacy of various surfactants with tea tree oil against E. faecalis.

MATERIALS AND METHOD

Aqueous solutions of Cetrimide, SDS, Tween 80 and its combination with Tea Tree Oil were prepared. Zone of inhibition was assessed by agar well diffusion assay and MIC by broth dilution assay. Statistical analysis was performed and mean and standard deviation was determined. Eighty teeth samples were prepared and inoculated with *E. faecalis* ATCC 29212 and were incubated for 4 weeks. The samples were assigned into four groups (n=20) for irrigation. They are, Group 1 – Cetrimide(0.024%) + (6%) Tea Tree Oil, Group 2 – Tween 80 (24%) + (6%) Tea Tree Oil, Group 3- SDS(0.18%) + (6%) Tea Tree Oil , Group 4 – (6%) Tea Tree Oil. The irrigants were activated for 3 mins using ultrasonics. Paper point sampling of canals were obtained before irrigation (PRE), All irrigants were delivered at a flow rate of 0.04 mL/s using a side-vented 30-gauge needle (Diadent). Irrigants were activated using passive ultrasonic irrigation

technique for 3 minutes. Post irrigation sample (POST-B) was taken following saline wash using paper points. Paper point samples were cultured on MHA for 24 hrs at 37˚C and Colony forming units were calculated and statistically analyzed.

RESULTS

The agar well diffusion assay was performed in triplicates in order to confirm the reproducibility of the results. The Mean diameter of the zone of inhibition of *E. faecalis* with Tea Tree oil, test surfactants i.e Cetrimide, SDS, Tween 80 and combination of Tea Tree oil with the surfactants were noted. Statistical analysis by one way ANOVA revealed that significant difference was observed between the study groups. Post Hoc Test: Tukey HSD reveled that Tween 80 and Tea Tree oil + Tween 80 showed the least inhibition. The surfactants, 2% Cetrimide and 2% SDS showed a wider zone of inhibition compared the surfactants combined with tea Tree oil (2% Tea Tree oil + 2% Cetrimide and 2% Tea Tree oil + 2% SDS). The minimum inhibitory concentration of the test irrigants were evaluated to be 1% Tea tree oil, 0.004% Cetrimide, 0.03% SDS and 4% Tween 80. There was a significant difference between pre and post test values in all the four groups. The Post test values were significantly lower compared to pre test values. The percentage difference between pre and post tests do not differ significantly among the four groups compared, p-value > 0.05 (ANOVA). Pair-wise comparison of the mean values between the groups indicate that the percentage difference between pre and post tests do not differ in all the six comparison groups.

CONCLUSION

Within limitations of the study, surfactants provided a boost to the antibacterial efficacy of 6% tea tree oil. Addition of a cationic surfactant like 0.024% cetrimide can further improve the antibacterial effects as compared to anionic and non-ionic surfactants in combination with tea tree oil.

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INSTITUTIONAL REVIEW BOARD
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EVALUATION REPORT OF THE RESEARCH PROPOSAL

Name, Designation & Dept of the Principal Investigator: Dr. Nivashini G.S.V, PG student, Conservative Dentistry & Endodontics.
Name, Designation & Dept of the Co- Investigator: Dr. A.Venkatesh, Professor, Conservative Dentistry & Endodontics.

Title of the proposed research: Antibacterial efficacy of various surfactants with Tea Tree oil against Enterococcus faecalis - An *In Vitro* study.

IRB - Approval Number: SBDCH - IRB/ 22-06/06.

The Institutional Review Board had reviewed your application with the necessary documents to conduct the study entitled "Antibacterial efficacy of various surfactants with Tea Tree oil aginst Enterococcus faecalis - An *In Vitro* study." on 7th **July 2022**.

The following documents were reviewed:

i. Research proposal submitted by the Principal Investigator.
ii. Patient Information Sheet and Informed Consent Form in English and/or vernacular language.
iii. Questionnaire / clinical trial protocol.
iv. Advertisements/ information brochure to be used for recruiting the patients for the purpose of your research/study.
v. Insurance Policy / Compensation for participation and for serious adverse events occurring during the study participation.
vi. Investigator's Agreement with the Sponsor(s).
vii. Principal Investigator's and Co-Investigators- *Curriculum Vitae*.
viii. Investigator(s) - self-declaration form

The following members of the Institutional Review Board were present at the IRB meeting at Lakshmi Ammal auditorium, SBDCH on 7th July 2022 at 10.00 a.m.

Dr. S. Jimson, Convener.
Dr. Badri Thiruvenkatachari, Chairman.
Dr. K. Padmavathy, Member secretary.

Dr. S. Raghavendra Jayesh, clinician- Prosthodontics, Dr. A. Ponnudurai, clinician-Pedodontics, Dr. A. Radhakrishnan, clinician-General Medicine, Dr. C. Sumathi Jones- Scientific Member -Pharmacology, Dr. Vijay Ebenezer, clinician- Oral surgery, Dr. N. Aravinda Babu- clinician-Oral pathologist, Dr. V. Prakash, clinician-Endodontics, Dr. T. Sarumathi, clinician-Oral Medicine & Radiology, Dr. Anitha Balaji, clinician-Periodontics, Dr. M. Anita, clinician-Public Health dentistry.

The Institutional Review Board approves the study to be conducted in the form presented by the PI.

The Institutional Review Board hereby advices that the PI / Co-I(s) / researcher, should get approval from the Institute's Ethics Committee prior to commencement of the study and should maintain "confidentiality" *vis-a-vis* subjects.

The Institutional Review Board expects to be informed about the half-yearly progress of the study, changes in the research/study protocol (if any) and to submit a copy of the final report of the research study.

MEMBER SECRETARY

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