AN IN VITRO EVALUATION OF ANTIMICROBIAL PROPERTY AND SMEAR LAYER REMOVAL EFFICIENCY OF FRAGARIA ANANASSA EXTRACT ON ROOT CANAL DENTIN

Dissertation submitted to

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In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH IV

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CERTIFICATE

This is to certify that this dissertation titled "**AN IN VITRO EVALUATION OF ANTIMICROBIAL PROPERTY AND SMEAR LAYER REMOVAL EFFICIENCY OF FRAGARIA ANANASSA EXTRACT ON ROOT CANAL DENTIN**" is a bonafide record work done by **Dr.B.VENKATESH** under our guidance during his postgraduate study period between 2010 - 2013.

This dissertation is submitted to **THE TAMILNADU Dr.M.G.R.MEDICAL UNIVERSITY**, in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY – CONSERVATIVE DENTISTRY AND ENDODONTICS, BRANCH IV**. It has not been submitted (partial or full) for the award of any other degree or diploma.

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ABSTRACT

BACKGROUND: Phytomedication a possible alternative in contemporary endodontics.

AIM: The aim of this study was to investigate the minimum inhibitory concentration of Fragaria Ananassa against E.Faecalis and to evaluate the MIC of F.A in removing smear layer along the length of the root dentin.

METHODOLOGY: Crude F.A extract was serially diluted in Neat, 1:2, 1:4 and 1:8 concentration. MIC was tested against standard strain of E.Faecalis in blood Agar suspended in BHI broth. 40 extracted human mandibular premolar tooth was selected. Divided into 4 groups. (10 tooth per group). Group 1 – 50% F.A., Group 2 – 17% EDTA, Group 3 – 5.25% Naocl, Group 4 – saline (control). Irrigation was performed with respective irrigants (10 ml) for every change of file for one minute (S1 – F3 protaper). Ultrasonic irrigation was done for one minute for every change of file. 10ml of distilled water was used as a final irrigant for one minute. Tooth was split and examining under SEM.

RESULTS: Group 1 showed superior smear layer removal along Apical, Middle, Coronal portion. Group 2's ability to remove smear layer in the apical portion was less than Group 1 whereas in middle and coronal portion it was comparable with group 1. Group 3's ability to remove smear layer was less than Group 1 and Group 2. Group 4 was least effective.

CONCLUSION: The MIC of F.A against E.Faecalis was 50%. 50% F.A was superior to 17% EDTA and 5.25% Naoacl in removing smear layer along the entire length of the canal.

KEY WORDS: Irrigation, Smear layer, Fragaria Ananassa, Root canal dentin.

Introduction

INTRODUCTION

In contemporary medicine the use of plants, herbs and their various components which have medicinal properties is gaining popularity. This trend is not new to Indian scenario because ancient Indians were using herbs and their products in native medicine. Even ancient romans were using native medicine for tooth whitening. The current technological evolution can highlight the therapeutic value of this native medicine in a scientific manner like their anti-inflammatory, anti-microbial and antifungal properties. Seeking a remedy by natural means is the trend of the day and endodontics is not an exception to that.[26] In the past attempts have been made to use them as a irrigant and intracanal medicament due to their high antimicrobial, anti-fungal, anti-inflammatory and anti-oxidant properties.[25] Some of the tested natural products include Morinda Citrifolia Juice (NONI)[26], Propolis, Arctium Lappa, Triphala and Green tea polyphenols (GTP), German chamomile and tea tree oil and ginger extract. The results have been encouraging. All the tested natural products revealed varying degree of antibacterial efficiency and varying ability to remove smear layer along the length of root dentin.[25] The major advantage of these natural alternatives are their

ease of availability, low cost, most importantly their high and excellent bio-compatibility and negligible side effects.[25]

The Success in endodontics primarily depends on complete eradication of micro-organisms from the root canal which is achieved by instrumentation and irrigation techniques to promote healing of the periapex. The current instrumentation techniques do not ensure complete removal of micro-organsims from the root canal. Moreover mechanical instrumentation leaves a smear layer.[2] There is consensus opinion today that this smear layer has to be removed.[23] Hence it is mandatory to remove smear layer and microorganisms from the root canal by effective irrigants and irrigation systems for effective disinfection.[29] Unfortunately there is no single irrigant which is available today which can totally eradicate both microorganisms and smear layer from the root canal. This scenario neccesiates the use of combination of endodontic irrigants in root canal cleaning.[32]

The irrigant which is being used for organic tissue dissolution is sodium hypochlorite in the concentration of 2%-5.25% for many decades. It has excellent tissue dissolution capability and antimicrobial effect but lacks the ability to remove smear layer.

Further it has several undesirable characteristics like tissue toxicity, allergic reactions and intolerable smell and taste.[26]

The irrigant generally used for removing smear layer is 17% EDTA which is a chelating agent. It is an excellent smear layer remover from coronal and middle third of root canal but not effective in apical third. Further EDTA has no ability to dissolve organic tissue and has no antimicrobial potential.[23]

The irrigant which is commonly used for antimicrobial effect is 2% chlorhexidine gluconate. CHX is a bis-bi-guanide with amphiphathic, antimicrobial, antiseptic effects. However literature shows the use of CHX can cause a precipitate of para-chloro aniline (PCA) which can affect the sealing ability of obturation material by acting as a chemical smear layer, PCA by itself is carcinogenic in nature. Furthermore CHX has very less ability to remove smear layer and dissolve organic tissue.[32] It is very much clear at present that there is no single irrigant which can remove both organic and inorganic tissue and which possess antimicrobial effect. In this scenario if a natural substance can be identified as a potent irrigant with the above mentioned features it will bring a revolution in endodontics.

FRAGARIA ANANASA (straw berries) are aromatic, refreshing, tantalizing,red beauty feast fruits to human eyes[28].They are predominantly cultivated in the belts of Maharastra (mahableshwar) Karnataka, Jammu and Kashmir and Himachal pradesh. They were an important part of the Nordic diet, they have also been used as natural antimicrobial pharmaceutical agents.[14] The garden strawberry is an octoploïd species belonging to the family of *Rosaceae.*It is not a real berry but an achene (false fruit).A strawberry consists of many tiny individual fruits embedded in a fleshy scarlet receptacle. The brownish or whitish specks, commonly considered seeds are the true fruits known as achenes.[33] The strawberry plant is a perennial plant characterised by an evolutionary morphology (vegetative growth, formation of runners, fructification).

The nutritional value of strawberries is mainly due to its content of Vitamin C.[21] Strawberry has a particularly rich secondary metabolite composition, along with other fruits of the Rosaceae family including apples, pears, plums, peaches and raspberries. The chemical profiles include hundreds of non-volatile and volatile compounds, the latter ones being responsible for the typical fruit aroma bouquet. These metabolites have been the subject of intensive

investigations for decades. The focus has been either on a wide-range non-targeted metabolite profiling, quantification of specific metabolite classes, or structural characterization of single phytochemicals.[31] The metabolites most frequently analyzed from strawberry are phenolic compounds because strawberries are rich in phenolic compounds (phytonutrients). They belong to four main groups:flavonoids, phenolic acids, lignans and polymeric tannins.[14]

Strawberry contain substantial amount of flavanoid anthocyanins and phenolic acids and a lesser level of ellagitanins and lignans.[14]

These phytonutrients are claimed to have potent health remedies. They give the characteristic colour to straw berry.[28] Out of this phytonutrients the two group of compounds which is of immense interest is anthocyanin (antioxidants) and ellagitanins. Anthocyanins have potent anti-inflammatory, anti-cancer, anti-rheumatoid,anti-osteoarthritis properties. Anthocyanins function similar to aspirin they are cyclooxygenase inhibitors. They reduce the transformation of arachydonic acid in to protoglandins (mediators of inflammation) similar to cox 1, 2 analgesics.Cox 1, 2 analgesics will produce mucous secreation in gastric mucosa but anthocyanins does not

produce this side effect as well. So it is superior than NSAIDS[28]. Ellagitanins on the other hand posses strong anti-oxidant property, they are selectively cytotoxic to tumour cells. More importantly they have strong antimicrobial property.[28] Literature survey reveals it can even selectively inhibit human pathogenic microorganism.[14]

Bulk of the composition of straw berry is organic acids among the organic acids citric acid forms the bulk, other major organic acid is maleic acid.[21] Both have been used for smear layer removal with maleic acid being effective along the entire length of the canal (coronal, middle, apical)[23] while citric acid being effective in coronal and middle third.[13] These features warrant immense potential in theraupatics.

The aim of this study was to investigate the efficiency of FRAGARIA ANANASSA JUICE as a potent root canal irrigant The objective of this study was to investigate the straw berry fruit juice's

a) MIC against E.Faecalis.
b) Ability to dissolve smear layer.

Review of literature

REVIEW OF LITERATURE

Marja p. kahkonen et al (1999)[18] examined antioxidative activity of 92 phenolic extracts from edible and non-edible plant materials by autoxidation of methyl linoleate and evaluated total phenolic content spectrometrically by folin-ciocalteu procedure. They concluded among edibile plants berries had remarkably high phenolic content (GAE > 20mg/g) and anti-oxidative activity. Among non-edible plants tree material had high high phenolic content and anti-oxidative activity.

Fugentasman et al (2000)[5]evaluated surface tension values of 9 potential endodontic irrigants without a surfactant and 1 potential endodontic irrigant with surfactant cetredixin by employing ring method on a DuNouytensiometer. They concluded cetrexidin had the lowest surface tension value followed by 2.5% and 5% NaoCl and 17% EDTA.Ringers solution, saline and distilled water had relatively high surface tension values.

Hatice dogan (2001)[7] evaluated the combined and single use of EDTA, RC-Prep and NaoCl on the mineral content of root dentin

by energy dispersion spectrometric microanalysis. They concluded using EDTA combined with NaoCl as a final irrigation and NaoCl alone changed the calcium/phosphorus level,significantly increasing the magnesium level as well. Usage of NaoCl as a final flush changed the effectiveness of chelating agent as well.

Leslawjuszczak et al(2003)[12] evaluated the rheological behavior of concentrated straw berry juice between a temperature range of 10-60°c and concentration range of 50-67.1°Bx. They concluded straw berry juice had Newtonian behavior and the viscosity ranged from 8.6-541.2 mPas.viscosity was temperature dependent.

Leonardo dos santos barroso et al (2004)[11] evaluated whether 10% citric acid solutions for endodntic use with and without microbiological stabilizer 0.1% sodium benzoate would be contaminated. They concluded 10%citric acid solutions without stabilizer were 100% contaminated by c.albicans, 80% by e.coli and 50% by e.faecalis. There was less contamination when a stabilizer was added. There was only 30% c.albicans growth.

Ertugrulercan et al (2004)[4]evaluated in an in-vivo study the anti-bacterial efficiency of 2% chlorhexidinegluconate and 5.25%NaoCl on teeth with necrotic pulp, periapical pathosis and both. Samples were collected before and after irrigation and subjected to microbiological processing, including anaerobic incubation on trypticase soy agar for 5-7 days.After counting of colony forming units they concluded both the irrigants effectively reduced the microorganisms.

Christopher p. McHugh et al (2004)[3] investigated the exact ph required to kill E.Faecalis in-vitro by positive growth using turbidity, visual scale and spectrophotometer.They concluded ph of 10.5-11.0 retarded bacterial growth whereas a ph of 11.5 and greater showed complete retardation of E.Faecalis.

Bettina basrani et al (2004)[1] investigated the physio-chemical properties of chlorhexidine and calcium hydroxide containing medication in gel form in different concentrations. The ph was measured by ph meter, contact-angle by goniometer and viscosity by Brookfield RVDV viscometer. They concluded CHX did not affect the ph and working time whereas it reduced the contact angle and increased the viscosity of calcium hydroxide.

Iris slutzky-goldberg et al(2004)[8] evaluated the effect of 2.5% and 6% NaoCl irrigation on bovine teeth at a depth of 500µm,1000µm and 1500µm from the lumen for 5,10 and 20 minute time period. They concluded there was decrease in micro hardness at all levels and all time periods. It was more marked for 6% NaoCl than 2.5% NaoCl.

Liisa j. Nohynek et al (2006)[14] investigated the antimicrobial effects of 12 Nordic berries against selected human pathogenic microbes by liquid culture analysis, adherence of bacterial cells to berry material by sonication and by fluorescence staining. The results showed straw berry exhibited very strong inhibition against bacilluscerus, helicobacter pylori and staphylococcus aureus. It showed strong inhibition against candida albicans and staphylococcu epidermidis. It also exihibited clear inhibition against campylobacter jejuni and clostridium perfringes.

G.morga et al (2006)[22] evaluated the compositional changes in strawberries due to dehydration, cold storage and freezing by osmotic dehydration,air drying and in combination. The results showed all samples exihibited sugar gain and small loses of ciric acid content.

Matthias zehnder et al (2006)[19] reviewed the intricasis of pulpal microenvironment and the resulting requirements for irrigation and irrigating solutions. They have reviewed about the desired irrigant action and choosing the main irrigant. They have reviewed in detail about hypochlrite,chelator agents and chlorhexidine. They have discussed about the suggested irrigating regimen and alternative concepts as well.

Peter E Murray et at (2008)[26] evaluated the efficiency of Morindacitrifolia in removing smear layer from root canal dentin .They concluded that the efficacy of MCJ was similar to NaoCl in conjunction with EDTA as an intracanalirrigant.

Marcia da ilva pinto et al (2008)[17] evaluated the total phenolics,antioxidant activity and its ability to inhibit α-amylase,α-glucosidase and ACE by DPPH radical scavenging assay of seven different Brazilian straw berry varieties and concluded that major phenolics were ellagicacid,quercetin and chlorogenic acid and they had an inhibitory action on α-glucosidase making it a potential dietary source of diabetes mellitus.

Ramar Perumal Samy et al (2008)[27] reviewed the therapeutic potential of plants as anti-microbials for drug discovery and concluded that it is potential alternative therapeutic modality for drug discovery.

Julio cesaremboavaspano (2009)[10] evaluated the concentration of calcium ions and Smear layer removing ability of 15%EDTA,10%citric acid,10%sodium citrate, apple vinegar,5% acetic acid,5% malic acid and 1% NaoCl by Flame atomic absorption spectrometry and SEM. They inferred that the use of 15%EDTA resulted in higher calcium ions concentration followed by 10%citric acid and both were very efficient in removing smear layer than the other groups.

Judit KRISCH et al (2009)[9] evaluated the antimicrobial action on Gram positive and negative organism (Bacillus cereus, B.subtilis, Campylobacterjejuni, E.coli, SalmonellaTyphimurium and Serratiamarcescens) and also evaluated the phenolic content from the Pomace of six common juice making fruits (fragaria x ananassa, prunuscerasus, ribesnigrum,R.rubrum,R.fruticosus,Rubusidaeus) by broth dilution assay and DPHH. They concluded that Ribes and

Rubus were efficient inhibitors, Fragaria inhibited B.cereus and E.coli to an extent.

NidamburVasudevBallal et al(2009)[23] evaluated the smear layer removing ability of 17%EDTA AND 7% maleic acid by SEM and concluded that a final irrigation with 7%maleic acid was very effective in removing smear layer from the apical portion of the root canal system than 17%EDTA.

Meena N Gulve et al(2010)[20] evaluated the antimicrobial efficacy of ginger extract and 2% NaoCl against E.Faecalis using Agar Diffusion Method.They concluded ginger extract showed better antimicrobial efficacy than 2%NaoCl.

Carmen Maria Ferrer-Luque et al (2010)[2] evaluated the antimicrobial activity of maleic acid alone and in combination with cetrimide against E.Faecalis biofilm in-vitro. They concluded that maleic acid alone and in combination with cetrimide started eradicating E.Faecalis from 30seconds.

D.R.Violich et al (2010)[29] have comprehensively reviewed the smear layer and its relevance to endodontics. They suggest that removal of smear layer enhances disinfection and the mode of

removal can be by chemical, ultrasonic and by laser techniques none of which is totally effective. They suggest the alternate use of EDTA and NaoCl as the best available option.

Mehmet ALI KOYUNCU et al (2010)[21] evaluated the changes in the organic acid content of two different straw berry varieties on cold storage by HPLC. They inferred that citric acid content decreased with increasing storage time as did the malic acid,tartaric,oxalic and fumaric acid content as well.

Madhupujar et al(2011)[16] evaluated the antimicrobial efficacy of Triphala, Green tea polyphenols and 3% NaoCl against E.Faecalis formed on tooth substrate. They concluded 3% NaoCl showed better antimicrobial efficacy than Triphala, Green tea polyphenols. ButTriphala and GTP too showed significant antimicrobial action.

NidamburVaudevBallal et al(2011)[24] investigated the chemical interaction of 7% maleic acid with 2% CHX and 2.5%NaoCl.They inferred that the chemical interaction of 7% maleic acid and 2% CHX did not produce any precipitates but reduced the availability of free chlorine when it was mixed with 2.5%NaoCl.

Madhupujar et al (2011)[25] comprehensively reviewed the use of herbal alternatives namely propolis,MCJ,Arctiumlappa,Triphala and GTP,German chamomile and tea tree oil.Th ey concluded that the use of these alternatives as intracanal medicaments and irrigants may be advantageous.

Subodh kumar Sarkar et al (2011)[28] evaluated the antimicrobial,nutritive,antioxidant and mineral composition of two varieties of fragariaananassa.They concluded it showed potent antimicrobial activity against gram positive and negative organisms and contained substantial quantity of sugar,antioxidants and minerals making it a rich dietary source.

Ligengwu et al(2012)[13] evaluated the efficacy of four decalcifying agents 17%EDTA,20%citric acid,Biopure MTAD and Smearclear's ability to remove smear layer. They concluded none effectively removed smear layer from apical portion,17% EDTA being better than MTAD and Smearclear.

Giampiero Rosi-Fedele et al (2012)[6] investigated the antagonistic interaction of NaoCl,EDTA,CHX and citric acid(CA).They inferred when NaoCl was mixed with chelators it

resulted in lss of free available chlorine,when mixed with CHX resulted in a precipitate called PCA.The combination of CHX and CA did not result in any interaction.

Liliana critinasoare et al(2012)[15] evaluated the antimicrobial and antioxidant properties of ragariaananassa, paeoniaofficinalis, hyacintusorientalis and scillabiflia. They concluded all the tested grups contain potent antioxidant contents and had antimicrobial activity againt E.coli, Pseudomonasaeruginosa, Salmonella abony, S.aureus,E.Faecalis, Brevibacteriumflavum, Sarcinasp.,Bacillus cereus, saccharomycescereviiae and Aspergillusniger.

Sharad Kamat et al[30] comprehensively reviewed the role of phytomedicine in dentistry and has highlighted its potential therapeutic application in relation to endodontics and dentistry in general.

Material and Methods

METHODOLOGY

The study was designed in two Phases

Phase 1 explored the anti-microbial property of FA Juice (Fragaria Ananassa) and its minimum inhibitory concentration against E.Faecalis (standard strain)

Phase 2 of the study examined the minimum inhibitory concentration of FA Juice as an endodontic irrigant to evaluate its efficiency in removing smear layer from the root canal walls

PHASE 1

Evaluation of Minimum Inhibitory Concentration of FA Juice

Materials

E-Faecalis – Standard Strain

500gms of fresh Straw Berry fruit

Blood agar plates

Brain Heart Infusion Broth

Methodology: Standard strain of E-Faecalis was cultured on Blood agar for 24 hours incubation. The growth was suspended in Brain Heart infusion broth. This was plated on blood agar. 500gms of fresh straw berry fruit was washed in sterile distilled water and the crude extract was prepared in a sterile blender. The extract was filtered by

biomembrane filter and the filtrate was serially diluted in saline to give a concentration of neat, 1:2, 1:4 and 1:8.

Discs were impregnated with these concentrations and incorporated on the blood agar with organism. This was incubated at 37º C for 24hrs. On examination anti-bacterial activity was expressed as the zone of inhibition produced by Straw Berry extract around the disc. The diameter was measured in millimeter's and the highest zone of inhibition was considered as having anti-bacterial activity at that concentration.

PHASE 2

Evaluation of Efficiency of 50% FA Juice to Remove Smear Layer from the Root Canal Walls

MATERIALS

1. Forty Freshly Extracted –Intact Human Mandibular Premolars with Single Canalpattern.
2. 50% Crude Fragaria Ananassa Juice
3. 17% EDTA -DE SMEAR, (ANA BOND STEDMAN PHARMA RESARCH PVT LTD KANCHIPURAM, TAMIL NADU, INDIA.)
4. 3% NaoCl (Prime Dental Products, Thane, Mumbai, India.)
5. Saline (Nirlife Health care, NIRMA LTD,GUJARAT,INDIA)
6. Distilled Water

ARMAMENTARIUM

1. Straight Hand Piece (NskEc,Japan)
2. Protaper Rotary File System (DentsplyMaillefer, Switzerland)
3. X-Smart Hand Piece (DentsplyMaillefer,Switzerland
4. Diamond Disc
5. Ultrasonic Irrigation Instrument (SUPRASSON P MAX, SATELEC, FRANCE)

6. Stop Clock
7. Plastic Containers
8. Chisel
9. Gloves
10. Face Mask
11. Protective Eye Wear
12. 10 ml Syringe With 26-Guage Needle

EQUIPMENTS

1. Scanning Electron Microscope (Hitachi S-3400n)

METHODOLOGY

Intact human mandibular premolars extracted for orthodontic reasons were collected. From this 40 teeth with single canal (verified with radiographs) and mature apices were selected for the study. The teeth were cleaned ultrasonically and stored in water containing 0.1% thymol until needed for the study, a period not exceeding one month.

The samples were decoronated at CEJ and randomly divided in to 4 groups with 10 teeth each

Group 1 - samples were irrigated with 10ml of 50% fragaria ananassa with each change of file for 1 minute.

Group 2 -samples were irrigated with 10ml of 17% EDTA with each change of file for 1 minute.

Group 3– samples were irrigated with 10ml of 3% NaoCl with each change of file for 1 minute.

Group 4– samples were irrigated with 10ml of saline with each change of file for 1 minute.

STUDY DESIGN

Instrumentation protocol:

A 10 size K-file was used to obtain a glide path. Working length was obtained using 15 size stainless steel K-file.Chemomechanical preparation was performed by nickel-titanium rotary protaper files (S1,S2,F1,F2,F3) in crown down sequence with irrigation using respective irrigants as mentioned previously.

Irrigation protocol during instrumentation:

10ml of respective irrigant was used to irrigate the root canal between each instrument with a contact time of 1 minute. A total of 50ml of irrigant was used in each root canal.Irrigation was carried out passively with a 26 - guage needle with tip being positioned 1mm short of working length. Deposited irrigant was activated ultrasonically for 1 minute

Post instrumentation final irrigation protocol:

Following completion of chemomechanical preparation a final irrigation was performed with 10ml distilled water for 1 minute to terminate the reaction.

Specimen preparation for SEM evaluation:

With diamond burs longitudinal grooves were made on the buccal and lingual outer surfaces of the samples without bur penetrating in to the lumen. The roots were split in to two halves with a chisel.

The split samples were placed in hot air oven to ensure complete dryness. One half of the samples were gold sputtered and

viewed under SEM at the coronal,middle and apical thirds of the root canal for the evaluation of the residual smear layer. Photomicrographs were taken at 1000x magnification and scores were recorded.

Scanning electron microscope (SEM):

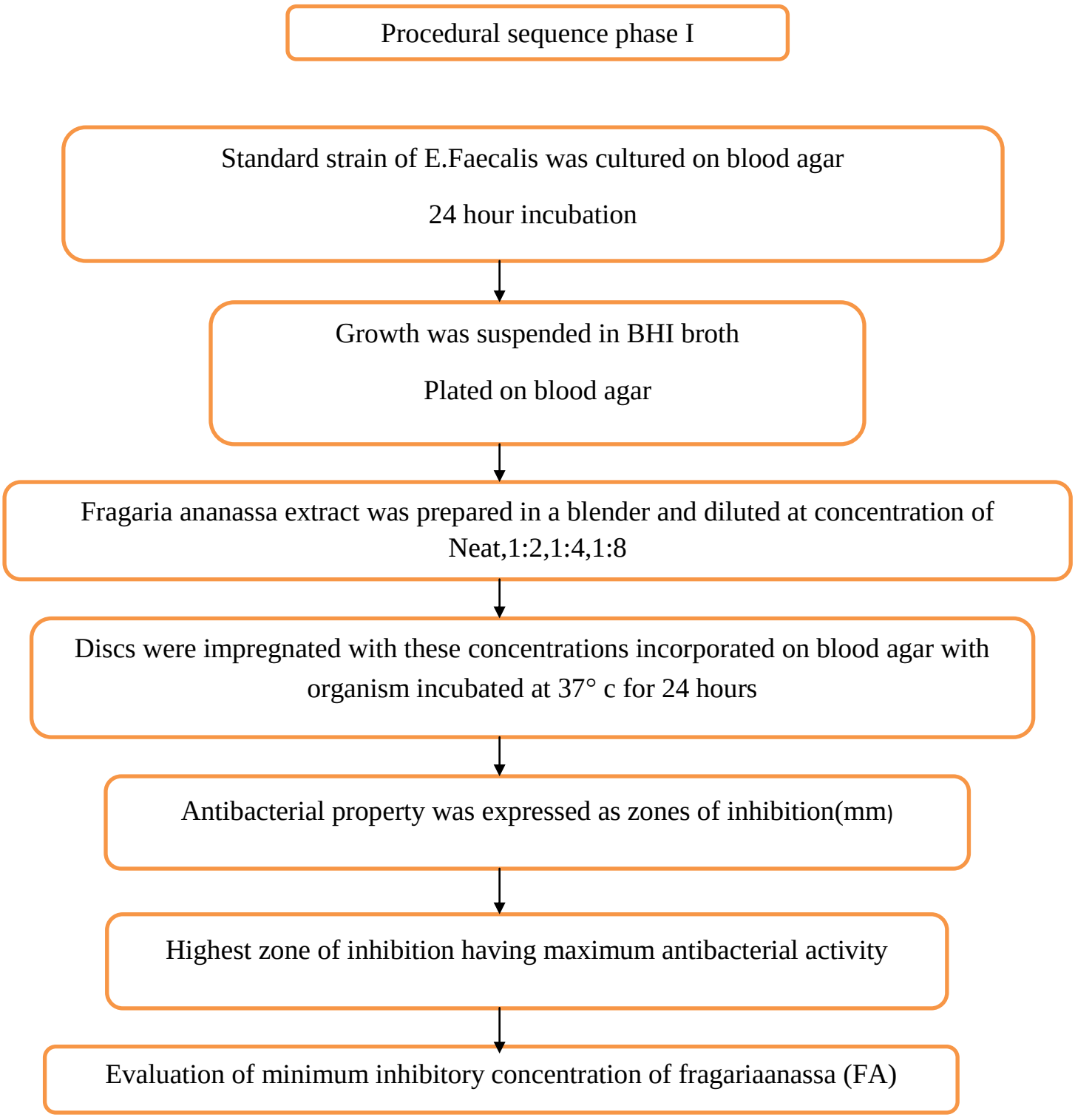
The SEM is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons.The electrons interact with electrons in the sample,producing various signals that can be detected and that contain information about the sample's surface topography and composition.The SEM allows a greater depth of focus compared to optical microscope as it can achieve a resolution better than 1nanometer.

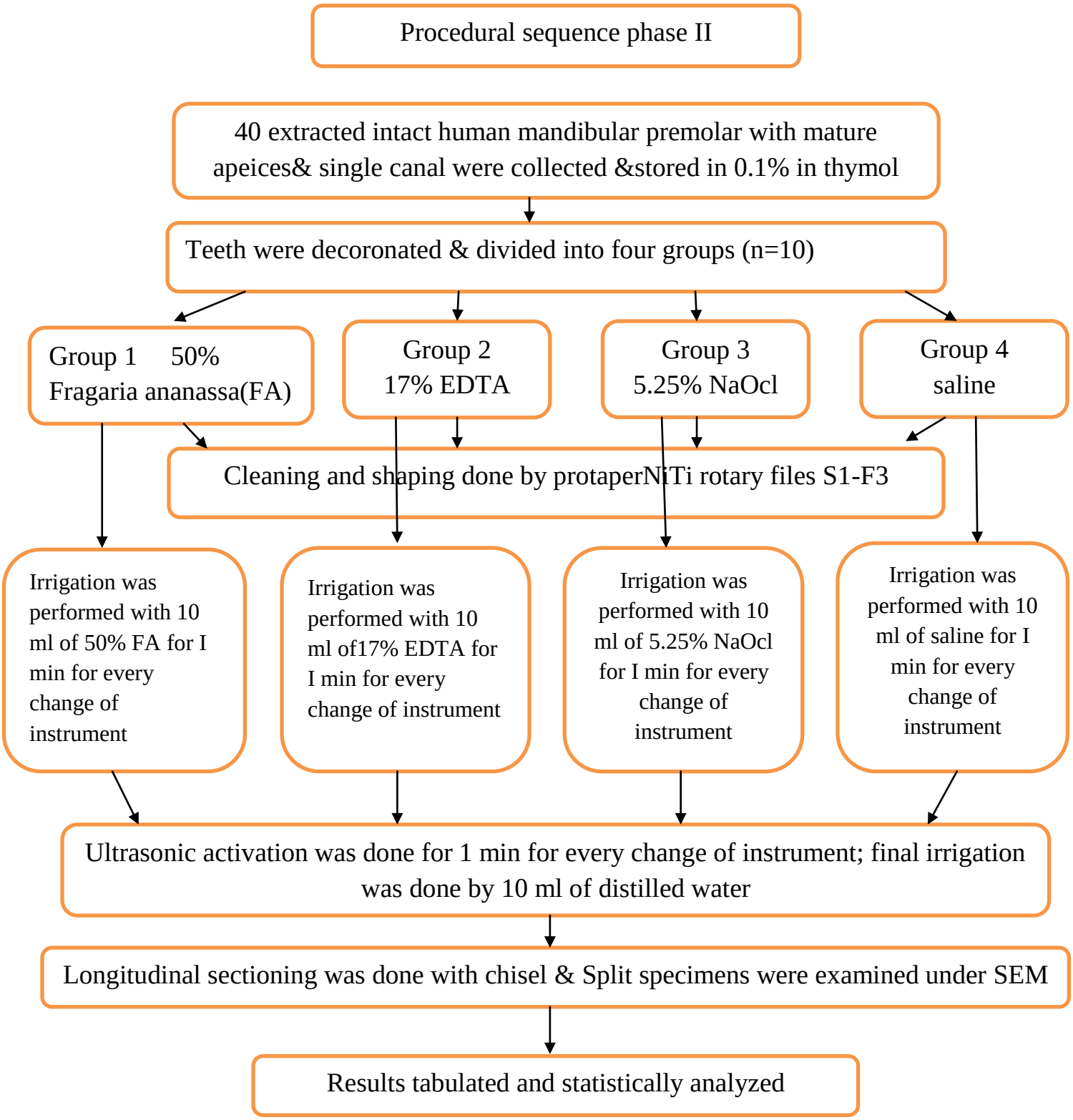
SCORING OF RESIDUAL SMEAR LAYER WAS DONE AS RECOMMENDED BY TORABINEJAD et al.

Score 1: no smear layer.

Score 2: moderate smear layer.

Score 3: heavy smear layer.


Procedural sequence phase I
Standard strain of E.Faecalis was cultured on blood agar
24 hour incubation
Growth was suspended in BHI broth
Plated on blood agar
Fragaria ananassa extract was prepared in a blender and diluted at concentration of Neat,1:2,1:4,1:8
Discs were impregnated with these concentrations incorporated on blood agar with organism incubated at 37° c for 24 hours
Antibacterial property was expressed as zones of inhibition(mm)
Highest zone of inhibition having maximum antibacterial activity
Evaluation of minimum inhibitory concentration of fragariaanassa (FA)


Procedural sequence phase II
40 extracted intact human mandibular premolar with mature apeices& single canal were collected &stored in 0.1% in thymol
Teeth were decoronated & divided into four groups (n=10)
Group 1 50% Fragaria ananassa(FA)
Group 2 17% EDTA
Group 3 5.25% NaOcl
Group 4 saline
Cleaning and shaping done by protaperNiTi rotary files S1-F3
Irrigation was performed with 10 ml of 50% FA for I min for every change of instrument
Irrigation was performed with 10 ml of17% EDTA for I min for every change of instrument
Irrigation was performed with 10 ml of 5.25% NaOcl for I min for every change of instrument
Irrigation was performed with 10 ml of saline for I min for every change of instrument
Ultrasonic activation was done for 1 min for every change of instrument; final irrigation was done by 10 ml of distilled water
Longitudinal sectioning was done with chisel & Split specimens were examined under SEM
Results tabulated and statistically analyzed

Figures

Figure 1: Fresh Fragaria Ananassa Fruit

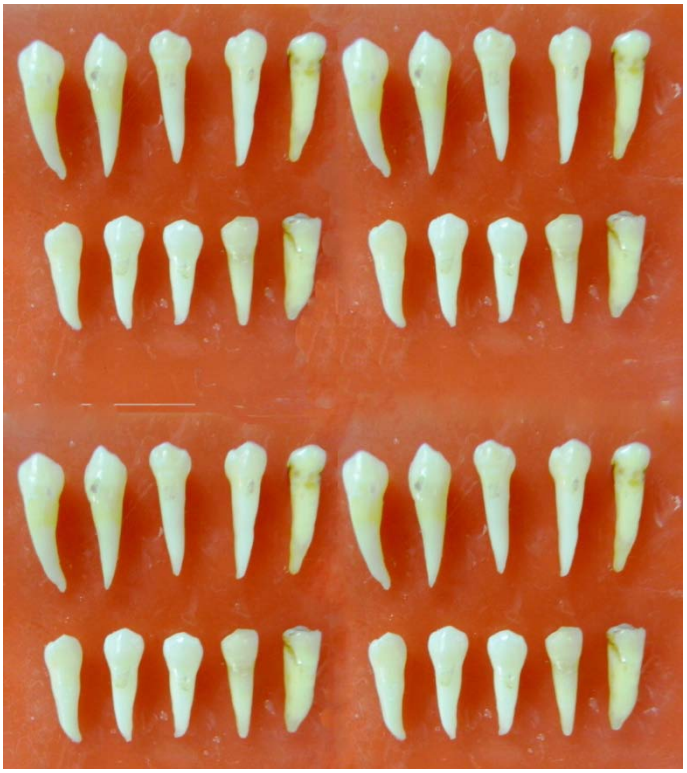


Figure 2: Tooth Samples

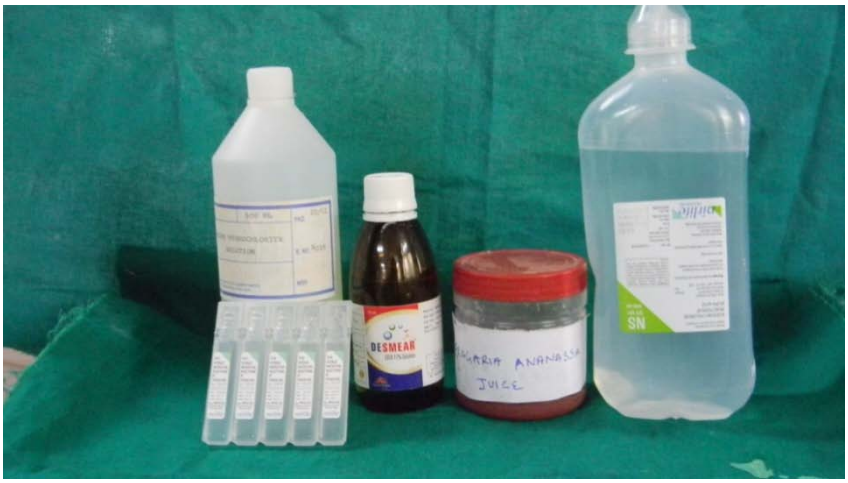


Figure 3: Materials for phase 2 of the study

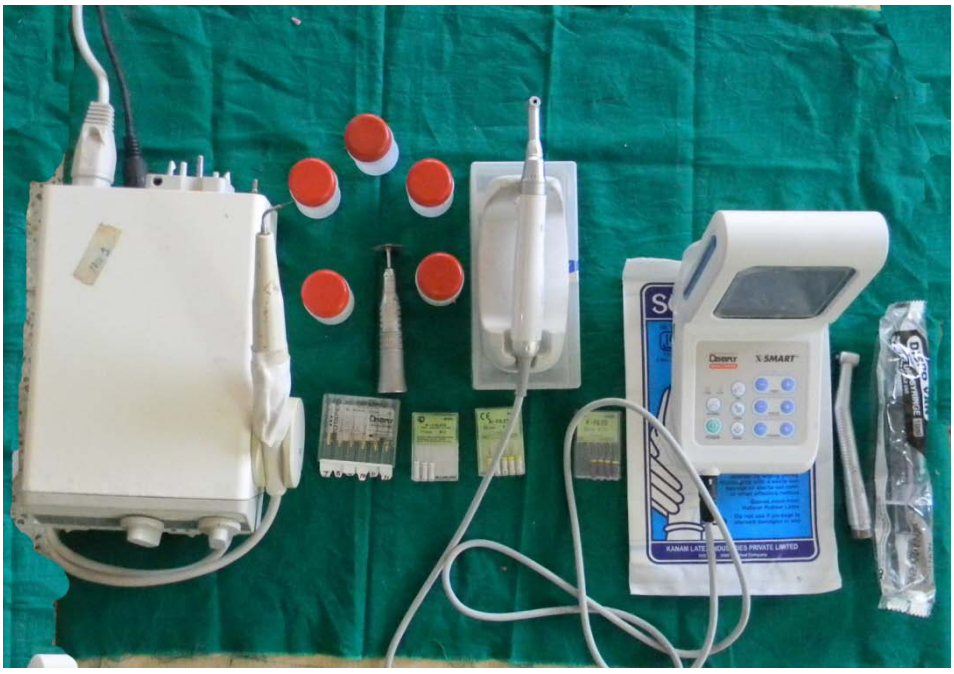


Figure 4: Clinical Armamentarium

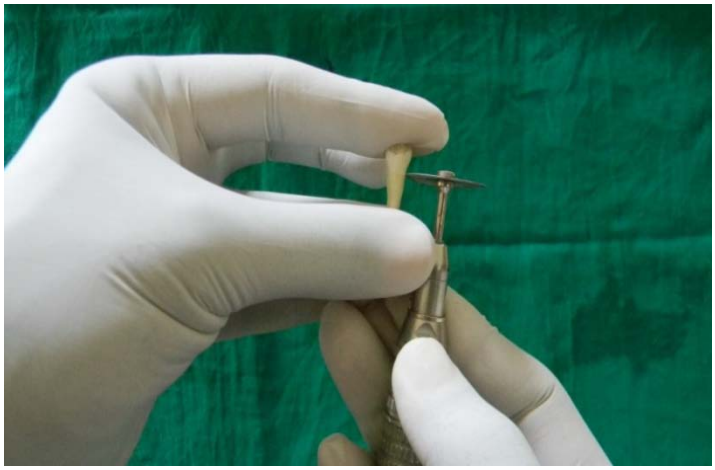


Figure 5: Crown Decoronation

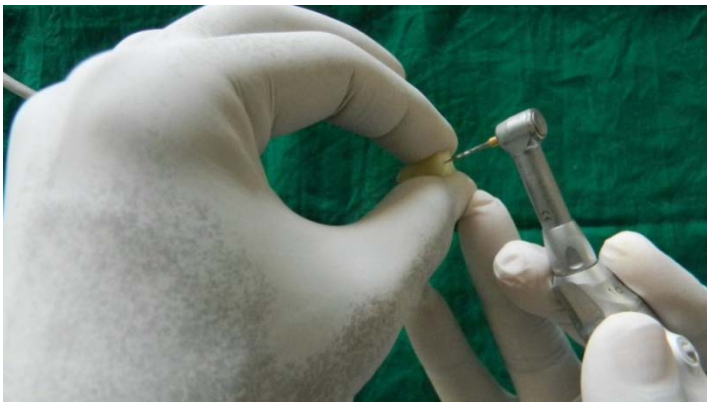


Figure 6: Cleaning and shaping by protaper files

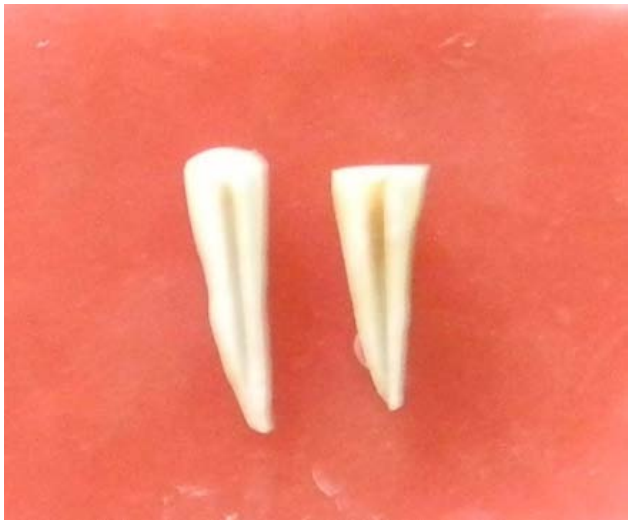


Figure 7: Split tooth samples

Results

RESULTS

Result for anti-bacterial activity and minimum inhibitory concentration of FA juice

Table 1: Dilutions tested for Straw Berry extract

E-Faecalis	Zone of Inhibition
Neat	10mm
1:2	8mm
1:4	–
1:8	–

Interpretation of result:

1. 1. Straw Berry extract seems to have inhibitory action against E-Faecalis in the dilution neat (100%) and 1:2 (50%).
2. Dilution 1:4 and 1:8 do not seem to have any inhibitory action.
3. The minimum inhibitory concentration of FRAGARIA ANANASSA EXTRACT against E.Faecalis was 50%

Results for smear layer removing ability of FA juice.

The recorded smear layer scores were subjected to statistical analysis to investigate the significant differences in smear layer scores within each group and between each group which was recorded by two blinded independent observers.

For comparing between groups and area one-way anova test followed by tukey HSD test was used.

For comparison between observer 1 and observer 2 independent sample ‘t’test was used.

Anova is a technique used to compare means of two or more sampled (using the F distribution). This technique can be used only for numerical data.The Anova tests the null hypothesis that samples in 2 (or) more groups are drawn from populations with the same mean values. Typically however, the one-way anova is used to test for deferrals among at least 3 groups, simple the two-group case can be covered by a ‘t’ test. It is a relatively robust procedure with respect to violations of the normality assumption. Generally the results of one-way Anova are considered highly reliable.

Tukey HSD Test:

It is a single step multiple comparison procedure and statistical test. It is used in conjunction with an Anova to find means that are significantly different from each other. It compares all possible pairs of means and is based on a standardized range distribution and identifies any difference between two means that is greater than the expected standard error. This method is basically conservative even when there are unequal sample sizes.

Independent sample 't'-Test:

It compares the mean scores of two groups on a given variable. It works on the assumption that (i) the dependent variable is normally distributed (ii) the two groups have approximately equal variable on the dependent variable. (iii) the two groups are independent of one another.

It tests the hypotheses (i) whether the means of the two groups are not significantly different (or) whether the means of the two groups are significantly different.

Table 2: Comparison between area of each Group Of Observer I

Area	GP1	GP2	GP3	GP4
Coronal	1.20 ± .632	1.10 ± .32	1.60 ± .84[a]	2.30 ± .95
Middle	1.10 ± .316	1.50 ± .85	2.40 ± .97[ab]	2.60 ± .84
Apical	1.30 ± .675	1.80 ± .92	2.60 ± .84[b]	3 ± .00
P value	0.733	0.128	0.042[☆]	0.120

Note: 1.☆Denotes significance at 5% level

2. Different alphabet between area denotes significant at 5% level using Tukey HSD test

Table 3: Comparison between area of each Group of Observer 2

Area	GP1	GP2	GP3	GP4
Coronal	1.10 ± .32	1.20 ± .63[a]	1.60 ± .84[a]	2.50 ± .85
Middle	1.20 ± .42	1.30 ± .67[ab]	2.20 ± .79[ab]	2.60 ± .70
Apical	1.30 ± .67	2.10 ± .99[b]	2.80 ± .63[b]	3.00 ± .00
P value	0.668	0.031☆	.006☆☆	.196

Note: 1. ☆Denotes significant at 5% level

2. ☆☆denotes significance at 1% level

Table4.Comparison betweengroup of each area of observer 1

Table – 3	GP1	GP2	GP3	GP4	P-Value
Coronal	1.20 ± .632[a]	1.10 ± .32[a]	1.60 ± .84[ab]	2.30 ± .95[b]	0.003☆☆
Middle	1.10 ± .316[a]	1.50 ± .85[ab]	2.40 ± .97[bc]	2.60 ± .84[c]	<0.001☆☆
Apical	1.30 ± .675[a]	1.80 ± .92[ab]	2.60 ± .84[bc]	3 ± .00[c]	<0.001☆☆

Note: 1. ☆Denotes significance at 5%level

2. ☆☆denotes significance at 1% level

3. Different alphabet between groups denotes significance at 5%level

Using Tukey HSD test

Table 5. Comparison betweengroup of each area of observer 2

Table – 3	GP1	GP2	GP3	GP4	P-Value
Coronal	1.10 ± .32[a]	1.20 ± .63[a]	1.60 ± .84[a]	2.50 ± .85[b]	0.001☆☆
Middle	1.20 ± .42[a]	1.30 ± .67[a]	2.20 ± .79[b]	2.60 ± .70[b]	<0.001☆☆
Apical	1.30 ± .67[a]	2.10 ± .99[ab]	2.80 ± .63[bc]	3.00 ± .00[c]	<0.001☆☆

Note: 1. ☆Denotes significance at 5%level

2. ☆☆denotes significance at 1% level

3. Different alphabet between groups denotes significance at 5%level usingTukey HSD test

Table 6: comparison between observer 1 and observer 2 of each area in to each group wise

Group	Area	Observer I	Observer II	P.value
I	C	1.20 ± 0.63	1.10 ± 0.32	0.660
	M	1.10 ± .32	1.20 ± .42	0.556
	A	1.30 ± .67	1.30 ± .67	1.000
II	C	1.10 ± .32	1.20 ± .63	0.660
	M	1.50 ± .85	1.30 ± .67	0.567
	A	1.80 ± .92	2.10 ± .99	0.492
III	C	1.60 ± .84	1.60 ± .84	1.000
	M	2.40 ± .97	2.20 ± .79	0.618
	A	2.60 ± .84	2.80 ± .63	0.556
IV	C	2.30 ± .95	2.50 ± .85	0.626
	M		2.60 ± .70	1.000
	A	3.00 ± .00	3.00 ± .00	1.000

Note : since all p-values are >0.05 there is no significant difference between observer 1 and observer 2.

Interpretation of results:

1. The smear layer removing ability of 50% FRAGARIA ANANASSA EXTRACT was superior compared to 17%EDTA, 5.25% NaoCl and saline along the entire length of root dentin (apical, middle and coronal).
2. 17%EDTA ability to remove smear layer was comparable with 50% FRAGARIA ANANASSA EXTRACT in coronal and middle portion whereas it less effective in removing smear layer from apical portion of root dentin.
3. 17%EDTA was better than 5.25%Naocl in removing smear layer.
4. Saline which was the control group was the least effective.

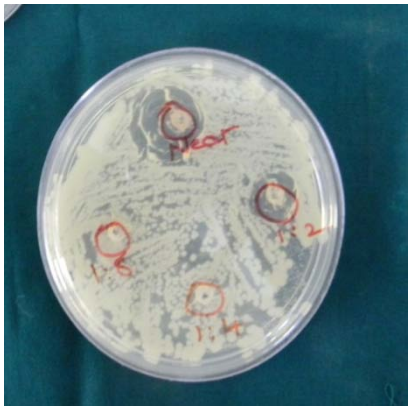


Figure 9: Agar plate showing zones of inhibition

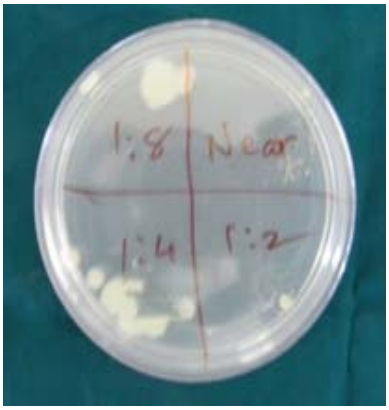


Figure 10: Bacterial growth on samples treated with FA extract

Figure 11: Neat and 1:2 dilution showing no turbidity

Figure 12: 1:4 and 1:8 dilution showing turbidity

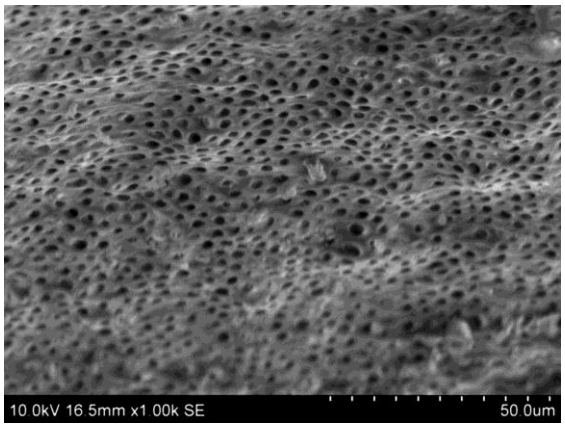


Image 1: SEM image of Coronal portion of Group 1 (50% FA)

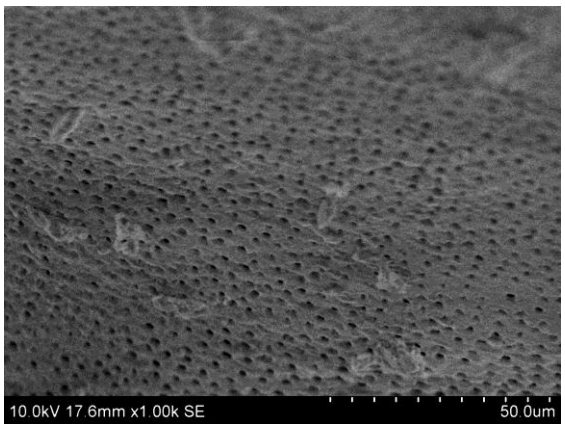


Image 2: SEM image of Middle portion of Group 1

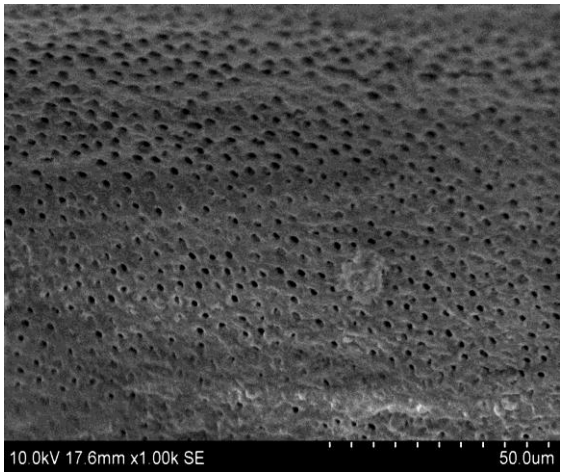


Image 3: SEM image of Apical portion of Group 1

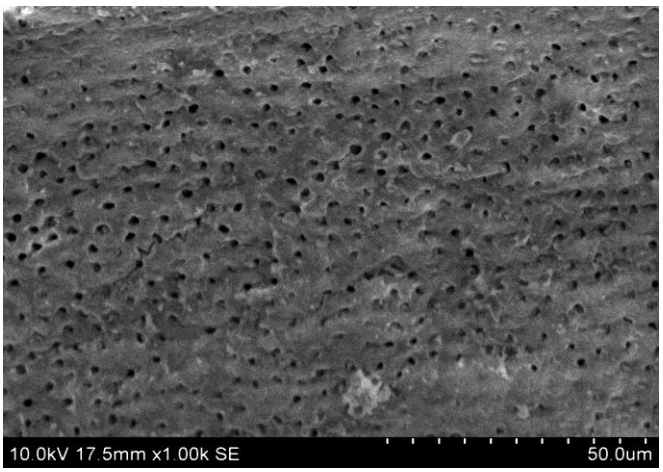


Image 4: SEM image of Coronal portion of Group 2 (17% EDTA)

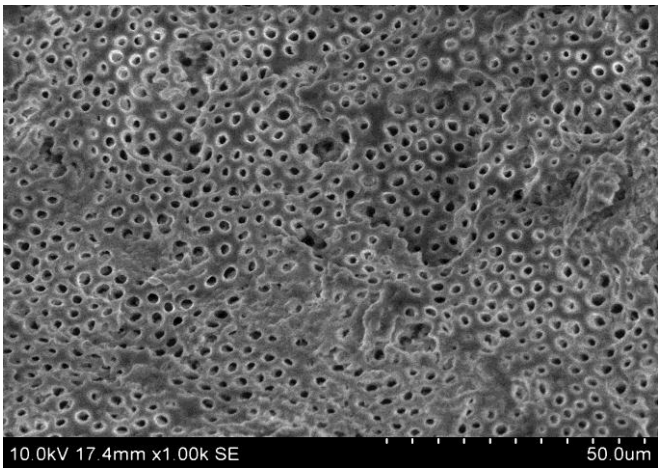


Image 5: SEM image of Middle portion of Group 2

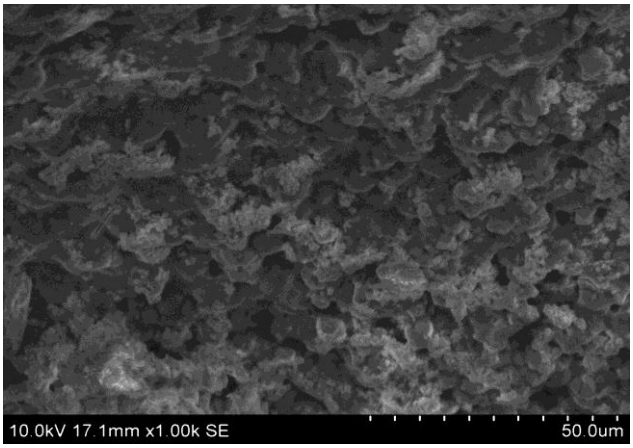


Image 6: SEM image of Apical portion of Group 2

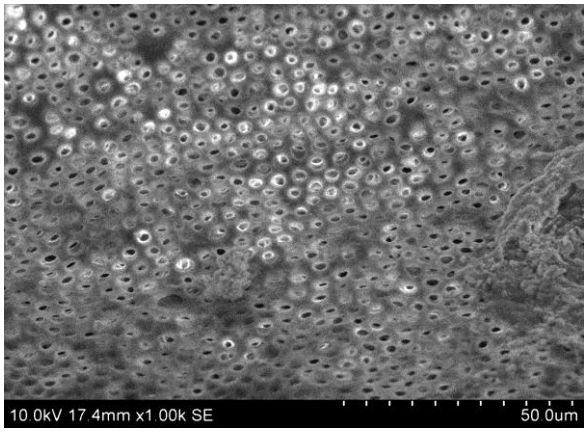


Image 7: SEM image of Coronal portion of Group 3 (5.25% NaoCl)

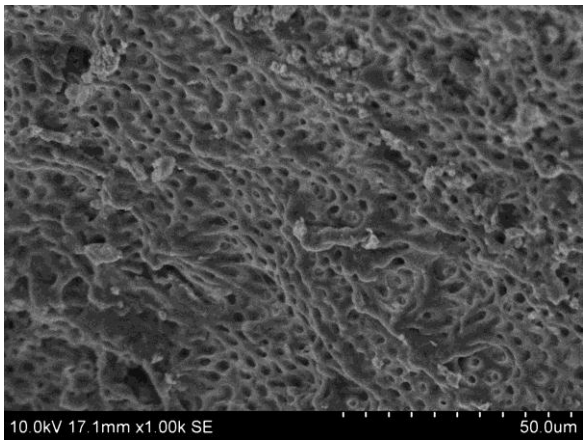


Image 8: SEM image of Middle portion of Group 3

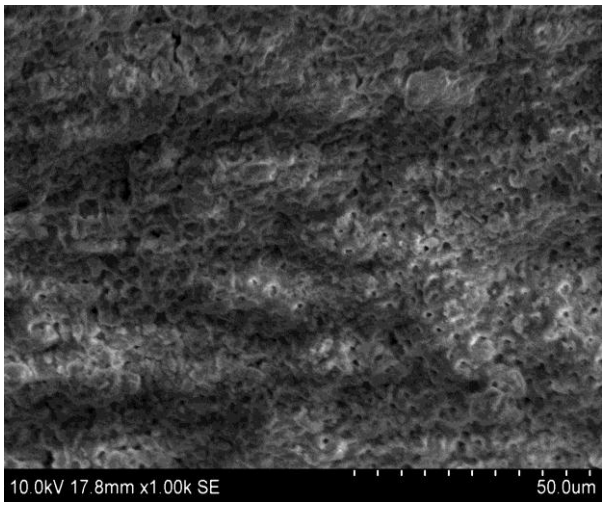


Image 9: SEM image of Apical portion of Group 3

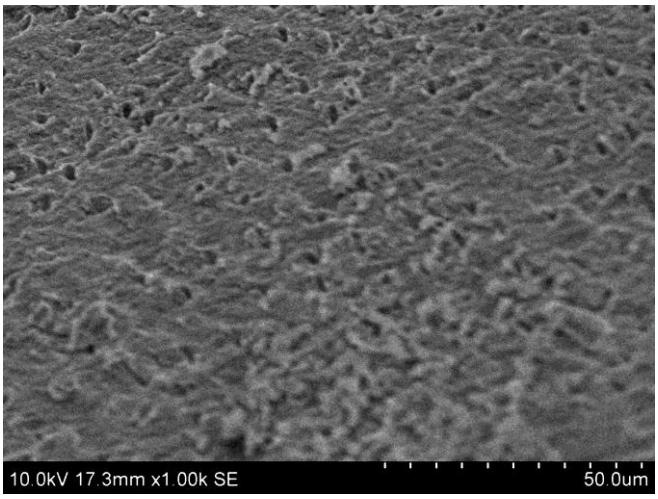


Image 10: SEM image of Coronal portion of Group 4 (saline)

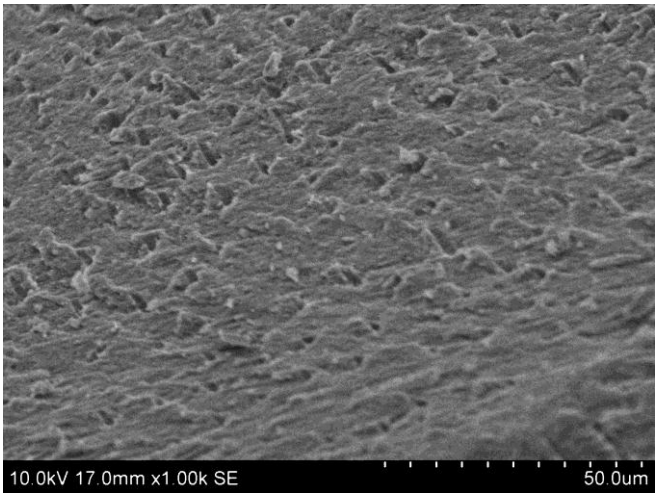


Image 11: SEM image of Middle portion of Group 4

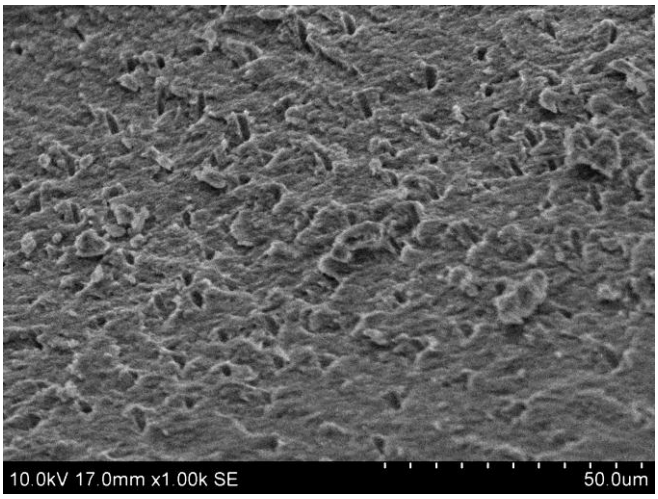
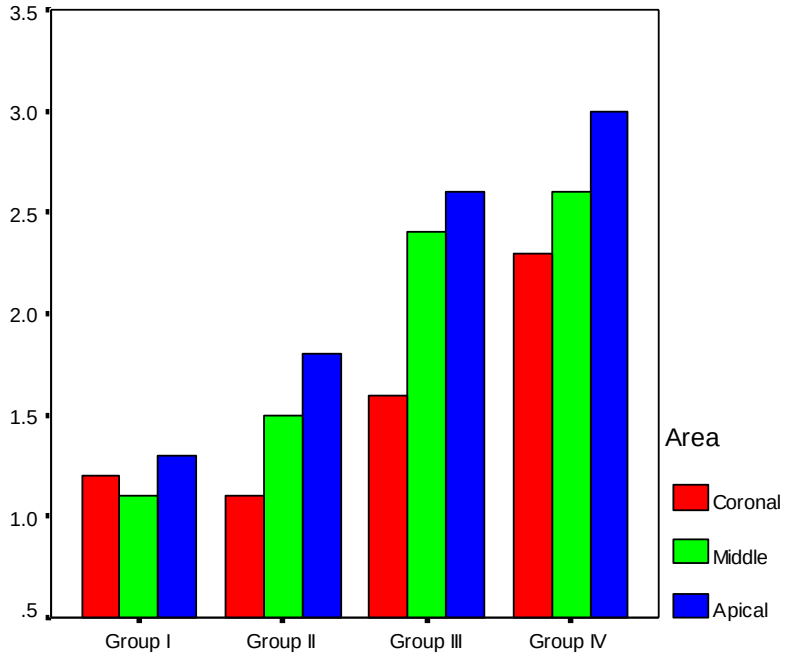
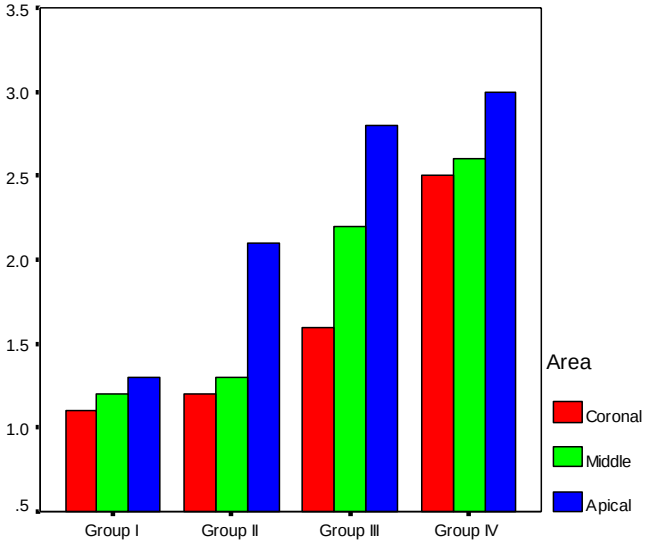


Image 12: SEM image of Apical portion of Group 4

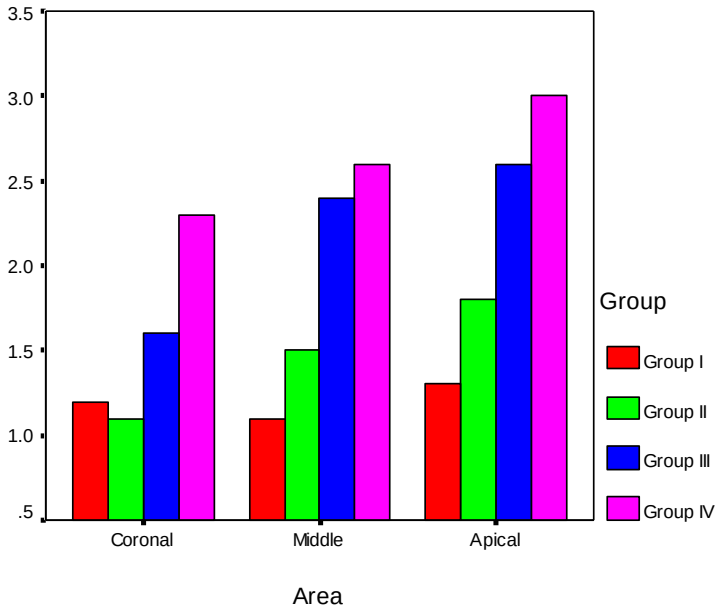
Graph 1: Comparison between area of each Group Of Observer I



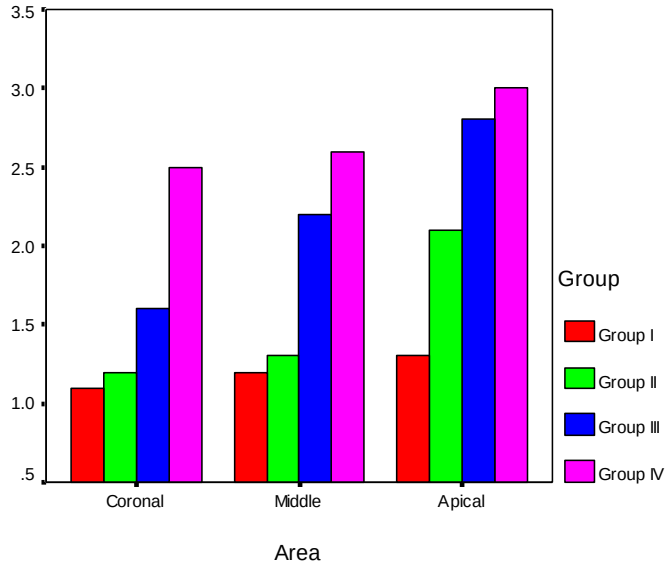
Graph 2: Comparison between area of each Group Of Observer I



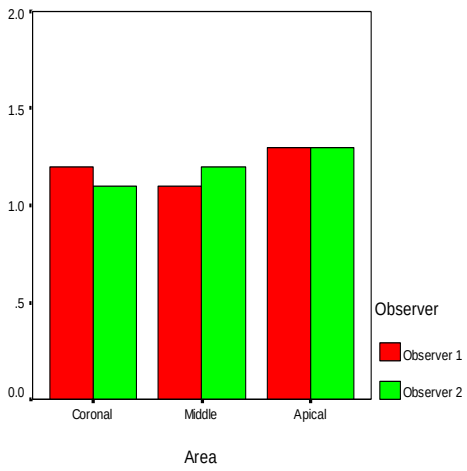
Graph 3: Inter group Comparison of coronel middle apical by observer 1comparison between group of each area of observer 1



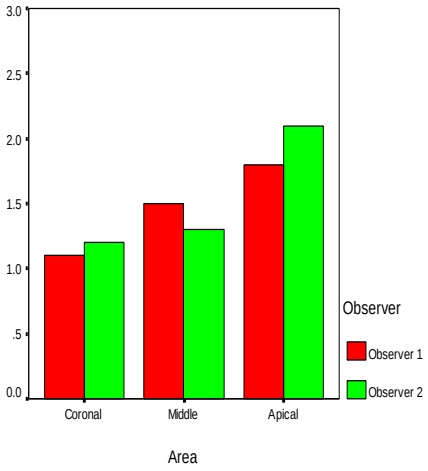
Graph 4: Inter group Comparison of coronel middle apical by observer 1 comparison between group of each area of observer 2



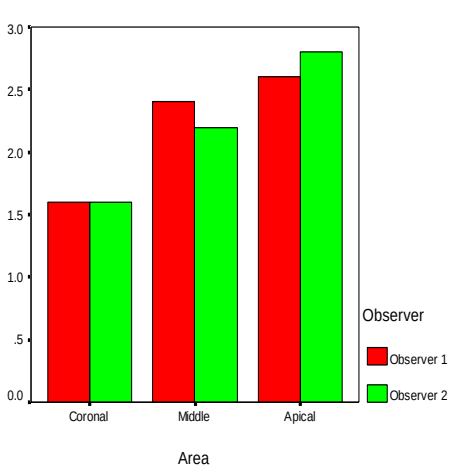
Graph 5 ,6,7 & 8: Comparison between observer 1 and observer 2 of each area in to each group wise



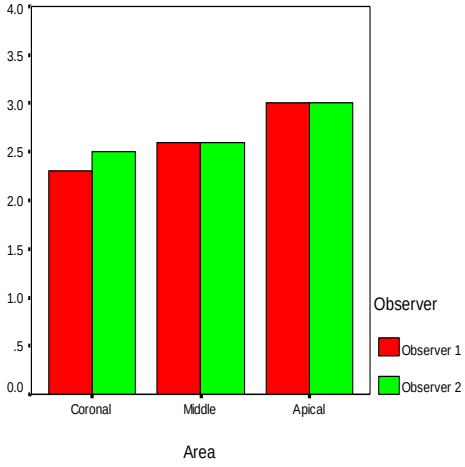
Group 1



Group 2



Group 3



Group 4

Discussion

DISCUSSION

Phytomedication in dentistry is a concept that has a fairly long history dating back to ancient times. They have been used as folk medicine with both eastern and western lineage. Their excellent biocompatibility, anti-inflammatory, anti-oxidant and antimicrobiological properties make them an interesting alternative to contemporary synthetic theraupatics which are known for their side effects.The rapid emergence of resistance to these products is a major drawback of them.[30] In endodontics natural substances have been evaluated as intracanal medicament and root canal irrigant in the past. Some of the tested natural products include Morinda Citrifolia Juice (MCJ,NONI)[1], Propolis,ArctiumLappa,Triphala and Green tea polyphenols(GTP),German chamomile and tea tree oil and ginger extract. The results have been encouraging.MCJ demonstrated adequate antimicrobial activity and was effective in removing smear layer when combined with a rinse of EDTA.[26] An in vitro evaluation of antimicrobial activity of Arctium lappa against microorganisms specifically found in endodontic infections showed a great microbial inhibition of Arctium lappa against the tested endodontic pathogens. The microbial inhibition potential of Arctium lappa observed in this

study opens perspective for its use as an intracanal medication. Triphala and Green tea polyphenols (GTP) in an in vitro evaluation showed statistically significant antibacterial activity although its antibacterial activity was not similar to 5% sodium hypochlorite.German chamomile and tea tree oil in an in vitro study showed ability to remove smear layer which was superior to NaOCl alone but less than NaOCl combined with EDTA. In an antimicrobial activity testing study Ginger extract showed significant inhibition of bacterial growth compared with sodium hypochlorite by agar diffusion test. A comparative evaluation on microbial efficacy of propolis, NaOCl and saline when used as intracanal irrigants indicated that the propolis has antimicrobial activity equal to that of NaOCl. The antibacterial efficacy of three commonly used intracanal medicaments with propolis against Enterococcus faecalis was compared. They concluded that propolis had good in-vitro antibacterial activity against Enterococcus faecalis in the root canals, suggesting that it could be used as an alternative intracanalmedicament.[25] The major advantage of these natural alternatives are their easy availability, low cost and most importantly their excellent bio-compatible nature with negligible side effects.[25]

The outcome of endodontic therapy primarily depends on complete eradication of micro-organisms from the root canal which is achieved by instrumentation of the root canal.[10]However contemporary instrumentation techniques does not ensure complete elimination of micro-organsims from the root canal since they leave considerable amount of root dentin untouched. Further mechanical instrumentation leaves a smear layer which necessitates the use of irrigants for it's removal.[2,6] It is widely accepted that organic remanants,smear layer and the micro-organisms have to be completely removed chemo-mechanically to attain success.[29]

Till date no irrigant has been identified which can perform these three important functions.[32] Sodium hypchlorite is the irrigant which is very widely accepted as the main endodontic irrigant due to its excellent tissue dissolving capacity and wide anti-microbial effect. It is highly alkaline(ph 11) in nature. At a ph of 11 it has more tissue dissolving capacity and at a ph of 6-7.5 it exhibits more anti-microbial effects. There is no denying in the fact that sodium hypochlorite is unchallengeable in its tissue dissolving ability but it possess certain undesirable effects as well which can lead to tissue toxicity when improperly used. It has very undesirable smell and taste

as well.Even a concentration of 0.125% of NaoCl when combined with chlorhexidine can lead to the formation of para-chloroaniline (PCA) which is a potent carcinogen. An antagonistic interaction between NaoCl and EDTA can lower the tissue dissolving ability of Naocl due to reduction in the availability of free chlorine. These are some of the controversial aspect of the currently used main synthetic endodontic irrigant NaoCl.[6,19,32]

The irrigant which has been widely used for smear layer removal is 17% EDTA. During instrumentation of root canal dentin is not shredded or cleaved but shattered to produce considerable amount of debris which is spread over the entire root surface. This covering of the root dentin by debris is called smear layer.Smear layer can be defined as an amorphous irregular layer containing inorganic dentin debris as well as organic materials like pulp tissue, odontoblasticprocess, necrotictissue, microorganisms and their metabolic products.[29] It was McComb & smith (1975) who first reported the presence of smear layer on the surface of instrumented root canal.The structure of smear layer can be divided in to two parts first a superficial smear layer, which is 1-2µm thick which is attached to the surface of root canal wall and the second one smear plugs

which is formed due to the forcing and packing of debris in to the tubules to a depth of 40µm.This packing is attributed to capillary action as a result of adhesive forces between the dentinal tubules and smear material. The thickness can vary depending on the type and sharpness of cutting instruments. Generally it is considered to be less with hand filling when compared to mechanized & motorized systems. It also depends on whether the canal is dry or wet. Increased centrifugal forces resulting from close proximity of the instrument to the dentinal wall can lead to thicker smear layer. In the beginning stages of instrumentation its content is more organic in nature containing necrotic and viable pulp tissue and progressively it becomes inorganic in later stages of instrumentation. The smear layer appears as a irregular granular layer under SEM. The appearance is attributed to the translocation and burnishing of the superficial components of the root walls during instrumentation.[29]

There is a consensus view now that this smear layer has to be effectively removed to attain a positive treatment outcome. The two popular means of smear layer removal are chemical and ultrasonic. The two aspects which play very critical role in chemical removal are ph and period of exposure. A number of chemicals have been tried for

smear layer removal the most commonly used are EDTA and ORGANIC ACIDS.[29]

EDTA is a chelating agent.It readily reacts with calcium ions in root dentin and forms soluble calcium chelates. EDTA very effectively decalcifies dentin in coronal and middle portion of root dentin but it is very ineffective in apical portion of root dentin the reason being attributed is because it basically acts by removing the mineral(calcium)portion and non-collagenous portion (NCP) of root dentin. The apical portion of root dentin is more sclerotic in nature and contains less NCP so the degree of decalcification in apical portion is very minimal. Further it works in neutral ph. However with increase in time ph decreases and there by efficiency decreases in removing calcium making it necessary for longer contact time.[23]

Among the organic acids citric acid is the one which is widely used. The other organic acids that are used are maleic acid, tannicacid. Lactic acid and polyacrylicacid. Smear layer components are very small particles with a large surface:mass ratio. So they are readily soluble in organic acids. Among the organic acids citric acid is effective in removing smear layer from coronal and middle portion

while maleic acid is very effective in removing smear layer from coronal, middle and apical portion of root dentin. The reason why maleic acid is able to remove from apical portion as well is its reduced surface tension (0.06345 N/m) and molecular mass (134.09 Dalton) than citric acid (192.13 Dalton) and hence it can easily diffuse in to even sclerotic dentin and can dissolve smear layer. The ph of citric acid(10-50%) is 1-2 where as the ph of maleic acid(7%) is 1. Hence maleic acid is more acidic and capable dissolving smear layer more than citric acid.[2,23,29] So it can be inferred that of all the irrigants used for smear layer removal 7% maleic acid is comparatively better in removing smear layer from all the three levels of root dentin.[23]

The irrigant that is widely and routinely used for anti-microbial effect is 2%chlorhexidine a bis-biguanide with antiseptic and anti-microbial properties with excellent substanstivityi.e it imparts residual anti-bacterial effect but chlorhexidine is a cation thatfore is more effective against gram negative than on gram positive micro organisms.More over when it combines with Naocl it can form PCA, Infactchlorhexidine by itself can undergo hydrolysis and can form PCA which is highly carcinogenic.[32] In this regard it becomes prudent

to use a combination of irrigation regimes and all the irrigating solution currently used in contemporary endodontics in addition to their beneficial effects have their own side effects as well.

In a situation of this nature if a non-synthetic substance can be identified as an irrigant with all ideal requsites it will have an positive impact on endodontics.

Strawberries are considered as one of the most magnificient fruits due to its rare color,extradinary aroma and wonderful taste.They are extraordinarily decorative and equally unique as well because of its potent health benefits. They are typically characterized by low calorific value(40 kcal/100g).[12] It is an hybrid species,not a botanical berry,but an aggregate accessory fruit meaning the fleshy part is derived not from the plants ovaries but from the receptacle that holds the ovaries.[33] It is consumed either as fresh fruit juice or it is used in prepared foods as preservative.

Of late the medicinal value of strawberry has been matter of intense scrutiny because of its high composition of micro-macro elements.[28] In fact the medicinal value of strawberry has been mentioned in ancient roman literature. European monks were using

the wild strawberry in their illuminated manuscripts. References about medicinal value of straw berry can also be found in Flemish. German art and English miniatures.

Straw berries are known source of vitamin c(potent antioxidant).Most of the health beneficial aspects of straw berry is due to its vitamin c content.[21] The other group of compounds that contribute immensely to its beneficial health effects are phenolic compounds(antioxidant).The phenolic compounds that are present in straw berry can be grouped in to flavonoids anthocyanins,phenolic acids(ellagitanins),lignans and polymeric tannins.[14]

The redox property of phenolics is responsible for the antioxidant ability of straw berries they act as reducing agents, singletoxygen quenchers and hydrogen donators. Phenolics also possess metal chelation property. Literature shows fresh straw berry extract contains 15 times more antioxidant capacity than trolox.The phenolic acids present in straw berry are derivatives of hydroxycinnamic acids and hydroxybenzoic acids. Because of these compounds straw berries exihibits a plethora of health benefits.[18] The claimed medicinal value of straw berries are

1. Boosts immunity,vitamin c is a immunity booster.it is a powerful,fast working anti oxidant.
2. Promotes eye health, the antioxidants prevent cataract, protects cornea and retina from free radicals released by sun's rays.
3. Helps to fight against cancer-ellagicacid, lutein and zeathancins are very powerful scavengers of free radicals.
4. It keeps wrinkles at bay.it induces collagen formation which increases elasticity and resilinence of skin.
5. It fights against bad cholesterol (LDL) ellagagic acid and other flavanoid phytochemicals inhibit human low density lipoprotein and liposome oxidation which reduces plaque build up in arteries.
6. They regulate blood pressure as they are medium source of potassium.Potassium acts as a buffer against sodium.Together with its cholesterol reducing property it is the most heart friendly fruit.
7. It helps in fighting against type 1 diabetes mellitus because it contains adequate amount of fibre which delays the absorption of sugar in to blood.

8. Low calories,fat free and low in both sugar and sodium fights against obesity.
9. They have been investigated in the treatment of gastrintetinal and urinary infections as well
10. They are potent anti inflammatoryagents.They block the conversion of arachydonic acid in to prostaglandins which are mediators of inflammation.
11. literature survey clearly shows that they have strong anti-microbial action against a variety of human pathogenic micro-organisms.Some of this organisms include enterococcus faecalis, candidaalbicans, staphylococcus aureus, staphylococcusepidermidis,bacilluscereus,Escherichiacoli,pseudomonasaeruginosa,salmonellaabony,sarcinaspecies,saccharomycescerevisiae and aspergillusniger. The anti-microbial effect of strawberries is most probably due to its composition of chemically complex compounds namely weak organicacids, low ph, phenolic acids, tannins (ellagitannins, ellagic acid) and their mixtures of different chemical forms. The exact mechanism of action is not exactly known but supposed to be by disintegratin of outer membrane (OM),immobilization of

bacterial cells there by preventing adhesion with substrate.[14,15] Even the pomace of straw berries are said to inhibit B.cereus and E.coli to an extent.[9]

With this compositional chemical profile, the vast plethora of health benefits it offers and keeping in line with the modern trend of seeking a remedy by natural substance it was decided to investigate and evaluate the efficiency of FRAGARRIA ANANASSA as a root canal irrigant which was the aim of this study.

The objective of this study was to investigate the efficiency of straw berry fruit juice's

a. MIC against E.FAECALIS.

b. Ability to dissolve inorganic tissue.

The study was designed in two phases. First phase explored the anti-microbial property FA Juice (Fragaria Ananassa) and its minimum inhibitory concentration against E.Faecalis (standard strain)

The second phase of the study examined the minimum inhibitory concentration of FA Juice as an endodontic irrigant to

evaluate its efficiency in removing smear layer from the root canal walls.

Although oral cavity is a privileged sanctuary for a wide variety of micro-organisms the number of bacterial species which inhabitate the root canal is very limited due lack of nutrient availability and low oxygen presence. These conditions lead to the predominance of facultative anaerobic microorganism enterococcus faecalis. It is highly dentinophilic in nature, Has the ability to invade up to 1000µ inside dentinal tubules because it can form chains. Has the ability to exihibit viable but not cultivable state (VBLC) state,has the ability to form bacterial bio films. Highly resistant to intra canal medicaments(ph) and irrigantsbecaue of a functioning intracellular proton pump.[3,34] Hence the anti-microbial action of any irrigant is generally investigated against E.faecalis, It is generally considered as a benchmark.so it was decided to investigate the MIC of FA against E.faecalis.

In phase 1 of the study Standard strain of E-Faecalis was cultured on Blood agar on 24 hours incubation. The growth was

suspended in Brain Heart infusion broth. This was plated on blood agar.

500gms of fresh straw berry fruit was washed in sterile distilled water and the crude extract was prepared in a sterile blender.The extract was filtered by biomembrane filter and the filtrate was serially diluted in saline to give a concentration of neat, 1:2, 1:4 and 1:8.

Discs were impregnated with these concentrations and incorporated on the blood agar with organism and was incubated at 37º C for 24hrs. On examination anti-bacterial activity was expressed as the zone of inhibition produced by Straw Berry extract around the disc. The diameter was measured in millimeter's and the highest zone of inhibition was considered as having anti-bacterial activity at that concentration.

The results of the phase 1 of the study showed raw Straw Berry fruit extract had inhibitory action against E-Faecalis in the dilution neat and 1:2 (50%).

E-Faecalis	Zone of Inhibition
Neat	10mm
1:2	8mm
1:4	–
1:8	–

Dilution 1:4 and 1:8 do not seem to have any inhibitory action. AsExplained earlier the anti-microbial action FA must be due to its composition of chemically complex compounds namely weak organic acids,lowph,phenolicacids,tannins(ellagitannins,ellagic acid) and their mixtures of different chemical forms(14,15). In the phase 2 of the study it was decided to investigate 50% FA JUICE ability to remove smear layer along the length of root dentin (coronal,middle and apical).

Intact human mandibular premolars extracted for orthodontic reasons were collected.From this 40 teeth with single canal (verified with radiographs) and mature apices were selected for the study.The

teeth were cleaned ultrasonically and stored in water containing 0.1% thymol until needed for the study,a period not exceeding one month.

The samples were decoronated at CEJ and randomly divided in to 4 groups with 10 teeth each

Group 1 was 50% FA

Group 2 was 17% EDTA

Group 3was 5.25% Naocl

Group 4 was saline (control)

Instrumentation protocol that was followed was a 10 size K-file was used to obtain glide path.Working length was determined using 15 size stainless steel K-file.The open-system design was followed.Chemomechanical preparation was performed by nickel-titanium rotary protaper files (S1,S2,F1,F2,F3) in crown down sequence with irrigation using respective irrigants 1mm beyond apex .Irrigation protocol that was followed during instrumentation was 10ml of respective irrigant (50% FA for group 1,17% EDTA for group 2,5.25% Naocl for group 3 and saline for group 4) between each instrument with a contact time of 1 minute. A total of 50ml of

irrigant was used in each root canal.Irrigation was carried out passively with a 26 guage needle with tip being positioned 1mm short of working length. Depositedirrigant was activated ultrasonically for 1 minute during each change of instrument. Post instrumentation final irrigation protocol was performed with 10ml distilled water for 1 minute to terminate the reaction following the completion of chemo-mechanical preparation. With diamond burs longitudinal grooves were made on the buccal and lingual outer surfaces of the samples without bur penetrating in to the lumen. The roots were split in to two halves with a chisel. The split samples were examined under SEM which is the best tool to evaluate smear layer removal.

The scoring criteria followed to evaluate smear layer removal for thi study was the one given by TORABINEJAD et al.

Score 1: no smear layer.

Score 2: moderate smear layer.

Score 3: heavy smear layer.

The scoring was done by two independent observers and the results were tabulated and statistically analysed.

First comparision between area of each group was done by observer 1 and observer 2

Secondcomparision between group of each area was done by observer 1 and observer2

Finally comparision between observer 1 and observer 2 of each area in to each group wise was done.

Results of the comparision between area of each group by observer 1 showed

In group 1 the smear layer removal in coronal and middle portion were same - 90% followed by apical portion 80%.

In group 2 the coronal portion showed more smear layer removal- 90% than middle portion -70% followed by apical portion- 50%.

In group 3 the smear layer removal in coronal portion was 60% followed by middle portion 30%. Apical portion portion showed negligible removal - 20%

In group 4 the smear layer removal in coronal and middle portion were 30% and 20% respectively there was no smear layer removal in apical portion- 0%.

Results of the comparision between area of each group by observer 2 showed

In group 1 the smear layer removal in coronal portion - 90% was more than middle portion. Middle and apical portion showed same smear layer removal - 80%

In group 2 the coronal portion showed more smear layer removal - 90% than middle portion - 80% followed by apical portion - 40%

In group 3 the smear layer removal in coronal portion was 60% followed by middle portion 20%, apical portion portion showed negligible removal -10%

In group 4 the smear layer removal in coronal and middle portion were 20% and 10% respectively there was no smear layer removal in apical portion 0%.

Results of the comparison of group of each area by observer 1 showed.

In coronal portion the smear layer removal in group 1 and group 2 were same 90% followed by group 3 which had 60% removal the least amount of removal was seen in group 4 which had 30% removal.

In middle portion the smear layer removal in group 1 and group 2 were 90% and 70% respectively followed by group 3 which had 30% removal the least amount of removal was seen in group 4 which had 20% removal.

In apical portion the smear layer removal in group 1 and group 2 were 80% and 50% followed by group 3 which had 20% removal the least amount of removal was seen in group 4 which had no removal at all.

Results of the comparision of group of each area by observer 2 showed

In coronal portion the smear layer removal in group 1 and group 2 were same 90% followed by group 3 which had 60% removal the least amount of removal was seen in group 4 which had 20% removal.

In middle portion the smear layer removal in group 1 and group 2 were same 80% followed by group 3 which had 20% removal the least amount of removal was seen in group 4 which had 10% removal.

In apical portion the smear layer removal in group 1 and group 2 were 80% and 40% followed by group 3 which had 10% removal the least amount of removal was seen in group 4 which had no removal at all.

The results of the comparision between observer 1 and observer 2 of each area in to each group wise shows.

In group 1 in coronal portion the smear layer removal score % given by observer 1 and 2 were same 90%,in middle portion it was

90% and 80% respectively but the difference is insignificant,in apical portion it was same 80%

In group 2 in coronal portion the smear layer removal score% given by observer 1 and 2 were same 90%,in middle portion it was 70% and 80% respectively but the difference is insignificant,in apical portion it was 50% and 40% again the difference is insignificant.

In group 3 in coronal portion the smear layer removal score% given by observer 1 and 2 were same 60%,in middle portion it was 30% and 20% respectively but the difference is insignificant, in apical portion it was 20% and 10% again the difference is insignificant.

In group 4 in coronal portion the smear layer removal score% given by observer 1 and 2 were 30% and 20% respectively, in middle portion it was 20% and 10% respectively but the difference is insignificant,in apical portion it was same 0%.

From the results of this study it can be inferred that 50%FA GROUP was superior to all other groups in removing smear layer from apical,middle and coronal portion of root dentin possibly due to the high organic acid content of FA. The organic acids which are present in strawberries are citric acid,malicacid,tartaricacid,oxalicacid

and fumaric acid.[21] Malic acid is highly unstable, it readily becomes fumaric acid and maleic acid which are cis and trans isomers.This maleic acid and citric acid have already been proved to be effective smear layer removers with maleic acid being effective along the entire length of the canal.[23] The synergistic action of these organic acids may have contributed to the superior ability of 50% FA GROUP in removing smear layer. Another aspect that may have contributed to it's superiority is surface tension. The average surface tension values of 50% FA,17%EDTA,5.25%Naocl and saline used in this study was found to be 39(dyne/cm),46(dyne/cm),43(dyne/cm) and 66(dyne/cm).This reduced surface tension value of 50%FA would have increased it's wettability there by increasing the penetrating ability and subsequently leading to superior results than other groups.

17% EDTA GROUP was equally effective in coronal portion with 50%FA GROUP but was less effective in middle and apical portion when compared to 50%FA GROUP which is in accordance with previous studies.[23]

5.25% Naoclgroup was less effective than 17%EDTA GROUP in coronal,middle and apicalportion as it is known to dissolve only the organic portion effectively which coincides with previous studies.[23]

Saline group which served as the control clearly indicated it had very negligible ability in removing smear layer along the entire length of the root dentin which is in line with results of previous studies.[23]

To an extent passive ultrasonic activation which was employed with each change of file would have aided in more smear layer removal especially in the apical portion as the magnitude and velocity of the file will be more in apical portion[29] but the difference in smear layer removal % in apical portion between group 1 and 2 clearly shows the role played by 50% FA AS A ROOT CANAL IRRIGANT.

Summary

SUMMARY

This study was aimed to evaluate the anti-microbial property and minimum inhibitory concentration of FRAGARIA ANANASSA EXTRACT against Enterococcus Faecalis.The MIC of FA EXTRACT was used to evaluate the smear layer removing ability along the length of root dentin (apical, middle & coronal). Standard strain of E-Faecalis was cultured on Blood agar on 24 hours incubation. The growth was suspended in Brain Heart infusion broth. This was plated on blood agar.

500gms of fresh straw berry fruit was washed in sterile distilled water and the crude extract was prepared in a sterile blender. The extract was filtered by biomembrane filter and the filtrate was serially diluted in saline to give a concentration of neat, 1:2, 1:4 and 1:8.

Discs were impregnated with these concentrations and incorporated on the blood agar with organism. This was incubated at 37º C for 24hrs. On examination anti-bacterial activity was expressed as the zone of inhibition produced by Straw Berry extract around the disc. The diameter was measured in millimeter's and the highest zone of inhibition was considered as having anti-bacterial activity at that

concentration. Intact human mandibular premolars extracted for orthodontic reasons were collected. From this 40 teeth with single canal (verified with radiographs) and mature apices were selected for the study.The teeth were cleaned ultrasonically and stored in water containing 0.1% thymol until needed for the study, a period not exceeding one month.

The samples were decoronated at CEJ and randomly divided in to 4 groups with 10 teeth eachGroup 1 was 50% FA, Group 2 was 17% EDTA,Group 3was 5.25% Naocl and Group 4 was saline(control).

Instrumentation protocol that was followed was a 10 size K-file was used to obtain the glide path.Working length was obtained using 15 size stainless steel K-file.Since a newer irrigant was investigated a open-system design was followed.Chemomechanical preparation was performed by nickel-titanium rotary protaper files (S1,S2,F1,F2,F3) in crown down sequence with irrigation using respective irrigants 1mm beyond apex .Irrigation protocol that was followed during instrumentation was 10ml of respective irrigant(50% FA for group 1,17% EDTA for group 2,5.25% Naocl for group 3 and

saline for group 4) was used to irrigate the root canal between each instrument with a contact time of 1 minute. A total of 50ml of irrigant was used in each root canal. Irrigation was carried out passively with a 26-guage needle with tip being positioned 1mm short of working length.Depositedirrigant was activated ultrasonically for 1 minute during each change of instrument. Post instrumentation final irrigation protocol was performed with 10ml distilled water for 1 minute to terminate the reaction following the completion of chemo-mechanical preparation. With diamond burs longitudinal grooves were made on the buccal and lingual outer surfaces of the samples without bur penetrating in to the lumen. The roots were split in to two halves with a chisel. The split samples were examined under SEM.

The scoring criteria followed for smear layer removal in this study was the one given by TORABINEJAD et al. The scoring was done by two independent observers. The results were tabulated and statistically analysed by one-way anova, tukey HSD and independent sample ‘t’test.

Conclusion

CONCLUSION

Within the limitations of this study, it can be concluded that:

1. The minimum inhibitory concentration of FRAGARIA ANANASSA EXTRACT against E.Faecalis was 50%.
2. The smear layer removing ability of 50% FRAGARIA ANANASSA EXTRACT was superior to 17%EDTA, 5.25% Naocl and saline along the entire length of root dentin(apical,middle and coronal).
3. 17%EDTA ability to remove smear layer was comparable with 50% FRAGARIA ANANASSA EXTRACT in coronal and middle portion but was less effective in removing smear layer from apical portion of root dentin.
4. 17%EDTA was better than 5.25%Naocl in removing smear layer.
5. Saline which was the control group was the least effective.
6. From the above study it can be inferred that 50% FRAGARIA ANANASSA EXTRACT can be considered as a potent alternative for EDTA to be used in conjuction with Naocl because of it’s anti-microbial and smear layer removing ability.

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