Comparative evaluation of antimicrobial activity of 3% naocl, 0.5 % metronidazole,12.5% curcuma longa and 25% mimusops elengi against enterococcus faecalis-an in vitro study

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Abstract
Aims: The increase of potential side effects and safety concerns of conventional medicaments and irrigants have led to the recent popularity of herbal alternatives. The present in vitro study was undertaken to evaluate the antimicrobial efficacy of 3% NaOCl, Metronidazole, Curcuma longa and Mimusops elengi against Enterococcus faecalis.
Settings and Design: The experimental materials were prepared and allotted into four groups (Group 1-NaOCl, Group 2-Metronidazole, Group 3-Curcuma longa, Group 4-Mimusops elengi).
Methods and Materials: Agar well diffusion test was done to evaluate the antimicrobial efficacy of various intracanal irrigants. Enterococcus faecalis was used as a test organism. The inoculums of Enterococcus faecalis was streaked on the agar plate. Using punch, wells each measuring 6mm in diameter and 4mm in depth were made and 50 micro liter of the experimental irrigant was pippeted into each well. The plates were placed in Carbon di oxide incubator at 37 degree Celsius for 48 hours and the zones of inhibition were measured.
Statistical analysis used: The results were statistically analysed using one way ANOVA and Tukey's HSD
Results: Highest inhibitory zone against E. faecalis was shown by 0.5% of metronidazole followed by 25% Mimusops elengi, 3% Sodium hypochlorite and the least is shown by 12.5 % Curcuma longa
Conclusions: Considering the undesirable side effects of sodium hypochlorite,metronidazole and herbal irrigants; mimusops elengi and Curcuma longa could be considered as an alternative

Keywords: Herbal root canal irrigants, Mimusops elengi, Curcuma longa, Metronidazole, Sodium hypochlorite, Toxicity, Root canal irrigants.

Introduction
Microorganisms are the common etiological factor in infecting the pulp space with diversity among the apical, middle and coronal thirds.[1] Ozok et al in a study identified 606 bacterial taxa at a species or higher taxonomic level belonging to 317 genera in the infected root canal system which revealed that the endodontic microbiome is more complex than previously described.[2] This supports the presence of a distinct ecological niche adjacent to the periapical inflammation.Further studies proved that there is a strong association of Enterococcus faecalis in both primary and secondary endodontic infections.[3]

E faecalis is an opportunistic pathogen associated with a high proportion of 29% to 77% in root canal treated teeth with persistent periapical disease. Although there are many factors responsible for its survival like an ability to grow in high salt concentrations (6.5% NaCl), a wide temperature range (10–60°C), 40% bile, a broad pH range, dentinal tubular penetration, presence of virulence factors endurance of long term starvation; high level of antibiotic resistance appears as a major concern.[4-10] Hence there is a need to find a potent antimicrobial irrigant that could combot the antibiotic resistance and prevent the side effects of the chemical irrigants.

Thus our study was aimed to evaluate the antimicrobial efficacy of 3% NaOCl, 0.5 % Metronidazole, 12.5% Curcuma longa and 25% Mimusops elengi against Enterococcus faecalis.

Materials and Methods
Procurement of material
The bark of Mimusops elengi and the rhizome of Curcuma longa were collected, washed with distilled water and dried under shade for 10-12 days. All the material was ground in an electric grinder to produce a powder.

Preparation of extracts
The plant materials were ground in an electric grinder to produce a coarse powder with a particle size of 300µm. 50mg of the powdered sample were accurately weighed and were extracted with 150 ml of ethanol.They were filtered using Whatman filter paper. The extracts were placed in the orbital shaker for 48 hrs with 150 -200 rpm to get the residues exhaustively extracted and were concentrated using rota-evaporator. The curcuma longa and mimusops elengi extracts were mixed with dimethyl sulfoxide at a concentration of 30mg/mL and were vortexed for maximum dissolution.

Minimal Inhibitory Concentration of extracts
Agar well diffusion method was used to find the Minimal Inhibitory Concentration of the extracts. Luria bertani Agar (LBA) plates were inoculated with test organism. The plates were evenly spread out and each well was loaded with 16.6µl(0.5mg), 33.3µl(1mg), 50µl(1.5mg), 66.6µl(2mg), 83.3µl(2.5mg) and 100µl(3mg) for Mimusops elengi and for curcuma longa, each well was loaded with 8µl(0.25mg), 16.6µl(0.5mg), 25µl(0.75mg), 33.3µl(1mg), 41.6µl(1.25mg) and 50µl(1.5mg). 10 mg of Tetracycline dissolved in 1 ml of

methanol was used as a positive control (c). The plates were incubated for 24h at 37°C. The development of inhibition zone around the well was measured (diameter) and recorded (Fig. 1)

The minimum inhibitory concentration was determined to be 1.25 mg/ml for Curcuma longa and 2.5 mg/ml for Mimusops elengi and hence the percentage was standardized as 12.5 % and 25% respectively to be used in our study.

Fig. 1

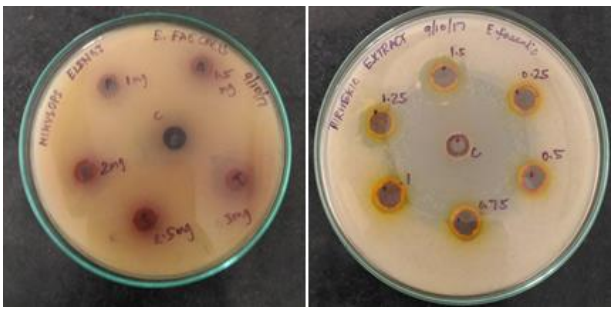


Fig. 1: Zone of inhibition tested against different concentrations of mimusops elengi and curuma longa

Microbial analysis Twenty samples were categorized into four groups with 5 samples in each for intracanal irrigation. The experimental materials were prepared and allotted into four groups (Group 1- 3% NaOCl, Group 2-0.5% Metronidazole, Group 3- 12.5% Curcuma longa, Group 4-25% Mimusops elengi)

Agar plates (LBA) were inoculated with the test organism (Enterococcus faecalis). The plates were evenly spread out. Then wells were prepared in the plates with a cork borer. Each well was loaded with 0.5 % metronidazole, 12.5% curcuma longa, 3% sodium hypochlorite and 25% mimusops elengi. The plates were incubated for 24hours at 37°C. The development of inhibition zone around the well was measured (diameter) and recorded. The results were analyzed using SPSS version 22 software (SPSS Inc, Chicago, IL, USA).One-way ANOVA and Multiple range test by TukeyHSD was used for mean comparison between the groups.

Results

Tables 1 reveals the mean and standard deviation of zone of inhibition of enterococcus faecalis observed for all groups. Table 2 reveals one way ANOVA (intergroup comparison) where the results were statistically significant($p<0.05$)

Highest inhibitory zone against E. faecalis was shown by 0.5% of metronidazole with a mean of 13.2 ±0.83 followed by 25% Mimusops elengi with 12.2±0.44, 3% Sodium hypochlorite with 11±1 and the least is shown by 12.5 % Curcuma longa with 10.2±0.44 (Bar Diagram 1)

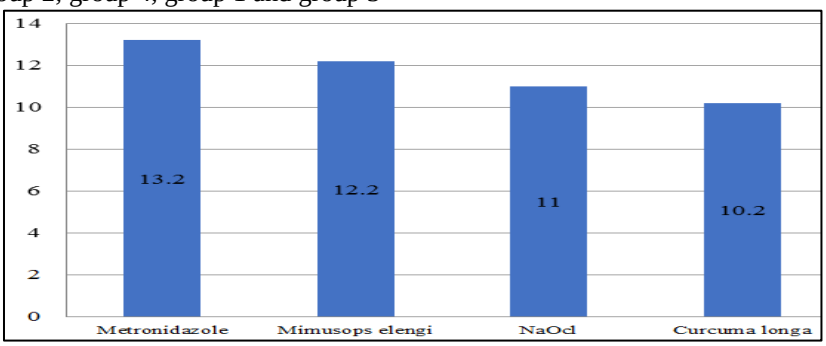
Table 1: Zone of inhibition before and after chemomechanical preparation using experimental and control groups as irrigant

Descriptives				
ZoneofInhibition				
	N	Mean	Std. Deviation	Std. Error
NaOCl	5	11.0000	1.00000	.44721
Metronidazole	5	13.2000	.83666	.37417
curcuma longa	5	10.2000	.44721	.20000
mimusops elengi	5	12.2000	.44721	.20000
Total	20	11.6500	1.34849	.30153

Table 2: One way anova

Zone of Inhibition					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	26.150	3	8.717	16.603	.000
Within Groups	8.400	16	.525		
Total	34.550	19			

Diagram 1: Mean of group 2, group 4, group 1 and group 3



Discussion

An optimal irrigant would have all of the characteristics considered beneficial in endodontics but none of the negative or harmful properties. Presently, no solution could be regarded as optimal.Sodium hypochlorite is the most commonly used irrigating solution owing to its antibacterial property and ability to dissolve tissues.[11] However due to its toxic reactions and antibiotic resistance it does not fulfill many important properties of an ideal irrigant.Hence there is search for an irrigant which is harmless and safe to host tissues; and also good in achieving the the maximum properties of an ideal irrigant.

Mimusops elengi is a large glaborous evergreen tree well documented for several medicinal properties like antinociceptive, diuretic effects, gastroprotective, antibacterial, antifungal, anticariogenic, free radical scavenging, antihyperglycemic etc and it is being focused for its chemical composition.[12] The preliminary phytochemical investigations of powdered bark shows the presence of Tannins and Phenolic derivatives, Steroids, flavanoids and Saponins type of major secondary metabolites.[13] The phytochemical agents such as Lupeol, saponin present in the bark show anti-inflammatory activity and tannins show antibacterial as well as antioxidant properties of this plant.[14]

Curcuma longa is a small perennial herb bearing rhizome and is comprised of a group of three curcuminoids: Curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin, as well as volatile oils (tumerone, atlantone, and zingiberone), sugars, proteins, and resins.[15] Curcumin, a yellow bioactive pigment, is the major constituent of turmeric which has a wide spectrum of biological actions such as anti-inflammatory, antioxidant, antifungal and antibacterial activities. The antibacterial activity is attributed to their antioxidant property and causing loss of integrity of bacterial membrane by their membrane permeabilization property. [16]

Enterococcus faecalis is tested in our study as it has been more frequently detected in persistent or secondary infections associated with the unsatisfactory outcome of the endodontic treatment [17] which could be attributed to its ability to ability to grow in high salt concentrations, a wide temperature range, tolerance to a broad pH range, as well as resist the intracanal procedures. [18] Commercial preparations were not used in our study since they are less efficient than herbal extracts and may cause discolourations. Since the ethanolic extracts were found to be more potent than aqueous extracts of all the medicinal plants,it has been used in our study.[19]

In our present study Metronidazole at 0.5% showed the maximum zone of inhibition with the mean of 13.2±0.83 which could be attributed to their single electron transfer reaction targeting the DNA synthesis of bacteria. Metronidazole has been established as an antimicrobial agent in various fields of medicine.[20] In dentistry metronidazole has shown its efficacy in the treatment of noma and cancrum oris, pericoronitis,osteomyelitis, chronic progressive periodontitis,dry socket and acute apical infections.[22] Our study has established its role as an effective intracanal irrigant. However metronidazole faces few limitations in its clinical applications as it shows no action against facultative anaerobes or aerobic bacteria and its unpleasant and metallic taste.However considering the toxicity of sodium hypochloride metronidazole could be used as an alternative with good antibacterial action and acceptable adverse effects.

This is followed by Mimusops elengi at 25 % which showed the mean zone of inhibition at 12.2±0.44 which could be attributed to its antibacterial and antioxidant properties due to the presence of tannins and saponins. Our study has been in accordance with Mistry et al who evaluated the antimicrobial activity of Azadirachta indica (Neem), Ocimum sanctum (Tulsi), Mimusops elelngi (Bakul), Tinospora cardifolia (Giloy) and Chlorhexidine Gluconate (CHX) on common endodontic pathogens like Streptococcus mutans, Enterococcus faecalis and staphylococcus aureus and concluded that methanolic extract of the herbal irrigants has considerable antibacterial activity against common endodontic pathogens. [22] In 2015 Mistry et al evaluated the antimicrobial activity of Azadirachta indica (Neem), Mimusops elengi (Bakul), and Chlorhexidine gluconate (CHX) on multispecies biofilm of common endodontic pathogens such as Streptococcus mutans, Enterococcus faecalis, Staphylococcus aureus and Candida albicans using an In vitro dentin disinfection model and he concluded as M. elengi is most promising among both the extracts as it has a significant difference in antimicrobial efficacy as compared to A. Indica and Control. [23] Also, alli et al gave the conclusion as crude extracts obtained from various portions of the plant Mimusops elengi may be used as drug to treat the disease caused by commonly isolated pathogens.[24] Hence the herbal irrigant mimusops elengi could be used as an alternative as it has pronounced antibacterial effects,non toxic,cost effective and no noticable resistance noted so far.

In our study 12.5% Curcuma longa showed antibacterial activity with the zone of inhibition at 10.2±0.44 which could be attributed to its antioxidant property. [16] Neelakantan et al [25] evaluated the antimicrobial efficacy of curcumin against Enterococcus faecalis biofilm formed on tooth substrate in vitro. Sodium hypochlorite (3%) showed maximum antibacterial activity against E.faecalis biofilm formed on the tooth substrate, followed by curcumin and CHX which is in accordance with our study.In an vitro study which evaluated the antimicrobial efficacy of curcumin against E. faecalis considering Sodium hypochlorite (3%) as references for comparison, NaOCl, achieved 100% killing of E.faecalis at 2 minutes, curcumin was able to achieve the same in 5 minutes.[26] In addition Curcuma longa has been well established in other fields of medicine as it has good Antioxidant, Anti-inflammatory, Hepatoprotective, Antiplatelet aggregation, Antimutagenic, Antimicrobial and Cardiovascular effects. In dentistry Curcuma longa has proved its therapeutic actions in Periodontal problems, Local drug delivery system, Subgingival irrigant, Pit and fissure sealant, Anticancer

properties Precancerous lesions.and the treatment of dental pain.[27] Hence curcuma longa could be used as an irrigant as it a cost effective,readily available potent antioxidant.

Hence to overcome the disadvantages of sodium hypochlorite like toxicity,allergic reactions, antibiotic resistance, chemical interactions with other irrigants; metronidazole and herbal irrigants could be considered as an alternative as it has negligible antibiotic resistance, non - toxic to tissues even if extruded beyond apex and also it could be was used as a final rinse to chemical irrigants without any interactions.[28]

In addition the herbal irrigant, M.elengi and C.longa has comparable antimicrobial activity to chemical irrigants as proved in our study,non-irritating to periapical tissues,smear layer removal [29,]contact time [16,]non - antigenic,nontoxic and non-carcinogenic property with no adverse effects on sealing ability of filling materials and inexpensive; thus achieving most of the ideal requirements of root canal irrigant.

Conclusion

Within the limitations of our study the herbal irrigants; 25% M.elengi and 12.5% C.longa shows considerable antibacterial activity against commonly isolated endodontic pathogen Enterococcus faecalis. Hence these methanolic extracts could be considered as an alternative to chemical irrigant to combat the antibiotic resistance. However further in vivo an long term studies are needed to fully utilize its role in clinical situations.

Source of funding

None.

Conflict of interest

None.

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