Original Article

A comparative assessment of pomegranate extract, sodium hypochlorite, chlorhexidine, Myrrh (Commiphora molmol), tulsi extract against *Enterococcus faecalis, Fusobacterium nucleatum* and *Staphylococci epidermidis*

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Abstract

Background: The presence of pathogenic bacteria, toxins and byproducts in the root canal system show a decisive part in success of endodontic therapy. Thus, the complete removal of this bacterium is highly desirable. Several intracanal medicaments were tried to disinfect the root canal before obturation.

Aim: The present study was conducted to compare Pomegranate, sodium hypochlorite, Chlorhexidine, Myrrh (Commiphora molmol), tulsi extract against *Enterococcus faecalis*, *Fusobacterium nucleatum* and *Staphylococci epidermidis*.

Settings and Design: Cross-sectional observational prospective study.

Materials and Methodology: Aqueous extract of 20% pomegranate peel, 20% pomegranate peel, 0.2% CHX, 2.5% sodium hypochlorite, Tulsi extract and Myrrh (Commiphora molmol) was used as agent against *E. faecalis*, *F. nucleatum* and *Staphylococci Epidermidis*. Zone of inhibition and minimum inhibitory concentration (MIC) was calculated and compared using analysis of variance and Mann–Whitney test. The information was statistically evaluated with SPSS software version 20 with $P < 0.05$.

Results and conclusion: The mean zone of inhibition against *E. faecalis*, *F. nucleatum* and *S. Epidermidis* was highest in chlorhexidine and sodium hypochlorite groups compared to herbal groups. MIC was least with group III followed by group II against all bacterial species ($P < 0.05$). Sodium hypochlorite found comparatively better followed by chlorhexidine and other agents against *E. faecalis*, *F nucleatum* and *S. epidermidis*.

Keywords: *Enterococcus faecalis, Fusobacterium nucleatum*, herbal, root canal, *Staphylococci epidermidis*

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INTRODUCTION

An effective endodontic therapy requires complete elimination of microorganisms from the root canal space (disinfection) followed by complete sealing of root canals.[1] The incomplete elimination of bacteria from canal or periradicular region is the reason for endodontics treatment failure. The presence of pathogenic bacteria, toxins and byproducts in the root canal system play a decisive role in infecting the radicular dentinal tubules in teeth. Other causes of endodontic failure are incomplete obturation, overextensions of root canal filling materials, missed canals, irreparable mishaps, improper, leaky coronal seal and iatrogenic procedural errors.[2]

Microbes associated with endodontic teeth infections are *Actinobacteria*, *Bacteroids, Fusobacteria, Proteobacteria,* Spirochetes*, Fungi*, viruses, Streptococci, Staphylococci, *Enterococcus faecalis*, *F. nucleatum*, Lactobacilli, Prevotella spp. etc. *E. faecalis* is commonly occurring bacteria in infected root canals of both primary and permanent teeth. The peculiarity of this bacterium is that it can resist even 100–10,000 folds in starvation stage and may survive.[3] Thus, the complete removal of this bacterium is highly desirable. Several studies have depicted that intracanal instrumentation does not completely remove all the microorganisms, hence various intracanal medicament studied in removing remaining bacteria such as chlorhexidine gluconate (CHX), Myrrh (Commiphora molmol), herbal medicines (tulsi extract, neem extract), ethylenediaminetetraacetic acid, *Punica granatum* and sodium hypochlorite (NaOCl).[4]

NaOCl is desired irrigant by maximum dentists as antimicrobial agents. CHX proved to be effective against *E. faecalis* and *F. nucleatum*.[5] Myrrh is an aromatic oleo-gum resin is obtained an exudate from the trunk of Commiphora molmol acts as anti-inflammatory agent against staphylococci, *Pseudomonas aeruginosa*, *Escherichia coli, E. Feacalis*. There are no available researches that can indicate antibacterial effect of myrrh alongside organisms causing periradicular lesions following to root canal treatment.[6]

The present study was conducted to compare pomegranate, sodium hypochlorite, chlorhexidine, Myrrh (Commiphora molmol), tulsi extract against *E. faecalis*, *F. nucleatum* and *S. epidermidis*.

MATERIALS AND METHODOLOGY

This research was conducted in the department of endodontics, after attaining the authorization from the ethics committee. All included patients were informed regarding the study and their approval was taken in written.

Study design

This study was a prospective, cross–sectional type.

Sample size

This study comprised of equal samples (total 40 samples with 10 per group) of group-I: Aqueous extract of pomegranate peel, group-II: 0.2% CHX, group-III: 2.5% sodium hypochlorite, group-IV: Tulsi extract and group-V: Myrrh (Commiphora molmol) (herbal groups) as agent which were tested against *E. faecalis*, *F. nucleatum* and *S. Epidermidis*.

Fifty-grams of myrrh resin was recovered by purification in 500 mL of 95% ethanol. Tulsi extract was acquired by thinly powdering the dried-out leaves then mixing it with 100% ethanol followed by filtration. All agents were stored in the refrigerator at 4°C until use. The standard strain of *E. faecalis* (ATCC 29212), *F. nucleatum* (ATCC 25586) and *S. epidermidis* (ATCC 14990) was used as a test strain.

Morphologically comparable colonies were chosen up from an agar medium, using a wire loop. To a test tube comprising 4 ml of sterile peptone water, growth of these strains was transported. Sheep blood agar was used to evaluate the antimicrobial activity against *E. faecalis*, *Fusobacterium nucleatum* and *S. epidermidis* following standard protocol. Following regulating the inoculums on blood agar plates, the McFarland tube lawn culture was done. A total of 250 wells were prepared with 50 for each group having diameter of 6 mm and depth of 5 mm. Each well was filled using a pipette with 50 μl of the test irrigants solutions.

Minimum inhibitory concentration (MIC**)** was defined as the smallest concentration of each irrigants in which no evident turbidity was noted. The MIC was evaluated with serial dilutions of aqueous extract of pomegranate peel, 0.2% CHX, 2.5% sodium hypochlorite, Tulsi extract and Myrrh (Commiphora molmol). Incubation of the well plate was done at 37°C for 24 h with 180 rpm rotation.

The agar plates were incubated in a CO2 incubator at 37°C for 48 h. The zones of inhibition were measured using a millimeter scale and by passing the scale through the diameter of the zones outlined and to the summit of absolute inhibition of growth on either side.

The obtained data were analyzed using analysis of variance and Mann–Whitney test with statistical software IBM SPSS Statistics for Windows, version 21.0. Armonk, NY, USA: IBM Corp . $P < 0.05$ was measured substantial.

RESULTS

Table 1 shows that mean zone of inhibition against *E. faecalis* in group I was 18.21 mm, in group II was 20.14 mm, in group III was 21.37 mm, in group IV was 19.73 mm and in group V was 15.12 mm. The disparity established to be considerable ($P < 0.05$) [Figure 1].

Table 2 shows that mean zone of inhibition against *F. nucleatum* in group I was 16.82 mm, in group II was 20.41 mm, in group III was 21.37 mm, in group IV was 19.86 mm and in group V was 18.27 mm. The disparity established to be considerable ($P < 0.05$) [Figure 2].

Table 3 shows that mean zone of inhibition against *S. Epidermidis* in group I was 118.17 mm, in group II was 22.51 mm, in group III was 22.74 mm, in group IV was 20.16 mm and in group V was 19.17 mm. The disparity established to be considerable ($P < 0.05$) [Figure 3].

Table 4 shows that MIC was least with group III followed by group II against all bacterial species ($P < 0.05$).

DISCUSSION

Actinobacteria, *F. nucleatum*, *Bacteroids*, *E. faecalis Firmicutes*, *S. Epidermidis*, *Proteobacteria*, Spirochetes and *Candida albicans* are commonly occurring species in failed cases of root canal. *E. faecalis* is considered to be prevalent bacterium that leads to the persistence of periradicular lesions even after root canal therapy.[7] It has been found that in 24%–76% failure cases of root canals *E. faecalis* could survive as a single organism or as a major component of the flora.

Table 1: Zone of inhibition against *Enterococcus faecalis*

Groups	Mean (mm)	SD	*P*
Group I	18.21	0.13	0.001
Group II	20.14	1.18	
Group III	21.37	1.97	
Group IV	19.73	0.14	
Group V	15.12	0.71	

ANOVA test, $P<0.05$ (significant). SD: Standard deviation

Table 2: Zone of inhibition against *Fusobacterium nucleatum*

Groups	Mean (mm)	SD	*P*
Group I	16.82	0.15	0.001
Group II	20.41	0.10	
Group III	21.37	1.02	
Group IV	19.86	1.04	
Group V	18.27	0.19	

ANOVA test, $P<0.05$ (significant). SD: Standard deviation

Table 3: Zone of inhibition against *Staphylococci epidermidis*

Groups	Mean (mm)	SD	*P*
Group I	18.17	0.19	0.001
Group II	22.51	0.13	
Group III	22.74	1.09	
Group IV	20.16	1.01	
Group V	19.17	0.23	

ANOVA test, $P<0.05$ (significant). SD: Standard deviation

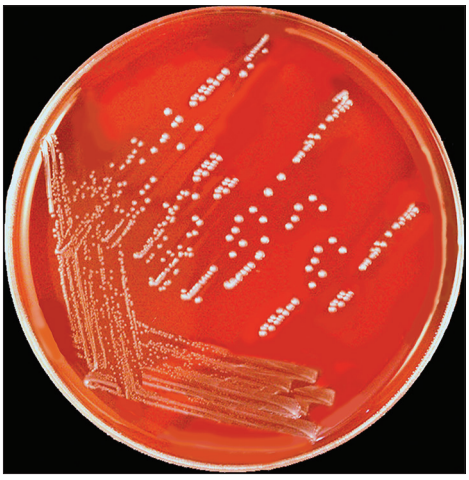

Figure 1: Image showing against *Enterococcus faecalis* in blood agar culture

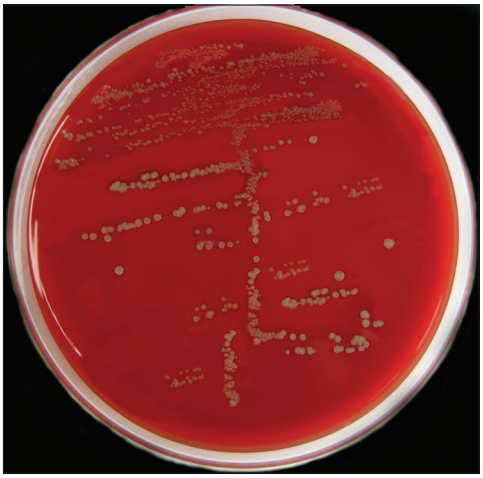

Figure 2: Image showing against *Fusobacterium nucleatum* in blood agar culturef

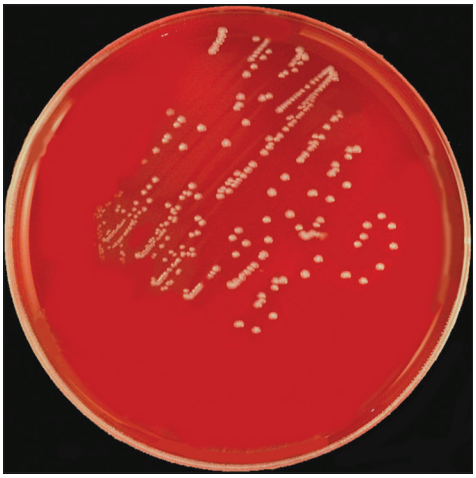

Figure 3: Image showing against *Staphylococci Epidermidis* in blood agar culture

Table 4: Minimum inhibitory concentration between each group (minimum inhibitory concentration) (μg/mL)

Groups	*Enterococcus faecalis*	*Fusobacterium nucleatum*	*Staphylococcus epidermidis*	*P*
Group I	350	320	385	0.024
Group II	50	55	51	
Group III	45	45	47	
Group IV	156	155	165	
Group V	200	201	205	

Mann-Whitney test, $P<0.05$ (significant)

It is more resistant to phagocytosis, and antimicrobial agents hence it can establish biofilm formation. *E. faecalis* is a Gram-positive bacterium with a dense peptidoglycan film that performs as an obstacle too many natural and synthetic antibiotics.[8] *F. nucleatum* is commonly found bacteria in dentinal tubules of untreated root canal with apical lesions. It is a Gram-negative bacterium with thin peptidoglycan layer. *S. epidermidis* is a facultative anaerobe that can grow through aerobic respiration or fermentation in anaerobic circumstances.[9] Hence, various studies have done with different intracanal medicaments to eliminate these infective bacteria.[4-9]

This research was conducted to associate 5 medicaments such as sodium hypochlorite, chlorhexidine, other herbal agents against common root canal species such as *E. faecalis*, *F. nucleatum* and *S. Epidermidis*. Herbal groups such as pomegranate extract, Tulsi extract and Myrrh (Commiphora molmol) are easily available and commonly used for their medicinal value.[2,3,9,10] Hence, these herbal agents were tried to evaluate their effect on root canal species in the present study.

Chum *et al*.[9] associated the antimicrobial effectiveness of octenidine dihydrochloride (OCT) with chlorhexidine and sodium hypochlorite against *Staphylococcus epidermidis* for root canal sterilization, and found a significant increase zone of inhibition seen with 3% NaOCl ($P < 0.05$) and there were no substantial variances among the CFU measurements of 3% NaOCl, 2% CHX, and 0.1% OCT indicating complete removal of *S. epidermidis* in all trials.

We found that mean zone of inhibition against Staphylococci and *E. faecalis* was maximum for sodium hypochlorite and chlorhexidine groups in contrast to herbal grouping.

Mallya *et al*. evaluated the antimicrobial effectiveness of granatum, 20% Punica 0.2%, 2.5% sodium hypochlorite and CHX alongside *E. faecalis*. They observed that mixture of sodium hypochlorite with *P. granatum* and CHX demonstrated the greatest mean zones of inhibition.[10]

In the present study, we found that MIC was least with group III followed by group II against all bacterial species ($P < 0.05$). Al-Madi *et al*. in their study compared antimicrobial efficiency of the extract of Commiphora molmol, against *F. nucleatum* and *E. faecalis* with sodium hypochlorite (NaOCl).[11] The mean bactericidal Concentration (MBC) outcome demonstrated that 2.5% sodium hypochlorite and 0.03 mg/μL myrrh extract drastically reduces bacterial growth after 30 and 60 min of dissimilar treatments of root canals, in contrast to the antibiotic group (positive group) and DMSO group (negative control).

Nourzadeh *et al*. estimated the antimicrobial outcome of *Myrtus communis L.* methanolic extracts, *Eucalyptus galbie,* sodium hypochlorite (NaOCl) and chlorhexidine (CHX) on *E. faecalis (E. faecalis),* and they concluded that *M. communis* L. and *E. galbie* were less effective than CHX and NaOCl.[12] Aravindraj *et al*. assessed the antimicrobial properties of different extracts of *P. granatum* in opposition to Streptococcus mutans, *Staphylococcus aureus*, *E. faecalis* Lactobacillus acidophilus, and Candida albicans, and they conclude that *P. granatum* demonstrate significant antifungal and antibacterial effects.[13] Shakouie *et al*. evaluated the antimicrobial action of Triphala (a plant-derived solution) with 0.5%, 1%, 2.5% and 5% concentrations of sodium hypochlorite (NaOCl), against *E. faecalis* (*E. faecalis*) and observed better antimicrobial activity with Triphala against *E. faecalis* in contrast to 0.5 and 1% NaOCl.[14] Chandrappa *et al*. evaluated the antimicrobial action of herbal remedies (neem extract, tulsi extract) and chlorhexidine against *E. faecalis* in Endodontics. They concluded that antimicrobial activity of herbal medicines is comparable to that of chlorhexidine.[15]

We found CHX and sodium hypochlorite are effective compared to herbal agents in removing common root canal bacterial compared to herbal medicaments. However, further studies are required to evaluate range of root canal pathogens such as candida albicans, *Actinobacteria* and spirochetes using other medicaments.

Antimicrobial efficacy of various intracanal medicaments helps to improve the successful outcome of endodontic treatment. Sodium hypochlorite found comparatively efficient against various microorganism. Drawback of

this study is that it was an *in vitro* but not *in vivo* study and sample size is small. Further long-term *in vivo* studies on other intracanal medicaments are needed.

CONCLUSION

We found sodium hypochlorite comparatively better followed by chlorhexidine and other herbal agents against *E. faecalis, F nucleatum* and *S. epidermidis*.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Tabassum S, Khan FR. Failure of endodontic treatment: The usual suspects. Eur J Dent 2016;10:144-7.
2. Rahmani AH, Alsahli MA, Almatroodi SA. Active constituents of pomegranates (*Punica granatum*) as potential candidates in the management of health through modulation of biological activities. Pharmacogn J 2017;9:689-95.
3. Bhardwaj R, Balasubramanyam BV, Mishra D, Papasani A. Preservative effect of pomegranate peel extract on the keeping quality of cream based fat spread. Int J Pure Appl Biosci 2017;5:3238.
4. Ghivari SB, Bhattacharya H, Bhat KG, Pujar MA. Antimicrobial activity of root canal irrigants against biofilm forming pathogens – An *in vitro* study. J Conserv Dent 2017;20:147-51.
5. Anuradha B, Rajamoni I, Lalitha MK, Sriram T. A new irrigant against *E. faecalis* in the root canal disinfection. Biosci Biotech Res Asia 2014;11:121-7.
6. Ganesh A, Veronica AK, Ashok R, Varadan P, Deivanayagam K. Quantification of *Fusobacterium nucleatum* at depths of root dentinal tubules in the tooth using real-time polymerase chain reaction: An *in vitro* study. Cureus 2019;11:e4711.
7. Frough-Reyhani M, Ghasemi N, Soroush-Barhaghi M, Amini M, Gholizadeh Y. Antimicrobial efficacy of different concentration of sodium hypochlorite on the biofilm of *Enterococcus faecalis* at different stages of development. J Clin Exp Dent 2016;8:e480-4.
8. Samiei M, Ghasemi N, Divband B, Balaei E, Hosien Soroush Barhaghi M, Divband A. Antibacterial efficacy of polymer containing nanoparticles in comparison with sodium hypochlorite in infected root canals. Minerva Stomatol 2015;64:275-81.
9. Chum JD, Lim DJZ, Sheriff SO, Pulikkotil SJ, Suresh A, Davamani F. *In vitro* evaluation of octenidine as an antimicrobial agent against *Staphylococcus epidermidis* in disinfecting the root canal system. Restor Dent Endod 2019;44:e8.
10. Mallya L, Shenoy R, Mala K, Shenoy S. Evaluation of the antimicrobial efficacy of 20% *Punica granatum*, 0.2% chlorhexidine gluconate, and 2.5% sodium hypochlorite used alone or in combinations against *Enterococcus faecalis*: An *in-vitro* study. J Conserv Dent 2019;22:367-70.
11. Al-Madi EM, Almohaimede AA, Al-Obaida MI, Awaad AS. Comparison of the antibacterial efficacy of Commiphora molmol and sodium hypochlorite as root canal irrigants against *Enterococcus faecalis* and *Fusobacterium nucleatum*. Evid Based Complement Alternat Med 2019;2019:6916795.
12. Nourzadeh M, Amini A, Fakoor F, Raoof M, Sharififar F. Comparative antimicrobial efficacy of Eucalyptus *g*albie and Myrtus communis L. extracts, chlorhexidine and sodium hypochlorite against *Enterococcus faecalis*. Iran Endod J 2017;12:205-10.
13. Aravindraj S, Preethi M, Sivapathasundharam B. Antimicrobial effects of *Punica granatum* extracts on *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus acidophilus*, *Enterococcus faecalis* and *Candida albicans*. Int J Curr Microbiol Appl Sci 2017;6:2762-74.
14. Shakouie S, Eskandarinezhad M, Gasemi N, Milani AS, Samiei M, Golizadeh S. An *in vitro* comparison of the antibacterial efficacy of triphala with different concentrations of sodium hypochlorite. Iran Endod J 2014;9:287-9.
15. Chandrappa PM, Dupper A, Tripathi P, Arroju R, Sharma P, Sulochana K. Antimicrobial activity of herbal medicines (tulsi extract, neem extract) and chlorhexidine against *Enterococcus faecalis* in endodontics: An *in vitro* study. J Int Soc Prev Community Dent 2015;5:S89-92.