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Comparison of antibacterial efficacy of herbal and non- herbal irrigants against e. Faecalis and c. Albicans: An in vitro study

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Abstract

Introduction: Comparative assessment of antimicrobial efficacy of herbal and non-herbal irrigants - An in vitro study

Aim: The aim of this study was to evaluate the antimicrobial efficacy of guava leaf extract, papaya leaf extract, 5.25% NaOCl, 2% CHX and distilled water against E. faecalis and C. albicans at different time intervals.

Material and methods: This study was conducted using 110 extracted permanent mandibular premolar teeth. They were decoronated, prepared upto 25 k-file and inoculated using broth of E. faecalis and C. albicans. These inoculated root sections were divided randomly into five irrigant groups. The seals were removed before instrumentation and further enlargement up to size 40 k-file in fixed period of 15 min was done by step back technique with simultaneous irrigation. The total volume of each irrigant was fixed (5 ml) with a fixed contact time of 20 min. First (S1) and second microbial sample recorded (S2) were taken immediately by inserting sterile paper points and colony counts were recorded**.** The colony forming units (CFU) obtained for each group were

counted thrice and the mean of all the three readings were taken as the final count for analysis.

Results: The colony forming units were significantly less in number in group C showing its higher antimicrobial efficacy at 20 mins and 72 hours of time intervals as compared to other irrigant groups.

Conclusion: Sodium hypochlorite has showed better antimicrobial efficacy followed by chlorhexidine, guava leaf extract and papaya leaf extract as an irrigant.

Keywords: Sodium Hypochlorite, Chlorhexidine, Guava leaf extract, Papaya leaf extract, E. faecalis, C. albicans

Introduction

The purpose of root canal treatment is to eradicate the bacteria from the root canal system[1]. Most prevalent micro-organism in infected root canals and in re-treatment cases of apical periodontitis is E. faecalis with a reported prevalence from 24% to 77%.

In addition, the incidence of C. albicans is mostly found in association with E. faecalis and also increases with secondary infections. Owing to multiple virulent factors, both of these micro-organisms have notorious reputation of being most persistent and resistant, especially in secondary infections[2].

Chemicomechanical preparation has a major role in achieving successful endodontic therapy[2].Mechanical instrumentation leaves around 40-50% of the root canal untouched[1].Therefore, to remove debris and necrotic pulp tissue and to eliminate micro-organisms that cannot be reached by mechanical instrumentation various substances have been used during and immediately after root canal preparation. Sodium hypochlorite (NaOCl) is an excellent organic solvent and an effective antimicrobial agent, it is known to be highly irritant to periapical tissues, mainly at high concentrations.

For this reason, the search for another irrigant with a lower potential to induce adverse effects is desirable. Chlorhexidine gluconate has been recommended as a root canal irrigant and many studies have demonstrated its substantivity, broad spectrum antimicrobial action and low grade of toxicity. However, the problem is inability of chlorhexidine to dissolve pulp tissue[3].

The trend of recent medicine attends to use biologic medication extracted from natural plants because the cytotoxic reactions of the most of the commercial intracanal medicaments used and their inability to eliminate bacteria from dentinal tubules [4].

Antibacterial and antioxidant actions of guava leaf extract on the other hand papaya leaf extract exhibiting greater activity toward bacteria and fungi found out that they both have antimicrobial actions against oral pathogens[4].

Thus, the present in vitro study was done to investigate the antimicrobial activity of herbal and non-herbal irrigants against culture of E. faecalis and C. albicans.

Material and methods

The present study was done on 110 intact extracted permanent single rooted human mandibular premolar teeth. All the teeth were decoronated with a diamond disc using a low speed straight handpiece to obtain root segments of 11 mm length, in which the patency was determined by inserting no. 15 k-file till its tip was just visible at the apex.

Working length in all root sections were taken as 10 mm and root canals were prepared up to size 25 with k-files with simultaneous irrigation of normal saline. The root canals were filled with 17 % EDTA for 3 mins to remove smear layer followed by 2 ml irrigation with normal saline. Root section surfaces were then coated

with two coats of acrylic varnish.

Before microbial inoculation, the root sections were individually placed in glass bottles, with numbering on each section and autoclaved twice with 24 hours interval in between, to sterilize the root canals, which was confirmed by culture methods. After confirming sterility of root canals, microbial inoculation in each root section was done.

Inoculation Procedure

One milliliter each of 24 hours old broth suspension of both E. faecalis and C**.** albicans were collected in a test tube. This broth was used first for taking baseline count and then for obtaining serial dilutions.

For baseline count - A 5 µL of each broth suspension was separately inoculated on respective culture media plates. The CFU/mL thus obtained was recorded thrice, and their mean was taken as the **baseline count** for the respective microorganism.

After that serial dilutions of 24 hours old broth suspension in normal saline to obtain 1:10-4 dilutions were done (Fig.1 &2). Root apices were sealed with a temporary cement, and 10 µL of this culture suspension was inoculated in each root canal through micropipette sterile tips, by placing them 1 mm short of working length, and simultaneously pulling upward to deliver the culture up to the coronal end of all root sections.

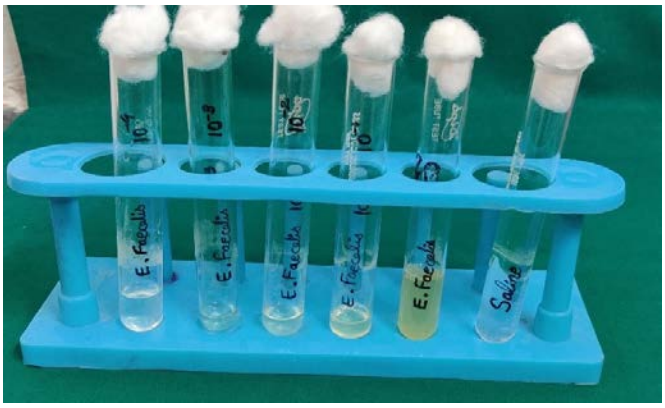


Fig. 1: Serial Dilutions for E. faecalis

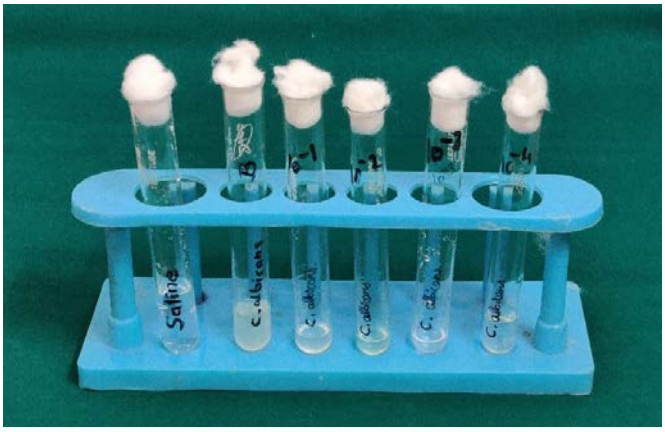


Fig. 2:- Serial Dilutions for C. albicans

These inoculated root sections were divided by simple random sampling into five irrigant groups (four experimental and one control) which are as follows:

GROUP A: (n=22) inoculated specimen irrigated using papaya leaf extract

GROUP B: (n=22) inoculated specimen irrigated using guava leaf extract

GROUP C: (n=22) inoculated specimen irrigated using sodium hypochlorite (5.25%)

GROUP D: (n=22) inoculated specimen irrigated using chlorhexidine (2%)

GROUP E: (n=22) inoculated control group, specimen irrigated using distilled water

There were 4 experimental irrigant groups (2 herbal and 2 non-herbal) and 1 control, all of which was evaluated at three time intervals i.e. at baseline (S0), immediately after chemico-mechanical preparation (S1) and after 72 hours of incubation (S2).

The cervical and apical seals were removed before instrumentation and further enlargement upto size 40 k-file in fixed period of 15 min by step back technique with simultaneous irrigation of total 3 ml of respective irrigant done.

On completion of the mechanical enlargement, 2 ml of irrigant solution was used to rinse off any debris in the canals, for another 5 min. Thus, the total volume of

each irrigant was fixed (5 ml) with a fixed contact time of 20 min. The canal was then flushed with saline and sample was taken immediately after 20 mins by inserting sterile paper points, which was transported in normal saline and incubated at 370c for 30 minutes. From this, 0.1 ml of sample was inoculated in BHI agar using pour plate method. It was incubated at 37°c for 24 hours after which colonies were counted using colony count plate (fig. 5)**.** The readings obtained were taken as **First microbial sample** (**S1)** (Fig. 3). These root specimens were sealed again and incubated for 72 hours at 37 ^{O}C. After 72 hours, temporary filling material was removed at apical end and using sterile paper point technique **Second microbial sample (S2)** was recorded in the same manner as first (Fig. 4)**.**

Fig. 3: CFU of E. faecalis at 20 mins in different irrigant groups.

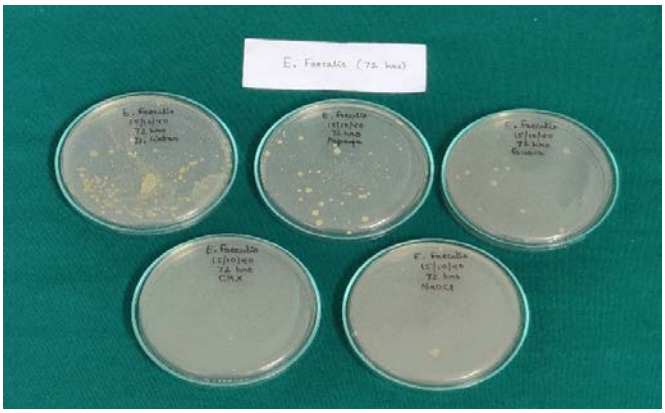
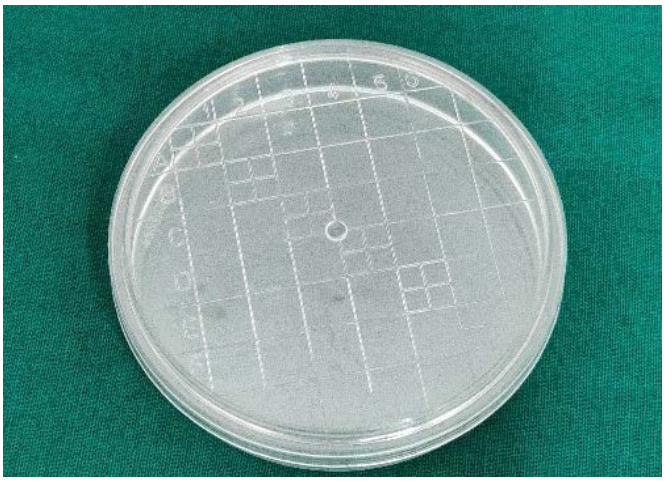


Fig 4: CFU of E. faecalis at 72 hours in different irrigant groups.

The colony forming units (CFU/mL) obtained for each group were counted thrice and then noted. The mean of all the three readings were taken as the final count for analysis. This data was subjected to statistical analysis. CFUs at baseline, S1 and S2 were tabulated and compared for each irrigant, against both the micro-organisms.



Results and Discussion

An important prerequisite for achieving long-term success of root canal treatment is complete debridement and effective disinfection of the root canal system5. Complete disinfection however is not always achievable through instrumentation alone due to the anatomical complexities such as fins, lateral or furcal canals, apical deltas, webs, and isthmus of the root canal6. Thus, effective disinfection in endodontics is only achieved by augmenting mechanical preparation with antimicrobial irrigants4.

Sodium hypochlorite is considered to be the gold standard for irrigation because of its antimicrobial activity and tissue dissolving capacity7. High pH of NaOCl interferes with the cytoplasmic membrane integrity and causes biosynthetic alterations in cellular metabolism, attributing to its antimicrobial nature8.

Chlorhexidine gluconate (2%) is a good disinfecting agent with a property of substantivity contributing to its

prolonged time of action and demonstrates broad spectrum antimicrobial activity7. It is a cationic bisguanide that seems to act by adsorption onto the cell wall of the microorganism and causing leakage of intracellular components1.

Due to the constant increase in the antibiotic resistant strains and the side effects caused by synthetic drugs, the trend has now shifted to the age-old herbal products9. The major advantages of herbal irrigants are safety, easy availability, increased shelf-life, cost effectiveness, and lack of microbial resistance so far10. Therefore, we used guava and papaya leaf extract as herbal irrigants in our study.

Guava leaf extract can inhibit the growth of bacteria because it contains active compounds, such as flavonoids, tannins and saponins11. The antimicrobial efficacy of **C. papaya** is due to presence of flavonoids (kaempferol and myricetin), alkaloids (carpaine, pseudocarpaine, dehydrocarpaine), phenolic compounds (ferulic acid, caffeic acid, chlorogenic acid), and cyanogenetic compounds (benzylglucosinolate)12.

Group C (NaOCl) showed **minimum** count of colony forming units/ml for E. faecalis and C. albicans suggesting higher antimicrobial efficacy at 20 mins and 72 hours of time intervals in comparison with all other irrigant groups whereas the **maximum** count was shown by control group, **Group E** (Distilled water) suggesting lower antimicrobial efficacy.

Conclusion

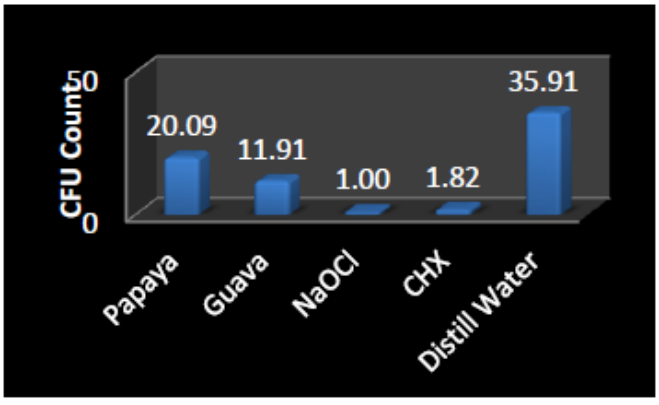
Within the limitations, based on the employed methodology and according to the results obtained in the present in-vitro study, following conclusions can be drawn (Graph 1, 2, 3, 4) (Table 1 & 2)

With respect to time intervals, all irrigants showed significant decrease in colony forming units against E. faecalis and C. albicans irrespective of control or experimental groups.

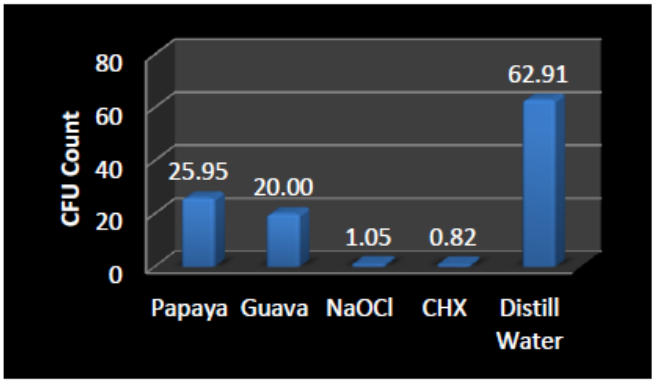
Among all groups, group C (NaOCl) has shown minimum colony forming units/ml after 20 mins and 72 hours for both E. faecalis and C. albicans, which was increased in group D (CHX), followed by group B (Guava leaf extract), group A (Papaya leaf extract), group E (Distilled water) respectively. Group C (NaOCl) signified the most effective antimicrobial agent for eradication of E. faecalis and C. albicans.

Group D (CHX) has shown decrease in colony forming units/ml after 72 hours of time interval proving its property of substantivity against both E. faecalis and C. albicans.

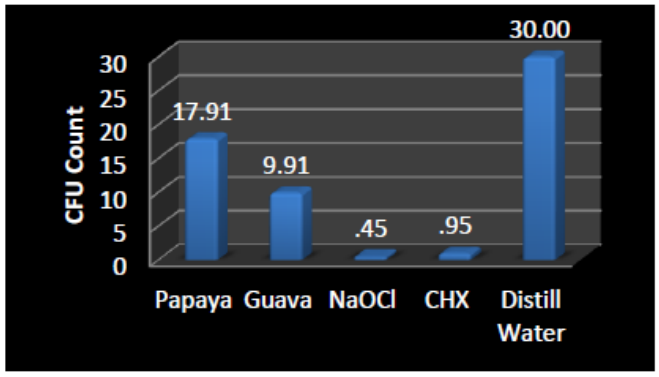
Group B (guava leaf extract) and group A (papaya leaf extract) showed better antimicrobial efficacy against E. faecalis and C. albicans compared to sodium hypochlorite and chlorhexidine. Further studies and extensive research are needed to validate these findings in regular clinical use.



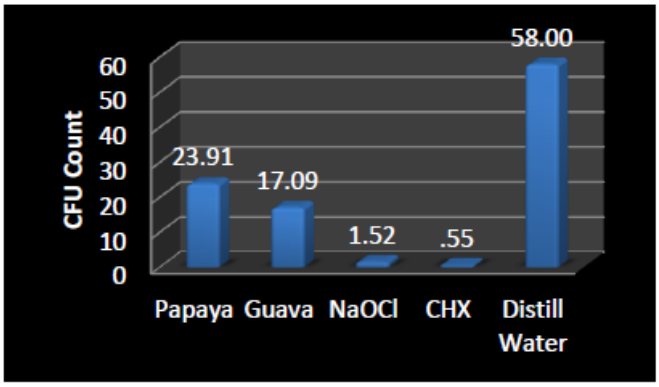
Graph 1: Comparison of CFU of E. faecalis in different irrigants at 20 minutes.



Graph 2: Comparison of CFU of E. faecalis in different irrigants at 72 hours.



Graph 3: Comparison of CFU of C. Albicans in different irrigants at 20 minutes.



Graph 4: Comparison of CFU of C. Albicans in different irrigants at 72 hours.

		Sum of Squares	df	Mean Square	F	Sig.	Result
Papaya	Between Groups	378.205	1	378.205	81.554	.000	Significant
	Within Groups	194.773	42	4.637			
	Total	572.977	43				
Guava	Between Groups	720.091	1	720.091	154.448	.000	Significant
	Within Groups	195.818	42	4.662			
	Total	915.909	43				
NaoCl	Between Groups	.023	1	.023	0.019	.890	Non Significant
	Within Groups	48.955	42	1.166			
	Total	48.977	43				
CHX	Between Groups	11.000	1	11.000	9.517	.004	Significant
	Within Groups	48.545	42	1.156			
	Total	59.545	43				
D. Water	Between Groups	8019.000	1	8019.000	1794.951	.000	Significant
	Within Groups	187.636	42	4.468			
	Total	8206.636	43				

Table 1: Comparison of CFU of E. faecalis within the groups at 20 minutes and 72 hours.

		Sum of Squares	df	Mean Square	F	Sig.	Result
Papaya	Between Groups	396.000	1	396.000	87.705	.000	Significant
	Within Groups	189.636	42	4.515			
	Total	585.636	43				
Guava	Between Groups	567.364	1	567.364	131.192	.000	Significant
	Within Groups	181.636	42	4.325			
	Total	749.000	43				
NaoCl	Between Groups	12.284	1	12.284	14.517	.000	Significant
	Within Groups	34.693	41	0.846			
	Total	46.977	42				
CHX	Between Groups	1.841	1	1.841	4.200	.047	Significant
	Within Groups	18.409	42	0.438			
	Total	20.250	43				
D. Water	Between Groups	8624.000	1	8624.000	1867.052	.000	Significant
	Within Groups	194.000	42	4.619			
	Total	8818.000	43				

Table 2: Comparison of CFU of C. albicans within the groups at 20 minutes and 72 hours.

References

1. Jayahari NK, Niranjan NT, Kanaparthy A. The efficacy of passion fruit juice as an endodontic irrigant compared with sodium hypochlorite solution: an in vitro study. Journal of Investigative and clinical dentistry. 2014 May; 5(2):154-60.

2. Choudhary E, Indushekar KR, Saraf BG, Sheoran N, Sardana D, Shekhar A. Exploring the role of Morinda Citrifolia and Triphala juice in root canal irrigation: An ex vivo study. Journal of conservative dentistry: JCD. 2018 Jul; 21(4); 443.
3. Ferraz CC, de Almeida Gomes BP, Zaia AA, Teixeira FB, de Souza Filho FJ. In vitro assessment of the antimicrobial action and the mechanical ability of chlorhexidine gel as an endodontic irrigant. Journal of Endodontics. 2001 Jul 1; 27(7):452-5.
4. Noushad MC, Balan B, Basheer S, Usman SB, Askar MM. Antimicrobial efficacy of different natural extracts against persistent root canal pathogens: An In vitro study. Contemporary clinical dentistry. 2018 Apr; 9(2):177.
5. Sinha DJ, Vasudeva A, Gowhar O, Garg P, Sinha A, Prakash P. Comparison of antimicrobial efficacy of Propolis, Azadirachta indica (Neem), Melaleuca alternifolia (Tea tree oil), Curcuma longa (Turmeric) and 5% Sodium hypochlorite on Candida albicans biofilm formed on tooth substrate: An In-vitro study. J Pharm Biomed Sci 2015; 05(06):469-474.
6. Hess W. Anatomy of root canals in the teeth of permanent dentition. New York: William Wood & co.1925: 82-85.
7. Jose J, Krishnamma S, Peedikayil F, Aman S, Tomy N, Mariodan JP. Comparative evaluation of antimicrobial activity of QMiX, 2.5% Sodium Hypochlorite, 2% Chlorhexidine, Guava Leaf extract and Aloevera extract against Enterococcus faecalis and Candida albicans–An in-vitro Study. Journal of clinical and diagnostic research: JCDR. 2016 May;10(5):ZC20.
8. Baumgartner JC, Cuenin PR. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. J Endod. 1992;18(12):605-12.
9. Prabhakar J, Senthilkumar M, Priya MS, Mahalakshmi K, Sehgal PK, Sukumaran VG. Evaluation of antimicrobial efficacy of herbal alternatives (Triphala and green tea polyphenols), MTAD, and 5% sodium hypochlorite against Enterococcus faecalis biofilm formed on tooth substrate: An in vitro study.J Endod 2010;36:83-6.
10. Pratishta J, Ranjan M. Role of herbs in root canal irrigation-A review. IOSR J Pharm Biol Sci 2014; 9:6-10.
11. Biswas B, Rogers k, McLaughlin F, Daniels D, Yadav A. Antimicrobial activities of leaf extracts of guava (Psidium guajava L.) on two gram-negative and gram-positive bacteria. International journal of microbiology. 2013 Oct 20;2013.
12. Anjum V, Ansari SH, Naquvi KJ, Arora P, Ahamed A. Development of quality standards of Carica papaya Linn. leaves.Pharm Lett 2013;5:370-6.