Original Article

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Dentistry Section

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

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ABSTRACT

Introduction: Debridement and disinfection of the root canal system is a critical step in endodontic treatment. Most of the irrigants presently used in the endodontic treatment can have an impact on the microbes surviving in the biofilm but none of them are able to do all of the required tasks. Researches are going on its full swing in order to produce an endodontic irrigant having ideal properties.

Aim: To compare the antimicrobial efficacy of different irrigants like QMiX, guava leaf extract, aloevera extract, 2.5% sodium hypochlorite and 2% chlorhexidine gluconate against *Enterococcus faecalis* and *Candida albicans*.

Materials and Methods: The antimicrobial activity was determined using agar diffusion test. The solutions were divided into five groups: Group I- QMiX, Group II- Guava leaf extract and Group III-Aloevera extract, Group IV–2.5% Sodium hypochlorite and Group V-2% Chlorhexidine. The zones of inhibition of growth were recorded.

Results: Statistical analysis was performed using one way ANOVA with post-hoc Tukey's HSD. Values obtained were statistically analyzed ($p<0.05$). QMiX showed maximum inhibitory effect against *Enterococcus faecalis* and *Candida albicans* followed by, 2% chlorhexidine, 2.5% sodium hypochlorite, guava leaf extract and aloevera extract. Results obtained were statistically significant.

Conclusion: Guava leaf extract showed significant inhibitory effects against *Enterococcus faecalis* and *Candida albicans*. QMiX demonstrated the best results among the tested solutions and can be considered as a potential alternative to existing root canal irrigants.

Keywords: Antimicrobial efficacy, Microbial infection, Root canal irrigation

INTRODUCTION

The contaminated root canal is a great source of aerobic, anaerobic, gram-positive and gram-negative bacteria and therefore endodontic infections are considered to be polymicrobial in nature. Microbial infection of root canal is the major reason behind the inflammatory reaction of periapical tissues which leads to apical periodontitis [1]. Chemomechanical preparation is considered as the prime procedure in endodontic treatment [2]. Anatomical complexities and microbiological factors often pose serious threats to adequate root canal disinfection [3]. It is a prerequisite to use endodontic irrigants in addition to mechanical preparation in order to ensure the success of root canal treatment.

Sodium hypochlorite (NaOCl) is the gold standard in root canal irrigation when used in concentrations ranging from 0.5-6%. The irrigant is well known for its antibacterial property and tissue dissolving capacity [4]. The actions and the toxicity of NaOCl are dose-dependent [5]. Another frequently used irrigant possess the property of substantivity is chlorhexidine gluconate [6]. Though 2% chlorhexidine demonstrated significant activity against *E. faecalis*, the reason behind its less acceptance might be due to the inability in dissolving necrotic tissue remnants coupled by poor anti microbicity when tested in vivo [7].

Various natural herbal extracts have also shown antibacterial activity suggesting their ability to be used as root canal irrigant. Antibacterial and antioxidant actions of guava leaf extract and aloevera extract have been studied and found out that they have antimicrobial actions against oral pathogens [8-9].

QMix™ 2 in 1 solution contains a mixture of a bisbiguanide antimicrobial agent (2% chlorhexidine), a polyaminocarboxylic acid calcium-chelating agent (17% EDTA), and a surfactant (N-cetyl-N,N,N-trimethylammonium bromide-0.001 to about 3.0 weight percent) and water. The solution has demonstrated substantial smear layer removal and antimicrobial properties [10].

AIM

The aim of the present study was to explore and compare the antimicrobial activity of newer irrigants like guava leaf extract, aloevera extract and QMiX with gold standards like sodium hypochlorite and 2% chlorhexidine against *E. faecalis* and *C. albicans*.

MATERIALS AND METHODS

This invitro study was conducted at the Tropical Institute of Ecological Sciences, Velloor, Kottayam after obtaining approval from Institutional Scientific Committee, Government Dental College; Kottayam. In the present study, the QMiX (Group I), guava leaf extract (Group II) and aloe vera extract (Group III) were selected as the experimental groups and 2.5% sodium hypochlorite (Group IV) and 2% chlorhexidine (Group V) as positive control groups. ATCC 29212 and ATCC 10231 were strains used in this study to check

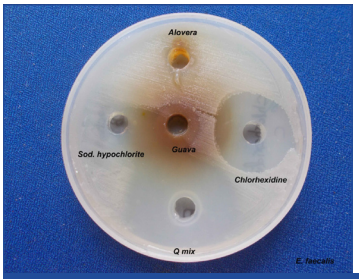
antimicrobial activity of *E. faecalis* and *C. albicans* respectively.

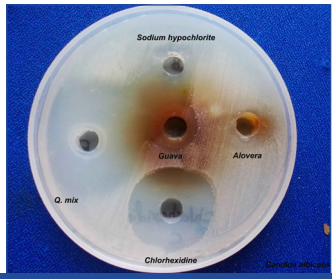
QMiX (Group I), manufactured by Dentsply, Tulsa Dental Specialties and control groups including 2.5% sodium hypochlorite (Group IV) and 2% chlorhexidine gluconate in liquid form (Group V) are commercially available.

Preparation of Guava Leaves Extract (Group II): Leaves of guava from the botanical garden of Tropical Institute of Ecological Sciences were dried in fresh open air protecting from direct exposure to sunlight. A 50gm of powdered leaves were taken into beaker containing 500 ml of sterile distilled water. Hot water extract was prepared by heating this in water bath till menstruum reduced to about 125ml which is about ¼th of the original volume. After the complete evaporation of the water content from extract, the resulting liquid was filtered using filter paper.

Preparation of Aloevera Extract (Group III): Aloevera leaves from the botanical garden of Tropical Institute of Ecological Sciences were cut into small pieces with a knife and grinded using an electric grinder into paste form. Aqueous extracts were prepared by dissolving the paste material in sterile distilled water in a ratio of 1:5 i.e., 20 gm of plant paste material in 100 ml of water in a sterile 250 ml flask. This was kept in refrigerator at 4˚C for 24 hours and was then filtered using filter paper. The extract was then again kept in the refrigerator at 4˚C before being reconstituted for further use.

Agar-diffusion test: Hundred microliters of test organisms *E.faecalis* and *C. albicans* suspensions were obtained from prepared cultures and inoculated in culture plates with previously set layers of Mueller Hinton Agar and Sabouraud Dextrose Agar respectively for each organism. Sterile spreader was used for inoculation of these organisms across respective media. Five uniform wells of size 6mm were prepared on the *E. faecalis* and *C. albicans* culture plates. Only 200µl of both experimental solution and positive control solution were added to the respective wells on each plate. These plates were incubated for 24 hours at 37°C in an incubator. After incubation period, plates were checked for zones of inhibition of bacterial growth and diameters of the zones achieved by each group against *E.faecalis* and *C. albicans* were recorded in centimeter (cm) [Table/Fig-1] and [Table/Fig-2].





[Table/Fig-1]: Figure of agar diffusion test against *E. faecalis*.
[Table/Fig-2]: Figure of agar diffusion test against *C. albicans*.

Agar diffusion tests were conducted for six times to achieve a statistically significant result.

RESULTS

Statistical Package for Social Sciences (SPSS) version 16 was used for the tabulation and statistical analysis of the results obtained. Analysis of variance (One Way ANOVA) was performed as parametric test to compare different groups [Table/Fig-3]. To elucidate multiple comparisons between groups, Tukey's HSD Tests were also performed along with ANOVA and post hoc tests [Table/Fig-4 and Table/Fig-5].

[Table/Fig-3] shows that there exist a significant difference between the diameters of zones of inhibition of bacterial growth obtained for QMiX, guava leaf extract, aloe vera extract and 2.5% sodium hypochlorite and 2% chlorhexidine against *E.faecalis* and *C.albicans* (p<0.05).

Organism	Irrigant	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		F value	p value
						Lower Bound	Upper Bound		
C. albicans	QMiX	6	3.6333	0.10328	0.04216	3.5249	3.7417	934.101	< 0.01**
	Guava Leaf extract	6	2.0000	0.08944	0.03651	1.9061	2.0939		
	Aloe vera extract	6	0.9167	0.07528	0.03073	0.8377	0.9957		
	2.5% NaOCl	6	2.0333	0.10328	0.04216	1.9249	2.1417		
	2% Chlorhe-xidine	6	3.2500	3.2500	0.02236	3.1925	3.3075		
	Total	30	2.3667	2.3667	0.18127	1.9959	2.7374		
E. faecalis	QMiX	6	3.5333	0.08165	.03333	3.4476	3.6190	635.558	< 0.01**
	Guava Leaf extract	6	1.9167	0.11690	.04773	1.7940	2.0394		
	Aloe vera extract	6	1.0333	0.08165	.03333	0.9476	1.1190		
	2.5% NaOCl	6	2.2167	0.07528	.03073	2.1377	2.2957		
	2% Chlorhe-xidine	6	3.1167	0.11690	0.04773	2.9940	3.2394		
	Total	30	2.3633	0.90572	0.16536	2.0251	2.7015		

[Table/Fig-3]: Analysis of variance (One Way ANOVA) of mean diameter of zone of inhibition of bacterial growth comparing antimicrobial activity tested irrigants.
* The mean difference is significant at the 0.05 level.
** Highly significant

Dependent Variable	(I) group	(J) group	Mean Difference (I- J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
E. faecalis	QMiX	NaOCl	1.316*	0.055	0.000	1.154	1.480
		CHX	0.417*	0.055	0.000	0.254	0.580
		Guava	1.617*	0.055	0.000	1.453	1.780
		Aloe vera	2.500*	0.055	0.000	2.337	2.663
	NaOCl	QMiX	1.316*	0.055	0.000	-1.480	-1.153
		CHX	-0.900*	0.055	0.000	-1.062	-0.737
		Guava	0.300*	0.055	0.000	0.137	0.463
		Aloe vera	1.183*	0.055	0.000	1.021	1.347
	Chlorh-exidine	QMiX	0.417*	0.055	0.000	-0.580	-0.253
		NaOCl	0.900*	0.055	0.000	0.737	1.063
		Guava	1.200*	0.055	0.000	1.037	1.363
		Aloe vera	2.083*	0.055	0.000	1.920	2.247
	Guava	QMiX	-1.617*	0.055	0.000	-1.780	-1.453
		NaOCl	-0.300*	0.055	0.000	-0.463	-0.137
		CHX	-1.200*	0.055	0.000	-1.363	-1.037
		Aloe vera	0.883*	0.055	0.000	0.720	1.047
	Aloe vera	QMiX	-2.500*	0.055	0.000	-2.663	-2.337
		NaOCl	-1.183*	0.055	0.000	-1.347	-1.020
		CHX	-2.083*	0.055	0.000	-2.247	-1.920
		Aloe vera	-0.883*	0.055	0.000	-1.047	-0.720

[Table/Fig-4]: Post Hoc Tukey's HSD test for inter comparison of antimicrobial activity different irrigants against *E. faecalis*.
* The mean difference is significant at the 0.05 level.

[Table/Fig-4 and 5] shows post hoc tests- Tukey's HSD for the inter comparison of the antimicrobial efficacy of different groups

Dependent Variable	(I) group	(J) group	Mean Difference (I- J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Candida albicans	QMiX	NaOCl	1.600*	0.050	0.000	1.4522	1.748
		CHX	0.383*	0.050	0.000	0.2355	0.532
		Guava	1.633*	0.050	0.000	1.4855	1.782
		Aloe vera	2.717*	0.050	0.000	2.5688	2.865
	NaOCl	QMiX	-1.600*	0.050	0.000	1.7478	-1.452
		CHX	-1.217*	0.050	0.000	-1.3645	-1.068
		Guava	0.033	0.050	0.963	-0.1145	0.182
		Aloe vera	1.117*	0.050	0.000	0.9688	1.265
	Chlorh-exidine	QMiX	-0.383*	0.050	0.000	-0.531	-0.235
		NaOCl	1.217*	0.050	0.000	1.068	1.364
		Guava	1.250*	0.050	0.000	1.102	1.398
		Aloe vera	2.333*	0.050	0.000	2.185	2.481
	Guava	QMiX	-1.633*	0.050	0.000	-1.781	-1.485
		NaOCl	-0.033	0.050	0.963	-0.181	0.115
		CHX	-1.250*	0.050	0.000	-1.398	-1.102
		Aloe vera	1.083*	0.050	0.000	0.935	1.231
	Aloe vera	QMiX	-2.717*	0.050	0.000	-2.865	-2.568
		NaOCl	-1.117*	0.050	0.000	-1.265	-0.968
		CHX	-2.333*	0.050	0.000	-2.481	-2.185
		Aloe vera	-1.083*	0.050	0.000	-1.231	-0.935

[Table/Fig-5]: Post Hoc Tukey's HSD test for inter comparison of antimicrobial activity different irrigants against *C. albicans*.
* The mean difference is significant at the 0.05 level

against *E. faecalis* and *C. albicans* respectively. Group I (QMiX) had statistically significant superior antibacterial activity against both *C. albicans* and *E. faecalis* than all other groups of tested irrigant solution. Group V (2% Chlorhexidine) showed greater antimicrobial activity when compared to guava leaf extract, aloevera extract and 2.5% sodium hypochlorite against both the pathogens. This was statistically significant. Group IV (2.5% Sodium hypochlorite) demonstrated a statistically significant superior antibacterial activity than aloevera extract and a statistically insignificant superior activity than guava leaf extract against the tested organisms. From the table it is clear that a significant greater antimicrobial activity shown by Group II (Guava leaf extract) when compared to Group III (Aloevera).

DISCUSSION

The major objective of root canal treatment is to eliminate the microorganisms from the root canal and to prevent their recontamination in the post treatment period. In the anatomically challenging areas like fins, lateral or furcal canals, apical deltas, webs and isthmus the biofilm may remain undisturbed after mechanical debridement. Therefore, in order to ensure complete cleanliness of the canal system it is necessary to use irrigant solutions to complement the action of the mechanical instruments [11].

Root canal infections are multibacterial with anaerobic bacteria being present in more than 70% of the bacteria isolated [12]. *E.feacalis* had frequently been isolated from root canals of failed endodontic treatment cases [13]. *C.albicans*, the common organism associated with therapy resistant apical periodontitis is more resistant to disinfecting agents used in endodontics [12]. In the present study, QMix, 2.5% sodium hypochlorite, 2% chlorhexidine and guava leaf extract were shown to inhibit the *E. faecalis* and *C. albicans* effectively. But aloevera extract had showed very minimal activity against *E. faecalis* and *C. albicans* in the present study.

Since its introduction NaOCl has been considered as an irrigant of choice for root canal irrigation because of its antimicrobial activity and tissue dissolving capacity. High pH of NaOCl interferes with the cytoplasmic membrane integrity and cause biosynthetic alterations in cellular metabolism attributing to its antimicrobial nature. Tissue dissolving action and dissolution rate of NaOCl is directly proportional to its concentration [14]. But not only its actions like antimicrobial activity, tissue dissolving capacity and smear layer removing ability but also the caustic potential and toxicity of NaOCl also increases with the increase in concentration [15].

Chlorhexidine gluconate (2%) is a good disinfecting agent with a property of substantivity contributing to its prolonged time of action [6]. On comparison with NaOCl, the irrigant is having less toxicity and foul taste. Chlorhexidine is proposed to be an alternative to NaOCl in open apex cases and NaOCl allergic patients. But the major disadvantage persisting is its inferior tissue dissolving action as a primary endodontic irrigant [16].

QMiX (Dentsply, Tulsa Dental) is a recently introduced irrigating solution which contains chlorhexidine, EDTA and a detergent (surface-active agent). The pH of the solution is considered to be slightly above neutral. The surface-active agent in the solution decreases the surface tension of solutions and thereby increases its wettability. This might be the reason behind better penetration of QMiX into the root canal and its good antimicrobial activity [17]. The biocompatibility of the solution had been studied by V Chandrasekhar et al., and they reported that the solution was less toxic when compared to 3% NaOCl, 2% chlorhexidine, and 17% EDTA when used in the rat tissue [18].

In an invitro study conducted in 2015, Ying Liu et al., reported that the antimicrobial efficacy of QMix was comparable to that of EDTA and chlorhexidine [19]. The decision to compare 2% chlorhexidine with QMiX though the percentage of chlorhexidine being the same for both was because of the fact that QMiX is much expensive than chlorhexidine and EDTA used separately. As ours is a Government institution catering economically backward patients in particular we were interested in an economically viable alternative to QMiX.

The flavonoids such as mosin glycosides, quercetin and quercetin glycosides may contribute to antibacterial action of guava leaf extracts [20]. The resistance to bacterial attacks suggested being as a result of the polygalacturonase inhibitory proteins in the plant cell walls of guava. The aqueous extracts of guava leaf can cause a marked reduction in the adhesion of the early organisms of plaque biofilm formation [21].

Chemical composition of the aloevera includes vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids. The latex compound present in aloe vera has bacteriostatic property [22]. These constituents may contribute to its antimicrobial activity against various microbes.

In the present study QMiX had obtained a mean diameter of 3.63cm and 3.53cm for *C. albicans* and *E. faecalis* respectively. The zones of inhibition of bacterial growth attained by QMiX were greater than that obtained for other extracts for the tested organisms and the result was statistically significant. This indicates that it has the highest efficacy against the tested organisms than other herbal and chemical agents. Our study has a correlation with the study conducted by Stojicic et al., [17], where they compared antibacterial activity of QMiX, MTAD, Chlorhexidine and NaOCl against plaque biofilm bacteria and found that at 3 minutes QMiX had killed more bacteria (65.3%) than any other solution tested.

In our study 2% chlorhexidine had shown superior significant activity against the tested pathogens than NaOCl and this was

similar to the study performed by Vianna et al., [23], where they obtained a largest mean microbial growth zone against *E. faecalis* by 2.0% chlorhexidine gel, and the smallest zones were obtained by 1% and 2.5% NaOCl.

Though the zones of inhibition of bacterial growth obtained by guava leaf extract against *C. albicans* and *E. faecalis* significantly less than QMiX and 2% chlorhexidine but it had shown an antibacterial activity almost similar to the 2.5% NaOCl in our study. This value had some sort of significance even though it was statistically insignificant as our study emphasized on whether herbal products would provide acceptable anti microbicity in routine endodontic practice as it is well known that herbal products are more bio friendly to human tissues.

Hence from the present study it can be evaluated that against both *C. albicans* and *E. faecalis*, QMiX was the best antimicrobial irrigant which is followed in descending order by the 2% chlorhexidine, 2.5% NaOCl, guava leaf extract and aloevera extract.

LIMITATION

Since our study being an in vitro study has significant limitation due to the fact that it does not take into consideration the dynamics involved inside the root canal. Moreover the defense mechanisms like biofilm protection employed by endodontic microflora is also not included in the study.

CONCLUSION

In conclusion, the present invitro study aimed to evaluate the antimicrobial potential of certain chemical agents like QMiX, 2% chlorhexidine and 2.5% NaOCl and herbal extracts viz. guava leaf extract and aloevera extract when used as root canal irrigants against *E. faecalis* and *C. albicans*. Among the herbal extracts, guava leaf extract had shown statistically significant activity against *Enterococcus faecalis* and *Candida albicans* which was less than that of QMiX, 2% chlorhexidine and 2.5% NaOCl. Within the limitations of this study, QMiX had demonstrated the best results amongst the five tested solutions. Hence this chemical solution can be considered as a potential alternative to other existing endodontic irrigants. But further evaluation of the antimicrobial efficacy of QMiX and guava leaf extract is highly recommended before extensive clinical usage.

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