Original Research

Comparative Evaluation of Antimicrobial efficacy of Guava Leaf Extract, Asafetida Extract and 2.5% Sodium Hypochlorite used as Endodontic Irrigant: An In-vitro study

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ABSTRACT

Background: Chemo-mechanical preparation for debridement and disinfection of infected root canal system plays a vital role in long term success of an endodontic therapy. It is a scientifically proven fact that most of the irrigants used for eradication of micro-organism from the root canal system can remove these microbes surviving in the biofilms but none them are able to do so successfully alone. Studies have found that natural medicinal herbs can be used as root canal irrigant and are found to be equally effective as compared to their counterparts. These herbal extracts being more bio-friendly having negligible side effects and can be considered an alternative to commonly used root canal irrigant. Hence; the present study was conducted for assessing antimicrobial efficacy of two different herbal extracts namely Guava Leaf extract and Asafetida extract along with 2.5% Sodium Hypochlorite when used as an endodontic irrigant against *Enterococcus faecalis*.

Materials and methods: The antimicrobial activity of irrigants used was determined using agar diffusion test. The group allocation was done based on the irrigants used into three groups: Group I- Guava Leaf extract, Group II- Asafetida extract and Group III- 2.5% Sodium Hypochlorite. The zones of inhibition of growth were recorded. The results obtained were tabulated and statistically analyzed by one way ANOVA with post-hoc Tukey's HSD and level of significance set at a p value<0.05.

Results: The mean zones of growth inhibition in 2.5% Sodium Hypochlorite (Group III) was significantly higher than that for Asafetida extract (Group II) (p<0.001). This difference was also statistically significant for Guava Leaf extract (Group I) and Asafetida extract (Group II) (p<0.001).

Conclusion: In the present study Guava leaf extract showed significant inhibitory effect against *Enterococcus faecalis*. However, Sodium Hypochlorite demonstrated the best antimicrobial efficacy and Asafetida extract showed the least. Moreover, considering side effects Sodium Hypochlorite, Herbal extracts like Guava leaf extract having significant antimicrobial activity can be considered as potential antimicrobial alternative to existing endodontic irrigants.

Key words: Antimicrobial efficacy, Anti-bacterial agents, Herbal, Root canal irrigants, Sodium Hypochlorite

Introduction:

Endodontic therapy provides opportunities to maintain teeth compromised by infection or trauma, in function and improve the health of dentition and a successful endodontic therapy requires proper chemo-mechanical preparation followed by, three dimensional obturation to attain adequate sealing of the disinfected root canal space.[1] Literatures show that infected root-canal is a great source of micro-organisms both aerobic and anaerobic, which play a vital role in initiating and sustaining the root canal infection and inflammation of peri-radicular tissues.[2] An anatomical and morphologic complexity of the infected root canal often makes its adequate disinfection a tough task.[3] Thus, eradication of micro-organisms from the infected root canal system is primary goal for long term success of any endodontic therapy and chemo-mechanical preparation involving use of various root canal irrigants serves this purpose. Bystrom A and Sundqvist G[4] found in their study that mechanical cleaning of the canal with normal Saline,

alone does not completely remove the bacteria from the canal and use of antimicrobial solution seems necessary to remove microorganisms from the root canal system.

Over the last many years, several materials, such as Sodium hypochlorite (NaOCl) and bisbiguanide antimicrobial agent Chlorhexidine, have been used for canal irrigation; but, none of them solely were able to completely remove the microorganisms from the root canal space.[5] However, Sodium hypochlorite (NaOCl) in concentrations of 0.5-5% has been a gold standard in root canal irrigation. Although, it has well known antibacterial and tissue dissolving properties, rather it is also known for undesirable tissue toxicity.[6] Since most chemical and synthetic drugs used as antimicrobial agents for root canal disinfection have toxic effect on human cells, use of herbal medicinal extracts, due to their biocompatibility and naturalness, have gained popularity in recent times.[7] The use of herbal plants for treatment, due to their antimicrobial, analgesic, anti-inflammatory and antioxidant properties have been there for thousands of years. These herbal products are natural and environmental-friendly and also been traditionally used by the society for treatment of gingival inflammation as home remedy. In recent years, many herbal extracts have used for root canal disinfection, debridement and smear layer removal.[8-10]

The microorganisms are a proven cause of persistent peri-radicular diseases, and subsequent failure of root canal treatment.[2] As a result, many studies are always carried out to detect antimicrobial efficacy of root canal irrigants. Considering the antimicrobial and antioxidant effect of various herbal extracts,[11-12] like Guava leaf extract, Asafetida extract and their possible use in root canal treatment due to their proven antimicrobial action, the present study was carried out to make a comparison of antimicrobial effects of Guava leaf extract, Asafetida extract and 2.5% Sodium Hypochlorite against oral pathogen *Enterococcus faecalis*.

Materials And Methods

The present study was conducted in the Department of Conservative Dentistry & Endodontics for assessing the antimicrobial efficacy of two different herbal extracts and compares it with traditionally used 2.5% Sodium Hypochlorite endodontic irrigant against oral pathogen *Enterococcus faecalis*. The antimicrobial activity of irrigants used was determined using agar diffusion test. The group allocation was done based on the irrigants used into three groups: Group I- Guava Leaf extract, Group II- Asafetida

extract and Group III- 2.5% Sodium Hypochlorite (positive control group). Standard strains of *Enterococcus faecalis* ATCC 29212 were used in the study to check antimicrobial activity of irrigants studied.

Preparation of Guava Leaves Extract (Group I):

Fresh leaves of Guava were taken and air dried in open while protecting them from direct sunlight. Dried leaves were then powdered and 50gm of this powder was taken and mixed with 500ml of sterile distilled water. This was heated for complete evaporation of water content and resulting liquid was filtered using filter paper to obtain desired Guava Leaf extract.

Preparation of Asafetida Extract (Group II):

Asafetida (Hing) is a dried latex (gum oleoresin) derived from the root of ferula. 50gm powder of this was taken and dissolved in 500ml of sterile distilled water to obtain a smooth solution. Later water evaporation and adding of 90% v/v pure alcohol was done simultaneously to obtain desired 30% concentration Asafetida extract.

Agar-diffusion test:

Hundred microliters of test organism *Enterococcus faecalis* (*E. faecalis*) suspension were obtained and inoculated in culture plates having previously set layers of Mueller Hinton Agar. Sterile spreader was used for inoculation of these micro-organisms across media. Three uniform cavities with a size of 6mm diameter and 3mm depth were made on each plate. These cavities were later filled respectively with 200µl of experimental solution and incubated at 37^0C for 24 hours. Plates were then checked for zones of inhibition of bacterial growth and diameters of zones achieved by *E. faecalis* in each group, which was recorded in centimeter (cm). Agar diffusion test was done 10 times to achieve statistically significant result.

Table 1 : Mean ± SD diameter of zones of inhibition of bacterial (*E. faecalis*) growth in centimeter (cm) comparing antimicrobial activity of experimental solutions

Irrigants	N	Mean ± SD	F value
Guava leaves extract (Group 1)	10	1.897±0.026	1253.919
Asafetida Extract (Group 2)	10	1.054±0.038	
2.5% NaOCl (Group 3)	10	2.163±0.076	

N: Sample size, SD: Standard deviation

Table 2 : Significance (p values) of mean difference of zones of inhibition of bacterial (*E. faecalis*) growth between groups (i.e., when comparing different irrigating solutions) by Tukey's HSD test

Irrigants	Tukey HSD Q statistic	p - value	Tukey HSD inference
Guava leaves extract (Group 1) vs Asafetida Extract (Group 2)	51.546	0.0010053	p<0.05 (highly significant)
Guava leaves extract (Group 1) vs 2.5% NaOCl (Group 3)	16.285	0.0010053	p<0.05 (highly significant)
Asafetida Extract (Group 2) vs 2.5% NaOCl (Group 3)	67.832	0.0010053	p<0.05 (highly significant)

p-value: Level of significance, HSD: Honestly significant difference, Q: Quantile

Results

The data obtained was tabulated and statistically analysed using using analysis of variance (ANOVA) and Tukey's HSD post-hoc test using SPSS 19 (Statistical Package for Social Sciences) (IBM Corporation, Chicago). The selected level of significance was set at a p value <0.05. The mean diameter and standard deviation values of zones of inhibition for bacterial (*E. faecalis*) growth in centimeter (cm) comparing antimicrobial activity of experimental solutions and descriptive statistics are presented in Table 1 and Table 2 respectively. Results obtained showed existence of significant difference in diameters of zones of inhibition of *E. faecalis* microbial growth obtained for Guava Leaf extract (Group I), Asafetida extract (Group II) and 2.5% Sodium Hypochlorite (Group III).

In Tukey's HSD post-hoc test for inter group comparison of antimicrobial efficacy, Group III- 2.5% Sodium Hypochlorite showed statistically significant and superior antimicrobial efficacy with greater zones of inhibition against *E. faecalis* than Group I- Guava Leaf extract and Group II- Asafetida extract. However, Guava Leaf extract (Group I) also demonstrated statistically significant and greater antimicrobial activity when compared to Asafetida extract (Group II).

Discussion

The main objective of a root canal treatment is complete disinfection of root canal space from the micro-organism and their by-products which solely is the main cause of pulpal and peri-radicular infection, thus preventing recontamination of root canal system.[5] However, anatomical root canal complexities like presence of lateral, accessory or furcal canals, apical deltas and isthmus often provide an undisturbed area for biofilm presence even after biomechanical preparation.[13] So, for the purpose of complete disinfection, biomechanical instrumentation of root canal space along with use of effective irrigating solution is always advisable for producing a debris free surface.[14] Hence; the present *in-vitro* study was conducted

for assessing antimicrobial efficacy of two different herbal extracts used as an endodontic irrigant.

Infected root canal space is host for micro-organisms both aerobic and anaerobic bacteria with *E. faecalis* most commonly being found and thought to be the main cause for endodontic failures.[15] In the present study 2.5% Sodium Hypochlorite [NaOCl] (Group III) and Guava Leaf extract (Group I) were shown to inhibit the *E. faecalis* effectively. However, Asafetida extract (Group II) showed very minimal activity against *E. faecalis* in the present study.

Since ages, chemicals like NaOCl has been an irrigant of choice commonly used in endodontic therapy due to its proven high anti-microbial efficacy and tissue dissolving capacity. The high anti-microbial activity of NaOCl is mainly attributed to its high pH that causes alteration in cytoplasmic membrane integrity and biosynthetic alteration in cellular metabolism.[16] However, due to its high caustic potential, toxicity, non-biocompatibility and sensitivity even to normal peri-radicular tissues,[17-18] over time, use of herbal extracts as root canal irrigants has gained attention. These herbal extracts are natural, biocompatible and non-caustic with relative very less side effects as compared to their counterparts.[9]

In the present *in-vitro* study, although Group III- 2.5% NaOCl showed superior antimicrobial efficacy with greater zones of inhibition against *E. faecalis* as compared to herbal extracts [Guava leaf extract (Group I) and Asafetida extract (Group II)]. However, both the herbal extracts used in the present *in-vitro* study showed an acceptable antibacterial activity and marked zones of inhibition against *E. faecalis*. Previous study by Mistry KS *et al.*[19] also evaluated antimicrobial activity of methanolic extracts of herbal plants and compared it with that of 5.25% NaOCl. They found that 5.25% NaOCl was the most effective antimicrobial agent having statistically significant difference against herbal extracts.

Moreover, in the present *in-vitro* study, Guava Leaf extract (Group I) demonstrated statistically significant and greater antimicrobial activity when compared to Asafetida extract (Group II). This can be attributed to the presence of flavonoids such as mosin glycosides, quercetin and quercetin glycosides in Guava leaf extracts. The higher bacterial resistance shown by Guava leaf extract may also be due to occurrence of polygalacturonase inhibitory proteins in the plant cell wall. So, the aqueous extracts of Guava leaf cause a marked reduction in early biofilm formation.[11,20] Hence, these may contribute to the enhanced

antimicrobial efficacy of Guava Leaf extract (Group I) in the present *in-vitro* study.

In the present *in-vitro* study, Group II-Asafetida extract showed least antimicrobial efficacy against *E. faecalis* when compared to Guava Leaf extract (Group I) and 2.5% NaOCl (Group III). However, although being a herbal plant extract, it did showed antimicrobial activity which can be due to its essence. Previous studies,[21-23] have shown that herbal essence can disrupt the lipid structures in the bacterial cell wall, resulting in cell wall lysis, causing cytoplasmic leakage, impairment in DNA transcription, disruption of protein synthesis and ultimately cell death.

Although the zones of inhibition of bacterial growth obtained by herbal extracts were found to be less than 2% NaOCl in the present *in-vitro* study. However, Group I-Guava leaf extract did showed almost similar antibacterial activity to 2% NaOCl. So, the results obtained in our study emphasized that herbal extracts, being more biocompatible and non-toxic, would also provide acceptable antimicrobial activity against *E. faecalis* in routine endodontic procedure.

Conclusion:

Based on the findings of the present *in-vitro* study, it can be concluded that herbal extracts like Guava leaf extract, Asafetida extract can be effectively used as root canal irrigant against *E. faecalis* during routine endodontic procedure. Among herbal extracts studied, guava leaf extract showed statistically significant antimicrobial activity against *Enterococcus faecalis* as compared to asafetida extract but was less than 2.5% NaOCl. Additionally, further studies are needed to evaluate antimicrobial efficacy of guava leaf extract to be used as root canal irrigant.

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