Research Article

Comparative evaluation of antimicrobial activity of 3% sodium hypochlorite, 2% chlorhexidine, and 5% grape seed extract against *Enterococcus faecalis* and *Candida albicans* - An *in vitro* study

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ABSTRACT

Aim: This study aims to compare the antimicrobial efficacy of different irrigants such as 3% sodium hypochlorite (NaOCl), 2% chlorhexidine gluconate, and 5% grape seed extract against *Enterococcus faecalis* and *Candida albicans*. **Background:** 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 to produce an endodontic irrigant having ideal properties; for many years, intracanal irrigants have been used as an adjunct to enhance antimicrobial effect of cleaning and shaping in endodontics. The constant increase in antibiotic-resistant strains and side effects of synthetic drugs has promoted researchers to look for herbal alternatives. For thousands of years, humans have sought to fortify their health and cure various illnesses with herbal remedies, but only few have been tried and tested to withstand modern scientific scrutiny. The present study was aimed to evaluate alternative and inexpensive simple and effective means of sanitization of the root canal systems. The antimicrobial efficacy of herbal alternatives as endodontic irrigants is evaluated and compared with the standard irrigants 3% NaOCl and 2% chlorhexidine. **Materials and Methods:** The irrigation solution was divided into three groups: Group I - 3% sodium hypochlorite, Group II - 2% chlorhexidine, and Group III - 5% grape seed extract. The zones of inhibition of growth were recorded against *E. faecalis* and *C. albicans*. Strains of these microorganisms were isolated and were subcultured. Disc diffusion was done for the primary evaluation of antimicrobial susceptibility, in which microorganisms were incubated in agar plates, following which 50 µL of each irrigant was added in sterile disc and placed on the plates. The plates were incubated and zone of inhibition around each disc was measured. **Results:** About 5% grape seed extract showed zones of inhibition, suggesting that they had antimicrobial properties. 5% grape seed extracts showed significantly greater zones of inhibition than 2% chlorhexidine. Henceforth, research should be directed toward the use as an irrigant in root canal treatment. **Conclusion:** Under the limitations of this study, it was concluded that 5% grape seed extract has a significant antimicrobial effect against *E. faecalis*. Microbial inhibition potential of grape seed extract observed in this study opens perspectives for its use as an intracanal irrigant.

KEY WORDS: *Candida albicans*, Endodontic irrigants, *Enterococcus faecalis*, Grape seed extract, Well diffusion method

INTRODUCTION

Pulpal and periapical pathologies are associated with microorganisms that are polymicrobial in nature. Ultimate aim of root canal treatment is to completely eradicate microorganisms from root canal system through mechanical and chemical debridement. Although mechanical debridement can reduce the bacteria from the root canal, chemical debridement is associated with high success rates in bacterial elimination from the root canal system.[1] Most persistent root canal infection in root canal system

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is *E. faecalis* that has the ability to survive harsh environment and it possesses a potential difficulty in removing from the root canal system.[2] Fungi also can seem in primary root canal infections, but they appear to occur more often in filled root canals of teeth in which treatment has failed. *Candida* albicans is by far the fungal species most commonly isolated from infected root canals. Candida species is one of the commonly isolated microorganism from root canals in 55% of the cases[3] and been associated with necrotic pulp in 15.3% of the cases. The objective of root canal treatment is to thoroughly clean the root canal system and debride it. This process is also called as chemicomechanical process in which the canal is derided with the help of chemical irrigants and mechanical procedures.[5,6] Most commonly used irrigant is sodium hypochlorite (NaOCl) which is used in different concentrations varying from 1% to 5%. NaOCl has many disadvantages such as unpleasant taste, cytotoxicity, and inability to dissolve the smear layer. Furthermore, various studies have shown that NaOCl has unable to penetrate at greater depths in dentin and long-term exposure of dentin to high concentrations of NaOCl can have a detrimental effect on dentin elasticity and flexural strength, thereby predisposing the tooth to vertical fracture. The constant increase in antibiotic-resistant strains and side effects caused by NaOCl has prompted researchers to look for herbal alternatives. Recently, much attention has been focused in the literature regarding antimicrobial regimens against endodontic microorganisms.

Antimicrobial solutions must possess many qualities such as the ability to penetrate the infected site, to suppress or destroy microbial growth, and to avoid the possible development of resistance to the agent. NaOCl is a commonly used irrigating solution that has been shown to have both antimicrobial and tissue-dissolving properties. However, there is concern about its possible toxic effect on the periapical tissues at higher concentrations. At lower concentrations, however, not only its tissue dissolving ability reduced but its antimicrobial effectiveness also reduced. The purpose of this study was to compare the *in vitro* effectiveness of 5% grape seed extracts and 3% NaOCl and 2% chlorhexidine (CHX) against *Enterococcus faecalis* and *C. albicans* by agar diffusion testing.

Aim

This study aims to evaluate the antimicrobial efficacy of 5% grape seed extract, 3% sodium hypochlorite, and 2% chlorhexidine against *E. faecalis and C. albicans*.

MATERIALS AND METHODS

Microorganisms Used

Clinical isolates of *E. faecalis* and *C. albicans* were used in the present study. These isolates were subcultured onto enterococcal confirmentional media and HiCrome Candida differential media, respectively. They were further confirmed by standard biochemical tests and stock culture and stored at −20°C for further use.

Preparation of Grape Seed Extract

Seeds of grape seed are brought from local markets which were dried in fresh open air protecting from direct exposure to sunlight. A 50 g of powdered were taken into beaker containing 500 ml of ethanol. After the complete evaporation of the ethanol content from extract, the resulting liquid was filtered and sterilized.

Preparation of Medium

Freshly prepared culture plates of Mueller-Hinton agar and Sabourand chloramphenicol agar (HiMedia) were prepared. McFarland standard 0.5 turbidity adjusted suspensions of *E. faecalis* and *C. albicans* were lawn cultured onto the media plates, respectively. After a brief drying, wells were made using sterile well cutter and added 10 ml 3% sodium hypochlorite, 2% CHX, and 5% grape seed extract in each one of the wells. These plates were incubated at 37°C for 24 h. Results were recorded by measuring the zone of inhibition of each irrigant against *E. faecalis* and *C. albicans* after 24 h.

Statistics

To compare the mean values between groups, one-way ANOVA is applied. SPSS version 22.0 is used to analyze the data. Significance level is fixed as 5% ($\alpha = 0.05$).

RESULTS

The mean values of growth inhibition produced by different test groups against the test microorganisms are given. Table 1 shows the mean zone of inhibition of 3% sodium hypochlorite, 2% chlorhexidine, and 5% grape seed extract, and there was statistically significant difference between the groups.

About 3% sodium hypochlorite performed better than all the test groups, followed by 5% grape seed extract and 2% chlorhexidine against all the tested microorganisms after 24 h incubation [Figures 1 and 2].

DISCUSSION

A variety of laboratory methods are available to evaluate the in vitro antimicrobial activity of an extract. The most known and basic methods are the disc diffusion method. It is commonly used due to providing direct estimation of its antimicrobial activity against a specific microorganism and added advantages of simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents,

Table 1: Mean zone of inhibition 3% hypochlorite, 2% chlorhexidine, and 5% grape seed extract. where 3% sodium hypochlorite shows highest mean zone of inhibition followed by 5% grape seed extract and 2% CHX

Variables	Group	Mean±SD	F value	*P* value
Candida albicans	Group-I	24.00±1.225	177.143	<0.001
	Group-II	12.00±0.707		
	Group-III	14.00±1.225		
Enterococcus faecalis	Group-I	35.00±2.000	393.781	<0.001
	Group-II	11.20±1.304		
	Group-III	14.20±0.837		

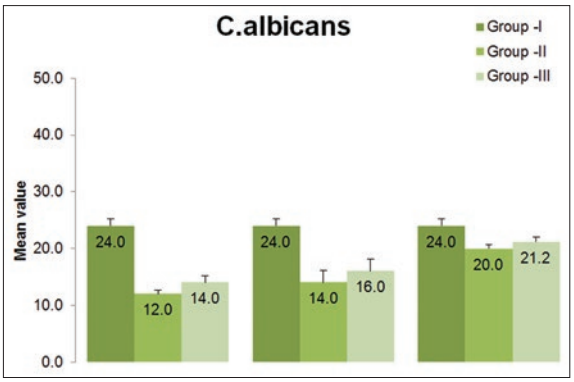

Figure 1: Mean zone of inhibition for *Candida albicans* for 3% hypochlorite, 2% chlorhexidine, and 5% grape seed extract. Where 3% sodium hypochlorite shows highest mean zone of inhibition followed by 5% grape seed extract and 2% chlorhexidine

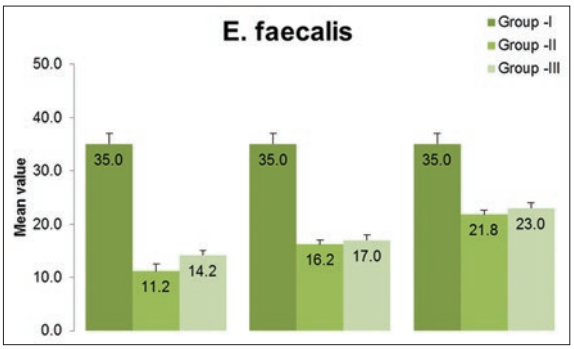

Figure 2: Mean zone of inhibition for *Enterococcus faecalis* for 3% hypochlorite, 2% chlorhexidine, and 5% grape seed extract. Where 3% sodium hypochlorite shows highest mean zone of inhibition followed by 5% grape seed extract and 2% chlorhexidine

and the ease of results interpretation. Although there are new technologies in the field of microbiology, disc diffusion is still one of the preliminary tests to assess the antimicrobial activity of a newer material. To further study, the antimicrobial effect of a material in depth, time-kill test, and flow cytofluorometric methods is recommended, which provide information on the nature of the inhibitory effect.

Several studies on the antimicrobial activity of irrigation solution in endodontics such as 0.5%, 1%, 2.5%, and 5% NaOCl are found in literature.[7-8] On the other hand, lack of studies on phytotherapic substances such as chlorophyll, propolis, *Morinda citrifolia*, neem, and grape seed extracts do not permit more objective conclusion about their use. Several pharmacological activities and medicinal applications of various parts of grape are known.

Interest on this substance is based on its properties such as antibacterial, antifungal, antiviral, and anti-inflammatory. Inclusion of *E. faecalis* in this study was based on the literature that relates these microorganisms to pulp infection, mainly in recalcitrant infection after endodontic treatment. Methodology of this study followed the standard established for agar dilution tests. Ideal irrigants should combine antimicrobial action and capacity to dissolve organic and in organic remnants. NaOCl in full concentration is well known for its bactericidal action and cytotoxicity. Moreover, its anti-adherence activity by altering bacterial adhesion and ability of organism to colonize also stimulated the study of this substance.

The use of grape seed extract as an irrigant might be advantageous due to its biocompatible, antioxidant and thus not likely to cause severe injuries to patients that might occur with NaOCl accidents. 2% CHX gluconate is an excellent disinfecting agent with a property of substantivity contributing to its prolonged time of action. On comparison with NaOCl, the irrigant is having less toxicity and foul taste. CHX is proposed to be an alternative to NaOCl in open apex cases and NaOCl allergic patients. However, the major disadvantage is persisting its inferior tissue dissolving action as an endodontic irrigant. Antimicrobial activity of grape seed extract is mainly due to the presence of polyphenolic compounds (causes loss of cell integrity) and also proanthocyanidins. 5% grape seed extract has moderate activity against *E. faecalis*. Grape seed extract has lower antimicrobial activity against *C. albicans*. Antimicrobial activity of grape seed extract is similar to 2% CHX against *E. faecalis*.

CONCLUSION

Within the limitations of the study, it can be concluded that 5% grape seed extract demonstrated antimicrobial activity against *E. faecalis* and *C. albicans*. Henceforth, further, dilution studies need to be carried out to find out the better activity of grape seed extract. This study

warrants the use of herbal-based non-irritant non-toxic irrigant in place of chemical ones.

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