Original Article

Evaluation of Antibacterial Properties of *Aloe vera* and Triphala as Endodontic Irrigants: A Microbiological Study

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Abstract

Background: Disinfection and shaping of the canal with a combination of chemical agents and endodontic instruments play important roles in the success of endodontic therapy. Root canal irrigants aid in achieving disinfection in canal systems inaccessible to biomechanical preparation. A wide variety of synthetic irrigants are available today but some may be ineffective, and there may be safety concerns and side effects. Herbal alternatives may be advantageous. **Aims and Objectives:** The aims of this study were to evaluate the antibacterial properties of herbal extracts, namely, *Aloe vera* and triphala as irrigants during endodontic treatment in comparison with the conventional irrigating solutions. **Materials and Methods:** Agar well diffusion assay and MIC methods were used to determine the efficacy of the experimental irrigant in removing *Enterococcus faecalis*. The difference between groups was statistically analyzed. **Results:** About 2% chlorhexidine (CHX) showed the highest zone of inhibition against *E. faecalis* followed by triphala. There was no significant difference between triphala and *A. vera*, but triphala showed more inhibition effect against *E. faecalis* due to chelating properties. CHX has significant antimicrobial efficacy against *E. faecalis*. Considerable reduction in growth of *E. faecalis* was seen in the herbal extract groups and considering their non-toxic nature and other physiological benefits, further studies are warranted. **Conclusion:** In this study, 2% CHX showed maximum antibacterial activity against *E. faecalis*. Triphala and *A. vera* did show antibacterial activity against the organism but at significantly reduced levels.

Keywords: Chlorhexidine, *Enterococcus faecalis*, Herbal irrigants.

Introduction

The goal of endodontic therapy is the removal of all vital or necrotic tissue, microorganisms, and microbial by-products from the root canal system. This may be achieved through chemomechanical debridement of the root canal.[1]

The complex nature of the root canal complex and the presence of many inaccessible areas requires a combination of mechanical instrumentation and irrigation to decrease the amount of bacteria/microorganisms in the system.[2]

Enterococcus faecalis is an enteric facultative Gram-positive bacterium which can grow independently in the root canal.[2] It is the most commonly isolated species from the root canals of the teeth, especially those with failed endodontic treatment.[3,4]

The bacterium possesses certain virulence factors such as lytic enzymes, cytolysin, pheromones, and lipoteichoic acid[5,6] which suppress the action of lymphocytes, potentially contributing to endodontic failure.[7]

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In this modern era of dentistry, we are looking toward herbal alternatives. Herbal medicines are drugs of plant origin which is used to attain or maintain a condition of good health and to treat diseases.[8]

It has been known for thousands of years that extracts of plant origin have therapeutic properties, and it has been found that natural plant extracts could be used as effective endodontic irrigants.[9] Some of the commonly used antibacterial agents are associated with increased antibiotic resistance and toxic and harmful side effects. There is a need for alternative agents which are affordable, non-toxic, and effective.[9]

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Traditional medicine is known to be fertile ground for the sourcing of modern medicines.[10] Medicines in that category are *Aloe vera* and triphala.[11] Keeping in view the advantages of using natural irrigants, the present research was undertaken to evaluate the antibacterial properties of herbal extracts, namely, *A. vera* and triphala, as irrigants during endodontic treatment, compared with conventional irrigating solutions.

Materials and Methods

The study was conducted in the department of microbiology. The samples were divided into a study group and a control group. *A. vera* and triphala were used in the study group, while in the control group, chlorhexidine (CHX) served as the positive control and saline as the negative control.

The bacterial stain used to identify *E. faecalis* was microbial type culture collection 439 (MTCC and Gene Bank, Chandigarh [UT], India). The analysis was carried out using the agar well diffusion assay method.[12]

The size of the sample to be used in the study was determined after consultation with statistician.

Preparation of aqueous solution of *A. vera*

A. vera gel was extracted from the plant's. The leaves of the plants were washed with distilled water and the surfaces of the leaves were disinfected with 70% of ethyl alcohol. After cutting and opening the leaves, the fresh pulp was collected and homogenized.

Eighty g of the gel were dissolved in 20 mL distilled water to prepare 80% *A. vera* solution.

Preparation of aqueous solution of triphala

Ripe fruits of *Terminalia chebula*, *Terminalia bellirica*, and *Embelica officinalis* were collected dried and powdered. Twenty-five g of the powder of each of the three fruits were separately passed through an 80# sieve and then mixed together in equal proportions to produce uniformly blended triphala churna.

This powder was then mixed with distilled water and allowed to stand for 7 days in a glass chamber, with occasional stirring. Triphala preparation was 20 g in 80 mL of distilled water.

Agar well diffusion assay method

The bacterium under test was *E. faecalis* (MTCC 439). Pure *E. faecalis* was grown on blood infusion agar plates. The micro-organisms were inoculated into tubes containing 5 mL 0.9% sterile saline solution. The suspension was adjusted using McFarland tubes to match the turbidity to 1.5×10^8 cfu/mL. The blood infusion agar plates were flooded with the test suspension.

Then, four wells (4 mm depth × 6 mm diameter) were cut in the agar, one for each test irrigating solution (*A. vera*, triphala, CHX, and saline). Into each well was placed 0.4 mL of one of the irrigants, using a sterile syringe.[2]

The plates were then incubated at 37°C under appropriate atmospheric conditions (80% N_2, 10% CO_2, and 10% H_2) for 7 days under anaerobic conditions in a CO_2 incubator. The diameters of the zones of inhibition of bacterial growth around the wells containing the test substances were recorded after the period of incubation.

The inhibitory zone was determined in millimeters by measuring the shortest distance between the outer margin of the well and initial microbial growth, a method previously established.[12] Each experiment was performed 6 times and the means and standard deviations of the measurements of the inhibitory zones were calculated.

Results

The means and standard deviations of the dimensions of the inhibition zones for each concentration of all the preparations are presented in Table 1.

The results were tabulated using Microsoft Excel software and an independent t-test was applied for statistical analysis which was carried out using SPSS v16 software by qualified statistician. Comparison of the results is recorded in Table 2.

Triphala was more efficacious an inhibitor than *A. vera* but the corelation suggests that neither are as efficacious against *E. faecalis* as CHX. Overall, 2% CHX effected the largest zones of growth inhibition in the well diffusion assay. Triphala and *A. vera* had significant inhibitory effects on *E. faecalis* when compared with the results of the control group.

Table 1: Mean and standard zone of inhibition

Materials	Sample number	Mean	Standard deviation
2% chlorhexidine	30	32.77	0.38
Triphala		24.08	0.33
Aloe vera		12.95	0.25

Table 2: Intergroup and intragroup comparison for statistical significance ($P<0.05$)

Group	*P*	R
Study group		
Group I		
2%CHX-Triphla	<0.05	−0.399
Group II		
2%CHX-*A. vera*	<0.05	−0.035
Group III		
Triphla-*A. vera*	<0.05	−0.59
Control group		
Group IV		
2%CHX-saline	<0.05	∞
Group V		
Triphla-saline	<0.05	∞
Group VI		
A. vera-saline	<0.05	∞

P: Probability value (paired *t*-test), R: Pearson correlation. *A. vera*: *Aloe vera*

Discussion

The present research compared the antibacterial properties of herbal with conventional endodontic irrigants. The agar diffusion test used in this study is useful for evaluating and comparing the *in vitro* antimicrobial activities of irrigants before performing more advanced tests; many studies have used this method for evaluation of antibacterial effects of various endodontic irrigants.[13]

The results obtained from this test must be interpreted with caution, as this assay may not demonstrate the full clinical potential of the material being tested. The MIC test is also a research tool to determine the *in vitro* activity of new medicaments and antimicrobials.

The bacterial species *E. faecalis* was selected as representing an organism commonly isolated from the root canals of teeth that have been previously root filled[14] and it prevalence in such infections ranges from 24% to 77%.[7]

There have been several studies which applied *E. faecalis* as a target micro-organism in the evaluation of the effects of antibacterial agents. The study conducted by Somayaji *et al*. compared the antimicrobial efficacy of sodium hypochlorite (NaOCl), triphala, and *Withania somnifera*, a combination of triphala and *W. somnifera* against *E. faecalis*. NaOCl, triphala, and *W. somnifera* failed to eliminate bacteria completely. However, considerable reduction in growth of *E. faecalis* was seen in the herbal extract groups though none were as effective as was seen in the NaOCl group. Another study was conducted to compare the antimicrobial activity of triphala (plant-derived solution) with 0.5%, 1%, 2.5%, and 5% concentrations of NaOCl against *E. faecalis*.[15] In our study, triphala exhibited better antimicrobial activity against *E. faecalis* compared with 0.5% and 1% NaOCl ($P < 0.05$).

E. faecalis has ability to invade and to live within dentinal tubules and can endure prolonged periods of starvation. *E. faecalis* may occur alone, in pairs or in short chains, can survive harsh environments such as extreme alkaline pH (9.6) and a temperature of 60°C for 30 min.[4]

The primary objective of endodontic therapy is microbial reduction or elimination to promote healing and reestablishment of health of the periradicular tissues.[16] A variety of chemicals have been introduced. While the commonly used endodontic irrigants have many potentially favorable properties, they can also cause cytotoxicity, allergic reaction, hypersensitivity, or immunosuppression.

According to the world health organization more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Plants used in traditional medicine contain a wide range of ingredients which are used to treat chronic as well as infectious diseases.[16]

Many drugs used in allopathic medicine have their origin in medicinal plants and traditional healers have relied on plant parts as folklore medicine since time immemorial.

Herbal products have a wide range of application in dentistry, such as anticaries activity; root canal irrigants; anti-collagenase activity; anti-microbial; and anti-oxidant effects and as a mouth rinse. The most commonly used herbal products are *A. vera* and triphala.[17]

A. vera is a kind of plant that its leaf is composed of two major parts. The outer green rind contains vascular bundles and the inner colorless part, named as aloe gel, includes the parenchymal cells which contain viscous clear liquid. The aloe gel consists of 99.5% water. The remaining solid material includes mineral, vitamins, enzymes, polysaccharides, phenolic compounds, and organic acids. It has been proved to have multiple therapeutic and antimicrobial properties, especially on *E. faecalis*. Furthermore, it has been successfully used for decontamination of gutta-percha. In the present *in vitro* study, *E. faecalis* which is a Gram-positive bacterial strain was used to evaluate the antibacterial effect of irrigating solutions due to its proved resistance to antimicrobial agents. Several studies have shown that *A. vera* extract, especially acts against Gram-positive bacteria. The antibacterial activity of *A. vera* is related to anthraquinones which are phenolic derivatives. According to Sureshchnadra *et al*., *A. vera* extract is effective against *E. faecalis* in agar medium. However, agar diffusion tests have some limitations, including the influence of substrate pH, incubation period, and diffusion capacity of the drug on the test results. Bhardwaj *et al*. assessed antibacterial activity of *A. vera* gel as long as 1, 3, and 5 days. *A. vera* showed good antibacterial activity just at the 1st day of incubation. They noted that *A. vera* had 75 potentially active constituents such as vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids which were possible reasons for its antimicrobial action.[11]

Triphala is a well-known Ayurvedic herbal formulation consisting of the dried and powdered fruits of three medicinal plants, namely, *T. bellirica*, *T. chebula,* and *E. officinalis* with tannic acid being its principal constituent. Other chemical constituents of triphala include quinones, flavones, flavonoids, gallic, and vitamin C. It has long been used in Indian traditional medicine (Indian system of medicine), for the treatment of headaches, constipation, and hepatic disorders. Initial studies have shown bacteriostatic or bactericidal effects of tannic acid on Gram-positive and Gram-negative pathogens, although the preparation is not as effective as is the CHX group. Compared with commonly used root canal irrigants, it is safe and is composed of compounds with appropriate physiologic effects in addition to its anti-oxidative and anti-inflammatory properties.

The most important advantages of triphala include easy access, low-cost, long-term stability, less toxicity, and absence of microbial resistance.[18] It has anti-cariogenic and thermogenic effects and can act as a probiotic. The antibacterial property of triphala extract has been shown in the present study by the, measurement of the zone of inhibition against *E. faecalis*, as has also been demonstrated by Shakouie *et al*.[17]

The study found *A. vera* to be the most effective herbal product when used as a root canal irrigant. However, this was an *in vitro* investigation which may not have demonstrated the full clinical potential of a tested material. Dentin can interact with herbal endodontic irrigants and the presence of a smear layer could delay any antimicrobial effects of the solutions. Further studies should be carried out under *in vivo* conditions to confirm the clinical efficacy of these herbal products as useful irrigants in endodontics.

Conclusion

In this study, 2% CHX showed maximum antibacterial activity against *E. faecalis*. Triphala and *A. vera* did show antibacterial activity against the organism but at significantly reduced levels. Nevertheless, herbal alternatives as root canal irrigants might prove to be advantageous considering the several undesirable characteristics of CHX. The non-toxic nature and other physiological benefits of these herbal extracts warrant further studies.

Further research is needed to conclusively recommend herbal solutions as root canal irrigants.

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