

The antibacterial effect of 10% Salvadorapersica (Siwak), 10% green tea, and 50% lemon juice on *Enterococcus faecalis*: an invitro study

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Abstract

Background and objective: Shaping and disinfection of the root canal with a combination of chemical agents and endodontic instruments play an important role in the success of endodontic therapy. This study aimed to evaluate the antibacterial properties of herbal alternatives as possible irrigates against *Enterococcus faecalis* during endodontic treatment compared with the conventional irrigation solutions such as NaOCl 5%.

Methods: A 10% Siwak extract, 10% green tea extract and 50% fresh lemon juice were prepared separately to make antibiotic disks and seeded on the culture media respectively and compared with 5% NaOCl, distilled water as the control. Plates were left to incubate for 48 hours at 37°C. Zones of inhibition of the bacterial growth were calculated to measure the antibacterial effect of the tested irrigants.

Results: All irrigants were active against this bacterium. The 5% NaOCl had the highest mean value (27.70±0.66), followed by 10% Siwak (Salvadorapersica) (21.60±0.7), 50% lemon juice (15.10±0.56), and 10% green tea (10.50±0.5).

Conclusion: Herbal extracts can be used as the possible irrigant to compensate 5% NaOCl during endodontic treatment.

Keywords: Siwak; Green tea; Lemon juice; NaOCl; *Enterococcus faecalis*.

Introduction

The main aim of endodontic treatment is to remove the diseased tissue, eliminate bacteria from the root canal system and prevent its recontamination. The essential procedures for the success of root canal treatment include the cleaning and shaping of the root canal system, as well as the proper filling of the canal. Failure may occur within the canal even when treatment is adequate. Therefore, disinfection and shaping of the canal with a combination of chemical agents and endodontic instruments play an important role in the success of endodontic therapy.¹ Endodontic success is depended on multiple factors, studies have shown that improper canal disinfection leads to recurrent root canal infections with cultivable micro-organisms, even after endodontic treatment and are most commonly associated with *Enterococcus*

faecalis.^{1,2} To reduce the resistance number of bacteria in the root canal system, irrigation is carried out to control the periapical disease. Because of the increased antibiotic resistance to these antimicrobial agents, toxic and harmful side effects of few common antibacterial agents. This is because of the use of a wide variety of synthetic antimicrobial agents over the last years as endodontic irrigants, thus, there is a need for alternative agents which are affordable, non-toxic and effective.³ Sodium hypochlorite (NaOCl) 5% is one of the most widely used endodontic irrigants because of its ability to destroy a broad spectrum of microbes. However, it has some undesirable characteristics such as tissue toxicity, allergic potential, disagreeable taste, and the inability to remove the smear layer. The high antimicrobial activity of herbal or natural

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products become more popular today due to biocompatibility, anti-inflammatory, and antioxidant properties.⁴ The mineral content and biologically active constituents of fruit juice exhibit a significant antibacterial effect. Hence, fruit juice with the property of bioavailability and retention of certain minerals by polyphenolic compounds can be recommended for their use as an alternative anti-infective agent in natural medicine for the treatment of infectious diseases.⁵ The bacterial enzyme (gyrase) is inhibited by green tea, which has antibacterial activity by binding to the ATP B subunit. Green tea exhibits antibacterial activity on *Enterococcus faecalis*. It is also found to be a good chelating agent.⁵ Commonly used over many years as a chewing stick, Salvadorapersica, commonly known as Siwak. Extracts of salvadorapersica have been shown to contain trimethylamine, salvadorine, chloride, fluoride, and traces of tannins, saponins, flavonoids and sterol, and these components have been shown to have the significant antibacterial effect. Also, these herbal products have fewer side effects and easily available at affordable cost.^{1,6} The inhibitory effect of some essential oils and extracts of spices, plants, or their major active constituents on bacteria like *Enterococcus faecalis* is documented by numerous studies.⁷ This study aimed to evaluate the antimicrobial effects of the herbal extract against *Enterococcus faecalis* as the endodontic irrigation solution.

Methods

Sample grouping:

Group 1: 5 % NaOCl irrigant was used to prepare ten antibiotic discs (as the positive control).⁵

Group 2: 50 ml fresh lemon juice was mixed with 50 ml distilled water and was further used for preparing antibiotic discs.¹

Group 3: 10% green tea extract irrigant solution was used to prepare antibiotic discs.¹

Group 4: 10% Salvadorapersica (siwak)

extract irrigant solution was used to prepare antibiotic discs.¹ Distill water was used as negative control.

Preparation of Fresh Citrus Fruit Juice

The fresh citrus fruit lemon was purchased from the local market. The procured fruit was washed at room temperature. The outer portion of these fruits was wiped with 70% alcohol, and fresh juice was collected, then diluted by 50% distilled water and then stored at 37C⁰ for farther study.⁶

Green Tea Preparation

Ten gram of the selected dry green tea leaf, steeped for 1.5- 2 minutes in 100 ml of distilled water. The coolest brewing temperature was below 70 C^o. The mixture was purified to obtain the 10% concentration solution of green tea.⁸

Salvadarapersica (siwak) Preparation

Sticks of siwak were cut with a knife to small pieces then ground into fine powder by a special machine, 125 gm of salvadorapersica powder was put in a beaker and added 1½ litter of sterile distilled water. The liquid was boiled at 60 C^o for 15 minutes in a closed container. After bench cooling, the liquid was filtered using filter paper (no1), and the solution left to dry in an incubator at 37°C for 24 hours to allow complete evaporation of water and obtain powder siwak. The powder was collected and kept in a tightly closed glass container and kept in the refrigerator until use.⁹

Preparation of Antibiotic Disks for Disk Diffusion Method

Preparation of disks from Salvadorapersica (Siwak), Green Tea, and Lemon juice extracts by distributing 25 filter paper discs (wattman No.2) of 6 mm in diameter into Petri plates and sterilized in the hot air oven at 150 C⁰ degree for one hour. Add 0.5 ml of appropriate freshly prepared (different extractions) to the sterilized discs shake well and stand for 30 minutes so that each disk absorbed 0.02ml of the (different extractions). Dry the disks at 37C⁰ in an incubator for two hours and stored in a refrigerator in a capped bottle.¹⁰

Isolation of *Enterococcus Faecalis*

Enterococcus faecalis was the most predominant species were isolated from cases suffering from root canal infection patients also have been found to be associated with chronic apical periodontitis the microbial samples were taken from root canal during endodontic treatment and culture methods were used to identify the *E. faecalis* present in the sample. Also, the isolated *Enterococcus faecalis* was identified by microscopic examination and biochemical reactions.

Micro-Organism Test (Culturing) and Growth Conditions

Antibacterial activities of the irrigants were evaluated against the *Enterococcus faecalis*. An antibiotic sensitivity test was performed on blood agar which, the surface was inoculated with 0.1 ml. of brain heart infusion (BHI) broth culture of *Enterococcus faecalis*. Prepared discs were seeded on each Petri dish. Plates were left to incubate for 24 hr. at 37 C. zones of inhibition of the bacterial growth were in millimeter calculated to measure the antibacterial effect of the tested irrigants.

Statistical Analysis

All data are expressed as means \pm S.E. ANOVA test was performed to find out the significant difference between the means

of the measure of the diameter of growth inhibition zones of the tested irrigants using statistical software GraphPad Prism.⁶ A *P* value of <0.05 was considered as statistically significant.

Results

The results obtained from disc diffusion method study were as follow by using (ANOVA) test Table 1: the antimicrobial sensitivity of irrigants against *Enterococcus faecalis* which recorded inhibition zone of 10 discs from each irrigant separately. Bacterial inhibition zone in mm diameters using 5.25% NaOCl, 50% lemon juice, 10% green tea, and 10% salvadorapersica (siwak) extract. It was found that all irrigants are active against this bacterium, NaOCl had the highest mean value (27.70 ± 0.66) followed by Siwak (*Salvadorapersica*) which had a mean value of (Mean 21.60 ± 0.7) followed by lemon juice which had mean value (15.10 ± 0.56) and Green tea which had lowest mean value about (10.50 ± 0.5) as shown in Table 1. Zones of growth inhibition were not seen around discs impregnated with distilled-water (as the negative control). Statistically, this difference is highly significant ($P < 0.001$) as shown in Table 1.

Table 1: Means of bacterial inhibition zones by irrigant type.

| Groups | N | Mean | (\pm SD) | <i>P</i> (ANOVA) |
|-------------|----|-------|-------------|------------------|
| NaOCl | 10 | 27.70 | (2.11) | |
| lemon juice | 10 | 15.10 | (1.79) | < 0.001 |
| Green tea | 10 | 10.50 | (1.58) | |
| Siwak | 10 | 21.60 | (2.22) | |
| Total | 40 | 18.72 | (6.85) | . |

Discussion

A successful endodontic treatment should reduce and remove the number of bacteria in the root canal system by irrigation and instrumentation.¹¹ The irrigation solution has an important role in the cleaning of the root canal by lubrication the files, flush out debris, and have an antimicrobial effect and tissue dissolution without damaging the periapical tissue thus control periapical diseases. *Enterococcus faecalis* is regarded to be a part of the endodontic microbial flora,¹ most common chemical irrigation solutions in endodontic treatment are chlorhexidine and NaOCl so in our present study we used 5.25% NaOCl which have the largest inhibition zone of bacterial growth (*Enterococcus faecalis*) in the agar media.^{12,13} The ability of NaOCl as a broad antibacterial spectrum, possessing some ability to inactivate endotoxin, and its effectiveness at dissolving tissue and removing the organic component of the smear layer are important features, but NaOCl 5.25% have some side effects, like tissue toxicity, and bad tests.¹⁴ The major advantages of herbal irrigants are safety, easy availability, increased shelf life cost-effectiveness and lack of microbial resistance so far, several studies have described the antioxidant properties of medicinal fruit juices which are rich in phenolic components. Natural antioxidant mainly comes from plants in the form of phenolic compounds such as a flavonoid, phenolic acids, tocopherols, etc.¹⁵ Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be microbial substances against wide arrange of microorganisms invitro, their activity is probably due to their ability to made a complex with bacterial cell wall.⁶ This study suggested that 50% of citrus fruit juices have a potential antimicrobial effect against selected enteric pathogens especially *E. faecalis*. According to this study, we can use fresh lemon juice as a compensating chemical irrigation solution.

This result is comparable to those found by Sudhir, Devendra, and Vijay.¹⁶ Green tea polyphenols are preferred over the traditional root canals irrigants due to their curative properties such as anti-oxidant, anti-inflammatory, and radical scavenging activities also its rich in citric acid which helps in removing the smear layer. Its chelating property make it an effective alternative to sodium hypochlorite.¹⁷ In the present study, green tea polyphenol showed significant antibacterial activity against *E. faecalis*. This result agrees with Al- Azzawi, but this percent of green tea has the lowest value of antibacterial effects in comparison to present solutions this results may be due to a low percent of green tea in solution or may be due to a mistake in application procedures.³ *Salvadorapersica* can be used as irrigation solution in endodontic treatment because it contains tri -methylamine, salvodorime chloride and fluoride in a large amount, which acts as antibacterial especially *E. faecalis*, also *salvadorapersica* antimicrobial efficacies being increases by increasing the percent up to 10%.^{18,19} In the present study, we can decide that possible use of *salvadorapersica* extract solution 10% as the substitute to NaOCl in irrigation during endodontic treatment.

Conclusion

We can use herbal extract (10% siwak, 50% lemon juice, and 10% green tea) as a possible irrigant solution to disinfect the root canal system especially against *Enterococcus faecalis* during endodontic treatment, but increasing the percentage of these irrigant solutions need further studies.

Competing interests

The authors declare that they have no competing interests.

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