Acta Scientific Dental Sciences (ISSN: 2581-4893)

Volume 6 Issue 5 May 2022

Research Article

Evaluation of Antimicrobial Efficacy of Punica Granatum, Green Tea, Chitosan and Sodium Hypochlorite Irrigants Against *E. faecalis*

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DOI: 10.31080/ASDS.2022.06.1355

Received: March 16, 2022

Published: April 07, 2022

Abstract

Aim: This study aimed to compare the antimicrobial efficacy of various irrigants- Punica granatum 20%, green tea extract 3%, chitosan 2% and sodium hypochlorite 3% against *E. faecalis.*

Materials and Methodology: *E. faecalis* strain ATCC 29212 colonies were collected from cultures grown on brain–heart infusion agar. Extracts of various test irrigants- Punica granatum 20%, green tea 3% and chitosan 2% were prepared. Wells of 6 mm diameter were cut in the prepared agar medium using sterile templates. 50 µL of the test reagents were inoculated into the wells under aseptic conditions. These plates were then incubated at 37° C for 24 hours. The zone of inhibition of growth around the wells was then measured. Data were analyzed using one-way analysis of variance and Tukey's post hoc test. Descriptive statistics was obtained using SPSS software (version 11.5) with P established at < 0.05

Results: The results indicated that Punica granatum showed the largest zone of inhibition with a mean value of 24mm followed by Sodium hypochlorite at 18.8mm, green tea at 13.8mm and chitosan extract showed a zone of 11.7mm respectively.

Conclusion: Punica granatum 20% had the greatest potential to be used as an endodontic irrigant against *E.faecalis* among the tested irrigants.

Keywords: Punica Granatum; Chitosan; Green Tea; Sodium Hypochlorite; *E. Faecalis*

Introduction

Success of endodontic treatment depends on the outright debridement and disinfection of the root canal system [1]. This is not routinely achieved as microflora may be found in root canals, dentinal tubules, apical ramifications, cementum or areas of root resorption, thereby restraining the access of the root canal by instruments and irrigants [2]. Irrigants have multiple functions and are necessary to have antimicrobial effects, dissolution of organic matter in the canal and flush out loose debris [2]. Enterococcus faecalis are highly resistant to disinfecting agents, instigating a persistent intra-radicular or extra-radicular infection. It plays an important role in the etiology of periradicular lesions after root canal treatment and is seen in 22-77% root canal failure cases [3]. It has demonstrated adherence to host cells, expressing proteins that permit it to compete with other bacterial cells and alter the host response. It can ingeniously invade the dentinal tubules surviving chemomechanical instrumentation and intracanal medication, thereby it can colonize the tubules and reinfect the obturated root canal system. Sodium hypochlorite has been considered the gold standard for irrigation owing to its ability to dissolve organic matter and its immense antimicrobial potential [4]. Nonetheless, there are certain major hindrances associated with the use of sodium hypochlorite i.e it irritates the periapical tissues, stains instruments, unpleasant taste, high toxicity, corrosion of instruments, inefficiency to remove smear layer, burning of neighboring tissues and decrease in elastic modulus and flexural strength of dentin [2]. Therefore to counter-

act these drawbacks caused by sodium hypoclorite, natural irrigants were introduced that do not possess much of side effects. *Punica granatum* which is an extract of pomegranate fruit peel (PPE) has been employed productively in medicine for its bactericidal, antifungal, antiviral, immune modulation, and anthelminthic effects [1]. The active components of PPE are flavonoids, tannins, and other phenolic compounds [1]. Green tea extract is gathered from the leaves of the plant *Camellia sinensis* which are high in flavonoids in the form of catechins. Catechins existing in green tea extracts have distinctive antioxidant, anti-inflammatory, antimicrobial and radical scavenging ability [4]. Chitosan is an indigenous polysaccharide comprising of copolymers of glucosamine and N-acetylglucosamine. The outcome of partial deacetylation of chitin is chitosan. It is biocompatible, biodegradable, bioadhesive and there is no proclaimed toxicity [2].

Therefore, the present *in-vitro* study was conducted to assess the antimicrobial efficacy of Punica granatum 20%, green tea extract 3%, chitosan 2% and sodium hypochlorite 3% irrigating solutions against *E. faecalis*.

Methodology

Preparation of punica granatum extract

The mesocarp of pomegranate was peeled and dried. The dried peel was then powdered. Initially 10grams of the material was boiled in 100ml of sterile distilled water for 15 minutes on a low flame and then allowed to cool for 45 minutes. The constituents of the flask were then filtered to remove any impurities present. To obtain 20% of Punica granatum, the extract was diluted with sterile water and stored in a sterile container in the refrigerator.

Preparation of green tea extract

Green tea powder was commercially available in capsule form. 3grams of this powder was taken and dissolved in 100ml of sterile boiling distilled water for 5minutes. The contents obtained were filtered using filter paper. The dilutions were kept overnight at 37°C.

Preparation of chitosan extract

For preparation of 2% chitosan solution, 2gm chitosan nanoparticles were diluted in 100 ml of 1% acetic acid. The mixture was then stirred for 2 hours in a magnetic stirrer. The obtained solution was then stored in the refrigerator until used.

Sodium hypochlorite

Commercially available 3% Sodium hypochlorite was used in this study.

Figure 1: Irrigating solutions.

The samples were divided into the following groups:

- Group A: 3% Sodium hypochlorite
- Group B: 20% Punica granatum (Pomegranate peel extract)
- Group C: 2% Chitosan
- Group D: 3% Green tea

E. faecalis culture preparation

E. faecalis (ATCC 29212) was inoculated into sterile BHI broth and incubated at 37° C for 4 hours. Culture suspensions were adjusted to Mc. Farland's opacity standard 0.5 to get a culture equivalent to 1.5×10^8 CFU/ml. 25µL of the adjusted culture was added to 25ml of freshly prepared BHI agar. Cooled to 50° C. the medium was then poured into a sterile petri plate (90mm) and allowed to set. Wells of 6 mm diameter were cut in the prepared agar medium using sterile templates.

50 µL of the test reagents were inoculated into the wells under aseptic conditions. The plates were then incubated at 37° C for 24 hours. The zone of inhibition of growth around the wells was measured using a vernier caliper and the antimicrobial effect of test reagents was estimated.

Data was analyzed using one-way analysis of variance (ANOVA) and Tukey's *post hoc* test. Descriptive statistics were obtained using SPSS software (version 11.5) with *P* established at < 0.05.

Results

A total of four groups of irrigating solutions were checked for competency on *E. faecalis*. The diameter of zones of inhibition was tabulated for all the test group. Using ANOVA and Tukey's *post hoc* test, data was statistically analyzed with significance level at $P < 0.05$. The results of the study indicated that Punica granatum

showed the largest zone of inhibition with a mean value of 24mm followed by Sodium hypochlorite at 18.8mm, green tea at 13.8mm and chitosan extract showed a zone of 11.7mm respectively.

Group	N	Mean in mm
A	20	18.8
B	20	24
C	20	11.7
D	20	13.8

Table 1

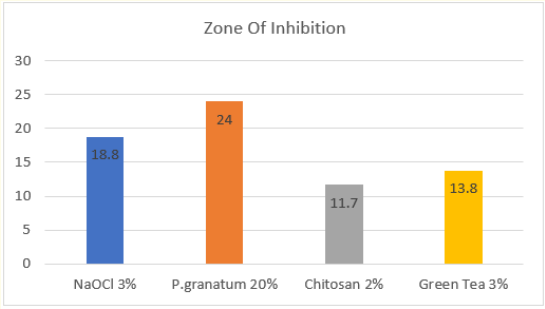

Figure 2: Mean zones of inhibition.

Discussion

The paramount goal of endodontic treatment is to achieve complete disinfection and prevent reinfection of the root canal system and periapical tissues. Every clinician's aim should be to eradicate or significantly reduce the microbiota and to flush out loose debris and remove organic and inorganic material from the root canal system. Chemomechanical preparation, including both mechanical instrumentation and chemical irrigation, is crucial for decreasing bacterial population. Enterococcus faecalis was chosen as the test organism for the present study, as it is the most commonly isolated microorganism from the root canals of teeth with failed endodontic treatment, found in about 4-44% of primary endodontic infections and 24-74% of persistent endodontic infections [5]. Enterococcus faecalis survives prolonged periods of starvation and is resistant to the common intracanal irrigants and medicaments, probably because it passively maintains pH homeostasis, which occurs as a result of ions penetrating the cell membrane and cytoplasmic buffering capacity [6]. The continual escalation in antimicrobial resistance and side effects of synthetic drugs, has made phytotherapuetic compounds become prominent due to their easy accessibility, cost effectiveness, low toxicity and lack of antimicrobial resistance [7]. In the current study, *Punica granatum* demonstrated the largest zone of inhibition against *E. faecalis*, among all the experimental irrigants tested. *Punica granatum* possesses antiviral, bactericidal, and antifungal properties due to the immune modulation and anthelminthic actions of pomegranate [4]. The possible reason for the antibacterial activity of Punica granatum might be due to the presence of phytocompounds in its peel, extracts like hydrolysable tannins, polyphenols and flavonoids; specifically punicalagin, gallagic acid, catechin, quercetin, glycosides, punicalin, rutin [8]. Polyphenols and tannin extracts present in *Punica granatum* help in the prevention of biofilm-related oral diseases such as dental caries and gingivitis/periodontitis [9-11]. The tannin present in PPE penetrates the cell wall of the microorganisms and binds to its surface, causing precipitation of proteins and abolishes enzymes such as glycosyl transferase which is a prominent enzyme for the breakdown of carbohydrates, which provides nutrition to organisms [12]. The phenolic compounds present in PPE make it burdensome for the existence of microorganisms due to the disruption of the bacterial cell wall [11]. Mallya., *et al*. [1] evaluated the antimicrobial efficacy of 20% *Punica granatum*, 0.2% chlorhexidine gluconate, and 2.5% sodium hypochlorite used alone or in combinations against *Enterococcus faecalis* and it was concluded that *Punica granatum* and CHX was the best combination of irrigants among experimental groups against *E. faecalis*. Sodium hypochlorite has been used as an irrigant for root canal treatment for more than 70 years [13]. NaOCl has potent dissolution action in both vital and necrotic tissues. The robust antibacterial effect of NaOCl against *E. faecalis* depends on the concentration and time of exposure. Despite NaOCl being the most commonly used root canal irrigant owing to its exclusive features, such as dissolution of organic tissue, carnage of microorganisms and lubricant action, its toxic effects on vital tissues and induction of inflammatory reactions in case of over extrusion cannot be overlooked [13-15]. Green tea possesses healing properties such as antioxidant activity, anti-inflammatory and radical scavenging properties [16]. It has also been employed as a chelating agent [17]. Availability, affordability, low toxicity and long shelf life are some of the other satisfactory properties of green tea as an irrigating agent. Prabhakar., *et al*. [18] evaluated the antimicrobial effects of green tea, Triphala, MTAD, and 5% NaOCl against *E. faecalis* and concluded that 5% NaOCl was the most effective antibacterial agent. The antimicrobial property of chitosan could be due to the mechanism of action of chitosan that maintains the positively charged NH3+ groups of glucosamine that interacts with the negatively charged surface components of bacteria, resulting in extensive cell surface attraction, leakage of intracellular substances, causing damage to vital bacterial activities [19]. In a study by Shaymaa., *et al.* Ca (OH) 2 combined with chitosan solutions were more effective in inhibiting the growth of *E. faecalis* when compared with Ca (OH) 2 mixed with saline [20].

Based on literature this was the first study which compared the antibacterial efficacy of these 3 natural irrigants- pomegranate peel, green tea and chitosan against the gold standard endodontic irrigant sodium hypochlorite.

Conclusion

Within the limitations of this study it was concluded that Punica granatum 20% had the greatest potential to be used as an endodontic irrigant against E.faecalis followed by the gold standard sodium hypochlorite 3%, subsequently green tea extract 3% and Chitosan 2% also demonstrated considerable antimicrobial activity.

Conflict of Interest

The authors deny any conflict of interest.

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