Antimicrobial Efficacy of Synthetic and Natural-Derived Novel Endodontic Irrigating Solution – An *In vitro* Study

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Abstract

Aim and Objective: This *in vitro* study was designed to comparatively evaluate the antimicrobial efficacy of peracetic acid (PAA – 0.5% and 1%), phytic acid (IP6 – 0.5% and 1%), and sodium hypochlorite (NaOCl – 3% and 5.25%) as irrigating agents against *Enterococcus faecalis*. **Materials and Methods:** Agar plates were prepared using Mueller-Hinton (MH) agar. *E. faecalis* injected in nutrient broth at 37°C. Well diffusion methods were used to derive results. Plates were inoculated for 36 h at 37°C, and microbial zones of inhibition were recorded. Statistical analysis was performed with repeated measures of analysis of variance. **Results:** PAA (1%) and IP6 (1%) showed larger zones of microbial inhibition. PAA (0.5%) was statistically significant ($P < 0.05$) when compared to NaOCl (3% and 5.25%). **Conclusion:** PAA (1%) was used as an irrigating agent in endodontic failure cases.

Keywords: *Enterococcus faecalis, peracetic acid, phytic acid, sodium hypochlorite*

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Introduction

Initial endodontic disease is caused by oral microorganisms, which are usually taking an authority to invade a root canal enclosing necrotic tissue and organize an infectious process. Elimination of these microorganisms from the root canal system is the basis for successful endodontic treatment.[1] Anaerobic bacteria mainly caused periapical diseases. Mostly black-pigmented Gram-negative species will cause signs and symptoms of these diseases.[2] However, after conventional root canal preparation, microorganisms may remain either within the dentinal tubules or bound within the apical dentin plug.[3]

One of the leading causes of root canal therapy failure is the persistence of microorganisms and their reinfection. Engstrom *et al*. recovered numerous species of bacteria from failed root canal cases. *Enterococcus faecalis* was one of the most prevalent bacteria,[4] and its frequency in search infections ranges from 24% to 77%.[5] It can survive very harsh environments, including extreme alkaline pH (9.6) at a temperature of 60°C for 30 min.[6] It had certain mordancy factors such as lytic enzymes, cytolysin, pheromones, and lipoteichoic acid[7] and burke the action of lymphocytes, potentially contributing to endodontic failure.[8] It can survive in well-instrumented and obturated root canal alone and can endure prolonged periods of starvation[5] and passively maintain pH homeostasis because of its functioning proton pump and synthesis a variety of stress protein when exposed to adverse environmental conditions.[9]

Because of the extremely complex root, canal anatomy cleaning of the root canal system using mechanical

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instrumentation alone is ineffective.[10] Around 40%–50% of root canal walls are remaining untouched by mechanical preparation.[11] Hence, cleaning and shaping, along with copious irrigation, further reduced microbes. The various commonly used irrigants are sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), citric acid, chlorhexidine (CHX), gluconate, hydrogen peroxide, povidone-iodine, etc.[12]

NaOCl has a great dentin-disinfecting potential and tissue-dissolving capacity,[13] since it has finite effect on dissolution of smear layer, dimeneralizing agents as adjuvants were used in endodontic therapy.[14] Exposed dentinal tubules may allow the hypochlorite solution to penetrate better into dentin, resulting in cleaner root canal.[15] The main drawback of this causes severe pain, inflammation, swelling, cellular destruction, and necrosis of the vital tissues when extrusion occurs through apices of the teeth.[16]

Hence, a constant increase in antibiotic-resistant strains and side effects caused by synthetic drugs, there is a need for alternative disinfecting measures to overcome the disadvantages. In this era of dentistry ,we are looking towards herbal alternatives like phytic acid(ip6, inositol hexakisphosphate).

Phytic acid (IP6, inositol hexakisphosphate) is a naturally occurring compound first identified in 1855 that was proposed to have superior properties as newer chelating agents. It is available in whole grains, cereals, legumes and rice bran seeds. It is economical, and it contains phytic as the primary storage form of phosphorus that contributes to a wide variety of cellular functions found in most mammalian cells at a concentration ranges from 10 to 100 mmol/l.[17]

IP6 has multiple negative charges, making it an effective chelator of multivalent cations such as calcium (Ca^{+2}), magnesium, and iron. IP6 is a highly negatively charged molecule that has an affinity to Ca^{+2}. The pH of 1% IP6 solution was around 1.2, and this acidity contributed to better Ca^{+2} extraction. Thus, both the acidity and chelate function of IP6 made it an effective smear layer removal agent. It did not show a negative effect on the viability and alkaline phosphatase (ALP) activity of MC3T3-E1 cells.[18] It is preventing the generation of reactive oxygen species responsible for cell injury and carcinogenesis. Based on these proposed properties, IP6 has the potential to replace EDTA as a root canal chelating agent.[18]

The search for alternative irrigating solutions has focused on substances with antibacterial effect and capacity to clean the dentin surface. A promising material to be used as a chelating agent with antimicrobial action is peracetic acid (PAA).[19] Despite being widely used in endodontics in Eastern Europe in the year 1980, PAA has only recently suggested as an auxiliary chemical solution with the potential to replace EDTA in the final irrigation procedure after biomechanical preparation.[20]

PAA action is both time and concentration dependent, so that longer exposure times and higher concentrations could show antimicrobial activity similar to that of NaOCl according to Ordinola-Zapata *et al*.[21] A study done by De-Deus *et al* has shown the biofilm dissolving ability of PAA.[22] PAA is a recognized disinfectant that exhibits antibacterial, sporicidal, antifungal, and antiviral properties used in hospitals and food industries.[23] It has a broad spectrum of action in the presence of heterogeneous organic matter, even at low concentrations of 0.5%.[24] The mechanism of the action of PAA decomposed into safe by-products such as acetic acid, and oxygen highlights the possibility as a final irrigant to dissolve the smear layer and antisepsis of the root canal system.[19]

As of now, there is no sufficient scientific research literature available that can evaluate the antimicrobial properties of IP6 and PAA, as root canal irrigants. The main purpose of this *in vitro* study was to evaluate the antimicrobial efficacy of PAA (0.5% and 1%), IP6 (0.5% and 1%), and NaOCl (3% and 5.25%) as root canal irrigants against *E. faecalis*.

Materials and Methods

Standard strains of *E. faecalis* (NCIM-5443) spores extracted from the National Collection of Industrial Microorganisms, Pune. The bacterium was grown and maintained on nutrient broth (High Media Laboratories, Mumbai). Initially, as per the manufacturer's instructions, the viability of spores was checked by reviving a pellet from the available vial of *E. faecalis* into 5 ml of nutrient broth at 37°C for 18 h followed by observing changes in turbidity to check bacterial growth.

Nineteen agar plates prepared using Mueller-Hinton (MH) agar mixed according to the manufacturer's directions and enough agar was poured to cover the surface of a 125-mm sterile Petri dish. The MH agar dishes were stored at room temperature for solidification. *E. faecalis* was inoculated into these MH agar plates and incubated at 37°C for 24 h.

Root canal irrigants used in this study contain 0.5% and 1% of PAA, 0.5% and 1% of IP6, and 3% and 5.25% of NaOCl (Leo Chemicals, Bangalore, Karnataka, India) obtained.

Wells were prepared using borates of sizes of 6-mm diameter and 3-mm depth which were punched with

the help of these borates in agar plates. Moreover, the respective tested solutions introduced with the help of sterile micropipette tips contain 10 µl of the respective tested solution introduced into these wells tested. Moreover, then, the plates were incubated at 37°C for 36 h.

All manipulations of the specimens were performed under a laminar flow chamber to avoid contamination, and then, the growth was observed.

Results

The mean values of Zones of Inhibition were calculated at in millimeter at 36hrs [Table 1] Statistical analysis was done using one-way ANOVA for intragroup comparison and *post hoc* test was used for intergroup comparison. Statistical analysis was made using one-way ANOVA and *post hoc* tests. It observed that PAA (1%) showed statistically ($P < 0.05$) more zone of microbial inhibition than PAA (0.5%), IP6 (0.5% and 1%), and NaOCl (3% and 5.25%). IP (1%) and PAA (0.5%) showed significantly larger zones of microbial inhibitions than NaOCl (3% and 5.25%) and IP6 (0.5%). The zone of inhibition between PAA (1%) and IP6 (1%) was not significant ($P > 0.05$), and the control group showed no microbial inhibition [Figures 1and 2].

Discussion

Failure of endodontic treatment can be attributed to the fact that mechanical instrumentation alone does not eliminate the bacterial load. The leading cause of endodontic failure may occur from residual bacteria within the root canal system and dentinal tubules resulting in recolonization after obturation.[25] Ideally, root canal irrigants should have a broad antimicrobial spectrum, especially against anaerobic and facultative microorganisms. When irritant comes in contact with vital tissues, it should be nontoxic, noncaustic to periodontal tissues, and had less potential for an anaphylactic reaction. It should dissolve necrotic pulp tissue remnants, inactivate endotoxins, prevent formation, or elimination of smear layer during instrumentation.[26-28]

Due to anatomical complexities or may be due to varying vulnerabilities of involved species, complete elimination of microorganisms from the root canal is not possible despite antimicrobial properties of chemomechanical preparation and intracanal medicaments.[29,30] The most commonly isolated species from failed endodontic treatment is *E. faecalis*.[31] The portal of entry into the root canal during treatment, between appointments, is after root canal completion.[7] *E. faecalis* could invade dentinal tubules and adhere to collagen in the presence of human serum.[32]

In this *in vitro* study, antimicrobial activity of various root canal irrigating agents was compared to eliminate the endodontic pathogen responsible for root canal failure. In the current study, NaOCl at 3% and 5.25% is a powerful antimicrobial agent against *E. faecalis.* It is showing fewer inhibition zones, which ranged from 28 mm to 31 mm.

The bacterial ability is due to the formation of hypochlorous acid (HOCl) wherein contact with organic debris. HOCl exerts its effects by oxidizing sulfhydryl groups within the bacterial enzyme system, which disrupts the metabolism of microbes resulting in the killing of bacterial cells.[33,34] NaOCl recommended for endodontic treatment; it has a more significant antibacterial effect at a higher concentration than the diluted level.[35] 5.25% NaOCl was efficacious in eliminating the bacterial strains commonly found in infected root canal but at this concentration has increased toxicity and can irritate the periapical tissues[36,37] and thus affect the prognosis of posttreatment recovery or even result in chronic postoperative pain. Hence, to

Table 1: Antimicrobial effect of irrigating agents on *Enterococcus faecalis*

Root canal irrigating agents	Concentration (%)	Mean zone of inhibition (mm)
PAA	0.5	38 [Figure 2d]
	1	48 [Figure 2a]
IP6	0.5	31 [Figure 2e]
	1	39 [Figure 2b]
NaOCl	3	28 [Figure 2f]
	5.25	31 [Figure 2c]
Control	0	0 [Figure 2g]

PAA=Peracetic acid; IP6=Phytic acid; NaOCl=Sodium hypochlorite

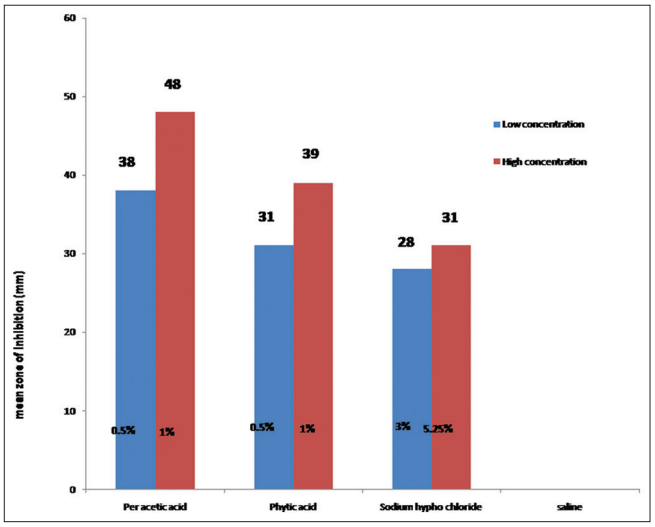

Figure 1: mean zone of inhibition

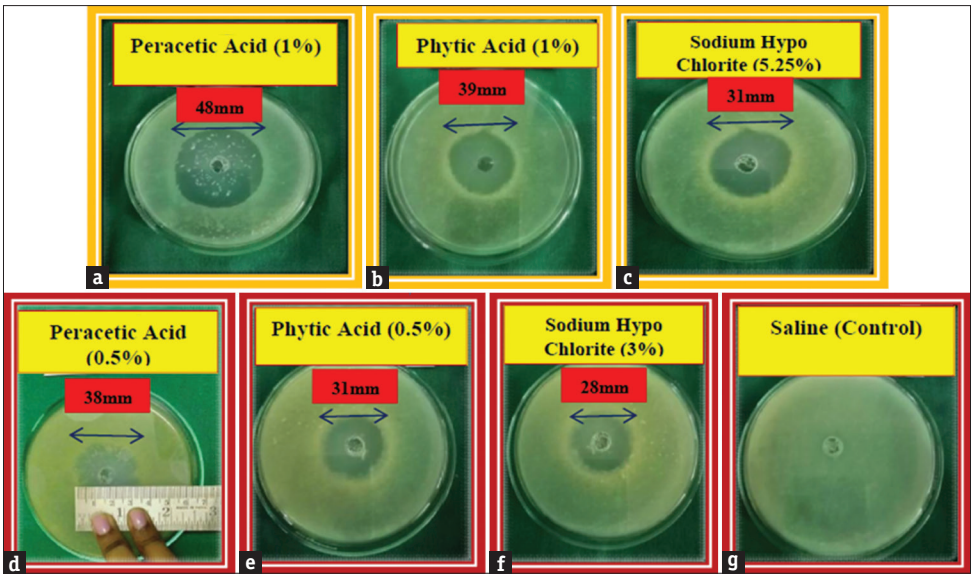

Figure 2: diameter of Zones of Inhibition in millimeters of various irrigating solutions against *E.faecalis* [a-g]

prevent NaOCl accidents, it should be used with caution in endodontic therapy.

In the present study, newer plant-based organic material is IP6, which is a natural extract from rice bran. Nassar *et al*. did a study state that the bactericidal effect of IP6 was much more significant than other organic acids. The antimicrobial effect was mainly explained by weak acid theory. Due to its unique structure of IP6 and wide acidity range, the antimicrobial activity is likely to be different.[38] A study done by Puvvada *et al*. states that 1% IP6 shows a more substantial zone of inhibition when compared to irritants such as NaOCl, CHX, and EDTA[17] against *E. faecalis*.

In the present study evaluating the antimicrobial efficacy against *E. faecalis,* it was showing a more substantial zone of inhibition IP6 (0.5% and 1%), which ranged from 31 mm to 39 mm. A study done by Nassar *et al*. states that the pH of a 1% IP6 solution was around 1.2, and this acidity contributed to better Ca^{+2} extraction. A study done by Nassar *et al* concluded that IP6 was able to remove the smear layer from flat coronal dentin surfaces and instrumented root canals and did not show negative effect on the viability and ALP activity of MC3T3-E1 cells.[18] It is binding of calcium with IP6 is pH dependent, which showed superior antimicrobial ability when compared to conventional root canal irrigants such as NaOCl, CHX, and EDTA.

In the present study, among irrigants, a 1% PAA shows a broad range of the antimicrobial spectrum, which showed the highest zone of microbial inhibition ranging from 48 mm to 45 mm. PAA is an equilibrium mixture between acetic acid and hydrogen peroxide in aqueous solution and has a strong oxidation potential,[39-41] and formed by-products have little or no toxicity.[42]

PAA is stable at pH equal to or less than its PKA of 8.2.[43] The mechanism behind the antimicrobial action of PAA possibly attributed to its effect on the lipoproteins in the cell membrane, which results in disruption of the lipoprotein cytoplasmic membrane or cell walls due to oxidative stress and subsequently denaturation of intracellular enzymes and other essential macromolecules.[44] A 0.5% PAA showed a higher antimicrobial efficacy compared to 17% EDTA in *ex vivo E. faecalis* model, a study done by Hartmann *et al*.[45] In another survey by Shirley de Souza Pinto *et al*., a 2% PAA ultimately killed *E. faecalis* after 15 s of exposure.[46] These results clearly show that PAA (1%) is the most effective root canal irrigant.

In a study conducted by Dornelles-Morgental *et al*., a 1% PAA solution was reported as having antimicrobial action against *E. faecalis* similar to that of 2.5% NaOCl and 2% CHX. Another property that potentially allows PAA to be recommended as an endodontic irrigating solution is its ability to dissolve smear layers. In a recently conducted study, a 1% PAA was significantly more effective in removing calcium hydroxide from root canals compared with 2.5% NaOCl or 17% EDTA + 2.5% NaOCl.

Because the use of PAA as a substitute for EDTA may be clinically advantageous because of the possibility of optimizing root canal disinfection, further studies are essential to confirm the antimicrobial efficacy of PAA that may use as an alternative to conventional root canal irrigants.

Conclusion

The present study confirms that PAA (1%) showed promising results in the elimination of *E. faecalis* one of the collective organisms responsible for root canal failure, suggesting that it is to be used as an endodontic irrigating solution and as an excellent alternative to all conventional root canal irrigants and shows the least resistance developed by the species. A 1% PAA showed more zone of inhibition, indicating that its antimicrobial efficacy is more when compared to other irritants. However, further research is needed to evaluate its biocompatibility and its action in dissolving organic tissues.

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Conflicts of interest

There are no conflicts of interest.

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