Antimicrobial Activity of Phytic Acid: An Emerging Agent in Endodontics

Rania Nassar1,2*, Mohannad Nassar3, Morgana E. Vianna2, Nerissa Naidoo1, Fatma Alqutami1, Eleftherios G. Kaklamanos4, Abiola Senok1 and David Williams2

1 College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, United Arab Emirates, 2 Oral and Biomedical Sciences, School of Dentistry, College of Biomedical and Life Sciences, Cardiff University, Cardiff, United Kingdom, 3 Department of Preventive and Restorative Dentistry, College of Dental Medicine, University of Sharjah, Sharjah, United Arab Emirates, 4 Hamdan Bin Mohammed College of Dental Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, United Arab Emirates

Background: Phytic acid (IP6) is a promising and emerging agent, and because of its unique structure and distinctive properties, it lends itself to several applications in dentistry. Recently, IP6 was proposed as a potential chelating agent in endodontics. However, there is limited knowledge regarding its antimicrobial and antibiofilm effectiveness. The aims of this study, were therefore to evaluate the antimicrobial and antibiofilm activities of IP6 against a range of microbial species and compare these with ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite (NaOCl). The contact time required for IP6 to exert its bactericidal effect on Enterococcus faecalis was also determined.

Methods: The inhibitory and biocidal activities of IP6, EDTA and NaOCl were assessed using a broth microdilution assay against 11 clinical and reference strains of bacteria and a reference strain of Candida albicans. The contact time required for various IP6 concentrations to eliminate planktonic cultures of E. faecalis was determined using a membrane filtration method according to BS-EN-1040:2005. IP6 bactericidal activity was also evaluated using fluorescent microscopy, and the antibiofilm activity of the test agents was also determined.

Results: IP6 was biocidal against all tested microorganisms. At concentrations of 0.5%, 1% and 2%, IP6 required 5 min to exert a bactericidal effect on E. faecalis, while 5% IP6 was bactericidal after 30 s. IP6 also eradicated biofilms of the tested microorganisms. In conclusion, IP6 had notable antimicrobial effects on planktonic cultures of E. faecalis and sodium hypochlorite (NaOCl). The contact time required for IP6 to exert its bactericidal effect on Enterococcus faecalis was also determined.

Keywords: antibacterial, antibiofilm activity, EDTA, dental biofilm, Enterococcus faecalis, phytic acid
1 INTRODUCTION

Microbial biofilms are integral to the failure of root canal treated teeth (Ricucci et al., 2016; Neelakantan et al., 2017; Pereira et al., 2017). Key objective of root canal treatment is therefore the reduction or elimination of biofilm from the root canal system through use of antimicrobial agents (Rahimi et al., 2014; Jhajharia et al., 2015). Ideally, root canal irrigants should have broad antibacterial efficacy, be able to remove the smear layer, dissolve organic tissue and should not be toxic to surrounding tissue (Haapasalo et al., 2014). Sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) are the main irrigants currently used in endodontics (Cheung and Stock, 1993; Hülsmann et al., 2003; Haapasalo et al., 2014). NaOCl exhibits bactericidal activity (Retamozo et al., 2010) and can dissolve vital and necrotic organic tissue (Naenni et al., 2004). However, NaOCl has no effect on the inorganic components of the smear layer that forms following mechanical instrumentation (Torabinejad et al., 2003a). As a result, a metal chelator is required as an adjuvant irrigating solution to fully remove the smear layer. Due to its ability to remove the smear layer, EDTA is the chelating agent of choice for this purpose (Violich and Chandler, 2010). However, EDTA has several drawbacks, including toxicity to periradicular tissue and an inability to eradicate bacteria which may have a negative impact on treatment outcome (Spangberg et al., 1973; Tanomaru Filho et al., 2002; Amaral et al., 2007). In addition, EDTA is considered a pollutant (Sillanpää, 1997; Oviedo and Rodriguez, 2003), and the relatively high EDTA concentration (15-17%) used in dentistry (Haapasalo et al., 2014) is a concern. Therefore, it is imperative to identify alternative chelating agents for root canal treatment.

Phytic acid (IP6) is the major storage form of phosphorus in plant seeds and grain and is extracted using a simple and cost effective process (Bloit et al., 2021). Due to its unique structure and distinctive properties, IP6 lends itself to several dental applications (Nassar et al., 2021), such as dental adhesives (Nassar et al., 2013; Forgione et al., 2021) and an endodontic irrigant (Nassar et al., 2015). The high negative charge density of IP6 facilitates chelating activity with cations, thus IP6 has been proposed as an alternative root canal chelating agent with an ability to remove the smear layer, whilst also being biocompatible with osteoblast-like cells (Nassar et al., 2015). Enterococcus faecalis is one of the most frequently encountered microorganisms in cases of endodontic treatment failure (Roças et al., 2004), where persistent infection is commonplace and possibly related to the ability of this species to form biofilms, invade dentinal tubules and resist bactericidal agents in endodontic procedures (Zhang et al., 2015; Alghamdi and Shakir, 2020). Streptococcus and Staphylococcus species are also often isolated from persistent root infections (Pinheiro et al., 2003; Murad et al., 2014). In addition to bacteria, fungi may also be involved in root canal infections and in such cases, Candida albicans is the species most frequently isolated (Persoon et al., 2017). In preliminary in vitro experiments, we reported that IP6 had bactericidal effects on planktonic E. faecalis (Nassar and Nassar, 2017). However, there is a paucity of knowledge on the antimicrobial spectrum of IP6 and its mechanism of action. There is therefore a need to study IP6’s antimicrobial activity against a range of Gram-positive and Gram-negative bacteria with different susceptibility profiles. As root canal infections are biofilm-associated (Ricucci and Siqueira, 2010), it is important that the irrigant eradicates biofilm and constituent bacteria. To the best of our knowledge, there are no reports of IP6’s antibiofilm activity, nor the contact time and concentration required for antimicrobial effects against E. faecalis. This study was therefore undertaken to investigate the antimicrobial and antibiofilm spectrum of IP6 and compare this with EDTA and NaOCl. In addition, the contact time required for different concentrations of IP6 to its exert bactericidal effects on E. faecalis was also determined.

2 MATERIAL AND METHODS

2.1. Experimental Agents and Test Microorganisms

Test agents included IP6 (50% (w/w) in H2O) (Sigma Aldrich), 5.25% NaOCl (Chlorox; Cerkamed, Poland) and EDTA (0.5 M, 18.61% (w/v), pH 8) (Sigma Aldrich). Two-fold serial dilutions (up to 512-fold dilution) of these test agents were prepared for susceptibility testing and the highest IP6 concentration was 40%. Susceptibility of both planktonic and biofilm cultures of reference and clinical microorganisms to the agents was undertaken. Reference strains were commercially sourced, while clinical strains were obtained from stock cultures at the School of Dentistry, Cardiff University (Table 1).

2.2. Antimicrobial Activity Against Planktonic Cultures

2.2.1. Minimum Inhibitory Concentration (MIC) of Test Agents

The antimicrobial activities of IP6, EDTA and NaOCl were assessed against planktonic cultures using a broth microdilution method. The antimicrobial activities of IP6, EDTA and NaOCl were assessed against planktonic cultures using a broth microdilution method.

### Table 1: List of the reference and clinical microorganisms used in this study.

| Microorganism                  | Stain reference/origin                  |
|-------------------------------|-----------------------------------------|
| Enterococcus faecalis (VRE)   | Clinical strain (Kuriyama et al., 2003) |
| Enterococcus faecalis (VSE)   | Oral strain (United Kingdom)             |
| Enterococcus faecalis (VRE)   | Hospital/Environnement strain (Kuriyama et al., 2003) |
| Enterococcus faecalis (VSE)   | Clinical strain (Japan)                  |
| Staphylococcus aureus (MSSA)  | ATCC 25923                               |
| Staphylococcus aureus (MRSA)  | ATCC 43300                               |
| Pseudomonas aeruginosa         | ATCC 9027                                |
| Escherichia coli               | ATCC 25922                               |
| Enterococcus faecalis (VSE)   | ATCC 29212                               |
| Enterococcus faecalis (VRE)   | ATCC 51299                               |
| Streptococcus mutans          | ATCC 25175                               |
| Candida albicans              | ATCC 90028                               |

VSE: Vancomycin Susceptible Enterococcus; VRE: Vancomycin Resistant Enterococcus; MSSA, Methicillin Resistant Staphylococcus aureus; MRSA, Methicillin Resistant Staphylococcus aureus; VRE, Vancomycin Resistant Enterococcus; MRSA, Methicillin Resistant Staphylococcus aureus; MSSA, Methicillin Susceptible Staphylococcus aureus.
assay to determine minimum inhibitory concentrations (MICs). Bacteria were cultured on Tryptone Soya Agar (TSA) for 24 h at 37°C, while *C. albicans* was cultured on Sabouraud Dextrose Agar (SDA) in 5% CO₂, at 37°C for 48 h. Isolated colonies were used to prepare bacterial suspensions in Mueller Hinton Broth (MHB), whilst *C. albicans* suspensions were prepared in RPMI-1640 medium (Lonza, USA). Microbial suspensions were adjusted to a turbidity equivalent to a 0.5 McFarland standard, and then diluted 100-fold in either MHB (for bacteria) or RPMI-1640 (for *C. albicans*). One hundred µl of each suspension was then added to a turbidity equivalent to a 0.5 McFarland standard, and then diluted 100-fold in either MHB (for bacteria) or RPMI-1640 for 24-72 h at 37°C. Tests were performed on a minimum of three different occasions. Control and validation tests were undertaken throughout the experiment according to BS-EN-1040:2005 (BSi, 2006). Purity checks of the bacterial suspensions and sterility of reagents were achieved by culturing on TSA. The log reduction (R) was calculated using the following equation: logR = logN₀ - logNₐ, where N₀ was the bacterial count (CFU/ml) in the test mixture at contact time of 0 (before treatment) and Nₐ was the surviving bacterial count (CFU/ml) after the contact time (post treatment). A concentration of IP6 was considered to exert bactericidal activity when it produced a log reduction of 5 or more (BSi, 2006).

### 2.3. Determination of Minimum Biofilm Eradication Concentration of Test Agents

Isolated colonies from 24 h agar cultures of bacteria and 48 h agar cultures of *C. albicans* were used to prepare microbial suspensions in MHB or RPMI-1640, respectively. The resulting suspension was adjusted to a turbidity equivalent to a 0.5 McFarland standard for bacteria and a 1.0 McFarland standard for *C. albicans*. One hundred µl of the microbial suspension was then added to the wells of a 96-well (flat-bottom) microtiter plate. Peripheral wells were unused to avoid potential edge effects. Microtiter plates were incubated for 24 h at 37°C to facilitate biofilm formation. After incubation, culture medium was aspirated, and each well washed twice with 150 µl of PBS to remove planktonic cells and any excess washing medium was aspirated. One hundred µl of MHB or RPMI-1640 was added to each well, together with 100 µl of diluted test agent. Controls included uninoculated wells with fresh culture medium only or untreated biofilms. The plates were incubated for 24 h. Antimicrobial solutions were removed, and the wells gently washed twice with 200 µl of PBS. Two hundred µl of MHB or RPMI-1640 were added to the wells and the plates were incubated for 24-72 h. Microbial growth was then assessed visually and by measuring turbidity at 630 nm using a plate reader. The minimum biofilm eradication concentration (MBEC) was determined relative to the turbidity of a growth control (100% growth) and a medium-only control (0% growth). The MBEC was determined when there was no visual turbidity.
or the spectrophotometric readings were ≥80% reduced relative to the untreated control. Three independent experiments, each including 3 replicates were performed.

3 RESULTS

3.1. Minimum Inhibitory Concentration (MIC)

All agents were effective at inhibiting planktonic growth of test microorganisms. MICs for IP6 were 0.156% for all bacteria except for S. mutans, which was 0.078%, and 1.25% for C. albicans. NaOCl inhibited bacteria and C. albicans growth at 0.041% and 0.005%, respectively. While the MICs for EDTA ranged between 0.018% and 0.145% (Table 2).

3.2. Minimum Bactericidal Concentration (MBC) of Test Agents

Table 2 shows the MBCs for test agents. IP6 was bactericidal against all bacteria at a concentration of 0.313%, which was generally two-fold higher than the MIC. Exceptions to this were for P. aeruginosa where the MBC and MIC were the same, and for S. mutans, where the MBC was 4-fold higher than the MIC. IP6 exerted biocidal activity against C. albicans at higher concentrations (10%) than the MIC. NaOCl was bactericidal at 0.041%, Whilst EDTA was not bactericidal, except for E. coli and P. aeruginosa at concentrations of 2.326% and 9.305%, respectively.

3.3. Fluorescent Microscopy

Fluorescent microscopy revealed that the number of dead (red stained) E. faecalis cells increased with duration of contact time with 0.313% IP6 (Figure 1). The emission of red fluorescence from cells stained with propidium iodide (PI) indicated loss of membrane integrity, as PI only penetrates cells with compromised membranes.

3.4. Antimicrobial Contact Time

Table 3 shows the log reduction of E. faecalis ATCC 29212 planktonic cultures after IP6 treatment at different contact times. All tested concentrations of IP6 were effective at killing the bacteria, but at different contact times. IP6 (0.5%) required 5 min to exert a bactericidal effect, while at 1% and 2% IP6 exerted a bactericidal effect, with total reduction of viable cells counts at 5 min. IP6 (5%) required 30 s to exert a bactericidal effect and 1 min to give total reduction of viable cells counts.

3.5. Minimum Biofilm Eradication Concentration (MBEC)

IP6 was able to eradicate established biofilms of all tested microorganisms with an MBECs ranged between 0.156% and 10%. The highest MBEC for IP6 was against C. albicans (Table 4). NaOCl eradicated biofilms of all tested organisms at concentrations between 0.041% and 0.164% (Table 4). EDTA did not eradicate biofilms of the tested organisms, except for E. coli at concentrations between 2.3% and 9.3% (Table 4).

4 DISCUSSION

An ideal endodontic irrigant should possess several properties including an ability to remove the smear layer, have biocompatibility with vital tissues, and should have a broad spectrum antimicrobial effect with a rapid action to minimize adverse effects on root canal dentin (Haapasalo et al., 2014). However, none of the currently used irrigants fulfill all of these properties (Calt and Serper, 2002).

Due to its broad spectrum antimicrobial activity and ability to dissolve organic tissue (Zehnder et al., 2005), NaOCl is the gold standard disinfectant in endodontic treatment, despite its well-recognised drawbacks (Zehnder, 2006). NaOCl lacks the ability to remove inorganic materials, hence the necessity to use chelating agents following NaOCl treatment (Zehnder, 2006). EDTA is typically used as a chelating agent in root canal treatment to remove the smear layer (Calt and Serper, 2002) with a recommended contact time of 1-5 min. Despite the popularity of EDTA, the associated disadvantages of this irrigant have led to the search for alternative agents (Haapasalo...
It has previously been shown that the gold standard irrigation protocol (NaOCl followed by EDTA) could not eradicate *E. faecalis* and *C. albicans* biofilms (Alshanta et al., 2020). This was not attributed to the buffering effect of dentine or the complex root canal anatomy, but rather to the high tolerance of biofilm to antimicrobials (Alshanta et al., 2020). It is clearly desirable therefore to identify a chelating agent with enhanced antimicrobial properties.

**TABLE 3** Log reduction of *E. faecalis* ATCC 29212 compared to the untreated control (log N₀) using IP6 for 30 s, 1 min or 5 min.

| Contact time | 0.5% IP6 | 1% IP6 | 2% IP6 | 5% IP6 |
|--------------|---------|-------|-------|-------|
| 30 s         | Log N₀  | Log R | Log N₀ | Log R | Log N₀ | Log R | Log N₀ | Log R |
|              | 7.13 (± 0.18) | < 3.91 | 7.13 (± 0.18) | < 3.91 | 7.13 (± 0.18) | < 3.91 | 7.08 (± 0.12) | 5.52 (± 0.73) |
| 1 min        | 7.13 (± 0.18) | < 3.91 | 7.13 (± 0.18) | < 3.91 | 6.99 (± 0.22) | < 3.78 | 7.23 (± 0.10) | 7.23 (± 0.10) |
| 5 min        | 7.04 (± 0.10) | 5.37 (± 0.43) | 7.13 (± 0.18) | 7.13 (± 0.18) | 7.13 (± 0.18) | 7.13 (± 0.18) | 7.23 (± 0.10) | 7.23 (± 0.10) |

Standard Error of Means are shown in parentheses.

**TABLE 4** Minimum biofilm eradication concentration (MBEC) of IP6, EDTA or NaOCl against test microorganisms.

| IP6                | EDTA               | NaOCl              |
|--------------------|--------------------|--------------------|
| Minimum (%)        | Maximum (%)        | Mode               | Minimum (%) | Maximum (%) | Mode |
| 0.313              | 2.50               | 1.25               | R           | 0.041       | 0.082          | 0.082 |
| E. faecalis (VRE)  | Clinical strain    | (Kuriyama et al., 2003) |           |       |
| 0.625              | 1.25               | 0.625              | R           | 0.02       | 0.082          | 0.082 |
| E. faecalis (VSE)  | Oral strain (UK)   | (Lins et al., 2019) |           |       |
| 0.313              | 2.50               | 0.313*              | R           | 0.041      | 0.164          | 0.041* |
| E. faecalis (VRE)  | Hospital/Environmenatal strain | (Kuriyama et al., 2003) |           |       |
| 0.625              | 2.50               | 0.625              | R           | 0.082      | 0.082          | 0.082 |
| S. aureus (MSSA) ATCC 25923 | |           |       |
| 0.625              | 2.50               | 0.625              | R           | 0.082      | 0.082          | 0.082 |
| P. aeruginosa ATCC 9027 | |           |       |
| 0.156              | 1.25               | 0.313*              | R           | 0.041      | 0.082          | 0.082 |
| E. coli ATCC 25922 | |           |       |
| 0.156              | 0.625              | 0.156*              | 2.326       | 9.305      | 2.326          | 0.082      | 0.082          | 0.082 |
| E. faecalis (VSE) ATCC 29212 | |           |       |
| 0.313              | 2.50               | 0.625              | R           | 0.041      | 0.082          | 0.041 |
| E. faecalis (VRE) ATCC 51299 | |           |       |
| 0.313              | 1.25               | 0.625              | R           | 0.041      | 0.082          | 0.082 |
| S. mutans ATCC 25175 | |           |       |
| 0.156              | 0.313              | 0.313               | R           | 0.041      | 0.082          | 0.082 |
| C. albicans ATCC 90028 | 1.25 | 10 | 1.25* | R | 0.041 | 0.082 |

VSE, Vancomycin Susceptible Enterococcus; VRE, Vancomycin Resistant Enterococcus; MRSA, Methicillin Resistant Staphylococcus aureus; MSSA, Methicillin Susceptible Staphylococcus aureus.

*Multiple modes exist. The lowest value is shown. R, resistance to all concentration tested.

It has previously been shown that the gold standard irrigation protocol (NaOCl followed by EDTA) could not eradicate *E. faecalis* and *C. albicans* biofilms (Alshanta et al., 2020). This was not attributed to the buffering effect of dentine or the complex root canal anatomy, but rather to the high tolerance of biofilm to antimicrobials (Alshanta et al., 2020). It is clearly desirable therefore to identify a chelating agent with enhanced antimicrobial properties.
IP6 is a natural organic acid that is extracted from rice bran (Graf, 1983). IP6 has multiple negative charges with an ability to chelate with multivalent cations (Luttrell, 1993; Torres et al., 2005) and has been proposed as a potential alternative to EDTA (Nassar et al., 2015). IP6 effectively removes the smear layer with minimal adverse effects on osteoblast cells (Nassar et al., 2015). Despite use of IP6 in several medical, dental, nutritional, and industrial applications, there is limited knowledge concerning its antimicrobial effectiveness and mode of action. Elucidating such effects might result in the development of novel IP6 products for a range of applications, including in endodontics (Nassar et al., 2021).

In general, EDTA has little or no antibacterial effect (Torabinejad et al., 2003b). Indeed, it has been suggested that EDTA does not exert an antimicrobial effect per se, but rather reduces the amount of microorganisms in root canal dentin via removal of the smear layer (Di Lenarda et al., 2000). The results of the present in vitro study showed that EDTA did not have bactericidal effect against planktonic cultures except for Gram-negative bacteria. This finding agreed with previously published results, where EDTA affected Gram-negative bacteria by combining with cations of the outer cell membrane, leading to altered permeability and metabolism (Vaara, 1992; George et al., 2009). In contrast, a bactericidal effect on Gram-positive bacteria, specifically E. faecalis, has either been absent or minimal (Torabinejad et al., 2003b; Arias-Moliz et al., 2008; Zhang et al., 2015), which is consistent with our present study. It was also reported that different concentrations of EDTA were not able to eradicate E. faecalis biofilms (Arias-Moliz et al., 2009) and this is in agreement with our results, where we revealed an inability of EDTA to eradicate established biofilms of E. faecalis and all tested organisms except E. coli. Moreover, our data showed that EDTA did not have a biocidal effect on C. albicans and was unable to eradicate its biofilms. As expected, NaOCl showed biostatic, biocidal and antibiofilm activity against all tested organisms, where the minimum concentration needed for NaOCl to eradicate established bacterial biofilms was the same, or only 2-fold greater than the concentration required to kill planktonic counterparts. However, the minimum NaOCl concentration needed for C. albicans biofilms eradication was 8-fold higher than the concentration needed to kill planktonic counterparts and this is indicative of higher tolerance of C. albicans biofilms to antimicrobials (Alshanta et al., 2019; Alshanta et al., 2020).

For the first time, the antimicrobial and antibiofilm effects of IP6 using a broad spectrum of microorganisms have been reported. To the best of our knowledge, no study has reported the optimal irrigation time required for different concentrations of IP6 to exert antimicrobial effects against E. faecalis. Our findings showed that IP6 had biostatic and biocidal activities against a variety of microbial strains, including Gram-positive and Gram-negative bacteria, drug resistant strains, and C. albicans. Furthermore, a bactericidal effect on E. faecalis, was achieved after a contact time of 5 min with 0.5%, 1% and 2% IP6. A reduced contact time of 30 s was required for 5% IP6 to be bactericidal. IP6’s rapid action highlights its potentials for use in strategies where rapid bactericidal activity is required. The broad-spectrum antibacterial activity of IP6 and its rapid action, may indicate that its bactericidal mechanism of action is through membrane disruption. Few studies have elucidated the antimicrobial aspects of IP6 and most, if not all, emanate from the food industry where IP6 is tested for its ‘environmentally friendly’ application for food preservation and safety, and prolonging food shelf-life (Kim and Rhee, 2016; Boukhris et al., 2020). Therefore, to aid comparison of our results with others, we included P. aeruginosa and E. coli in our experiments. Kim and Rhee (2016) showed that IP6 exerted its bactericidal effects at higher concentrations compared to our results, and this may be explained by the different E. coli strains used in the studies. Meanwhile, Boukhris et al. (2020) found that IP6 exerted inhibitory effects on S. aureus, E. coli and P. aeruginosa at lower concentrations than those reported in this current study. It has been suggested that IP6 exerts its bactericidal effect on E. coli through disruption of the cell membrane (Kim and Rhee, 2016), thus causing excessive cell permeability, which then leads to changes in cell morphology and reduction in intracellular ATP concentration (Zhou et al., 2019). Deferoxamine, a drug that has shown the ability to disrupt biofilms integrity (Ishida et al., 2011), was found to have properties similar to IP6, where both have significant iron-chelating properties (Hawkins et al., 1993). Studies have shown that iron-chelating agents are effective against pathogenic organisms (Coraca-Huber et al., 2018; Scott et al., 2021), presumably because iron is an important component for bacterial proliferation and biofilm formation (Lin et al., 2012; Farrand et al., 2015). Hence, it is plausible to speculate that iron-chelation by IP6 might play a role in its broad-spectrum antibacterial and antibiofilm activity reported in this study. Further in vitro and in vivo studies are, however, required to fully understand its mechanism.

The failure of root canal treatment is not uncommon and likely due to the high tolerance of associated biofilms to antimicrobials (Ricucci et al., 2013). Therefore, it is imperative for root canal irrigants to eradicate these recalcitrant biofilms. For the first time, IP6 has been shown to be effective in eradicating biofilms of a variety of Gram-positive, Gram-negative bacteria and C. albicans. The superiority of IP6 over EDTA in terms of its broad antimicrobial and antibiofilm activity has been demonstrated. The fact that IP6’s antimicrobial activity required minimal time, coupled with previous data showing its ability to remove smear layer while being more biocompatible than EDTA (Nassar et al., 2015), indicates that IP6 is potentially an ideal root canal chelating agent. In this present in vitro study, a single-species biofilm model was employed to investigate IP6 antibiofilm activity, however, since most root canal infections are polymicrobial in nature, our future investigations will aim to investigate IP6 activity against complex polymicrobial biofilms. Further in vitro and in vivo studies are also warranted to determine optimal concentrations for the clinical use of IP6.

CONCLUSION

In summary, within the limitation of this in vitro study, our data revealed the antimicrobial efficiency of IP6 against planktonic...
cultures of microorganisms with a rapid bactericidal action against *E. faecalis* planktonic cultures. Furthermore, for the first time we reported the antibiofilm efficacy of IP6 against a range of microorganisms with clinical relevance in dentistry and other human infections.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

**AUTHOR CONTRIBUTIONS**

RN, MN, MV, AS and DW conceived the study. RN designed and undertook laboratory experiments, analysed the data and wrote the first draft of the manuscript. FA contributed to data collection. MN, AS and DW contributed to data analysis. RN, MN, MV, NN, EK, AS and DW contributed to critical review and interpretation. All authors contributed to manuscript revision and approved the submitted version.

**REFERENCES**

Alghamdi, F., and Shakir, M. (2020). The Influence of Enterococcus Faecalis as a Dental Root Canal Pathogen on Endodontic Treatment: A Systematic Review. *Cureus* 12 (3). doi: 10.7759/cureus.7257

Alshanta, O. A., Shaban, S., Nile, C. J., McLean, W., and Ramage, G. (2019). Antimicrobial Activity of Antibiotics Against Coagulase-Negative Staphylococci. *Pathog. Dis.* 76 (5), ft052. doi: 10.1093/femspd/ft052

Di Lenarda, R., Cadenaro, M., and Shaizero, O. (2000). Effectiveness of 1 Mol L–1 Citric Acid and 15% EDTA Irrigation on Smear Layer Removal. *Int. Endod. J.* 33 (1), 46–52. doi: 10.1111/j.1365-2591.2000.00273.x

Farrand, A. J., Friedman, D. B., Reniere, M. L., Ingmner, H., Frees, D., and Skaar, E. P. (2015). Proteomic Analyses of Iron-Responsive, Clp-Dependent Changes in Staphylococcus Aureus. *Pathog. Dis.* 73 (3), ftv004. doi: 10.1093/femspd/ftv004

Frogione, D., Nassar, M., Seseogullari-Dirihan, R., Thithaweeraset, S., and Tezvergil-Mutluay, A. (2021). The Effect of Phytic Acid on Enzymatic Degradation of Dentin. *Eur. J. Oral. Sci.* 129 (2), e12771. doi: 10.1111/eos.12771

George, S., Hamblin, M. R., and Kishen, A. (2009). Uptake Pathways of Anionic and Cationic Photosensitizers Into Bacteria. *Photochem. Photobiol. Sci.* 8 (6), 788–795. doi: 10.1039/b809624d

Graf, E. (1983). Applications of Phytic Acid. *J. Am. Oil Chemists’ Soc.* 60 (11), 1861–1867. doi: 10.1007/BF02901539

Haapasalo, M., Shen, Y., Wang, Z., and Gao, Y. (2014). Irrigation in Endodontics. *Br. Dental J.* 216 (6), 299–303. doi: 10.1038/sj.bdj.2014.204

Hawkins, P. T., Poyner, D. R., Jackson, T. R., Letcher, A. J., Lander, D. A., and Irvine, R. F. (1993). Inhibition of Iron-Catalysed Hydroxyl Radical Formation by Inositol Polyphosphates: A Possible Physiological Function for Myo-Inositol Hexakisphosphate. *Biochem. J.* 294 (3), 929–934. doi: 10.1042/ bj2940929

Hülsmann, M., Heckendorff, M., and Lennon, A. (2003). Chelating Agents in Root Canal Treatment: Mode of Action and Indications for Their Use. *Int. Endod. J.* 36 (12), 810–830. doi: 10.1034/j.1365-2591.2003.00754.x

Ishida, S., Arai, M., Niikawa, H., and Kobayashi, M. (2011). Inhibitory Effect of Cyclic Trihydroxamate Siderophore, Desferrioxamine E, on the Biofilm Formation of Mycobacterium Species. *Biochim. J.* 294 (3), 929–934. doi: 10.1042/ bj2940929

Jhajharia, K., Parolia, A., Shetty, K. V., and Mehta, L. K. (2015). Biofilm in Endodontics: A Review. *J. Int. Soc. Prev. Community Dent.* 5 (1), 1. doi: 10.1016/j.jspcd.2015.07.001

Kim, N. H., and Rhe, M. S. (2016). Phytic Acid and Sodium Chloride Show Marked Synergistic Bacterialicidal Effects Against Nonadapted and Acid-Adapted Escherichia Coli O157: H7 Strains. *Appl. Environ. Microbiol.* 82 (4), 1040–1049. doi: 10.1128/AEM.03307-15

Kuriyama, T., Williams, D. W., Patel, M., Lewis, M. A. O., Jenkins, L. E., Hill, D. W., et al. (2003). Molecular Characterization of Clinical and Environmental Isolates of Vancomycin-Resistant Enterococcus Faecium and Enterococcus
Antimicrobial Activity of Phytic Acid

Ricucci, D., and Siqueira, J. F. Jr. (2010). Biofilms and Apical Periodontitis: Study of Prevalence and Association With Clinical and Histopathologic Findings. J. Endod. 36 (8), 1277–1288. doi: 10.1016/j.joen.2010.04.007

Ricucci, D., Loghin, S., and Siqueira, J. F. Jr. (2013). Exuberant Biofilm Infection in a Lateral Canal as the Cause of Short-Term Endodontic Treatment Failure: Report of a Case. J. Endod. 39 (5), 712–718. doi: 10.1016/j.joen.2012.12.008

Copyright © 2021 Nassar, Nassar, Vianna, Naidoo, Alqutami, Kaklamanos, Senok and Williams. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.