International Journal of Applied Dental Sciences 2017; 3(2): 19-22

ISSN Print: 2394-7489
ISSN Online: 2394-7497
IJADS 2017; 3(2): 19-22

www.oraljournal.com
Received: 06-02-2017
Accepted: 07-03-2017

Dr. Saketh Puvvada
Post Graduate, Dept. of Conservative Dentistry and Endodontics

Dr. Prasanna Latha
Professor, Dept of Conservative Dentistry and Endodontics

Dr. Jayalakshmi KB
Prof and HOD, Dept of Conservative Dentistry and Endodontics

Dr. Arul Selvan K
Prof and HOD, Dept of microbiology

Correspondence
Dr. Saketh puvvada
Post Graduate, Dept. of Conservative Dentistry and Endodontics

Comparative assessment of chelating and antimicrobial efficacy of phytic acid alone and in combination with other irrigants

Dr. Saketh Puvvada, Dr. Prasanna Latha, Dr. Jayalakshmi KB and Dr. Arul Selvan K

Abstract
Chemomechanical debridement is an integral part in endodontics. Mechanical debridement results in the formation of smear layer on root canal surfaces whereas chemical debridment results in removal of smear layer resulting higher success of root canal treatment. This present study evaluates the chelating and antimicrobial ability of phytic acid alone and in combination with different irrigating solutions. Chelating ability was assessed by calcium titration method and antimicrobial efficacy was assessed by agar diffusion method. In this study the chelating ability of phytic acid in combination with sodium hypochlorite gave the best result when compared to phytic acid alone. Phytic acid alone showed more zone of inhibition indicating its antimicrobial efficacy is more when compared with other irrigants. However the antimicrobial efficacy of phytic acid with other irrigants gave better results when compared to individual irrigants.

Keywords: Phytic acid, chelating ability, antimicrobial efficacy, sodium hypochlorite

1. Introduction
The aim of mechanical preparation in endodontic therapy is to maximally reduce microbial count and necrotic remnants of pulpal tissue in the root canal system. Around 40-50% of the root canal walls are untouched in mechanical preparation [1]. Irrigating solutions are therefore required in association with mechanical preparation for better results [2]. Two frequently used irrigants in endodontics are aqueous solutions of sodium hypochlorite (NaOCl) and ethylenediamine tetraacetic acid (EDTA) [3]. A 0.5% NaOCl solution buffered with sodium bicarbonate was basically charecterized for the cleansing of open wounds in WorldWar I. Current endodontic practice, NaOCl solutions are used at concentrations ranging from 0.5 to 5.25% [4]. Chlorinated soda irrigants were introduced to the dental literature in 1936. Sodium hypochlorite has great dentine-disinfecting potential and tissue-dissolving capacity [5]. Since it has finite effect on the dissolution of smear layer, demineralizing agents as adjuvants were used in root canal treatment [6].
Exposed dentinal tubules may allow the hypochlorite solution to penetrate better into dentine, resulting in cleaner root canals [7]. Paralleling irrigation regimen of sodium hypochlorite and EDTA are more capable than NaOCl alone in decreasing the bacterial load in the root canals. [8]. The sodium salts of EDTA are non-colloidal organic chelating agents, which have the capability to form nonionic chelates with metallic ions such as Ca_2. EDTA solutions are most active at a pH between 7 and 8, and are believed to be more tissue-friendly than other acid-based demineralizing agents [9]. EDTA is not readily biodegradable, there are evidences about the leakage of this irrigant into the periapical tissue. Because of these involvements, the extrusion of EDTA beyond the root canal should be avoided [10]. Recent advances and scientific research in endodontics lead to introduction of newer chelating agents for smear layer removal in root canal systems. Phytic acid is a new chelating agent proposed to have superior properties.
Plant seeds and bran contains phytic as the major storage form of phosphorus (IP6, inositol hexakisphosphhate) that contributes to a wide variety of cellular functions. It is also present in mammalian cells in which its concentration ranges from 10-100mmol/L.

Extraction of phytic acid from rice bran is economical. Phytic acid has multiple negative charges, making it an efficient chelating agent of multivalent cations such as calcium (Ca+2), magnesium, and iron. [11]. On the basis of these proposed properties, we postulate that IP6 has the potential to replace EDTA as a root canal chelating agent [11]. Till date there is no sufficient scientific research literature available that can evaluate biochemical properties of phytic acid in conjunction with other root canal irrigants and its effect on root canal systems. Hence main aim of this study is to evaluate the chelating ability and antimicrobial efficacy of phytic acid alone and in combination with other root canal irrigants.

2. Materials and Methods

2.1 Chelating Ability of Phytic Acid

1. The chelating ability of phytic acid was tested with a calcium titration method, using Murexide as an indicator.
2. In a 50 ml test tube, 0.3 g of the 1% phytic acid was mixed with 20 ml of deionised water
3. The solution was rendered alkaline by the addition of 5ml of a 2:1, 0.1 M glycine/0.1MNaOH buffer added
4. Subsequently, Murexide was added, and the solution was titrated with a 500 ppm calcium solution till its colour changed from purple to red, indicating the presence of non-chelated calcium ions

2.2 Chelating Ability of Phytic Acid Along With Naocl

1. A freshly prepared 9:1 mixture of 1% phytic acid and 5% NAOCL was subjected to same procedure.
2. Calcium chelating potential of both the pure phytic acid and phytic acid/ NAOCL was compared by calculating the amount of chelated calcium.

2.3 Anti-Microbial Efficacy

1. E. faecalis (29212),was inoculated in to 5ml of sterile Brain-heart Infusion Broth incubated at 37degree C for 4 hours
2. 20 micro litres of adjusted culture was inoculated in to 30 ml of sterile BHI agar at 50 degree C. and mixed well and poured in to sterile petri-dish
3. The medium is allowed to set.
4. Then using sterile micro tips 6mm in diameter wells were made in the agar surface.
5. Using sterile micro tips the wells are inoculated with the respective solutions.
6. The plates were incubated at 37 degree C for 24 hrs
7. The growth was observed on agar surface and zone of inhibition of growth around the cells was measured.

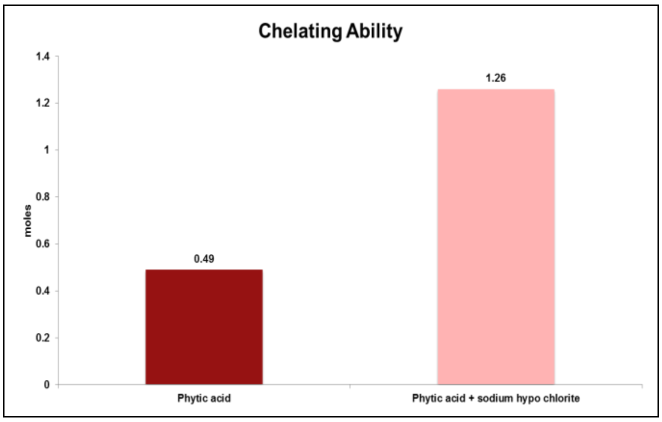
3. Results

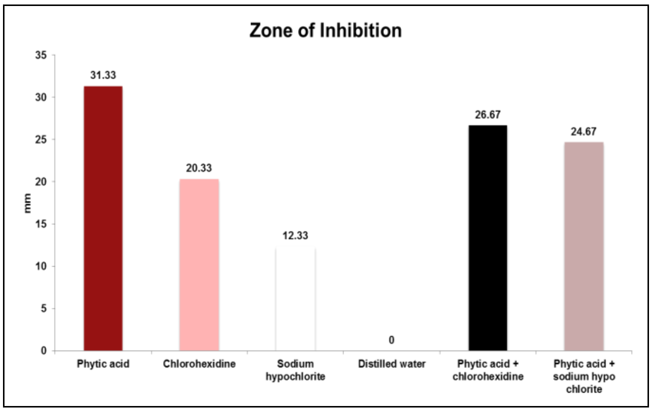
In present study chelating ability found between phytic acid and phytic acid along with sodium hypochlorite

Group I- phytic acid
Group II- Phytic acid + sodium hypochlorite
Antimicrobial efficacy
Group A- phytic acid
Group B- chlorhexidine
Group C- sodium hypochlorite
Group D- phytic acid + sodium hypochlorite
Group E- phytic acid + chlorhexidine
Group F-Distilled water

3.1 Statistical analysis

Kruskal wallis





	N	Mean (SD)	Range	Median (Q1-Q3)	Kruskal wallis test	
					Chi square value	p-value
Phytic acid	6	31.33 (0.58)	31 - 32	31 (31 - 32)	16.734	0.005*
Chlorohexidine	6	20.33 (0.58)	20 - 21	20 (20 - 21)		
Sodium hypochlorite	6	12.33 (0.58)	12 - 13	12 (12 - 13)		
Distilled water	6	0.00 (0.00)	0 - 0	0 (0 - 0)		
Phytic acid + chlorohexidine	6	26.67 (0.58)	26 - 27	27 (26 - 27)		
Phytic acid + sodium hypo chlorite	6	24.67 (0.58)	24 - 25	25(24 - 25)		

	N	Mean(SD)	Range	Median(Q1-Q3)	Mann whitney U test	
					U statistic	p-value
1.00	5	0.49 (0.02)	0.48 - 0.52	0.50 (0.48-0.51)	0.00	0.008*
2.00	5	1.26 (0.05)	1.20 - 1.30	1.30 (1.20-1.30)		

Comparison groups	p-value
1 vs 2	0.04*
1 vs 3	0.04*
1 vs 4	0.03*
1 vs 5	0.04*
1 vs 6	0.04*
2 vs 3	0.04*
2 vs 4	0.03*
2 vs 5	0.04*
2 vs 6	0.04*
3 vs 4	0.03*
3 vs 5	0.04*
3 vs 6	0.04*
4 vs 5	0.03*
4 vs 6	0.03*
5 vs 6	0.04*

4. Discussion

Root canal treatment is an important part of common dental practice. Though the success rate of root canal treatment is 95% failures are uncommon. This is attributed to many etiological factors such as intra-radicular or secondary infections. [12] Bacteria plays an important role in the failure of root canal treatment hence there is need to use antimicrobial agents during the treatment. Enterococcus faecalis, a gram positive facultative anaerobic bacterium, is the most common microorganism associated with endodontic treatment failure and enigma of infections. This bacteria is known for its capability to resist many of the clinically used irrigants. [12] Sodium hypochlorite (NaOCl) is the main irrigant used in root canal treatment, even though it has many advantages it also has several drawbacks which are mainly related to its toxicity on vital tissues and corrosion of metals. Ethylenediaminetetra acetic acid (EDTA) and chlorhxidne (CHX) are also used as irrigants. EDTA is mainly used as a chelating agent to remove the smear layer and the CHX is used for its antimicrobial effectiveness and substantively to dentin. Nonetheless, these two irrigants are synthetic materials that may have negative affect on the vitality of healthy tissue around the teeth, thus affecting the prognosis of post-treatment recovery or even result in chronic post-operative pain. Newer plant based organic material is phytic acid which is a natural extract from rice bran is used. Kim *et al*. reported that the bactericidal effects of IP6 were much greater than those of other organic acids under the same experimental conditions.

The antimicrobial effect of organic acids is mainly explained by the weak acid theory, but due to the unique structure of IP6 and a wide acidity range, the mechanism of the antimicrobial activity of phytic acid is likely to be different. Little information is available on the direct interaction of phytic acid and sodium hypochlorite in the endodontic literature. [12] There is no reported literature on the chelation and antimicrobial potential of a direct phytic acid alone and with NaOCl combination. Grawehr *et al* evaluated the antimicrobial efficacy of sodium hypochlorite when combined with EDTA. This stated that the antimicrobial efficacy of sodium hypochlorite decreased when combined with EDTA. This is due to less available chlorine in sodium hypochlorite when EDTA is added.[3] Nassar *et al* appraised the antimicrobial activities of 5% IP6, 5% NaOCl, 17% EDTA, 37% PA and 2% CHX against E. faecalis were determined using disk diffusion test. The results of agar diffusion test showed statistically significant differences between the groups. PA showed a larger zone when compared to other tested materials ($p< 0.05$). There was no statistical significant difference between NaOCl, EDTA and CHX (p=0.098). IP6 showed the smallest zone of inhibition when compared to all groups ($p<0.05$) [12]. In the present study antimicrobial efficacy of 1% Phytic acid, 5% NaOCl, 2% CHX, 1% phytic acid and 5% sodium hypochlorite, 1% phytic acid and 2% CHX, distilled water against E. feacalis. Phytic acid alone showed more zone of inhibition indicating that its antimicrobial efficacy is more when compared to other irrigants. However the antimicrobial efficacy of phytic acid with other irrigants gave better results when compared to individual irrigants when used alone. Present study is in contrary to the study done by Nassar *et al*, in which phytic acid showed larger zone of inhibition when compared to irrigants like NaOCl, CHX, EDTA etc.

Chelating agents are an integral part of root canal therapy. Irrigating solutions should be biocompatible and also help in removing smear layer. Nassar *et al* studied the chelating ability of phytic acid, EDTA and also evaluated the biocompatibility of the same. He observed the effect of phytic acid led to cleaner root canals and widely opened dentinal tubules when compared with EDTA. He concluded that phytic acid showed better biocompatibility to MC3T3-E1 odontoblast cells which may contribute to periapical bone healing [12]. Present study evaluated the chelating ability of 1% Phytic acid, 1% phytic acid and 5% sodium hypochlorite. The chelating ability of phytic acid in combination with sodium hypochlorite gave the best result when compared to phytic acid alone. Phytic acid has a strong binding affinity to important minerals, such as calcium, iron, and zinc, although the binding of calcium with phytic acid is pH-dependent leading to better chelating ability than conventional chelating agents. This is in accordance with the present study and a study done by Nassar *et al* in which phytic acid found to be more effective in removing smear layer from NaOCl treated flat coronal dentin surfaces than EDTA. Hence phytic acid alone or in combination with other conventional root canal irrigants showed superior antimicrobial and chelating abilities when compared to conventional root canal irrigants such as NaOCl, CHX, EDTA etc.

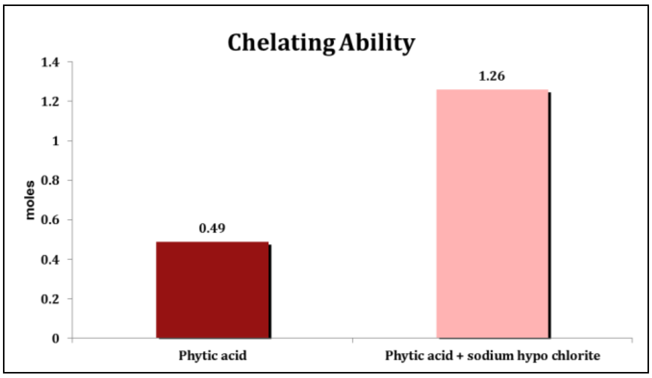
Further studies are essential to confirm antimicrobial efficacy and chelating ability of phytic acid, where phytic acid may be used as an alternative to conventional root canal irrigants for compromised patients for whom its usage is narrowed.

Table 1.

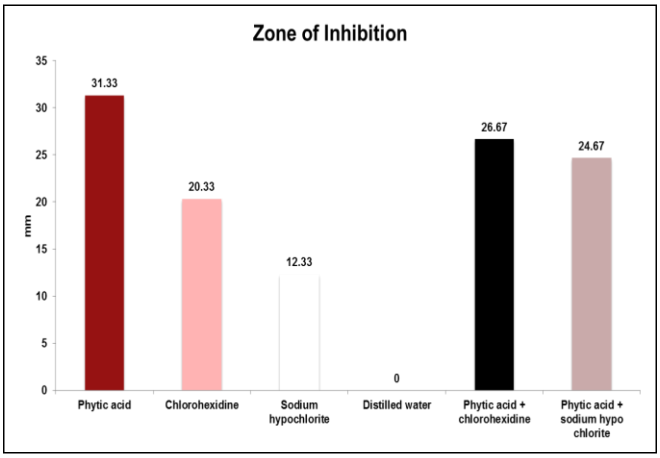
	N	Mean (SD)	Range	Median (Q1-Q3)	Kruskal wallis test	
					Chi square value	**p-value**
Phytic acid	6	31.33 (0.58)	31 - 32	31 (31 - 32)	16.734	0.005*
Chlorohexidine	6	20.33 (0.58)	20 - 21	20 (20 - 21)		
Sodium hypochlorite	6	12.33 (0.58)	12 - 13	12 (12 - 13)		
Distilled water	6	0.00 (0.00)	0 - 0	0 (0 - 0)		
Phytic acid + chlorohexidine	6	26.67 (0.58)	26 - 27	27 (26 - 27)		
Phytic acid + sodium hypo chlorite	6	24.67 (0.58)	24 - 25	25(24 - 25)		

Table 2.

	N	Mean(SD)	Range	Median(Q1-Q3)	Mann whitney U test	
					U statistic	p-value
1.00	5	0.49 (0.02)	0.48 - 0.52	0.50 (0.48-0.51)	0.00	0.008*
2.00	5	1.26 (0.05)	1.20 - 1.30	1.30 (1.20-1.30)		



Graph 1.



Graph 2.

5. Conclusion

The chelating ability of phytic acid in combination with sodium hypochlorite gave the best result when compared to phytic acid alone.

Phytic acid alone showed more zones of inhibition indicating its antimicrobial efficacy is more when compared to other irrigants.

However the antimicrobial efficacy of phytic acid with other irrigants gave better results when compared to individual irrigants when used alone.

6. References

1. Peters OA, Laib A, Gohring TN, Barbakow F. changes in root canal geometry after preparation assessed by high-resolution computed tomography. Journal of Endodontics 2001; 27:1-6
2. Baker NA, Eleazer PD, Averbach RE, Seltzer S. Scanning electron microscopic study of the efficacy of various irrigating solutions. Journal of Endodontics, 1975; 1:127-35.
3. Grawehr M, Sener B, Waltimo T, Zehender M. Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions. International Endodontic Journal. 2003; 36:411-415.
4. Dakin HD. On the use of certain antiseptic substances in treatment of infected wounds. British Medical Journal 1915; 2:318-20.
5. Zehnder M, Kosicki D, Luder H, Sener B, Waltimo T. Tissue dissolving capacity and antimicrobial effect of buffered and unbuffered hypochlorite solutions. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics 2002; 94:756-62.
6. Nygaard Ostby B. Chelation in root canal therapy. Odontologisk Tidskrift 1957; 65:3-11.
7. Goldman M, Goldman LB, Cavaleri R, Bogis J, Lin PS. The efficacy of several endodontic irrigating solutions: a scanning electron microscopic study. Part 2. Journal of Endodontics. 1982; 8:487-492.
8. Bystorm A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. International Endodontic Journal. 1985; 18:35-40.
9. Nikiforouk G, Sreebny L. Demineralization of hard tissues by organic chelating agents at neutral pH. Journal of Dental Research. 1953; 32:859-67.
10. Segura JJ, Calvo JR, Guerrero JM. The disodium salt of EDTA inhibits the binding of vasoactive intestinal peptide to macrophage membranes: endodontic implications. J Endod. 1996; 22:337-40.
11. Nassar M, Hiraishi N, Tamura Y, Otsuki M, Aoki K, Tagami J. Phytic Acid: An Alternative Root Canal Chelating Agent. J Endod. 2015; 41:242-247.
12. Nassar RI, Nassar M. Antimicrobial effect of phytic acid on Enterococcus faecalis. The International Arabic Journal of Antimicrobial Agents. 2017; 6:1-7.