ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 8, Issue 4, 2015

ISSN - 0974-2441

Review Article

EFFECT OF VARIOUS IRRIGANTS ON DENTAL BIOFILM: A REVIEW

PREETHA S, JAMES D RAJ

Department of Endodontics, Saveetha Dental College, Poonamallee, Chennai, Tamil Nadu, India. Email: jamesdraj@gmail.com

Received: 11 April 2015, Revised and Accepted: 12 May 2015

ABSTRACT

Objective: The objective of the article is to describe in brief the various endodontic irrigants on dental biofilm.

Method: Articles based on various endodontic irrigants and dental biofilm were searched in an iterative manner from journals,books and sites such as PubMed.

Result: It was found out that sodium hypochlorite is more effective on dental biofilms than chlorhexidine, MTAD, EDTA, herbal irrigants and new irrigants such as Qmix and tetraclean.

Conclusion: Disinfection of root canal system is done using mechanical instrumentation,chemical irrigation along with medication.Sodium hypochlorite an excellent antibacterial agent,is the most commonly used and is more effective than other irrigants on dental biofilm.

Keywords: Irrigants, Dental biofilm, Antimicrobial resistance, Sodium hypochlorite, Chlorhexidine, Tetraclean, Herbal irrigants.

INTRODUCTION

The effectiveness of biomechanical preparation underpins the success and longevity of root canal treatments [1]. Elimination of microorganisms from a root canal is a complicated task. Bacteria within a biofilm have increased resistance to the host defense mechanisms, antibiotics, and shear forces compared with isolated bacterial cells [2].

A favorable outcome with root canal treatment is high when the infection is eradicated before obturation. However, if microorganisms persist at the time of obturation or penetrate the canal after obturation, there are high chances of treatment failure [3]. This article describes in brief, the various endodontic irrigants used in root canal treatments and its effect on the endodontic biofilm.

BIOFILM

Microbial biofilm is defined as a sessile, multicellular microbial community characterized by cells that are firmly attached to a surface and enmeshed in a self-produced matrix of extracellular polymeric substances [4]. Endodontic biofilms are divided as intracanal, periapical, and external root (cementum) biofilms [5].

Intracanal biofilm

Using transmission electron microscopy Nair described intracanal biofilm. He examined the root canal content of teeth with gross coronal caries and to which periapical inflammatory tissue was attached upon extraction. He observed that the major bulk of organisms existed as a loose collection of cocci, rods, filaments, and spirochetes. Most of these organisms appeared suspended in a moist canal space while dense aggregates were also found sticking to canal walls and forming thin to thick layers of bacterial condensations. Amorphous material filled the inter-bacterial spaces and this was interpreted as an extracellular matrix of bacterial origin [6].

George *et al*. observed that when *Enterococcus faecalis* cells were grown under the aerobic nutrient rich condition, they produced irregularly shaped amorphous macro-structures of 500-1000 µm in dimension. These structures were identified as aggregates of bacterial cells [7].

Distel *et al*. found that pure cultures of *E. faecalis* inoculated to calcium hydroxide medicated or non-medicated root canals were able to form a biofilm structure on the canal walls [8].

External root surface (cementum)

These biofilms were reported in teeth with asymptomatic apical periodontitis and in teeth with chronic apical abscesses associated with sinus tract [5].

Tronstad *et al*. identified cocci and rods with the presence of fibrillar forms in the apex of roots [9]. Lomçali *et al*. reported that lacunar resorption zones were frequently observed on the apical root surfaces of teeth with chronic apical periodontitis. Bacteria and yeast cells were detected in some lacunae. Periapical bacterial plaques, which had microorganisms embedded in an extracellular matrix was found. This coating was considered to be a combination of bacterial by products and local inflammatory components. These findings suggest that host defense mechanisms may be unable to hold back the microorganisms outside the apical foramen may not be eliminated by conventional endodontic procedure and systemic antibiotic usage [10].

Periapical biofilms

These biofilms are found in the periapical region of endodontically involved teeth and may or may not be dependent on root canal infections [5]. Members of the genus *Actinomyces* and species *Propionobacterium propionicum* have been found in asymptomatic periapical lesions refractory to endodontic treatment [11].

Actinomycotic colonies may live in equilibrium with the host tissues without necessarily inducing an acute response, but rather maintains a chronic periapical inflammation. Very high numbers of *Actinomyces* cells are usually necessary to form persistent infections [12].

The perpetuation of the chronic periapical lesion may be due to the low pathogenicity of these microorganisms and the consequent minimal host response.

MECHANISM OF ANTIMICROBIAL RESISTANCE

Biofilm formation is a stepwise process that includes deposition of conditioning film, adhesion, and colonization of planktonic microorganisms in a polymeric matrix, coadhesion of other organisms and detachment of biofilm microorganisms into their surrounding [13].

Biofilm is made of numerous microcolonies of organisms in an aqueous solution surrounded by a matrix of glycolax, this type of growth

provides resistance to antimicrobial agents, communicate between the bacterial population, exchange of genetic material, increase in the local concentration of nutrients and production of growth factors.

This unique type of growth gives antimicrobial resistance and makes the organisms to grow in tough environmental conditions [14].

IRRIGANTS

Sodium hypochlorite (NaOCl)

NaOCl (household bleach) is the most commonly used irrigant in root canal treatment. It is used in dilutions ranging from 0.5% to 5.25%. It is an antiseptic and a lubricant. Free chlorine in NaOCl dissolves vital and necrotic tissues by breaking down proteins into amino acids.

Increasing its volume increases its effectiveness and decreasing the concentration of the solution reduces its toxicity, antibacterial effect, and ability to dissolve tissues [15].

Its advantages are its ability to dissolve organic substances present in the root canal system and its cheap. However, it is cytotoxic when it is injected into periradicular tissues, foul smell, and taste; and causes corrosion of metal objects [16]. It does not kill all bacteria and does not remove smear layer [17,18].

Spratt *et al*. showed that NaOCl was the most effective antimicrobial irrigant [19]. Ozok *et al*. compared the growth and susceptibility of mono and dual species biofilms of *Fusobacterium nucleatum* or *Peptosteptococcus micros* at 24 or 96 hrs, *in vitro* to different concentration of NaOCl [20].

It was revealed that although at 24 hrs the dual species biofilms had similar viable counts to those of mono species, they were more resistant to NaOCl. At 96 hrs, both microorganisms had higher viable count, and the dual species were more resistant to NaOCl than mono species. Time-dependent synergy in growth and resistance to NaOCl were showed by mixed species biofilms of *F. nucleatum* and *P. micros*.

In 2007, Giardino *et al*. did a comparative evaluation of antimicrobial efficacy of NaOCl, a mixture of tetracycline isomer, an acid, and a detergent (MTAD), and tetraclean against *E. faecalis* biofilm generate on cellulose nitrate membrane filters. They reported that only 5.25% NaOCl can disintegrate and remove the biofilm every time [21].

A similar study conducted by Dunavant *et al*. in 2006 showed that 1% NaOCl and 6% NaOCl were more efficient in eliminating *E. faecalis* biofilm than other endodontic irrigants [22]. Lundstrom *et al*. conducted a study to assess the bacterial efficacy of 0.04% stabilized chlorine dioxide, 3% NaOCl, 2% chlorhexidine (CHX), and sterile distilled water in a polymicrobial biofilm model. He reported that NaOCl possessed higher bacterial activity than that of stabilized chlorine dioxide against *Streptococcus sanguinis*, *Actinomyces viscosus*, and *Prevotella nigrescens* [23].

A study of the effect of exposure to irrigant solutions on apical dentin biofilms in vitro with different concentrations of NaOCl, 2% CHX, and BioPure MTAD on intracanal contents from 10 patients diagnosed with chronic apical periodontitis revealed that 6% NaOCl was the only irrigant capable of both rendering bacteria non-viable and physically removing the biofilm [24].

Since extrusion of NaOCl into periapical tissues can result in severe injury to the patient, it should be used carefully [25].

Ethylenediaminetetraacetic acid (EDTA)

Since hypochlorite is active only against organic material, other irrigants must be used for the complete removal of smear layer and dentin debris.

EDTA is commonly used as 17% neutralized the solution. Although it is biocompatible, it is immediately reduces the available chlorine in solution making NaOCl ineffective in bacteria and necrotic tissues [26]. EDTA has little or no antibacterial effective [27].

Yoshida *et al*. conducted a clinical evaluation of the efficacy of EDTA solution as an endodontic irrigant in 189 single rooted infected teeth using 15% EDTA solution with ultrasonic agitation without antibacterial intracanal medicaments in between appointments. They reported that 15% EDTA was more effective than a saline solution as root canal irrigant [28].

Calt and Serper showed that 10 ml irrigation with 17% EDTA for 1 minute was effective in the removal of smear layer [29]. Longer exposure of 10 minutes can cause excessive peritubular and intertubular dentin [30].

In a study conducted by Paul *et al*. in 2013, comparison of efficacy of different irrigants with EDTA, EDTA with ultrasonics, citric acid, MTAD, and mixture of tetracycline isomer revealed that none of the irrigants were completely effective [31].

CHX

CHX has a broad spectrum antibacterial action, sustained action, and low toxicity, so it is widely used in dentistry [15].

Its advantages over NaOCl that it does not have a foul smell and does not cause injury to the surrounding tissues, but it is unable to kill all bacteria and cannot remove the smear layer. It is used in concentration of 0.2-2% [32].

In 2007, an *in vitro* study conducted by Oliveira *et al*. on the antibacterial efficacy of endodontic irrigants against *E. faecalis* revealed that 2% CHX gluconate gel, and 5.25% NaOCl were effective in eliminating *E. faecalis* even after 7 days after instrumentation [33].

In another *in vitro* assessment between 2% CHX gel and liquid against 5.25% NaOCl it demonstrated that although 5.25% reduced *E. faecalis* colony forming units (CFU) immediately after instrumentation but was not able to keep root canal free of detectable *E. faecalis* in the final sample whereas 2% CHX liquid reduced CFU in post-treatment and final microbiological samples [34].

Studies by Delany *et al*., Shah *et al*., Vahdaty *et al*., and Heling *et al.* demonstrated 0.2% CHX was effective in removing aerobic bacteria in 80% of the cases and anaerobic bacteria in 76% of the cases [35-38].

MTAD

Biopure MTAD is a mixture of tetracycline isomer, acetic acid, and a detergent [39]. It is superior to CHX in antimicrobial activity [18]. It is also biocompatible, has a sustained bacterial activity and enhances bond strength [40].

Giardino *et al*. showed that MTAD was not able to remove bacterial biofilms [41]. Stojicic *et al*. demonstrated that MTAD was unable to kill all plaque bacteria in 30 seconds, and some *E. faecalis* cells were able to survive even after 3 minutes exposure [42].

A comparative study by Paul *et al*. revealed that MTAD showed excellent results in the removal of the smear layer in the apical third as compared to EDTA, EDTA with ultrasonication, citric acid [31]. Effectiveness of EDTA and MTAD on debris and smear layer removal using self-adjusting file revealed no significant difference whereas with ultrasonication, MTAD appeared to cause less dentinal erosion while allowing proper removal of smear layer and debris [43,44].

Tong *et al*. said that adding nisin to MTAD enhanced its effectiveness against *E. faecalis* biofilm [45].

OTHER IRRIGATING SOLUTIONS

The other irrigating solutions used are sterile water, physiologic saline, iodine compounds, urea peroxide, hydrogen peroxide, citric acid.

Spratt *et al*. concluded that iodine and NaOCl were more effective than CHX against *Streptococcus intermedius*, *F. nucleatum*, and *E. faecalis* but not against *Prevotella intermedia* and *P. micros*. Iodine and NaOCl showed 100% bacteria elimination after 1 hr incubation for all used strains [19].

Water and saline can get contaminated and does not have antimicrobial activity. A study to evaluate, through scanning electron microscopy, the micromorphology of dentinal walls of primary anterior teeth with focus on the presence of smear layer after endodontic debridement and final irrigation with different systems revealed that NaOCl promoted formation of smear layer during shaping and the use of EDTA and citric acid facilitated smear layer removal [46].

NEWER IRIGATING SOLUTIONS

Tetraclean

It is a mixture of antibiotic, an acid and a detergent-like MTAD, but the concentration of antibiotic, doxycline (50 mg/ml), and type of detergent is different [47].

Giardino *et al*. compared the antimicrobial efficacy of MTAD, tetraclean, cloreximid (CHX digluconate and cetrimide) and NaOCl on *E. faecalis*, *Porphyromonas gingivalis* and *P. intermedia*; and concluded that 5.25% NaOCl showed a high antimicrobial activity against anaerobic bacteria, MTAD, and tetraclean showed a high action against strictly anaerobic and facultative anaerobic bacteria while chloremimid (CHX + cetrimide) showed the lowest antibacterial activity [21].

Qmix

It is a mixture of EDTA, CHX, and a detergent. In a study conducted in 2013, it was revealed that Qmix™ 2 in 1 was less toxic to rat subcutaneous tissue than 3% NaOCl, 2% CHX, and 17% EDTA [48].

In another study, using Qmix™ 2 in 1 revealed that Qmix was superior to EDTA in smear layer removal and exposure of dentinal tubules in root canal system in single-rooted teeth [49].

Ozonated water

It is a chemical compound containing three oxygen atoms. It is a powerful antimicrobial agent against bacteria, fungi, protozoa, and virus but rarely used as irrigant [4]. Huth *et al*. demonstrated that the efficacy of ozonated water and 2.5% NaOCl were about the same when the specimen was irrigated with sonication [50].

However, another study by Hems *et al*. found that NaOCl was superior to ozonated water in killing *E. faecalis* in biofilm and broth culture [51].

HERBAL IRRIGANTS

Herbs such as green tea, *Azadirachta indica*, *Salvadora persica* solution (miswak-siwak), triphala, German chamomile, tea tree oil, *Psidium guajava* can be used as irrigants. Triphala and green tea polyphenols (GTP) contains a beneficial physiological effect, as well as it was being an antioxidant, anti-inflammatory, and radical scavenging activity [52].

In an *in vitro* study based on herbal irrigants, it was evaluated that 5% NaOCl showed maximum antibacterial activity against *E. faecalis* biofilm formed on tooth substrate. Triphala, GTPs, and MTAD showed statistically significant antibacterial activity [53].

REFERENCES

1. Jaju S, Jaju PP. Newer root canal irrigants in horizon: A review. Int J Dent 2011;2011:851359.
2. Jenkinson HF, Lappin-Scott HM. Biofilms adhere to stay. Trends Microbiol 2001;9(1):9-10.
3. Sjögren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J 1997;30(5):297-306.
4. Mohammadi Z, Soltani MK, Shalavi S. An update on the management of endodontic biofilms using root canal irrigants and medicaments. Iran Endod J 2014;9(2):89-97.
5. Ingle JI, Bakland LK, Baumgartner JC. Endodontics. 6th ed. Hamilton, Ontario: BC Decker; 2008. p. 85-268.
6. Ramachandran Nair PN. Light and electron microscopic studies of root canal flora and periapical lesions. J Endod 1987;13(1):29-39.
7. George S, Kishen A, Song KP. The role of environmental changes on monospecies biofilm formation on root canal wall by *Enterococcus faecalis*. J Endod 2005;31(12):867-72.
8. Distel JW, Hatton JF, Gillespie MJ. Biofilm formation in medicated root canals. J Endod 2002;28(10):689-93.
9. Tronstad L, Barnett F, Cervone F. Periapical bacterial plaque in teeth refractory to endodontic treatment. Endod Dent Traumatol 1990;6(2):73-7.
10. Lomçali G, Sen BH, Cankaya H. Scanning electron microscopic observations of apical root surfaces of teeth with apical periodontitis. Endod Dent Traumatol 1996;12(2):70-6.
11. Siqueira JF. Periapical actinomycosis and infection with propionibacterium propionicum. Endod Top 2003;6(1):78-95.
12. Figdor D, Sjögren U, Sörlin S, Sundqvist G, Nair PN. Pathogenicity of *Actinomyces* israelii and *Arachnia propionica*: Experimental infection in guinea pigs and phagocytosis and intracellular killing by human polymorphonuclear leukocytes *in vitro*. Oral Microbiol Immunol 1992;7(3):129-36.
13. Svensäter G, Bergenholtz G. Biofilms in endodontic infections. Endod Top 2004;9(1):27-36.
14. Magar S, Palekar A, Magar S, Mosby S. Endodontic biofilm a review. NJDSR 2014;2:1.
15. Johnson WT, Noblett WC. Cleaning and shaping. In: Endodontics: Principles and Practice. 4th ed. Philadelphia, PA: Saunders; 2009.
16. Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. *In vitro* antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis*. Int Endod J 2001;34(6):424-8.
17. Shuping GB, Orstavik D, Sigurdsson A, Trope M. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. J Endod 2000;26(12):751-5.
18. Shabahang S, Torabinejad M. Effect of MTAD on Enterococcus faecalis-contaminated root canals of extracted human teeth. J Endod 2003;29(9):576-9.
19. Spratt DA, Pratten J, Wilson M, Gulabivala K. An *in vitro* evaluation of the antimicrobial efficacy of irrigants on biofilms of root canal isolates. Int Endod J 2001;34(4):300-7.
20. Ozok AR, Wu MK, Luppens SB, Wesselink PR. Comparison of growth and susceptibility to sodium hypochlorite of mono- and dual-species biofilms of *Fusobacterium nucleatum* and *Peptostreptococcus* (micromonas) micros. J Endod 2007;33(7):819-22.
21. Giardino L, Ambu E, Savoldi E, Rimondini R, Cassanelli C, Debbia EA. Comparative evaluation of antimicrobial efficacy of sodium hypochlorite, MTAD, and Tetraclean against *Enterococcus faecalis* biofilm. J Endod 2007;33(7):852-5.
22. Dunavant TR, Regan JD, Glickman GN, Solomon ES, Honeyman AL. Comparative evaluation of endodontic irrigants against *Enterococcus faecalis* biofilms. J Endod 2006;32(6):527-31.
23. Lundstrom JR, Williamson AE, Villhauer AL, Dawson DV, Drake DR. Bactericidal activity of stabilized chlorine dioxide as an endodontic irrigant in a polymicrobial biofilm tooth model system. J Endod 2010;36(11):1874-8.
24. Clegg MS, Vertucci FJ, Walker C, Belanger M, Britto LR. The effect of exposure to irrigant solutions on apical dentin biofilms *in vitro*. J Endod 2006;32(5):434-7.
25. Hülsmann M, Hahn W. Complications during root canal irrigation – Literature review and case reports. Int Endod J 2000;33(3):186-93.
26. Zehnder M. Root canal irrigants. J Endod 2006;32(5):389-8.
27. Torabinejad M, Shabahang S, Aprecio RM, Kettering JD. The antimicrobial effect of MTAD: An *in vitro* investigation. J Endod 2003;29(6):400-3.
28. Yoshida T, Shibata T, Shinohara T, Gomyo S, Sekine I. Clinical evaluation of the efficacy of EDTA solution as an endodontic irrigant. J Endod 1995;21(12):592-3.
29. Calt S, Serper A. Time-dependent effects of EDTA on dentin structures. J Endod 2002;28(1):17-9.
30. .Calt S, Serper A. Smear layer removal by EDTA. J Endod 2000;26:459-61.
31. Paul ML, Mazumdar D, Niyogi A, Baranwal AK. Comparative evaluation of the efficacy of different irrigants including MTAD under SEM. J Conserv Dent 2013;16(4):336-41.
32. Estrela C, Silva JA, de Alencar AH, Leles CR, Decurcio DA. Efficacy of sodium hypochlorite and chlorhexidine against *Enterococcus*

faecalis – A systematic review. J Appl Oral Sci 2008;16(6):364-8.
33. Oliveira DP, Barbizam JV, Trope M, Teixeira FB. *In vitro* antibacterial efficacy of endodontic irrigants against Enterococcus faecalis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103(5):702-6.
34. Dametto FR, Ferraz CC, Gomes BP, Zaia AA, Teixeira FB, de Souza-Filho FJ. *In vitro* assessment of the immediate and prolonged antimicrobial action of chlorhexidine gel as an endodontic irrigant against *Enterococcus faecalis*. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;99(6):768-72.
35. Delany GM, Patterson SS, Miller CH, Newton CW. The effect of chlorhexidine gluconate irrigation on the root canal flora of freshly extracted necrotic teeth. Oral Surg Oral Med Oral Pathol 1982;53(5):518-23.
36. Shah N, Singh S, Gupta U. Antimicrobial potency of Iodine-potassium Iodide, chlorhexidine and sodium hypochlorite as root canal irrigants: An *in vitro* study. Endodontology 1990;2(2):19-23.
37. Vahdaty A, Pitt Ford TR, Wilson RF. Efficacy of chlorhexidine in disinfecting dentinal tubules *in vitro*. Endod Dent Traumatol 1993;9(6):243-8.
38. Heling I, Sommer M, Steinberg D, Friedman M, Sela MN. Microbiological evaluation of the efficacy of chlorhexidine in a sustained-release device for dentine sterilization. Int Endod J 1992;25(1):15-9.
39. Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, *et al*. A new solution for the removal of the smear layer. J Endod 2003;29(3):170-5.
40. Johnson WT, Noblett WC. Cleaning and shaping. In: Torabinejad M, Walton RE, editors. Endodontics: Principles and Practice. 4th ed. Philadelphia, PA: Saunders; 2008.
41. Williamson AE, Cardon JW, Drake DR. Antimicrobial susceptibility of monoculture biofilms of a clinical isolate of *Enterococcus faecalis*. J Endod 2009;35(1):95-7.
42. Stojicic S, Shen Y, Qian W, Johnson B, Haapasalo M. Antibacterial and smear layer removal ability of a novel irrigant, QMiX. Int Endod J 2012;45(4):363-71.
43. Adigüzel O, Yiğit-Özer S, Kaya S, Uysal İ, Ganidağli-Ayaz S, Akkuş Z. Effectiveness of ethylenediaminetetraacetic acid (EDTA) and MTAD on debris and smear layer removal using a self-adjusting file. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112(6):803-8.
44. Dadresanfar B, Khalilak Z, Delvarani A, Mehrvarzfar P, Vatanpour M, Pourassadollah M. Effect of ultrasonication with EDTA or MTAD on smear layer, debris and erosion scores. J Oral Sci 2011;53(1):31-6.
45. Tong Z, Zhou L, Li J, Jiang W, Ma L, Ni L. *In vitro* evaluation of the antibacterial activities of MTAD in combination with nisin against *Enterococcus faecalis*. J Endod 2011;37(8):1116-20.
46. Pitoni CM, Figueiredo MC, Araújo FB, Souza MA. Ethylenediaminetetraacetic acid and citric acid solutions for smear layer removal in primary tooth root canals. J Dent Child (Chic) 2011;78(3):131-7.
47. Giardino L, Ambu E, Becce C, Rimondini L, Morra M. Surface tension comparison of four common root canal irrigants and two new irrigants containing antibiotic. J Endod 2006;32(11):1091-3.
48. Chandrasekar V, Amulya V, Rani VS, Prakash TJ, Ranjani AS, Gayathri C. Evaluation of biocompatibility of a new root canal irrigant Qmix™2 in an *in vivo* study. J Conserv Dent 2013;16(1):36-40.
49. Alkahtani A, Alkahtany SM, Mahmood A, Elsafadi MA, Aldahmash AM, Anil S. Cytotoxicity of QMix™ endodontic irrigating solution on human bone marrow mesenchymal stem cells. BMC Oral Health 2014;14:27.
50. Huth KC, Quirling M, Maier S, Kamereck K. Effectiveness of ozone against endodontopathogenic microorganisms in a root canal biofilm model. Int Endod J 2009;42:3-13.
51. Hems RS, Gulabivala K, Ng YL, Ready D, Spratt DA. An *in vitro* evaluation of the ability of ozone to kill a strain of *Enterococcus faecalis*. Int Endod J 2005;38:22-9.
52. Vani T, Rajani M, Sarkar S, Shishoo CJ. Antioxidant properties of the ayurvedic formulation triphala and its constituents. Int J Pharm 1997;35(5):313-7.
53. Prabhakar J, Senthilkumar M, Priya MS, Mahalakshmi K, Sehgal PK, Sukumaran VG. Evaluation of antimicrobial efficacy of herbal alternatives (triphala and green tea polyphenols), MTAD, and 5% sodium hypochlorite against *Enterococcus faecalis* biofilm formed on tooth substrate: An *in vitro* study. J Endod 2010;36(1):83-6.