Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/jobcr

Original Article

Comparative antimicrobial efficacy of herbal alternatives (*Emblia officinalis*, *Psidium guajava*), MTAD, and 2.5% sodium hypochlorite against *Enterococcus faecalis*: An in vitro study

Sandeep Dubey

Senior Lecturer, Conservative Dentistry and Endodontics, College of Dental Sciences and Hospital, Indore, Madhya Pradesh 453331, India

ARTICLE INFO

Article history:

Received 12 October 2015

Accepted 28 December 2015

Available online 20 January 2016

Keywords:

Agar disk-diffusion method

*Emblia officinalis**Psidium guajava*

ABSTRACT

Aim: The objective of this study was to evaluate the antimicrobial efficacy of herbal alternatives (*Emblia officinalis*, *Psidium guajava*), BioPure MTAD, and 2.5% sodium hypochlorite against *Enterococcus faecalis*.

Materials and method: The testing of the antimicrobial efficacy of selected medicaments against *E. faecalis* was done by the agar disk-diffusion method. Whatman paper discs of 6 mm diameter were prepared and soaked with the test solution. These discs were then placed onto the previously seeded agar Petri plates. Later, these plates were incubated for 48 h at 37 °C under the appropriate gaseous conditions in a CO₂ incubator. A zone of inhibition was recorded in millimeter for each plate and the results were analyzed statistically.

Result: MTAD was found to be superior in its antibacterial abilities against *E. faecalis* compared with the other irrigants used. All the other tested irrigants showed significant zone of inhibition.

Conclusions: BioPure MTAD offers better antibacterial efficacy than NaOCl. *E. officinalis* and *P. guajava* are effective antibacterial agents against *E. faecalis* and can be used to reduce root canal microflora and root canal failures.

© 2016 Craniofacial Research Foundation. Published by Elsevier B.V. All rights reserved.

1. Introduction

Achieving predictable long-term success of endodontic therapy is to eliminate the bacteria and their by-products from the pulp space, which can be achieved by hand or rotary instrumentation of root canal system.

Although mechanical instrumentation can remove a large number of microbial flora from the root canal system, bacteria remaining in the intricacies of the canal due to complex root canal system anatomy can cause failure of endodontic therapy or sustain periradicular inflammation.¹

Therefore, mechanical instrumentation of the root canal is accompanied by use of different irrigating solutions having

E-mail address: sandeepdubey.mds@gmail.com

<http://dx.doi.org/10.1016/j.jobcr.2015.12.010>

2212-4268/© 2016 Craniofacial Research Foundation. Published by Elsevier B.V. All rights reserved.

antimicrobial effect on microorganisms as one of the desirable property.^{2,3} Sodium hypochlorite (NaOCl) is the most commonly used endodontic irrigant because of its antimicrobial potential⁴ and its ability to dissolve organic matter.⁵ However, it is not only irritant to the vital periradicular tissues, but also inherently possesses certain disadvantages such as inability to remove the smear layer, unpleasant taste, high toxicity,⁶ corrosive to instruments, reduction in elastic modulus, and flexural strength of dentin.⁷

Recently, a new root canal irrigant, BioPure MTAD, was introduced which is a mixture of a tetracycline isomer, an acid, and a detergent. It can safely remove the smear layer, has antimicrobial property,⁸ and has been suggested to be more efficient than sodium hypochlorite in disinfecting the pulp space.^{8–10}

Herbal alternatives have been used in medical and dental science for hundreds of years and have become even more acknowledged today due to their high antibacterial activity, analgesic, biocompatibility, anti-inflammatory, and anti-oxidant properties.¹¹

Emblica officinalis, commonly known as Nelli, Amla, Amalaki, or Indian gooseberry, is a deciduous tree, the fruits of which are highly valued in traditional medicine.¹² They have the potential to prevent dental caries by inhibiting the virulence factors of *Streptococcus mutans* and *Lactobacillus*;¹³ however their role in endodontics have not been evaluated to date.

Psidium guajava, commonly known as guava, belongs to the family Myrtaceae. Different parts of the plant are used in the indigenous system of medicine for the treatment of various human ailments. Pharmacological investigations indicated that its bark, fruit, and leaves possess antibacterial, hypoglycemic, anti-inflammatory, analgesic, antipyretic, spasmolytic, and CNS depressant activities.¹⁴ There is no much documentation of *P. guajava* associated to microbial flora related to endodontics.

2. Materials and method

To evaluate and compare antibacterial effect of irrigants anaerobic facultative bacteria, *Enterococcus faecalis* (ATCC 29212) was employed. *E. faecalis* strains were allowed to grow in brain heart infusion broth (Difco Co, Becton Dickinson, Sparks, MD) which was supplemented with hemin (5 mg/l) and menadione (0.5 mg/l).

2.1. Preparation of herbal extracts

The fruits of *E. officinalis* were collected and were dried under shade for one month and grounded with the help of an electrical grinder. Powdered fruit material was extracted using Soxhlet extractor for 6 h using methanol as the solvent system not exceeding its boiling point. The extracts were then filtered using Whatman filter paper no. 1 and dried under reduced pressure using rota-vapour at 4 °C for 10 min.

The leaf samples of *P. guajava* were washed in tap water, shade dried, for 10–12 days and placed into an electric grinder to be grounded into powder. Whatman filter paper no. 3 was used to pack the leaf powder and extracted under soxhlet extractor in methanol at 80 °C for 12 h. The extracts were then

filtered using Whatman filter paper no. 1, and dried using rota-vapour. The dried extracts of *E. officinalis* and *P. guajava* were stored at 4 °C and were further dissolved in 5% dimethyl sulphoxide (DMSO).

2.1.1. Preparation of BioPure MTAD

The BioPure MTAD (Dentsply Tulsa Dental) was prepared as per the manufacturer's instructions, by mixing the BioPure MTAD powder with the liquid content.

The inoculum for *E. faecalis* strain was prepared by picking up three to five colonies (with the help of a previously sterilized, circular loop) and dissolving them into the test tube containing 5 ml of 0.85% saline solution; these organisms were adjusted to 0.5 ml McFarland scale (1.5×10^8 CFU/ml) and streak on Muller–Hilton–Agar plate (MHA plate) for *E. faecalis*, in a form that lawn growth can be observed.

Discs of 6-mm diameter were prepared from Whatman paper no. 1 (Whatman, Maidstone, Kent, UK), which were sterilized by a hot air oven. Prepared discs were then saturated with 50 µl of each tested irrigant and aseptically transferred to the previously inoculated agar plate with bacteria.

Plates were then incubated for 48 h in a CO₂ incubator (Jouan, Saint Herblain, France) at 37 °C under the appropriate gaseous conditions, in an atmosphere of 10% CO₂. All assays were repeated four times to ensure reproducibility. Microbial zones of inhibition were measured in millimeters.

3. Results

The results were tabulated and statistically analyzed using analysis of variance (ANOVA). The results are expressed as the mean $\pm$ one standard deviation. Values of $P < 0.05$ were considered statistically significant. Table 1 shows the diameters of the zones of microbial growth inhibition (in mm) for the tested irrigants against *E. faecalis*. MTAD showed the

Table 1 – Comparison of the antimicrobial effect of tested irrigants against *Enterococcus faecalis*.

Test solution	Mean $\pm$ SD	Result
BioPure MTAD	19.93 $\pm$ 0.11	Sensitive
2.5% NaOCl	17 $\pm$ 0.50	Sensitive
<i>Emblica officinalis</i>	18.97 $\pm$ 0.55	Sensitive
<i>Psidium guajava</i>	14.83 $\pm$ 0.29	Sensitive
DMSO	00 $\pm$ 0.00	Resistant

Fig. 1 – Mean value of zone of inhibition against *Enterococcus faecalis* by tested irrigants.

Fig. 2 – (A) Zone of inhibition against *E. faecalis* by *Emblica officinalis*. (B) Zone of inhibition against *E. faecalis* by *Psidium guajava*.

largest zone of inhibition size against *E. faecalis* amongst the tested irrigants (Figs. 1 and 2).

4. Discussion

E. faecalis was selected in the present study because it has been detected in 63% of post-treatment diseases and based on its proved resistance to wide range of antimicrobial agents.¹⁵ Furthermore, *E. faecalis* plays a major role in the etiology of persistent periradicular lesions after root canal treatment. ATCC 29212 strains of *E. faecalis* which has been the standard strain used in the previous researches were selected for this study.

Agar disk-diffusion test employed in this study is a well-established technique for antibacterial study.^{16,17} This method has been employed frequently in the recent researches. Advantage of this method is that antimicrobial resistance of medicaments can be detected by challenging bacterial isolates with antimicrobial discs without any change in the chemical properties of medicaments.¹⁸ Moreover, this is a less technique-sensitive method and is easy to employ.

According to results of current study, BioPure MTAD showed superior antibacterial activity against *E. faecalis* amongst tested irrigants which can be attributed to carry over effect of doxycycline. Furthermore, previous studies have shown antimicrobial effect of doxycycline against wide range of oral microbial flora.^{19–21} It has also been claimed that the acidic pH of citric acid and its chelating property are effective in destroying bacterial isolates although the exact mechanism is not known.²²

In the present study, 2.5% NaOCl showed significant antibacterial efficacy against *E. faecalis*, but it was lower than MTAD which is in accordance with previous studies conducted by Davis et al. and Mohammadi et al.^{23,24} However, our results are contrary to some in vitro studies which have shown higher antibacterial efficiency of NaOCl than BioPure MTAD against *E. faecalis*.^{25,26} The inconsistency in the results may be caused by different concentration of NaOCl, variance in strains tested, differences in methodology, and possibly due to the bacterial species and/or slightly different incubation conditions employed.

The results obtained from the present study represent excellent antimicrobial activity of *E. officinalis* against *E. faecalis* which confirms its potential to be used as a root canal irrigant in endodontic therapy. The antimicrobial activity exhibited by the *E. officinalis* could be attributed to the tannins, flavonoids, and saponins present in its fruits.²⁷ The fruits contain two types of tannins Emblicanin A and B, which have antimicrobial activities.²⁸ Mode of antimicrobial action of tannins may be related to their ability to inactivate microbial adhesions, enzymes, cells envelop transport proteins, etc. They also combine with polysaccharides present in cell wall.²⁹

The results of the present investigation were in accordance with the previous studies,^{30,31} which reported that *E. officinalis* possess potent antibacterial activity against wide range of microbes.

In the present study, *P. guajava* leaves showed decent antimicrobial effect against *E. faecalis*. Flavonoids present in *P. guajava* leaves such as quercetin, morin-3-O-arabinoside, morin-3-O-lyxoside, and quercetin-3-O-arabinoside are responsible for its antimicrobial activity.³² Flavonoids are hydroxylated phenolic substances, synthesized by plants in response to bacterial infection. Their antimicrobial activity is probably due to their ability to complex with extracellular and soluble proteins, and to complex with microbial cell walls.²⁹ Previous studies have demonstrated antimicrobial activity of *P. guajava* leaves against gram-positive and gram-negative bacteria.^{29,33}

5. Conclusion

With the limitations of this study, it can be concluded that:

1. MTAD was found to be superior in its antibacterial abilities against *E. faecalis* compared with the other irrigants used.
2. *E. officinalis* performed antimicrobial activity better than sodium hypochlorite against *E. faecalis*.
3. *P. guajava* also exhibited good antimicrobial action.

However, further preclinical and clinical researches are required on *E. officinalis* and *P. guajava* to evaluate their antimicrobial action against other microbes, biocompatibility,

and their safety before they can conclusively be recommended as intracanal irrigating solutions, but in vitro antimicrobial observations of these irrigants appear promising.

Conflicts of interest

The author has none to declare.

REFERENCES

- Young GR, Parashos P, Messer HH. The principles of techniques for cleaning root canals. *Aust Dent J*. 2007;52 (1 Suppl.):S52–S63.
- Pataky L, Iványi I, Grigár A, Fazekas A. Antimicrobial efficacy of various root canal preparation techniques: an in vitro comparative study. *J Endod*. 2002;28:603–605.
- Paiva SS, Siqueira JF, Rôças IN, et al. Molecular microbiological evaluation of passive ultrasonic activation as a supplementary disinfecting step: a clinical study. *J Endod*. 2013;39:190–194.
- Siqueira Jr JF, Batista MM, Fraga RC, de Uzeda M. Antibacterial effects of endodontic irrigants on black-pigmented gram-negative anaerobes and facultative bacteria. *J Endod*. 1998;24:414–416.
- Naenni N, Thoma K, Zehnder M. Soft tissue dissolution capacity of currently used and potential endodontic irrigants. *J Endod*. 2004;30:785–787.
- Kleier DJ, Averbach RE, Mehdipour O. The sodium hypochlorite accident: experience of diplomates of the American Board of Endodontics. *J Endod*. 2008;34:1346–1350.
- Sim TP, Knowles JC, Ng YL, Shelton J, Gulabivala K. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. *Int Endod J*. 2001;34:120–132.
- Torabinejad M, Shabahang S, Aprecio R, Kettering JD. The antimicrobial effect of MTAD: an in vitro investigation. *J Endod*. 2003;29:400–403.
- Shabahang S, Pouresmail M, Torabinejad M. In vitro antimicrobial efficacy of MTAD and sodium hypochlorite. *J Endod*. 2003;29(7):450–452.
- 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):852–855.
- Cogulu D, Uzel A, Sorkun K, et al. Efficacy of propolis as an intracanal medicament against *Enterococcus faecalis*. *Gen Dent*. 2006;54:319–322.
- Scartezzini P, Antognoni F, Raggi MA, Poli F, Sabbioni C. Vitamin C content and antioxidant activity of the fruit and of the ayurvedic preparation of *Emblica officinalis* Gaertn. *J Ethnopharmacol*. 2006;104(1):113–118.
- Hasan S, Danishuddin M, Adil M, Singh K, Verma PK, Khan AU. Efficacy of *E. officinalis* on the cariogenic properties of *Streptococcus mutans*: a novel and alternative approach to suppress quorum-sensing mechanism. *PLoS ONE*. 2012;7: e40319.
- Begum S, Hassan SI, Siddiqui BS, Shaheen F, Ghayur MN, Gilani AH. Triperpenoids from the leaves from *Psidium guajava*. *Phytochemistry*. 2002;61:399–403.
- Hancock HH, Sigurdsson A, Trope M, Moiseiwitsch J. Bacteria isolated after unsuccessful endodontic treatment in a North American population. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2001;91:579–586.
- Poggio C, Colombo M, Scribante A, Sforza D, Bianchi S. In vitro antibacterial activity of different endodontic irrigants. *Dent Traumatol*. 2012;28:205–209.
- Carson KR, Goodell GG, McClanahan SB. Comparison of the antimicrobial activity of six irrigants on primary endodontic pathogens. *J Endod*. 2005;31:471–473.
- Aravind. Gopikrishna V, Kandaswamy D, Jeyavel RK. Comparative evaluation of the antimicrobial efficacy of five endodontic root canal sealers against *Enterococcus faecalis* and *Candida albicans*. *J Conserv Dent*. 2006;9:2–12.
- Van der Vyver PS, Botha FS, Herbst D, Ismail M. Antimicrobial efficacy and irrigating potential of irrigation solutions using different activation methods. *SADJ*. 2009;64 (56):58–60.
- Le Corn DW, Vertucci FS, Rojas MF, Progulske-Fox A, Belanger M. In vitro activity of amoxicillin, clindamycin, doxycycline, metronidazole, and moxifloxacin against oral *Actinomyces*. *J Endod*. 2007;33:557–560.
- Lakhssassi N, Elhajoui N, Lodter JP, Pineill JL, Sixou M. Antimicrobial susceptibility variation of 50 anaerobic periodontal pathogens in aggressive periodontitis: an interindividual variability study. *Oral Microbiol Immunol*. 2005;20:244–252.
- Portenier I, Waltimo T, Orstavik D, Haapasalo M. Killing of *Enterococcus faecalis* by MTAD and chlorhexidine digluconate with or without cetrimide in the presence or absence of dentin powder or BSA. *J Endod*. 2006;32:138–141.
- Davis JM, Maki JJ, Bahcall JK. An in vitro comparison of the antimicrobial effects of various endodontic medicaments on *Enterococcus faecalis*. *J Endod*. 2007;33(5):567–569.
- Mohammadi Z, Shahriari S. Residual antibacterial activity of chlorhexidine and MTAD in human root dentin in vitro. *J Oral Sci*. 2008;50(1):63–67.
- Dunavant TR, Regan JD, Glickman GN, Solomon ES, Honeyman AL. Comparative evaluation of endodontic irrigants against *Enterococcus faecalis* biofilms. *J Endod*. 2006;32(6):527–531.
- Krause TA, Liewehr FR, Hahn CL. The antimicrobial effect of MTAD, sodium hypochlorite, doxycycline, and citric acid on *Enterococcus faecalis*. *J Endod*. 2007;33(1):28–30.
- Javale P, Sabnis S. Antimicrobial properties and phytochemical analysis of *Emblica officinalis*. *Asian J Exp Biol Sci*. 2010;91–95.
- Jyothi KS, Subba Rao B. Screening of antibacterial activity of *Emblica officinalis* fruits. *Pharmacol Online*. 2011;3:848–852.
- Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev*. 1999;12:564–582.
- Vijayalakshmi S, Arunkumar V, Anju D, Gunasundari P, Moorthy P, Chandrasekharan AK. Comparative antimicrobial activities of *Emblica officinalis* and *Ocimum sanctum*. *Anc Sci Life*. 2007;27(2):1–6.
- Saeed S, Tariq P. Antibacterial activities of *Emblica officinalis* and *Coriandrum sativum* against Gram negative urinary pathogens. *Pak J Pharm Sci*. 2007;20(1):32–35.
- Rattanachaikunsopon P, Phumkhachorn P. Contents and antibacterial activity of flavonoids extracted from leaves of *Psidium guajava*. *J Med Plants Res*. 2010;4: 393–396.
- Perez Gutierrez RM, Mitchell S, Solis RV. *Psidium guajava*: a review of its traditional uses, phytochemistry and pharmacology. *J Ethnopharmacol*. 2008;117:1–27.