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Original Article

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

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ABSTRACT

Aim: The objective of this study was to evaluate the antimicrobial efficacy of herbal alternatives (*Emblica 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 CO_2 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.

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

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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 potential[4] 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 × 108 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 CO_2 incubator (Jouan, Saint Herblain, France) at 37 °C under the appropriate gaseous conditions, in an atmosphere of 10% CO_2. 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 ± 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 ± SD	Result
BioPure MTAD	19.93 ± 0.11	Sensitive
2.5% NaOCl	17 ± 0.50	Sensitive
Emblica officinalis	18.97 ± 0.55	Sensitive
Psidium guajava	14.83 ± 0.29	Sensitive
DMSO	00 ± 0.00	Resistant

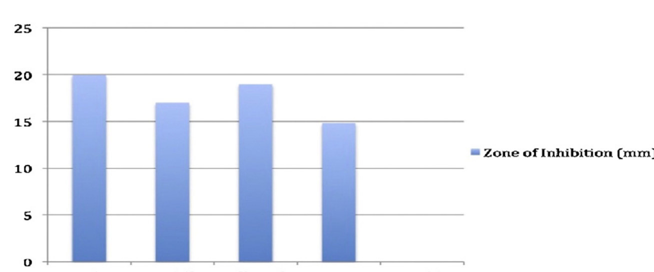

Fig. 1 – Mean value of zone of inhibition against *Enterococcus faecalis* by tested irrigants.