



# Comparitive Evaluation of Dentinal Tubule Disinfection using Propolis, Morinda Citrifolia, Aloe vera.

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## Abstract

Disinfection of the root canal is one of the most important steps in root canal preparation. To disinfect the root canal various chemical disinfecting solutions are available. These chemical agents can cause adverse tissue reactions when they are used. Herbal disinfectants seem to overcome the adverse reactions of chemical reagents and can provide effective disinfection in the root canal. This study evaluates the dentinal tubules disinfection capacity of commercially available solutions containing herbal components (Propolis, Morinda citrifolia and Aloe vera) against *Enterococcus faecalis*.

**Key words:** Root canal disinfection, Herbal irrigants, Propolis, Morinda citrifolia, Aloe Vera

## INTRODUCTION

Root canal debridement is one of the prime importances in long term success of the root canal system. Irrigants are said to augment mechanical debridement by flushing out debris, dissolving tissue, and disinfecting the root canal system<sup>1</sup>. Chemical debridement is required for teeth with complex internal anatomies such as fins or other irregularities that may be missed by instrumentation<sup>2</sup>.

Root canal systems are harboured by enormous amount of endodontic microflora that are resistant to irrigants and intracanal medicaments currently available. Search for the biocompatible and effective irrigants has led to the development of herbal irrigants and intracanal medicaments in endodontics.

Persistent endodontic infection may be associated with retention of microorganisms in the dentinal walls that harbour microorganisms. Routine root canal preparation procedures, can remove micro organisms in the root canal wall but those in the dentinal tubules are removed with the help of irrigants and intracanal medicaments.

*Enterococcus faecalis* is commonly isolated in failed endodontic treatment, as it has the ability to thrive in harsh conditions. *E.faecalis* has demonstrated resistance to irrigants and intracanal medicaments that is commonly used in endodontic practice<sup>3</sup>.

Murray et al used Morinda citrifolia as an endodontic irrigant against endodontic pathogen and concluded that 6 % was effective in disinfecting the canal space<sup>4</sup>. Aloe Vera contains the mucilaginous extract called Aloe Vera gel that contains total leaf extracts accounting for its antibacterial properties<sup>5</sup>. Propolis otherwise called as honey bee wax contain flavonoids contributing for its anti-inflammatory and anti-bacterial activity<sup>6</sup>.

This study was undertaken to evaluate the disinfection of dentinal tubules when contaminated with *E. faecalis* using commercially available preparation containing Propolis, Morinda Citrifolia, Aloe Vera.

## MATERIALS AND METHODOLOGY:

The model proposed by Haapasalo and Ørstavik was modified. Fifty freshly extracted single rooted teeth were used for this study. Rotary diamond disk was used to decoronate the teeth 5 mm below cemento-enamel junction and the apical part of the root to obtain 6 mm of the middle third of the root. Cementum was removed from the root surface. Gates Glidden drills no. 3 used in a slow speed handpiece to standardize the internal diameter of the root canals. The dentin blocks were placed in 17% ethylene diamine tetra acetic acid (EDTA) in an ultrasonic bath for 5 min followed by 3% Sodium Hypochlorite (NaOCl) for 5 min to remove the organic and inorganic debris.

The traces of chemicals used were removed by immersing the blocks in an ultrasonic bath containing distilled water for 5 minutes. All the blocks were sterilized in an autoclave for 2 cycles. The first cycle was at 121°C and the second was with the blocks immersed in 1 mL of Tryptone soya (TS) broth in individual microcentrifuge tubes. This allows better penetration of the broth into the dentinal tubules. All the blocks were coated externally with paraffin wax.

*E. faecalis* (ATCC 29212) was grown in TS agar for 24 hours. The culture was suspended in 5 mL of TS broth and incubated for 4 hours at 37°C and its turbidity was adjusted to 0.5 McFarland standards. Each dentin block was placed in presterilized microcentrifuge tubes containing 1 mL of the TS broth. Fifty microliters of the inoculum containing the *E. faecalis* was transferred into each of the microcentrifuge tubes. At the end of 24 hours the dentin blocks were transferred into fresh broth containing *E. faecalis*. All procedures were carried out under laminar flow. Purity of the culture was checked by subculturing 5 µL of the broth from the incubated dentin blocks in TS broth on TS agar plates. Contamination of the dentin specimens were carried out for a period of 21 days.

### Antimicrobial assessment

At the end of 21 days the blocks were irrigated with 5 mL of sterile saline to remove the incubation broth. The blocks were assigned into 6 groups (n = 10 dentin blocks).

- Group 1: Saline (Negative control)  
 Group 2: 3% Sodium Hypochlorite (Positive control)  
 Group 3: 25 % Propolis in alcohol free base - Manuka Health Bio30  
 Group 4: 66% Morinda Cirifolia - Divine Noni gold  
 Group 5: 70% Aloe vera - Vitro Naturals Aloe Health

A 27-gauge irrigating needle was used for irrigation in groups A, B, C, D and E. The syringes were pre-measured to ensure that the correct amount of irrigant was used on each occasion. Conventional syringe irrigation was carried out using digital pressure with the forefinger only. The needle was moved back and forth in the canal gently whilst the irrigation was being performed; ensuring that the needle did not bind in the canal. A total of 4 mL of irrigant was used for the protocol in various groups used in the study. Harvesting of the dentin was carried out at 2 depths (200 and 400  $\mu\text{m}$ ) with Gates Glidden drills no.4 and 5, respectively. The collected dentin shavings were transferred into 1 mL of sterile TS broth and incubated in an anaerobic environment at 37°C for 24 h. After 24 h, the contents of each tube was serially diluted, 100  $\mu\text{L}$  of the broth in 100  $\mu\text{L}$  of sterile saline for 5 times. Fifty microliters of the dilution was then plated on TS agar plates and incubated for 24 h and the Colonies were counted.

#### Statistical analysis:

The data were statistically analyzed using Kruskal wallis and Posthoc (multiple pairwise comparison) to check the difference in bacterial inhibition between the groups. To compare the bacterial inhibition between groups at various intervals Friedman repeated measures ANOVA and Posthoc (multiple pair wise comparison).

#### RESULTS:

The current study showed that all the four irrigants studied exerted antibacterial activity. Contamination of the dentin blocks was confirmed when debris samples harvested from the saline group (negative control) yielded positive growth. The inhibition of growth in all the groups was statistically significant in comparison to the control group (saline). Statistically significant difference in the reduction of CFU/ml of *E.faecalis* was seen for 3% NaOCl when compared with other groups (p value < 0.01). But there was no statistical significant difference among herbal irrigants ie.comparison of CFU/mL between Propolis, Morinda citrifolia, Aloe Vera. Statistically significant difference in the CFU/ ml of *E.faecalis* was seen at different levels of root canal space (coronal > middle > apical) for hebal irrigants and saline.

**Mean colony counts for different irrigants**

Groups	Coronal	Middle	Apical
Saline	3.82 x 10 <sup>5</sup>	3.32 x 10 <sup>5</sup>	2.45x x 10 <sup>5</sup>
Sodium Hypochlorite	0	0	0
Propolis	2.17 x 10 <sup>5</sup>	1.90 x 10 <sup>5</sup>	1.77 x 10 <sup>5</sup>
Morinda Citrifolia	2.03 x 10 <sup>5</sup>	1.67 x 10 <sup>5</sup>	1.83 x 10 <sup>5</sup>
Aloe Vera	3.43 x 10 <sup>5</sup>	2.03 x 10 <sup>5</sup>	1.87 x 10 <sup>5</sup>

#### DISCUSSION:

Currently, the use of natural extracts in dentistry has gained importance both to patients and endodontic professionals, as the shift is toward natural health remedies Hence the use of an irrigant made of natural extracts is of great significance<sup>7</sup>. The use of this irrigants during biomechanical preparation seems to be associated with increased anti-microbial property<sup>8,9,10</sup>. But, herbal products are not widely used in practice due to manipulation difficulties. So this study evaluated the antimicrobial efficacy of some of the commercially available health mixtures containing herbal components in them, against *E.faecalis*.

In vitro model developed by Haapasalo and Ørstavik has been used to assess the efficacy of endodontic medicaments in the disinfection of dentinal tubules<sup>11</sup>. Lynne et al<sup>12</sup> modified this model to include quantitative analysis of bacteria in the dentin tubules to define a percentage of reduction in colony forming units in infected dentin before and after the application of intracanal medication preparations. *E. faecalis* was chosen as the test organism because it has long been implicated in persistent root canal infections and has been identified as the species most commonly recovered from root canals of teeth with post treatment disease<sup>13</sup>.

As expected 3% sodium hypochlorite demonstrated highest antimicrobial activity followed by herbal irrigants. Bioactive compounds found within herbal irrigants are the reason for demonstrating such antimicrobial activity<sup>14</sup>. Morinda citrifolia juice has a broad range of therapeutic effects including antibacterial, antifungal, antiviral, antitumor, anti-helminthic, analgesic, hypotensive, anti-inflammatory, and immune enhancing effects<sup>4</sup>. The beneficial antimicrobial effects may be the result of acubin, L-asperuloside, alizarin, scopoletin and other anthraquinones<sup>15</sup>.

Propolis, also known as bee glue and bee propolis, is a brownish resinous substance collected by bees, mainly from plants. It is a potent antimicrobial, antioxidant, and anti-inflammatory agent<sup>16</sup>. It is composed of resin and balsams (50-60%), pollen (5-10%), and other constituents like amino acids, minerals, vitamins A and B complex, and highly active biochemical substance known as bioflavonoids (vitamin P), phenols, and aromatic compounds<sup>17,18</sup>.

Aloe Vera contains mucilaginous extract called as Aloe Vera gel. Total leaf extracts contain anthraquinones<sup>19</sup>. It has a well-established antimicrobial activity ascribed to compounds that are now specifically identified as *p*-coumaric acid, ascorbic acid, pyrocatechol and cinnamic acid<sup>20</sup>.

CFU/ml was more in apical third of the root canal space compared to that of the coronal third, which can be attributed to the flow and viscous nature of irrigants. Sodium hypochlorite is less viscous compared to the herbal irrigants and can flow till the apical region, where as natural irrigants are more viscous.

Further research should be directed at the use of herbal irrigants in endodontics by increasing the concentration or by incorporating nanoparticle technologies to increase its efficacy.

#### CONCLUSION:

3% Sodium hypochlorite showed the maximum antimicrobial activity against *E. faecalis*. Among the natural irrigants, all of them exhibited antibacterial activity against *E. faecalis*, but it was not comparable with that of 3% sodium hypochlorite.

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