Abstract
Kandaswamy D, Venkateshbabu N, Gogulnath D, Kindo AJ. Dentinal tubule disinfection with 2% chlorhexidine gel, propolis, Morinda citrifolia juice (MCJ), 2% povidone iodine (POV-I), and calcium hydroxide on Enterococcus faecalis-infected root canal dentine at two different depths (200 μm and 400 μm) and three time intervals (day 1, 3 & 5).

Methodology One hundred and eighty extracted human teeth were infected for 21 days with E. faecalis. Samples were divided into six groups. Group I (Saline) (Negative control), Group II (Propolis), Group III (MCJ), Group IV (2% Povidone Iodine), Group V (2% Chlorhexidine Gel), Group VI (Calcium hydroxide). At the end of 1, 3, and 5 days, the remaining vital bacterial population was assessed. Dentine shavings were collected at two depths (200 μm and 400 μm), and total numbers of colony forming units were determined. The values were analysed statistically with one-way analysis of variance followed by Tukey multiple comparison test. The paired t-test was used to check for differences in growth at different time intervals within groups and for differences at the two depths (P < 0.01)

Results The number of colony-forming units was statistically significant in all groups compared to the control group (Saline). Group V (chlorhexidine gluconate) (100%) produced better antimicrobial efficacy followed by 2% POV-I (87%), propolis (71%), MCJ (69%), and calcium hydroxide (55%). There was no significant difference between propolis and MCJ and no significant difference between data at 200 μm and 400 μm.

Conclusion Propolis and MCJ were effective against E. faecalis in dentine of extracted teeth.

Keywords: calcium hydroxide, chlorhexidine gel, E. faecalis, morinda citrifolia juice, povidone iodine, propolis.
Enterococcus faecalis is more likely to be found in cases with post-treatment infection (Sirén et al. 1997). Starvation conditions appear to increase the resistance of E. faecalis substantially (Portenier et al. 2005). It is probable that the physiological state of the cells particularly in retreatment cases approximates to the starvation phase (Stuart et al. 2005).

Calcium hydroxide has been advocated as an intracanal medicament because of its bactericidal properties (Froeman & Barnes 1990). Its high pH (of about 12.5) has a destructive effect on bacterial cell membranes and protein structure (Spangberg 1994). However, calcium hydroxide is not effective in eliminating bacteria from dentinal tubules. Gomes et al. (2003) reported that E. faecalis present in the dentinal tubules was resistant to calcium hydroxide over 10 days.

Two per cent chlorhexidine gluconate (CHX) has been used as an irrigant and intracanal medicament in endodontics. CHX is a bis-bis-guanide (Carlo et al. 2006) that acts by adsorbing onto the cell wall of microorganism resulting in leakage of intracellular components. CHX has a broad-spectrum antimicrobial activity (Delany et al. 1982), targeting both gram-positive and gram-negative microbes and biocompatible (Yesilsoy et al. 1995).

Propolis, also known as bee glue and bee propolis, is a brownish resinous substance collected by bees, mainly from plants. It is used to reinforce the combs and to keep the hive environment aseptic (Uzel et al. 2005). It is a potent antimicrobial, antioxidant, and anti-inflammatory agent. The main chemical elements present in propolis are flavonoids, phenolics, and various aromatic compounds. Flavonoids are well-known plant compounds that have antioxidant, antibacterial, antifungal, antiviral, and anti-inflammatory properties (Gopikrishna et al. 2008).

Morinda citrifolia (Rubiaceae) (MCJ), commercially known as noni, is indigenous to tropical countries and is considered as an important folk medicine. Its juice has a broad range of therapeutic effects including antibacterial, antifungal, antiviral, antitumor, antihelmintic, analgesic, hypotensive, anti-inflammatory, and immune enhancing effects (Murray et al. 2008). Murray et al. (2008) compared the effectiveness of MCJ with sodium hypochlorite and chlorhexidine gluconate to remove the smear layer from the walls of instrumented root canals. They concluded that 6% MCJ could be used as an endodontic irrigant in combination with EDTA followed by a final flush with 6% MCJ (Murray et al. 2008).

The American Heart Association (AHA) recommend the use of topical antimicrobials such as povidone-iodine (POV-I) as an adjunct to systemic antibiotic cover to enhance the efficiency of current prophylactic measures (Dajani et al. 1997). POV-I, which is used widely as a topical antiseptic agent, is an iodophore in which iodine is linked to povidone (polyvinylpyrrolidone), a dextran-like molecule (Fleischer & Reimer 1997). POV-I appears to be active against all microorganisms, including gram-positive and gram-negative bacteria, spores, mycobacteria, fungi, viruses, and protozoa (Cherry et al. 2007).

This study was undertaken to evaluate the disinfection of dentinal tubules when contaminated with E. faecalis using propolis, MCJ, POV-I, 2% CHX gel when compared to calcium hydroxide.

### Materials and methods

#### Preparation of dentine specimens

The model proposed by Haapasalo & Ørstavik (1987) was modified. One hundred and eighty single-rooted human mandibular premolar teeth freshly extracted for orthodontic reasons from 90 individuals were selected. A rotary diamond disc was used to decoronate the teeth below the cementoenamel 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 (Mani Inc, Tachigi-ken, Japan) in a slow-speed handpiece was used to stan-

### Table 1

<table>
<thead>
<tr>
<th>Medicament</th>
<th>N</th>
<th>Mean 200 μm</th>
<th>Standard deviation 200 μm</th>
<th>Median 200 μm</th>
<th>Interquartile ranges 200 μm</th>
<th>Mean 400 μm</th>
<th>Standard deviation 400 μm</th>
<th>Median 400 μm</th>
<th>Interquartile ranges 400 μm</th>
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<tbody>
<tr>
<td>Propolis</td>
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<td>3.3 3.2</td>
<td>0.48 0.42</td>
<td>3 3</td>
<td>1.0 0.25</td>
<td>3.3 3.2</td>
<td>0.48 0.51</td>
<td>3 3</td>
<td>1.0 1.0</td>
</tr>
<tr>
<td>Morinda</td>
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<td>3.2 3.4</td>
<td>0.20 0.51</td>
<td>3 3</td>
<td>1.0 1.0</td>
<td>3.1 3.2</td>
<td>0.17 0.42</td>
<td>3 3</td>
<td>0.25 0.25</td>
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<tr>
<td>2% POV-I</td>
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<td>3.1 3.2</td>
<td>0.17 0.42</td>
<td>3 3</td>
<td>0.25 0.25</td>
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</tr>
<tr>
<td>CHX</td>
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<td>0.00 0.00</td>
<td>0 0</td>
<td>0 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca(OH)₂</td>
<td>10</td>
<td>3.3 3.3</td>
<td>0.15 0.48</td>
<td>3 3</td>
<td>1.0 1.0</td>
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</tbody>
</table>

POV-I, povidone Iodine; CHX, chlorhexidine gluconate.
standardize the internal diameter of the root canals. The specimens were placed in an ultrasonic bath of 17% ethylenediaminetetraacetic acid for 5 min followed by 3% NaOCl for 5 min to remove organic and inorganic debris. The traces of chemicals used were removed by immersing the dentine specimens in an ultrasonic bath containing distilled water for 5 min. All the specimens were sterilized in an autoclave for two cycles. The first cycle was at 121 °C and the second was with the specimens immersed in 1 mL of tryptone soya (TS) broth in individual microcentrifuge tubes.

Contamination of the specimens

The test organism used for this study was E. faecalis, which is a gram-positive facultative anaerobic bacterium that is common in root filled teeth with post-treatment infection. E. faecalis (ATCC 29212) was grown in tryptone soya agar for 24 h. The culture was suspended in 5 mL of TS broth and incubated for 4 h at 37 °C and its turbidity adjusted to 0.5 McFarland standard. Each dentine block was placed in pre-sterilized microcentrifuge tubes containing 1 mL of the TS broth. Fifty microlitres of the inoculum containing the E. faecalis was transferred into each of the microcentrifuge tubes. At the end of 24 h, the dentine specimens 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 dentine specimens in TS broth on tryptone soya agar plates. Contamination of the dentine specimens was carried out for a period of 21 days.

Antimicrobial assessment

At the end of 21 days, the specimens were irrigated with 5 mL of sterile saline to remove the incubation broth. They were assigned into seven groups (n = 30 dentine blocks). Group 1, saline (negative control); Group 2, propolis; Group 3, MCJ; Group 4, 2% POV-I; Group 5, 2% CHX gel; Group 6, calcium hydroxide. Calcium hydroxide (Sigma-Aldrich, Mumbai, India) was mixed with sterile saline in a ratio of 1.5 : 1(wt/vol) to obtain a paste-like consistency (Krithikadatta et al. 2007). Methyl cellulose was used as a thickening agent for Groups 2, 3, and 4.

The medicaments were placed inside the canals and sealed at both ends with paraffin wax. They were incubated in an anaerobic environment for 37 °C. At the end of 1, 3, and 5 days an assessment of microbial cells was carried out with 10 specimens at each time interval. Harvesting of dentine was carried out at two depths (200 and 400 µm) with Gates Glidden drills no 4 and 5, respectively. The collected dentine 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

Table 2 Mean, standard deviation, median, and interquartile range for various intracanal medicaments at Log Day 3 at 200 & 400 µm

<table>
<thead>
<tr>
<th>N</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Median</th>
<th>Interquartile ranges</th>
</tr>
</thead>
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<tr>
<td></td>
<td>200 µm</td>
<td>400 µm</td>
<td>200 µm</td>
<td>400 µm</td>
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<td>10</td>
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<td>2.7</td>
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</tr>
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<td>Morinda</td>
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<td>1.4</td>
<td>2.7</td>
<td>0.51</td>
</tr>
<tr>
<td>CHX</td>
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<td>0</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Ca(OH)₂</td>
<td>10</td>
<td>3.3</td>
<td>3.3</td>
<td>0.48</td>
</tr>
</tbody>
</table>

POV-I, povidone iodine; CHX, chlorhexidine gluconate.

Table 3 Mean, standard deviation, median, and interquartile range for various intracanal medicaments at Log Day 5 at 200 & 400 µm

<table>
<thead>
<tr>
<th>N</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Median</th>
<th>Interquartile ranges</th>
</tr>
</thead>
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<td></td>
<td>200 µm</td>
<td>400 µm</td>
<td>200 µm</td>
<td>400 µm</td>
</tr>
<tr>
<td>Propolis</td>
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<td>0.60</td>
</tr>
<tr>
<td>Morinda</td>
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<tr>
<td>2% POV-I</td>
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<tr>
<td>CHX</td>
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<tr>
<td>Ca(OH)₂</td>
<td>10</td>
<td>2.7</td>
<td>2.5</td>
<td>0.48</td>
</tr>
</tbody>
</table>

POV-I, povidone iodine; CHX, chlorhexidine gluconate.
diluted, 100 μL of the broth in 100 μL of sterile saline five times. Fifty microlitres of the dilution was then plated on TS agar plates and incubated for 24 h. Colonies were counted and readings were tabulated.

Statistical analysis

The data were statistically analysed with one-way analysis of variance followed by Tukey multiple comparison means to check the difference in bacterial inhibition between groups (P < 0.01). The paired t-test was used to check for differences in growth at different time intervals within groups and for differences at the two depths (P < 0.01).

Results

The number of colony-forming units in all the experimental groups was significantly lower in comparison with the control group (Saline). Group V (CHX) (100%) demonstrated better antimicrobial efficacy followed by 2% POV-I (87.07%), propolis (71%), MCJ (69%), and calcium hydroxide (55%). No statistical difference was seen between propolis and MCJ. There was no difference in the data between 200 μm and 400 μm (Tables 1,2,3).

Discussion

The use of a biocompatible intracanal medicament possessing antimicrobial properties between appointments may reduce or eliminate bacteria in the root canal system and significantly increase the success of root canal treatment (Bystro¨m et al. 1985). The in vitro model developed by Haapasalo & Ørstavik (1987) has been used to assess the efficacy of endodontic medicaments in the disinfection of dentinal tubules. Lynne et al. (2003) modified this model to include quantitative analysis of bacteria in the dentine tubules to define a percentage of reduction in CFU in infected dentine before and after the application of intracanal medicaments. The model has clear limitations because it does not reflect the situation in apical dentine, which is mostly sclerotic (Paque et al. 2006). E. faecalis was chosen as a test organism because it is a facultative organism that is non-fastidious, easy-to-grow, and efficiently and rapidly colonizes tubules (Ørstavik & Haapasalo 1990). It has been used extensively in endodontic research because it has been found to be present in 63% of teeth with post-treatment disease (Hancock et al. 2001).

In the present study, 2% CHX gel provided 100% inhibition of E. faecalis at depths of 200 μm and 400 μm from day 1 to day 5. The possible reason could be the bactericidal dosage of 2% and increased diffusion of the medicament into the dentinal tubules (Krithikadatta et al. 2007). Basrani et al. (2003) observed that 2% CHX gel was a better antimicrobial when compared to 0.2% CHX gel or calcium hydroxide mixed with 0.2% chlorhexidine. The result of the present study was similar to that of Krithikadatta et al. (2007), Gomes et al. (2003), and Siqueria & Uzeda (1997).

POV-I produced 68% and 72% inhibition of E. faecalis at depths of 200 μm and 400 μm from day 1 to day 5. The possible reason might be attributed to the povidone molecule, by virtue of its affinity for cell membranes, delivers diatomic free iodine directly to the bacterial cell surface where it exerts its antibacterial effects (Schreier et al. 1997). POV-I appears to be active against all microorganisms, including gram-positive and gram-negative bacteria, spores, mycobacteria, fungi, viruses, and protozoa (Cherry et al. 2007).

Propolis produced 66% and 70% inhibition of E. faecalis at depths of 200 μm and 400 μm from day 1 to day 5. The possible reason for the antimicrobial action of propolis might be attributed to its flavanoid content (Grange & Davey 1990). Grange & Davey (1990) assessed the bacteriocidal ability of propolis against a wide range of gram-positive and gram-negative organisms. They reported complete inhibition of cultures of Staphylococcus aureus, including MRSA strains. The results of the present study was similar to the study of Awawdeh et al. (2008) who compared the antimicrobial activity of propolis with calcium hydroxide as intracanal medicament against E. faecalis and that propolis was effective in eliminating the microorganism.

MCJ showed 41% inhibition of E. faecalis at depths of 200 μm and 400 μm from day 1 to day 5. The possible reason for the antimicrobial action of MCJ might be attributed to its flavanoid content followed by POV-I, propolis and calcium hydroxide as a short-term intracanal medicament against E. faecalis followed by POV-I, propolis, MCJ, and Ca(OH)2

References