Antibacterial Effect of *Azadirachta indica* (Neem) or *Curcuma longa* (Turmeric) against *Enterococcus faecalis* Compared with That of 5% Sodium Hypochlorite or 2% Chlorhexidine *in vitro*

Dakshita Joy Sinha, Kanwar D.S. Nandha, Natasha Jaiswal, Agrima Vasudeva, Shashi Prabha Tyagi and Udai Pratap Singh

Department of Conservative Dentistry and Endodontics, Kothiwal Dental College and Research Centre, Moradabad, U.P. 244001, India

Received 28 September, 2015/Accepted for publication 30 August, 2016

**Abstract**

The purpose of this study was to compare the antibacterial properties of *Azadirachta indica* (neem) or *Curcuma longa* (turmeric) against *Enterococcus faecalis* with those of 5% sodium hypochlorite or 2% chlorhexidine as root canal irrigants *in vitro*. The activity of neem, chlorhexidine, sodium hypochlorite, or turmeric against *E. faecalis* was measured on agar plates using the agar diffusion method. The tube dilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the irrigants used. Chlorhexidine or neem exhibited the greatest antibacterial activity when used as endodontic irrigants against *E. faecalis*, followed by sodium hypochlorite. No statistically significant difference was observed between neem, sodium hypochlorite, or chlorhexidine. The MIC of neem was 1:128, which was similar to that of chlorhexidine. The MBC for each of these irrigants was 1:16. Neem yielded antibacterial activity equivalent to 2% chlorhexidine or sodium hypochlorite against *E. faecalis*, suggesting that it offers a promising alternative to the other root canal irrigants tested.

Key words: Chlorhexidine — *Enterococcus faecalis* — Herbal irrigants — Sodium hypochlorite

**Introduction**

The elimination of microorganisms from the root canal is crucial to successful endodontic treatment, which emphasizes the need for chemo-mechanical preparation. Instrumentation and irrigation promote significant microbial reduction during the stages of cleaning and shaping. Complete eradication of intra-canal infection, however, is still unachievable by the methods currently available, and remaining microorganisms may
cause re-infection of the root canal space\textsuperscript{(15)}. The most common Enterococcus species isolated from non-healing endodontic cases is Enterococcus faecalis\textsuperscript{(16)}, and its prevalence in failed endodontic cases ranges from 24 to 77\%\textsuperscript{(25)}. It has the ability to bind to dentin, invade dentinal tubules, and survive starvation by genetic polymorphism. It possesses a number of virulence factors, including lytic enzymes, cytolsin, aggregation systems, pheromones, and lipoteichoic acid. It can also suppress the action of lymphocytes\textsuperscript{(17,28)}. The most effective way to eliminate E. faecalis from the root canal space and dentinal tubules is to apply sodium hypochlorite or chlorhexidine in gel or liquid form. Both of these irrigants possess certain disadvantages, however. Sodium hypochlorite has an unpleasant taste, high toxicity, stains instruments, burns surrounding tissue\textsuperscript{(18)}, corrodes instruments\textsuperscript{(19)}, cannot remove smear layers\textsuperscript{(20)}, and reduces the elastic modulus and flexural strength of dentin\textsuperscript{(8,19)}. Chlorhexidine is a broad-spectrum antimicrobial agent. Its antimicrobial action is related to its cationic bisbiguanide molecular structure. At low concentrations, it is bacteriostatic, while at high concentrations, it is bactericidal, resulting in coagulation and precipitation of cytoplasm. It carries the property of substantivity\textsuperscript{(10)}. The major advantages of chlorhexidine over sodium hypochlorite are its lower cytotoxicity and lack of foul smell and bad taste. It has certain disadvantages, however: for example, it cannot dissolve organic substances, necrotic tissue, or smear layers\textsuperscript{(21)}. Due to the disadvantages of these two conventional irrigating solutions, therefore, it is necessary to find a better alternative, which has led to the search for an herbal alternative.

Azadirachta indica (neem) is the most commonly used traditional medicinal plant in India\textsuperscript{(26)}. Each part of the tree has been explored in phytotherapy. More than 140 biologically active compounds that exhibit immunomodulatory, anti-inflammatory, antifungal, antibacterial, antiviral, antioxidant, antimutagenic, or anticarcinogenic properties have been isolated from this plant. Its antimicrobial properties are due to the presence of alkaloids, glycosides, saponins, flavonoids, steroids, anthraquinone, tannic acid, and active constituents such as nimbidin, nimbin, nimbolide, gedunin, azadirachtin, mahmoodin, margolone, and cyclic trisulphide. These active constituents uncouple mitochondrial oxidative phosphorylation, thus inhibiting the respiratory chain. This results in anti-adherence activity by altering microbial adhesion and the ability of microorganisms to colonize, causing maximum reduction in adherence to dentin\textsuperscript{(5,26)}.

Curcuma longa (turmeric) belongs to the Zingiberaceae family. It is a native of southeast Asia and is cultivated mainly in India. It has a wide spectrum of action, including anti-inflammatory, antioxidant, antibacterial, antifungal, antiprotozoal, and antiviral activities. The main yellow bioactive component of C. longa is curcumin (diferuloylmethane). It is a natural polyphenolic flavonoid and is a known cyclooxygenase-2 inhibitor\textsuperscript{(16)}. To our knowledge, no studies to date have compared the antimicrobial efficacy of these herbal products with that of sodium hypochlorite or chlorhexidine. The purpose of the present study, therefore, was to compare the antimicrobial potential of these new herbal irrigants with that of sodium hypochlorite or chlorhexidine against E. faecalis.

**Materials and Methods**

Enterococcus faecalis, a facultative anaerobic bacteria, was selected as the test microorganism. An agar disc diffusion test was conducted to evaluate and compare the antimicrobial efficacy of the targeted medicaments. The E. faecalis (ATCC 29212, HiMedia, Pune, India) strains were reactivated in a brain heart infusion (BHI) broth (Sigma Aldrich, Bengaluru, India).

Cultures of E. faecalis were maintained in the BHI broth and agar. The cultures were grown overnight at 37°C in the BHI broth on a rotary shaker (150 rpm) and bacterial
growth determined by change in turbidity after 24 hr\(^9\).

Inoculums of the \textit{E. faecalis} bacterial strains were prepared by harvesting 4 to 5 colonies with a circular, previously sterilized loop and dissolving them into their respective test tubes containing saline solution to produce a turbidity of 0.5 on the McFarland scale. A sterile cotton swab was then rolled in the suspension to streak the plate surface of a Mueller-Hinton agar plate (HiMedia, Pune, India) for growth of \textit{E. faecalis}\(^3\).

Discs of 6 mm in diameter were prepared from Whatman paper No.1 (Wipro GE Healthcare Pvt. Ltd., Ahmedabad, India) and sterilized in a hot air oven. These discs were then saturated with 50 \(\mu\)l of each irrigant and aseptically transferred to the agar plate previously incubated with bacteria. The procedure was carried out under laminar flow conditions.

Alcoholic extract of neem was prepared by adding 25 g fresh neem leaf powder of 99% purity (The Indian Neem Tree Company, Mumbai, India) to 50 ml absolute ethanol (Sterling Chemicals and Alcohols Pvt. Ltd., Mumbai, India). The mixture was then macerated for 1–2 min and the resulting extract filtered through muslin cloth for coarse residue and then through filter paper for finer residue\(^20,22-24\).

A total of 40 g turmeric powder (RYM Exports, Mumbai, India) was dissolved in 500 ml ethanol. Extract was then filtered through muslin cloth for coarse residue and filter paper for finer residue\(^28\).

Other irrigants used were commercially available 2% chlorhexidine solution (Ceraxidin-C, IMICRYL Corporation, Konya, Turkey) and 5% sodium hypochlorite (WERAX, SDD, Izmir, Turkey). Saline was taken as negative control (0.9% Sodium chloride; 9 g/liter).

The plates were then incubated at 37\(^\circ\)C under aerobic conditions. All assays were repeated 4 times to ensure reproducibility. After 24 hr, microbial zones of inhibition were measured in millimeters\(^3\). A one-way analysis of variance (ANOVA) and the Bonferroni multiple comparison test were used for inter-group comparisons in the statistical analysis. A p value of <0.05 was considered statistically significant. The statistical analysis was performed with SPSS (PC. Version 10 software) (IBM, NY, USA).

1. Minimum inhibitory concentration

\textit{Enterococcus faecalis} bacterial strains were grown in BHI broth for 24 hr at 37\(^\circ\)C. Serial dilution of all the test irrigants (neem, turmeric, 5% sodium hypochlorite, or 2% chlorhexidine) was performed as follows: 100 \(\mu\)l each dilution was added to 100 \(\mu\)l Mueller-Hinton broth (HiMedia, Pune, India). A total of 5 \(\mu\)l bacterial suspension adjusted to 0.5 on the McFarland scale was then added to the broth and the mixture incubated at 37\(^\circ\)C for 24 hr. After 24 hr, the tubes were visually checked for turbidity (bacterial growth). The lowest dilution inhibiting growth was taken as the minimum inhibitory concentration (MIC). A loopful of each broth dilution was obtained and streaked onto a flat-bottomed Mueller-Hinton agar plate (HiMedia). The growth of bacteria was checked after incubation for 24 hr at 37\(^\circ\)C under laminar flow and aerobic conditions. The lowest dilution at which bacterial growth was halted was taken as the minimum bactericidal concentration (MBC)\(^11\).

Results

Chlorhexidine yielded the largest zone of inhibition, followed by neem (Fig. 1). A significant difference was observed between the zone diameters obtained with neem, chlorhexidine, or sodium hypochlorite and that with turmeric. No statistically significant difference was observed between neem, chlorhexidine, and sodium hypochlorite, however (p<0.05) (Table 1). No statistically significant difference was observed between groups 1, 3, and 4, which is why all have been denoted with an ‘a’. A statistically significant difference was observed between group 2 and groups 1, 3, and 4, however, which is why it is denoted with a ‘b’. A statistically significant
difference was observed between group 5 and groups 1, 2, 3, and 4, which is why it is denoted with a ‘c’. The MIC represents the lowest concentration at which an irrigant inhibited growth by absence of turbidity. The MBC represents the lowest concentration at which an irrigant halted growth. The results for MIC and MBC showed that neem (1:128 and 1:16, respectively) and chlorhexidine (1:128 and 1:16, respectively) were strong anti-bacterials, even after dilution (Table 2).

Discussion

Enterococcus faecalis is a saprophytic component of enteric flora. It was selected as the test organism in this study as it is the most common bacterium isolated during endodontic retreatment of apical periodontitis, either as a single organism or as a major component of the flora. The agar diffusion method used for preliminary assessment here is commonly used in evaluating antibacterial activity, and is accepted as an adequate way of comparing the antibacterial effect of different root canal irrigants.

Sodium hypochlorite, the most commonly used irrigant in clinical practice, exhibits strong antibacterial activity. The present

![Zones of inhibition](image)

**Fig. 1** Zones of inhibition yielded by N = Azadirachta indica (Neem); CHX = Chlorhexidine; H = Sodium hypochlorite; C.l. = Curcuma longa (Turmeric); S = Saline

<table>
<thead>
<tr>
<th>Group</th>
<th>Irrigant</th>
<th>Mean + / − S.D (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Azadirachta indica</em> (Neem)</td>
<td>14.42 + / − 0.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td><em>Curcuma longa</em> (Turmeric)</td>
<td>9.62 + / − 0.47&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Sodium hypochlorite</td>
<td>14.37 + / − 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>Chlorhexidine</td>
<td>14.5 + / − 0.37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>Saline</td>
<td>0.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

p<0.05

**Table 2** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Neem, Chlorhexidine, Sodium hypochlorite, and *Curcuma longa*

<table>
<thead>
<tr>
<th>Irrigant</th>
<th>MIC (mg/liter)</th>
<th>MBC (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Azadirachta indica</em> (Neem)</td>
<td>1 : 128</td>
<td>1 : 16</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>1 : 128</td>
<td>1 : 16</td>
</tr>
<tr>
<td>Sodium hypochlorite</td>
<td>1 : 64</td>
<td>1 : 8</td>
</tr>
<tr>
<td><em>Curcuma longa</em> (Turmeric)</td>
<td>1 : 8</td>
<td>1 : 2</td>
</tr>
</tbody>
</table>
results showed that it exhibited strong antibacterial efficacy, similar to that of chlorhexidine or neem. As such, this was in accordance with the results of an earlier study by Sinha et al. (2021).

Chlorhexidine possesses strong antibacterial properties. It is a positively-charged hydrophobic and lipophilic molecule which interacts with negatively-charged phospholipids and lipopolysaccharides on the cell membrane of bacteria. This alters osmotic equilibrium, thereby allowing chlorhexidine molecules to penetrate. Here, chlorhexidine exhibited strong antibacterial efficacy, which is in accordance with the results of an earlier study by Basson and Tait (2018). Although the above-mentioned conventional irrigants show strong antibacterial activity, each has certain drawbacks, which has resulted in the search for better alternatives, especially herbal.

In the present study, neem exhibited strong antibacterial activity against *E. faecalis* similar to that of chlorhexidine or sodium hypochlorite. Ethanolic extract of neem was chosen here, as earlier studies found that it was better than aqueous extract (2014, 2020). The present results also support those of another earlier study by Bohora et al. (2021) showing that neem exhibited a significant antibacterial effect against *E. faecalis*.

In the present study, turmeric exhibited only limited antibacterial efficacy, significantly lower than that of the other three irrigants tested. This was in contrast to the results of an earlier study by Neelakantan et al. (2015). This difference may be due to differences in the testing methodology. On the other hand, the present results are in agreement with those of another study by Suvarna et al., which showed that ethanolic extract of turmeric exhibited no activity against *E. faecalis* (2020). Absolute alcohol was not selected as one of the groups in the present study as previously published data revealed that it yields no zones of inhibition (2019). As expected, saline yielded no zones of inhibition in the present study.

The MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. As such, it is an important diagnostic measure in confirming the resistance of microorganisms to an antimicrobial agent and in monitoring the activity of new antimicrobial agents. The present results on MIC revealed that chlorhexidine and neem were strong antibacterials against *E. faecalis*, even after dilution. Sodium hypochlorite also exhibited strong antibacterial efficacy on dilution.

Earlier research on the effects of neem or turmeric has compared them with either chlorhexidine or sodium hypochlorite. To our knowledge, this is the first time that these herbal irrigants have been compared with chlorhexidine or sodium hypochlorite against *E. faecalis* in the same study.

**Conclusion**

Within the limitations of this study, it can be concluded that neem is as effective as chlorhexidine or sodium hypochlorite against *E. faecalis*. Turmeric also exhibited limited antibacterial action against *E. faecalis*. This suggests that neem offers an alternative to sodium hypochlorite or chlorhexidine in the treatment of endodontic infection. We believe that further in vivo and long-term studies are warranted.

**Acknowledgements**

We would like to thank Dr. Jagvir Singh (M.Sc. Microbiology, Ph.D.) for helping us with this study.

**References**


Correspondence:
Dr. Kanwar Deep Singh
Department of Conservative Dentistry and Endodontics,
Kothiwal Dental College and Research Centre,
Moradabad, U.P. 244001, India
E-mail: kanwar37@gmail.com