Antibacterial efficacy of curcumin, allicin, gingerol and cinnamon against Enterococcus faecalis: An in vitro study

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Abstract

Introduction: Bioactive compounds from plants have potential antimicrobial activity. The aim of this in vitro study was to evaluate the antimicrobial efficacy of curcumin, allicin, gingerol and cinnamon compared to 4% sodium hypochlorite (NaOCl) against Enterococcus faecalis and its biofilm.

Methods: The dry herbal compounds were diluted with dimethyl sulfoxide (DMSO). Antimicrobial activity was evaluated using agar diffusion test, minimum inhibitory concentration (MIC) assay, minimum bactericidal concentration (MBC) test, time kill study, and biofilm susceptibility assay. The zone of inhibition (ZOI) was determined using agar diffusion test on Muller Hinton (MH) agar plates. MIC was evaluated using the tube dilution method. Root canals of extracted human anterior teeth were instrumented, split into two halves, autoclaved, and incubated with brain heart infusion broth containing E. faecalis for 21 days to form a biofilm. The susceptibility of the biofilm to the test solutions was evaluated by counting bacterial colonies on MH agar.

Results: NaOCl exhibited potent antimicrobial activity under all tested parameters. Allicin showed a significantly greater ZOI, while curcumin showed the least MIC among the tested herbal extracts (P < 0.05). MBC varied widely among the groups with no significant difference between allicin and cinnamon (P > 0.05). Gingerol and cinnamon were significantly superior to the other groups killing E. faecalis within 4-4.2 min (P < 0.05). Curcumin, gingerol, and cinnamon were equally efficacious as NaOCl in completely eradicating E. faecalis biofilm (P > 0.05).

Conclusion: NaOCl emerged as the most efficacious antibacterial agent and all herbal extracts showed significant antibacterial activity against E. faecalis.

Implication for health policy/practice/research/medical education: This in vitro study showed the promising antibacterial effects of curcumin, allicin, gingerol, and cinnamon against Enterococcus faecalis. Future studies should focus on harnessing the advantages of these natural medicaments in endodontic disinfection.


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Introduction

Microorganisms form the primary etiological factors in the development of pulpal and periapical pathology (1). The main objective of endodontic therapy is to eliminate infected root canal contents using appropriate instrumentation, irrigation, and disinfection protocols, which will eventually enable a relatively bacteria-free canal (2). Studies have indicated that apical periodontitis is a biofilm-related disease (3). While the primary colonizers are planktonic, they slowly get organized into a structured biofilm, adhering to the main canal, lateral canals, isthmuses and apical ramifications (4). Routine instrumentation and irrigation can remove planktonic and superficial bacteria in a biofilm effortlessly; however,
a strategic treatment planning is needed to effectively disrupt and eliminate bacteria present in deeper layers of the biofilm (2).

Persistent endodontic infections result from residual bacteria that manage to survive chemomechanical preparation (1). *Enterococcus faecalis*, a facultative anaerobic gram-positive bacterium, is the most commonly isolated species in infections involving root-filled teeth (5,6). It is capable of establishing a biofilm in the root canal, which enables it to resist intracanal antimicrobials and survive harsh, non-nutrient conditions (7).

Sodium hypochlorite (NaOCl) remains the most commonly used endodontic irrigant owing to its excellent antimicrobial and tissue dissolving properties (8). It is also associated with some disadvantages such as an unpleasant taste, toxicity, painful inflammatory reaction following accidental periapical extrusion and deleterious effects on flexural strength of root dentin (9,10). Hence, an increasing inclination of research towards natural remedies could be evidenced lately.

Natural spices such as turmeric (11), garlic (12), ginger (13), and cinnamon (14) have expressed bactericidal activity against root canal pathogens. *Curcuma Longa* Linn., commonly known as turmeric and *Zingiber officinale* Roscoe or ginger, both belonging to the Zingiberaceae family, exhibit broad spectrum biologic activities inclusive of antimicrobial, anti-inflammatory, and antioxidant activities (11,13). *Allium sativum* (garlic) of the Liliaceae family contains allicin, a key component responsible for its antibacterial and antifungal properties (12). *Cinnamomum zeylanicum* or cinnamon belonging to the Lauraceae family exhibits significant antibacterial and antifungal properties (14). Though various isolated studies have demonstrated the effectiveness of these herbal alternatives against *E. faecalis* (15-18), there are no studies in the literature providing a comprehensive comparison of the antibiofilm activity of these agents with NaOCl against *E. faecalis* biofilm. Hence, this *in vitro* study aimed to comparatively assess the antibacterial efficacy of curcumin, allicin, gingerol, and cinnamon in comparison to NaOCl against *E. faecalis* and its biofilm formed on root dentin surface of extracted human teeth using agar diffusion test, minimum inhibitory concentration (MIC) assay, minimum bactericidal concentration (MBC) test, as well as time kill study and biofilm susceptibility assay.

**Materials and Methods**

**Preparation of test solutions**
Curcumin, allicin, gingerol, and cinnamon were purchased as dry compounds from ATOZ Pharmaceuticals Pvt. Ltd., Chennai, India, and their purity was assessed to be >98% by high-performance liquid chromatography (Dynamic Bio-Medical Systems, New Delhi, India). Ten milliliters of dimethyl sulfoxide (DMSO) (Sigma Aldrich Chemicals Pvt. Ltd., Bangalore, India) was diluted using 90 mL of distilled water. Twenty grams each of curcumin (group 1), allicin (group 2), gingerol (group 3), and cinnamon (group 4) was dissolved in 10% DMSO solution to make 20% of the respective test solutions. 4% NaOCl (group 5) served as positive control and saline (group 6) served as negative control.

A pure culture of *E. faecalis* test strain ATCC 29212 was cultured in Brain Heart Infusion (BHI) broth (HiMedia Laboratories Pvt. Ltd., Mumbai, India), and incubated at 37 °C for 24 hours. Broth turbidity was balanced spectrophotometrically to 560 nm optical density corresponding to McFarland 0.5 scale (1.5 × 10⁶ colony forming units per mL).

**Agar diffusion test**
Under sterile conditions, the fresh inoculum of *E. faecalis* was swabbed over 20 mL of Muller Hinton agar (MHA) plate (HiMedia Laboratories Pvt. Ltd., Mumbai, India). Labelled wells measuring 6 × 4 mm were punched into agar medium, with each well receiving 10 µL of the test solution via a micropipette and incubated at 37 °C for 24 hours. The shortest distance spanning the outer margin of the well to the initial line of microbial growth was noted as the zone of inhibition (ZOI) (13).

**Minimum inhibitory concentration assay**
MIC was determined using tube dilution method. Double dilution was prepared from a higher (100 mg/mL) to a lower dilution sequentially. The first test tube received 500 µL of BHI broth followed by 500 µL of the test solution. Later, the concentrations were reduced from higher dilution to half in subsequent tubes from 1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9 µg/mL by diluting with distilled water. 50 µL of standardized *E. faecalis* suspension was inoculated in each tube and incubated overnight at 37 °C. The lowest dilution without any visible turbidity, indicating *E. faecalis* growth prevention was recorded as MIC (13).

**Minimum bactericidal concentration test**
Subcultures were made from samples obtained from MIC assay test tubes with no visible turbidity. These test sample solutions were inoculated into freshly prepared BHI agar plates and incubated for 24 hours at 37 °C. The lowest concentration of the test solution that allowed less than 0.1% *E. faecalis* growth was noted as MBC (13).

**Time kill study**
The time required to eliminate *E. faecalis* following its 30 min exposure to bactericidal concentration of the test solution was evaluated by time kill analysis. At 2 min regular intervals, a loop full of sample was inoculated into BHI agar plates and incubated at 37 °C for 24 hours and microbial growth was noted (19). Agar diffusion test, MIC, MBC and time kill analysis were done in triplicate.
Biofilm formation on tooth substrate
Sixty-one freshly extracted human maxillary anterior teeth were cleansed to remove surface tissue tags and calculus and stored in 0.2% thymol solution. Maintaining a fixed length of 14 mm from the apex, the remaining portion of the tooth was sectioned using a slow speed bur under copious water cooling. The root canals were instrumented in a crown down manner and the canals enlarged up to #20 K-file and subsequently finished with rotary F3 Protaper file (Dentsply Maillefer, Ballaigues, Switzerland). Two milliliters of 4% NaOCl was used between each instrument change. The teeth were cut into mesial and distal halves. The sectioned halves were ultrasonicated to remove the smear layer and sterilized in an autoclave at 121 °C for 20 minutes. Later, the samples were transferred to BHI broth and incubated at 37 °C for 24 hours. The sterility of the tooth samples was checked by observing changes in the turbidity of the broth solution after incubation.

The outer root surfaces of the sterile tooth sections were coated with nail varnish and the inner root surfaces were inoculated with BHI broth containing Enterococcus faecalis (1.5×10⁶ CFU/mL) and incubated for 21 days at 37 °C with the culture medium replaced every alternate day. Samples from each broth was taken using sterile paper points and inoculated in BHI agar plates and incubated for 24 hours at 37 °C to inspect bacterial viability (20).

Enterococcus faecalis biofilm identification
The tooth sections were cleansed with sterile phosphate buffered saline (PBS) thrice to eliminate planktonic bacteria and excess medium. Following sputter coating with gold/palladium (208 HR High Resolution Sputter Coater, Ted Pella Inc, CA, USA), the development of biofilm was verified in two samples using a scanning electron microscope (SEM, JEOL JSM 35CF, Tokyo, Japan) operating at 15 kV at 2500x magnification.

Biofilm susceptibility assay
The sectioned samples were randomly divided into six groups of 20 samples each and were treated with the test solutions for 10 min. The biofilm on the root canal surface was excavated with sterile small size pedodontic periosteal elevator. The biofilm samples from each group were inoculated in MHA plates and incubated at 37 °C for 48 hours. Enterococcus faecalis colonies were calculated using automated colony counting system.

Statistical analysis
The data obtained from agar diffusion test, MIC, MBC, time kill analysis, and biofilm susceptibility assay were tabulated and statistically analyzed using one-way ANOVA and Kruskal-Wallis test (P<0.05). The data from biofilm susceptibility assay was analyzed by one-way ANOVA followed by Bonferroni post-hoc test (P<0.05).
NaOCl, confirming complete eradication of *E. faecalis* biofilm in these groups.

**Discussion**

Natural products are much sought after alternate treatment options for managing dental problems. Their ease of availability, antimicrobial efficacy, lack of microbial resistance, and safety with lesser side effects make these a widely explored area in research (21). Literature is abundant with studies of various herbs possessing proven antimicrobial properties, but extensive comparison of multiple herbs and their effectiveness is sparsely reported. This study aimed to compare the antimicrobial efficacy of curcumin, allicin, gingerol, and cinnamon in comparison with NaOCl, the most widely used endodontic irrigant, against *E. faecalis*. DMSO, which was used to dissolve the natural products in this study, is an organic solvent with amphitropic property. It is commonly used as a drug solvent, which enhances the permeability of the drugs across biological membranes. It also enables an effective penetration of the drug into the bacterial cellular system. DMSO might have potentiated the maximum efficacy of the herbs dissolved in it, thereby facilitating better penetration of the herbal extracts into the bacterial cells in the biofilm assay (22).

Agar diffusion is commonly used for assessing antimicrobial activity as it allows direct comparisons of the test agents against targeted microorganisms. Dilution method used here to calculate MIC serves as a reference method to test the susceptibility of bacteria to multiple antimicrobials at once (23). Time-kill studies provide descriptive (qualitative) information on pharmacodynamics of drug interactions (19). Biofilm susceptibility assay is an assessment method to analyze the antibacterial properties of various endodontic irrigants (20). In the present study, saline was used as negative control and sodium hypochlorite as positive control to compare the efficacy of herbal extracts. Samples irrigated with saline exhibited no antibacterial property, whereas sodium hypochlorite eliminated *E. faecalis* strain and biofilm. The predominant antibacterial property of sodium hypochlorite is due to the formation of hypochlorous acid (HOCl) when it encounters organic debris. HOCl oxidizes sulfhydryl groups within bacterial enzymes and disrupts its metabolism (24).

The main antibacterial constituent of *Allium sativum* (garlic) is the oxygenated sulphur compound, allicin (thio-2-propene-1-sulfinic acid S-allyl ester). It exerts its antimicrobial activity by reacting with thiol groups of microbial enzymes such as alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase (25). In the current study, though *Allium sativum* exhibited a higher ZOI compared to the other herbal extracts, it could not eradicate *E. faecalis* growth after 48 hours of incubation, which agrees with the results of Eswar et al (26) and Alrazhi et al (27), who stated that allicin did not completely eradicate *E. faecalis* strains.

*Curcuma longa* L, or turmeric, an herb predominantly cultivated in South East Asian countries contains phenolic component curcuminoids, responsible for its biological properties (28). In the current study, among the herbal extracts compared, curcumin had the least MIC. It also showed antibacterial efficacy by eliminating *E. faecalis* biofilm, which is similar to the results observed with

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**Figure 2.** Graphical representation of the mean zone of inhibition, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and time kill analysis of the herbal solutions and sodium hypochlorite (NaOCl) against *Enterococcus faecalis*. Statistical analysis was done using one-way ANOVA and Kruskal-Wallis test. In agar diffusion test, *a* vs allicin, curcumin, gingerol and cinnamon, *b* vs NaOCl, curcumin, gingerol and cinnamon, *c* vs NaOCl and allicin (*P* < 0.05). In MIC, *a* vs curcumin, allicin, gingerol and cinnamon, *b* vs NaOCl, allicin, gingerol and cinnamon, *c* vs NaOCl, curcumin, gingerol and cinnamon, *d* vs NaOCl, and allicin (*P* < 0.05). In MBC, *a* vs allicin, cinnamon, curcumin and gingerol, *b* vs NaOCl, curcumin and gingerol, *c* vs NaOCl, allicin and cinnamon (*P* < 0.05). In time kill study, *a* vs gingerol, allicin, cinnamon and curcumin, *b* vs NaOCl, allicin and curcumin, *c* vs NaOCl, gingerol, cinnamon, and curcumin, *d* vs NaOCl, gingerol, cinnamon, and allicin (*P* < 0.05).
previous studies (4,11). Curcumin present in *Curcuma longa* inhibits the assembly of protein-filamentating temperature-sensitive mutant Z (PtsZ) protofilaments and increases the GTPase activity of PtsZ. The disruption of GTPase activity has been proved lethal for bacteria (28). Paschoal et al hypothesized that curcumin affects bacterial DNA intercalation by generating strand breaks in the nucleic acid, which results in genetic mutation (29).

In the present study, though *Zingiber officinale* Roscoe or ginger showed the least ZOI against *E. faecalis*, it showed a minimal time, next to NaOCl, in time kill study and was also effective in eliminating *E. faecalis* biofilm, the results of which coincide with previous reports (13,30). The active components present in gingerol are volatile essential oils and phenolic compounds. Among the volatile essential components, gingerol, shagelol, and sesquiterpenoids have been accounted for their antimicrobial activities (13).

*Cinnamomum zeylanicum* or cinnamon exerted a significantly lesser time compared to other herbal extracts in time-kill study against *E. faecalis*. The two major components of cinnamon responsible for bactericidal activity are cinnamaldehyde and terpenes. Cinnamaldehyde (65-80% aromatic aldehyde) is an active electronegative compound, which hinders the activity of amino acid decarboxylation by interfering with electron transfer reactions, thus decreasing cellular glutathione levels in the bacterial cell wall, which leads to energy deficit and thereby, microbial cell death. Terpenes act predominantly through the disruption of the cell membrane due to the presence of lipophilic compounds (14,31). These antibacterial activities of cinnamon could have been the possible reason for the complete eradication of *E. faecalis* biofilm from the root canal surface in the present study.

Despite the antimicrobial benefits of NaOCl, in case of an inadvertent extrusion, the caustic nature of NaOCl might irreversibly damage periapical tissues. Active oxygen species, such as O and -OH ions, have bactericidal effects and induce deleterious tissue reactions, such as inflammation, radiation damage, cell aging, and mutation (9). On the other hand, even though the time required for herbal extracts were 4 to 8 minutes, they were able to eliminate *E. faecalis*. These results were comparatively similar to sodium hypochlorite in biofilm susceptibility test except allicin extract. Thus, further *in vitro* characterization studies incorporating curcumin, cinnamon, and gingerol with various requisite ingredients to enhance their use as root canal irrigants can be considered. Their uses as a part in the synthesis of nanoparticles exhibiting accelerated antimicrobial activity also need to be explored (32). Future studies should assess their effects on smear layer removal and fracture resistance of root dentin. Further *in vivo* studies should be carried out to validate the effectiveness of these herbal extracts as irrigants against other primary and secondary endodontic pathogens.

**Conclusion**

Within the limitations of this *in vitro* study, it can be concluded that NaOCl emerged the most effective antibacterial agent against *E. faecalis* in all the tested parameters. The herbal extracts showed noteworthy antibacterial property against *E. faecalis*. Considering their efficacy, these herbal agents are promising additives in endodontic disinfection.

**Authors’ contributions**

VH, SM, SV, and PVP conceived, planned, designed, guided, and edited the manuscript. VH and SV contributed to data analysis. VH and PVP collected the materials, interpreted the data and drafted the manuscript. All authors read and confirmed the final version of the manuscript and agreed its publication.

**Conflict of interests**

The authors declare no competing interests.

**Ethical considerations**

The study protocol was presented to the Institutional Review Board and approval was obtained (SRMU/M&HS/ SRMDC/2013/M.D.S.-PG Student/304).

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**Table 1. Mean colony forming units (log CFU/mL) of all groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Standard error</th>
<th>Lower bound</th>
<th>Upper bound</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td>0.0000</td>
<td>0.00000</td>
<td>0.00000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>Allicin</td>
<td>4.1600</td>
<td>0.20736</td>
<td>0.09274</td>
<td>3.9025</td>
<td>4.4175</td>
<td>3.90</td>
<td>4.40</td>
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<tr>
<td>Zingerol</td>
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<td>0.00000</td>
<td>0.00000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>0.0000</td>
<td>0.00000</td>
<td>0.00000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>NaOCl</td>
<td>0.0000</td>
<td>0.04928</td>
<td>0.00000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Saline</td>
<td>7.9200</td>
<td>3.10292</td>
<td>0.56651</td>
<td>7.4863</td>
<td>8.3537</td>
<td>7.50</td>
<td>8.40</td>
</tr>
<tr>
<td>Total</td>
<td>2.0133</td>
<td>3.10292</td>
<td>0.56651</td>
<td>0.8547</td>
<td>3.1720</td>
<td>0.00</td>
<td>8.40</td>
</tr>
</tbody>
</table>

The data were analyzed statistically by one-way ANOVA followed by Bonferroni post-hoc test. *vs* curcumin, zingerol, cinnamon, NaOCl, and saline, *vs* curcumin, allicin, zingerol, cinnamon, and NaOCl (P < 0.05). NaOCl: sodium hypochlorite.
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References

Herbal alternatives against Enterococcus faecalis


