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Antimicrobial Efficacy of Indigenous Irrigants Compared with Commercially Available Irrigants - An In Vitro Study

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Abstract

Background: Successful endodontic treatment depends on complete eradication of microorganisms from the root canal system. Conventional irrigants such as sodium hypochlorite and chlorhexidine are widely used; however, their cytotoxicity and other disadvantages have encouraged the search for herbal alternatives with better biocompatibility.

Aim: To evaluate and compare the antimicrobial efficacy of green tea extract and green coffee extract with commercially available endodontic Irrigants.

Materials and Methods: An in vitro agar well diffusion study was conducted using four test materials: 2% chlorhexidine solution, 3% sodium hypochlorite solution, aqueous extract of green coffee, and aqueous extract of green tea. Four bacterial strains associated with oral infections—Escherichia coli, Pseudomonas aeruginosa,

Staphylococcus aureus, and Streptococcus oral is—were used for antimicrobial susceptibility testing. Zones of inhibition were measured in millimetres and expressed as mean ± standard deviation.

Results: Green tea extract demonstrated the highest antimicrobial activity among the herbal irrigants, with maximum inhibition against E. coli (32.66 ± 0.47 mm). Chlorhexidine showed strong broad-spectrum activity against all tested organisms. Green coffee extract demonstrated moderate antibacterial efficacy, whereas sodium hypochlorite showed comparatively lower inhibition under the agar diffusion conditions.

Conclusion: Green tea extract exhibited promising antimicrobial activity against the tested bacterial strains and may warrant further investigation as a potential herbal adjunct to conventional root canal irrigants. Further in vivo and clinical studies are required to establish its effectiveness and safety in pediatric Endodontics.

Keywords: Green Tea Extract, Green Coffee Extract, Chlorhexidine, Sodium Hypochlorite, Herbal Irrigants, Pediatric Endodontics.

Introduction

Preservation of primary teeth until their normal exfoliation is essential for maintenance of arch integrity, mastication, speech, esthetics, and proper eruption of permanent successors. Dental caries remains one of the major causes of premature loss of primary teeth, and pulpal involvement frequently necessitates endodontic treatment in pediatric patients.[7]

The primary objective of endodontic therapy is complete elimination of microorganisms and necrotic debris from the root canal system while preserving the structural integrity of the tooth. Mechanical instrumentation alone is insufficient for complete debridement because of the complex root canal anatomy and presence of inaccessible canal irregularities. Therefore, chemical irrigants play an indispensable role in reducing microbial load and disinfecting the canal system.[6,7]

An ideal endodontic irrigant should possess broad-spectrum antimicrobial activity, tissue-dissolving capacity, smear layer removal ability, low toxicity, lubricating properties, and substantively. Sodium hypochlorite is considered the gold standard irrigant because of its excellent antimicrobial and tissue dissolution properties. However, its unpleasant taste, cytotoxicity, risk of tissue injury, and potential adverse effects on periapical tissues limit its clinical use, especially in pediatric dentistry.[5, 7, 13] Chlorhexidine is another widely used irrigant because of its broad-spectrum antimicrobial activity and substantivity.

Nevertheless, it lacks tissue-dissolving ability and is ineffective in smear layer removal. These drawbacks have led researchers to explore herbal alternatives that may provide effective antimicrobial action with improved biocompatibility.[4,8]

Herbal irrigants have gained increasing attention because of their safety profile, availability, reduced toxicity, and therapeutic properties. Green tea extract contains catechins, particularly epigallocatechin gal late (EGCG), which exhibit potent antibacterial and antioxidant effects. Green coffee bean extract contains chlorogenic acid, caffeic acid, and polyphenolic compounds with proven antimicrobial potential.[1,9,15]

Considering the increasing demand for biocompatible and naturally derived irrigants in pediatric endodontics, the present study was undertaken to evaluate and compare the antimicrobial efficacy of green tea extract and green coffee extract with commercially available irrigants such as Chlorhexidine and sodium hypochlorite.

Aim and Objectives

Aim

To evaluate the antimicrobial efficacy of indigenous herbal irrigants and compare them with commercially available endodontic irrigants.

Objectives

1. To evaluate the antimicrobial efficacy of green tea extract as an endodontic irrigant.
2. To evaluate the antimicrobial efficacy of green coffee extract as an endodontic irrigant.
3. To evaluate the antimicrobial efficacy of 2% Chlorhexidine solution.
4. To compare the antimicrobial efficacy of all test irrigants against selected oral bacterial strains.

Materials and Methods

Study Design

An in vitro experimental study was conducted in the Department of Pedodontics and Preventive Dentistry in collaboration with the Department of Microbiology at Himachal Dental College and Hospital, Sundernagar, Himachal Pradesh, India.

Test Materials

The following irrigants were evaluated:

Figure 1. Test materials evaluated: (a) 2% chlorhexidine solution; (b) 3% sodium hypochlorite solution; (c) green coffee powder; and (d) Wagh Bakri green tea.

Aqueous extracts of green tea and green coffee were prepared using Soxh let extraction (Figure 1). Fifty grams of plant material was placedina Whatman No.1 filter paper thimble and extracted with 500 Ml distilled water at 80°C. Extraction continued until the solvent became colorless, indicating exhaustive extraction.

The obtained extracts were concentrated on a heated water bathuntila semi - solid crude extract was obtained. The extracts were then stored in sterile airtight containers (Figure 2). Standard stock solutions of100mg/mL were prepared using dim ethyl sulfoxide (DMSO).

Table 1: Details of extracts used for antimicrobial susceptibility testing.

Aqueous extract prepared	Concentration	Designated as
Green coffee extract	100 mg/ Ml in DMSO	C
Green tea extract	100mg/ mL in DMSO	D

Figure 2: Prepared green coffee and green tea extracts.

Sample	Material	Concentration
A	Chlorhexidine solution	2%
B	Sodium hypochlorite solution	3%
C	Green coffee extract	100 mg/mL in DMSO
D	Green tea extract	100 mg/mL in DMSO

Preparation of Herbal Extracts

Aqueous extracts of green tea and green coffee were prepared using Soxhlet extraction. Fifty grams of plant material was placed in a Whatman No.1 filter paper thimble and extracted with 500 mL distilled water at 80°C. Extraction continued until the solvent became colorless, indicating exhaustive extraction.

The obtained extracts were concentrated on a heated water bath until a semi-solid crude extract was obtained. The extracts were then stored in sterile airtight containers (Figure 9). Standard stock solutions of 100 mg/mL were prepared using dimethyl sulfoxide (DMSO). (Figure 10).

The prepared extracts and their concentrations used for antimicrobial susceptibility testing are summarized in Table 1.

Microorganisms Used

Four bacterial strains commonly associated with oral and endodontic infections were selected

1. Escherichia coli
2. Pseudomonas aeruginosa
3. Staphylococcus aureus
4. Streptococcus oralis

Table 2: Samples used for antimicrobial assay and manufacturing details.

Test	Material/Brand	Concentration	Manufactured by
A	Chlor X	2% chlorhexidine solution	EPIP, Bari Brahmana, Jammu, India
B	Sodium hypochlorite	3% sodium hypochlorite solution	Prime Dental Products Pvt Ltd, Maharashtra, India
C	Green coffee powder	Aqueous extract; 100 mg/mL in DMSO	Life Science Ayurveda Co., Delhi, India
D	Wagh Bakri Green Tea	Aqueous extract; 100 mg/mL in DMSO	Gujarat Tea Processors and Packers Ltd., Gujarat, India

Samples and concentrations evaluated for antimicrobial activity are shown in Table 2.

Preparation of Inoculum

Fresh bacterial cultures were transferred into sterile saline and vortexed thoroughly. Turbidity was adjusted to match the 0.5 McFarland standard corresponding to approximately 1 × 108 CFU/mL.

Antimicrobial Susceptibility Testing

Antimicrobial activity was evaluated using the agar well diffusion method. Mueller–Hinton agar plates were prepared and inoculated with bacterial suspensions using sterile cotton swabs to obtain uniform bacterial lawns.

Wells were prepared in the agar plates, and the respective irrigants were introduced into the wells using micropipettes. Plates were incubated at 37°C for 24 hours. Zones of inhibition around the wells were measured in millimetres.

Controls

- Negative control: DMSO

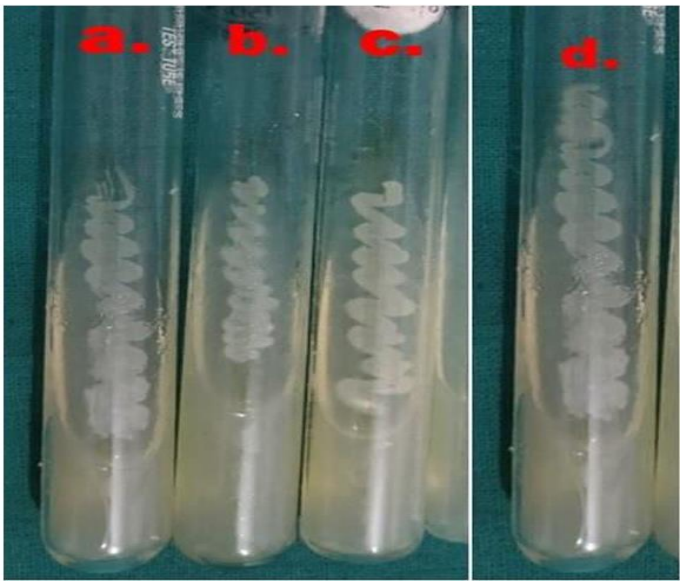


Figure 3: Pure culture slants of the bacterial isolates used for antimicrobial susceptibility testing.

Green tea extract exhibited the highest antimicrobial activity among the herbal agents tested. Maximum inhibition was observed against Escherichia coli (32.66 ± 0.47 mm), followed by Pseudomonas aeruginosa and Streptococcus oralis (23.66 ± 0.47 mm each)

Graph 1: Antimicrobial assay of sample D (aqueous green tea extract, 100 mg/mL in DMSO).

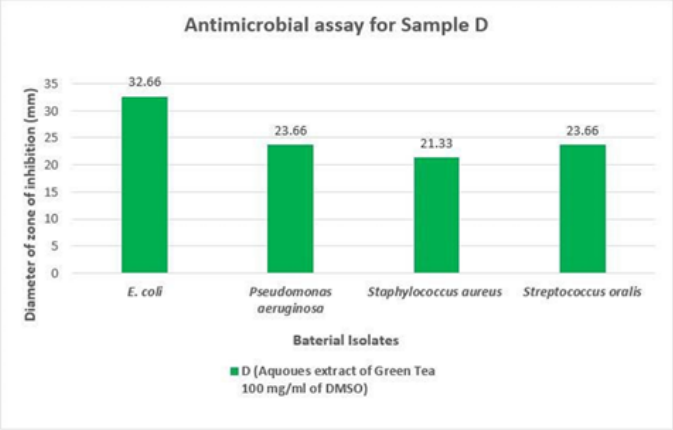
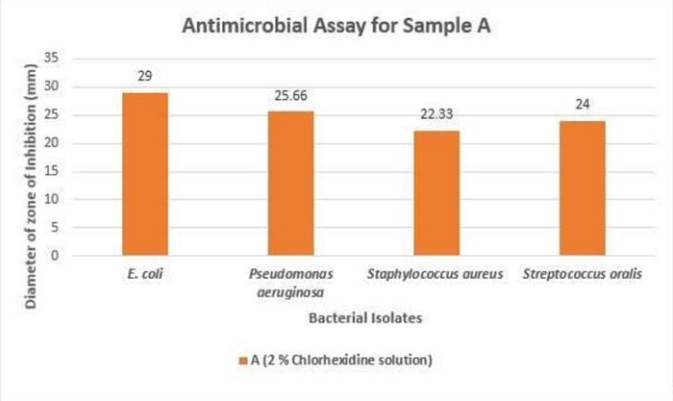


Table 3.

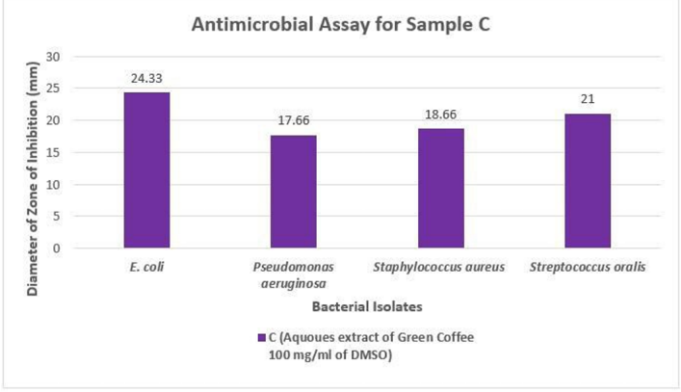
Test material	E. coli	P. aeruginosa	S. aureus	S. oralis
2% chlorhexidine solution	29 ± 0.82	25.66 ± 0.47	22.33 ± 0.47	24 ± 0.82
3% sodium hypochlorite solution	Nil	16 ± 0.47	14 ± 0.82	16.33 ± 0.47
Green coffee extract	24.33 ± 0.47	17.66 ± 0.47	18.66 ± 0.47	21 ± 0.82
Green tea extract	32.66 ± 0.47	23.66 ± 0.47	21.33 ± 0.47	23.66 ± 0.47

Chlorhexidine demonstrated strong broad-spectrum antimicrobial activity against all tested organisms, with zones of inhibition ranging from 22.33 ± 0.47 mm to 29 ± 0.82 mm (Table 4; Graph 1).

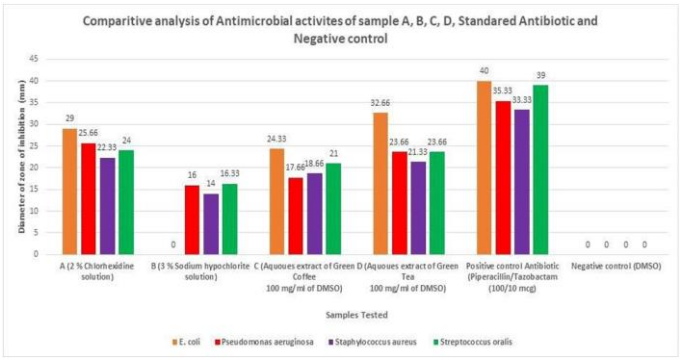
Graph 2: Antimicrobial assay of sample A (2% chlorhexidine solution).



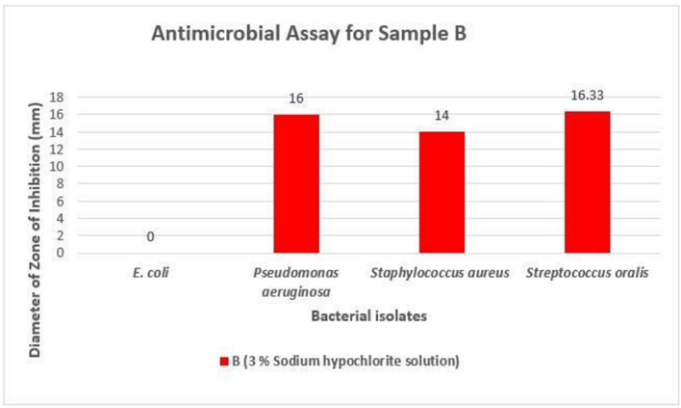
Graph 3: Antimicrobial assay of sample C (aqueous green coffee extract, 100 mg/mL in DMSO).



Graph 4: Comparative analysis of antimicrobial activity of samples A–D, positive antibiotic control and negative control.

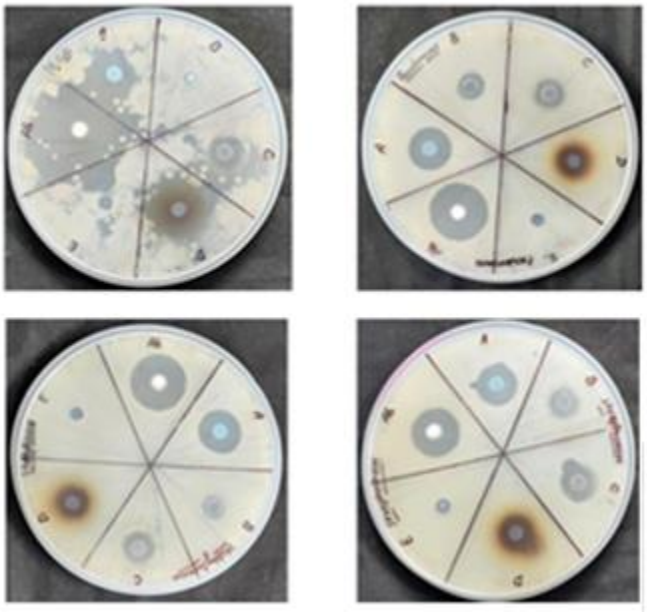


Graph 5: Antimicrobial assay of sample B (3% sodium hypochlorite solution).



Green coffee extract demonstrated moderate antibacterial activity against all microorganisms. The maximum zone of inhibition was observed against E. coli (24.33 ± 0.47 mm).

Hypochlorite demonstrated comparatively lower antimicrobial activity under the conditions of the agar diffusion assay, with no detectable inhibition against E. coli and measurable inhibition against the other three isolates. The negative control (DMSO) showed no inhibition against any bacterial isolate, confirming that antimicrobial activity was attributable to the active agents. The overall comparative profile is presented in Graph 5. Figure 4: Representative agar well - diffusion plates demonstrating zones of inhibition produced by the test materials against the bacterial isolates.



Results

All four test materials demonstrated varying degrees of antibacterial activity against the selected bacterial strains in Table 3. Pure culture slants of the microorganisms used for antimicrobial assay are shown in Figure. The comparative antimicrobial efficacy of the test materials against all bacterial isolates is presented in Table. Green tea extract exhibited the highest antimicrobial activity among the herbal agents tested. Maximum inhibition was observed against Escherichia coli (32.66 ± 0.47 mm), followed by Pseudomonas aeruginosa and Streptococcus oralis (23.66 ± 0.47 mm each) (Graph 4).[1,2,11,12] Chlorhexidine demonstrated strong broad-spectrum antimicrobial activity against all tested organisms, with zones of inhibition ranging from 22.33 ± 0.47 mm to 29 ± 0.82 (Graph 1).[9,15]

Green coffee extract demonstrated moderate antibacterial activity against all microorganisms. The maximum zone of inhibition was observed against E. coli (24.33 ± 0.47 mm) (Graph 3).[4,8] Sodium hypochlorite demonstrated comparatively lower antimicrobial activity under the conditions of the agar diffusion assay (Graph 2).[5,13]

The negative control (DMSO) showed no inhibition against any bacterial isolate, confirming that antimicrobial activity was attributable to the active agents.[1,9,1 ,14,15] A comparative analysis of antimicrobial activity among all experimental groups and controls is presented in Graph 5. Green tea extract demonstrated substantial measurable antimicrobial activity against all tested bacterial strains.

Discussion

Complete eradication of microorganisms from the root canal system remains a major challenge in Endodontics. Mechanical instrumentation alone cannot eliminate bacteria from inaccessible canal irregularities, fins, and dentinal tubules. Therefore, irrigants with effective antimicrobial properties are indispensable for successful endodontic therapy.

Green tea extract may be considered a promising investigational herbal adjunct to conventional root canal irrigants, subject to further safety and clinical evaluation.

In the present study, green tea extract demonstrated remarkable antimicrobial efficacy against all tested microorganisms. The potent antibacterial action of green tea can be attributed to catechins, especially epigallocatechin gallate (EGCG), which disrupt bacterial cell membranes and inhibit bacterial enzymatic activity.

The results of this study are consistent with previous studies conducted by Raj and Priyadharshni, Ramezanali et al., and Hegde and Kamath, who reported significant antibacterial activity of green tea against oral pathogens and Enterococcus faecal is. Green coffee extract also demonstrated moderate antibacterial activity against both Gram-positive and Gram negative bacteria.

Chlorogenic acid and phenolic compounds present in green coffee are believed to alter bacterial membrane permeability and interfere with microbial metabolism.

The findings support continued investigation of green tea and green coffee extracts as potential biocompatible adjuncts in pediatric endodontics.

However, because this was an in vitro agar diffusion study, the results should not be interpreted as evidence that herbal irrigants are clinically superior or ready to replace conventional irrigants. Sodium hypochlorite showed comparatively lower inhibition zones in the agar diffusion assay. This finding may be related to the limited diffusion capacity and instability of sodium hypochlorite in agar-based methods despite its known clinical efficacy.

The findings of the present study suggest that herbal irrigants, particularly green tea extract, possess significant antimicrobial potential and may serve as adjuncts or alternatives to conventional chemical irrigants. Herbal agents may offer advantages such as reduced toxicity, improved biocompatibility, antioxidant activity, and fewer adverse reactions.

However, the present study was limited to in vitro evaluation. Further studies involving minimum inhibitory concentration, cytotoxicity assessment, bio film disruption analysis, and randomized clinical trials are necessary before routine clinical application.

Conclusion

Within the limitations of the present in vitro study, the following conclusions were drawn.

1. Green tea extract demonstrated significant antimicrobial activity against all tested oral bacterial strains.
2. Chlorhexidine showed strong and consistent broad-spectrum antibacterial efficacy.
3. Green coffee extract exhibited moderate antimicrobial activity and showed potential as a herbal irrigant.
4. Sodium hypochlorite demonstrated comparatively lower inhibition in the agar diffusion assay.
5. Green tea extract may be considered a promising herbal alternative or adjunct to conventional root canal irrigants in pediatric endodontics.

Further in vivo and clinical investigations are recommended to validate the efficacy and safety of herbal irrigants before their routine clinical use.

Clinical Significance

The use of herbal irrigants such as green tea and green coffee may provide a safer and more biocompatible alternative to conventional irrigants in pediatric Endodontics while maintaining adequate antimicrobial efficacy.

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