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Original Research Article

In Vivo Comparative Evaluation of the Antimicrobial Efficacy of Cocoa Pod Husk Extract and 2% Chlorhexidine as Endodontic Final Irrigants: A Microbiological Study

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Abstract

Background: Effective root canal irrigation reduces microbial load. Chlorhexidine is a widely used irrigant; emerging herbal extracts of Cocoa Pod Husk have shown promising results in previous in-vitro studies.

Materials and Methods: A total of 30 patients with single-rooted teeth requiring treatment for irreversible pulpitis without periapical lesions were selected. The baseline microbial samples were collected after access cavity preparation and isolation, by placing paper points into the canal (pre-treatment sample). Chemo-mechanical preparation was performed using rotary files with 3.25% sodium hypochlorite, 17% EDTA, and saline as irrigants. Group 1 received Saline, Group 2 received 2% Chlorhexidine gluconate, and Group 3 received Cocoa Pod Husk Extract 6.25% as the final irrigant. Canals were closed temporarily, and microbial samples were collected again at 24 hours post-treatment. All the samples were sent for microbial culture, and Colony Forming Units (CFU) were noted.

Result: Group 2 (Chlorhexidine) and Group 3 (Cocoa Pod Husk Extract) both achieved significantly greater bacterial reduction compared to Group 1 (Saline).

Conclusion: 6.25% Cocoa Pod Husk Extract exhibits antimicrobial efficacy comparable to that of chlorhexidine gluconate 2%.

Keywords: Antimicrobial efficacy, Chlorhexidine, Cocoa Pod Husk Extract, Plant extracts, Root canal irrigants

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1. Introduction

Endodontics is the branch of dentistry that is concerned with the morphology, physiology, and pathology of the human dental pulp and periradicular tissues. The objective of endodontic treatment is to eliminate microorganisms and prevent subsequent reinfection to preserve the tooth.[1] Persistent microbial biofilms, anatomical complexities, and dentinal tubule invasion often limit the effectiveness of mechanical preparation alone.[2] Therefore, irrigation forms a critical adjunct to chemo-mechanical debridement. Researching disinfection strategies is pivotal because effectively eliminating bacteria and their byproducts during Root Canal Treatment (RCT) remains a challenge.

Chemical substances are used in combination with the mechanical action of endodontic instruments. Sodium Hypochlorite (NaOCl) is the most widely used irrigant because it dissolves pulp tissue and organic components within the root canal system, in addition to its strong antimicrobial efficacy.[3] However, it cannot dissolve inorganic components, and it has proteolytic action on dentinal collagen, causing tooth weakness and predisposing it to fracture.[4] Chelating agents and acid solutions have been recommended for removing the smear layer from instrumented root canals, including Ethylene diaminetetraacetic acid (EDTA) and citric acid. They also detach biofilms adhering to the root canal walls.[5] 17% EDTA for 1 minute is effective in the removal of smear layer, but a 10-minute application can cause excessive peritubular and intertubular dentinal erosion. Increasing contact time and concentration of EDTA from 10% to 17%, as well as pH of 7.5 versus pH 9.0, has been shown to increase dentin demineralization.[6]

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Chlorhexidine Gluconate (CHX) is an endodontic irrigant with efficient antibacterial activity in an endodontic environment, when used at concentrations of 2% to 20%.[7] It cannot remove necrotic residues and should not be used as a substitute for sodium hypochlorite (NaOCl). Moreover, it tends to form a toxic precipitate containing para-chloro-aniline when mixed with NaOCl. This precipitate is cytotoxic and affects the sealing ability of root canal fillings. Its effectiveness is also reduced in the presence of organic residues inside the canal. While it possesses antimicrobial properties, it still has the potential to be cytotoxic.[8] Given these significant limitations and potential adverse effects of chlorhexidine, there is a compelling need to explore alternative materials for endodontic irrigation. The search for safer, more effective, and biocompatible options has intensified, leading to investigations of the rich potential of herbal extracts.

Traditional medicine systems have long utilised plant-derived compounds for their antimicrobial, anti-inflammatory, and wound-healing properties. Neem, Tulsi, Aloe Vera, Green Tea, Propolis, Noni fruit, chamomile, Turmeric, Grape seed, gallic acid, Cinnamon, clove oil, Garlic extract, pomegranate extract have demonstrated effective antimicrobial activity against common endodontic pathogens, while also offering antioxidant benefits that help counteract the oxidative stress induced by chemical irrigants.[9]

Cocoa Pod Husk is a natural ingredient that can be processed as an alternative material. The utilization of Cocoa Pod Husk, which constitutes approximately 70–80% of the total fruit biomass, represents a critical challenge and opportunity for sustainable waste valorisation within the global cocoa supply chain. Cocoa Pod Husk Extract (CPHE) is now recognised as a rich, untapped bioresource.[10]

Numerous *in-vitro* data demonstrate the potential of this agent, but as in-vitro studies only use a particular strain of bacteria, an *in-vivo* clinical study on patients is essential due to the complex, polymicrobial population of bacteria residing in the root canal system, and the host-pathogen interactions, along with their effect on pain, cannot be accurately replicated in laboratory settings. This approach aims to establish the material's viability as a biocompatible, natural alternative for controlling complex endodontic infections.

2. Material and Methods

The present *in vivo* study was conducted in the Department of Conservative Dentistry and Endodontics at Tertiary care Hospital. Herbal Cocoa Pod Husk Extract was obtained from CytoGene Laboratories, Lucknow. Institutional ethical clearance was obtained from the Institutional Research & Development Committee (IRDC) and the Institutional Human Ethics Committee (IHEC).

2.1. Study design

The present *in-vivo* study aimed to compare the antimicrobial efficacy associated with the use of Chlorhexidine and Cocoa Pod Husk Extract as final irrigants during root canal treatment. A total sample size of 30 patients was found to be sufficient. Each group consisted of 10 patients; 2 samples were collected from each patient (pre- and post-operative).

2.2. Preparation of Cocoa Pod Husk Extract

40-45 grams of mature Cocoa Pod Husk were collected, washed with distilled water to remove soil, debris and surface contaminants and segmented into 2-3 cm pieces. They were dried at 40–50 degrees Celsius and milled into coarse powder. Water was added in the ratio of 1:5 W/V and soaked for 60 minutes at 30–40 °C with intermittent mixing. Filtration was done using membrane filters. Hydraulic pressure extraction was done by applying a gradual pressure of 40-60 MPa. 30 ml of extract was collected, and the concentration was standardized to 6.25% W/V.

2.3. Group allocation

1. Group 1 (n=10) – Saline 0.9%.
2. Group 2 (n=10) – Chlorhexidine 2%.
3. Group 3 (n=10) – Cocoa Pod Husk Extract 6.25%.

2.4. Inclusion criteria

1. Patients aged 25–50 years.
2. Single-rooted teeth exhibiting a single canal.
3. Tooth diagnosed with irreversible pulpitis without any associated periodontal involvement.

2.5. Exclusion criteria

1. Presence of periapical radiolucency greater than 2 mm on radiographic examination.
2. Re-treatment cases.
3. Patients currently taking antibiotics or analgesics.
4. Teeth with immature apex, calcified canals, or significant anatomical complexities.
5. Teeth exhibiting internal or external root resorption.
6. Pregnant or lactating women.

Patients reporting to the OPD of the Department of Conservative Dentistry and Endodontics, Tertiary care Hospital, were screened. Clinical and radiographic examination was done, and 30 patients were selected as per the inclusion criteria. They were thoroughly informed about the study, and informed consent was taken.

2.6. Treatment procedure

Local Anesthesia 2% Lignocaine with 1:80,000 adrenaline (Lignox 2%, Indoco Remedies Ltd, India) was administered. Isolation was done using a rubber dam (GDC Dental Instruments, India), and aseptic precautions were taken. Access opening was done using a high-speed air turbine handpiece (Being Foshan Medical Equipment Co., Ltd., China) with sterile distilled water as coolant. Working length was determined using digital RVG and Apex locator (Woodpex V, Guilin Woodpecker Medical Instruments Co., Ltd., China).

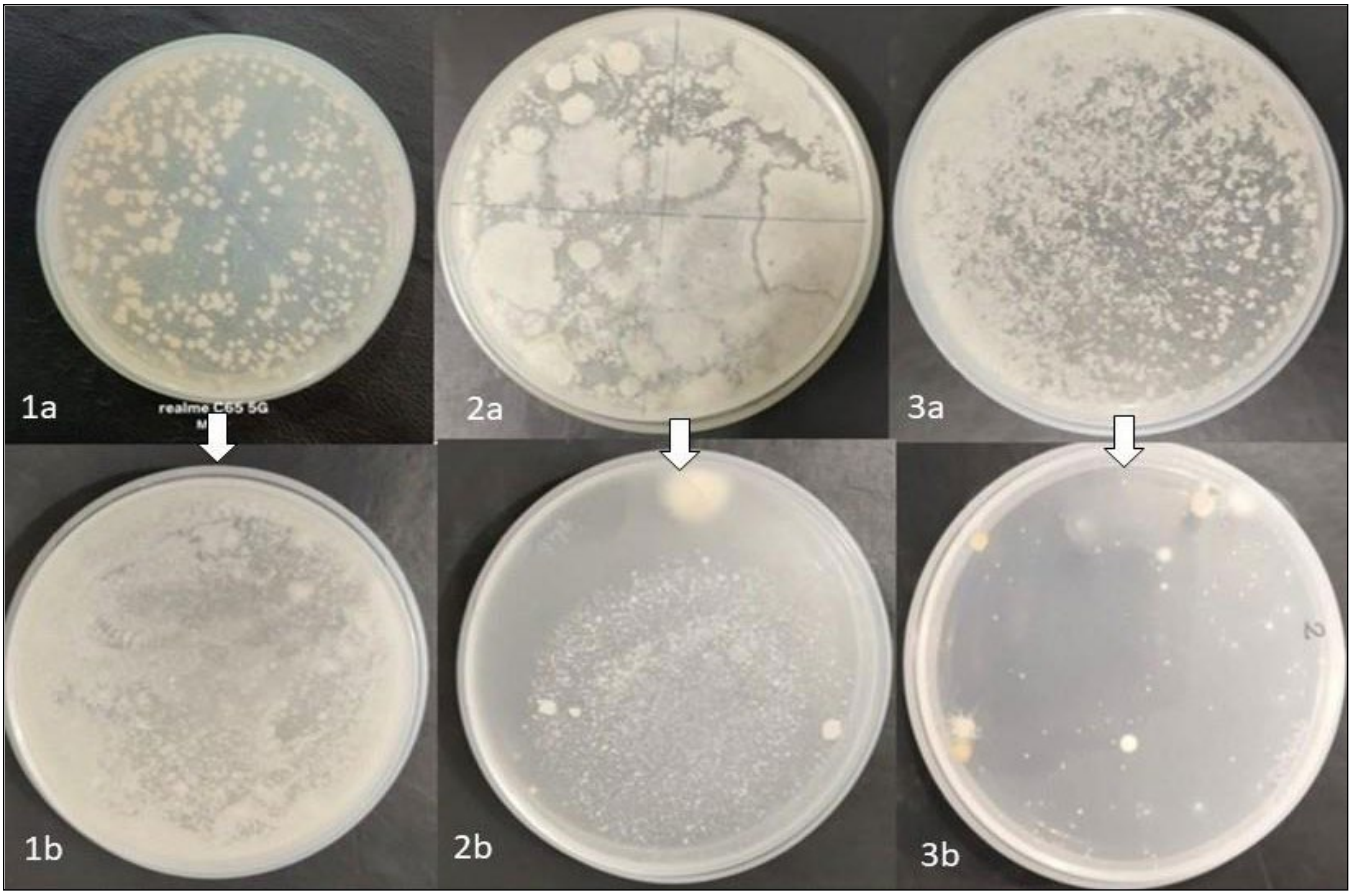

Figure 1: Representative images of bacterial colony growth on agar plates used for quantitative microbial assessment (CFU/mL) before and after irrigation protocols. **1a:** Group 1 (Saline) pre-treatment culture demonstrating baseline microbial load. **1b:** Group 1 (Saline) post-treatment culture. **2a:** Group 2 (Chlorhexidine) pre-treatment culture sample. **2b:** Group 2 (Chlorhexidine) post-treatment culture sample showing marked reduction in colony-forming units. **3a:** Group 3 (CPHE) pre-treatment culture sample. **3b:** Group 3 (CPHE) post-treatment culture demonstrating substantial reduction in bacterial growth

The first sample was collected by a sterile paper point of size 25 (Meta Biomed, South Korea) inserted into the canal and removed after 60 seconds. The paper point was transferred to the Alicote vial containing nutrient broth immediately. The sample was sent for microbial evaluation. Biomechanical preparation was completed with rotary instruments up till 25/6% taper file (Hyflex CM, Coltene Whaledent Pvt Ltd, Switzerland).

Irrigation was done using side vented needle in Group 1-Saline (NS) (Nirlife, Aculife Healthcare Private Limited, India), Group 2- Chlorhexidine (CHX) (HexaChlor, SafeEndo Dental India Pvt. Ltd., India) or Group 3 Cocoa Pod Husk Extract (CPHE) as per the allocated group. Irrigant was kept in the canal for 60 seconds and then rinsed with normal saline. Canals were dried with a paper point, and a temporary seal was given. The patient was recalled after 24 hours for post-treatment sample collection, following the same protocol used for the pre-treatment sample.

2.7. Statistical analysis

The collected data were entered into Microsoft Excel and analysed using Statistical Package for the Social Sciences (SPSS) software version 21.0 (IBM Corp., NY, USA). Microbial load was expressed as colony-forming units per millilitre (CFU/mL). Descriptive statistics, including mean, Standard Deviation (SD), minimum, and maximum values, were calculated for all three groups at both pre-treatment and post-treatment time intervals. For intra-group comparison, a paired Student's t-test was used. For inter-group comparison, the difference between pre-treatment and post-treatment values was calculated for each patient, and these mean reductions were compared using one-way Analysis of Variance (ANOVA). The level of statistical significance was set at $p < 0.05$.

3. Results

All three groups demonstrated a reduction in mean Colony forming Units (CFU) counts following treatment. Group 1 showed a reduction in mean CFU from approximately 802.20 CFU/mL (pre-treatment) to 492.97 CFU/mL (post-treatment), representing a reduction of about 38.5%, which was statistically significant ($p < 0.05$). Group 2 demonstrated a reduction from approximately 820.49 CFU/mL to 259.60 CFU/mL, corresponding to a 68.3% reduction, which was statistically significant ($p < 0.05$). Group 3 showed a decrease from approximately 805.36 CFU/mL to 271.36 CFU/mL, representing a 66.5% reduction, which was also statistically significant ($p < 0.05$) as seen in **Figure 1** and **Table 1**.

Table 1: Microbial load (Mean ± SD) among three Groups using ANOVA and corresponding percentage reduction from baseline values

Groups	Irrigant	Mean Pre-treatment microbial count (cfu/mL)	Mean Post-treatment microbial count (cfu/mL)	Percentage reduction in microbial count
Group 1	Saline	802.20 ± 45.06	492.97 ± 61.37	38.5%
Group 2	Chlorhexidine	820.49 ± 95.95	259.60 ± 46.65	68.3%
Group 3	CPHE	805.36 ± 90.60	271.36 ± 17.37	66.5%

SD: Standard deviation

Table 2: Level of significance (p-values) for intergroup comparison in microbial counts using Tukey's HSD Post Hoc test

Group comparisons	Test irrigants	Tukey's HSD Q statistic	p-value	Level of significance
Group 1B vs Group 2B	Saline vs Chlorhexidine	16.17	<0.001	Highly significant
Group 1B vs Group 3B	Saline vs CPHE	15.36	<0.001	Highly significant
Group 2B vs Group 3B	Chlorhexidine vs CPHE	0.81	0.72	Not significant

p: Level of significance, **HSD:** Honestly significant difference, **Q:** Quantile

Paired t-test analysis revealed that the reduction in microbial count within each group was statistically significant. When the mean reduction in CFU counts was compared among the three groups using one-way ANOVA, a statistically significant difference was observed ($p < 0.05$). Tukey's HSD Post Hoc test revealed that Groups 2 and 3 demonstrated significantly greater microbial reduction compared to Group 1. No statistically significant difference was observed between Group 2 and Group 3 ($p > 0.05$) as seen in **Table 2**.

4. Discussion

The primary goal of endodontic irrigation is to eliminate microbial contamination within the complex anatomy of the root canal system, which plays a critical role in achieving long-term endodontic success.[1] In the present *in-vivo* study, the quantitative assessment of overall reduction in viable microbes after use of interventional irrigants was evaluated by Total Bacterial Count using Colony Forming Unit count, as endodontic infections are polymicrobial in nature, involving a complex consortium of aerobic and anaerobic bacteria rather than a single pathogenic species.

In the present *in-vivo* study, at baseline, all three groups demonstrated comparable microbial loads, indicating uniform distribution before treatment: Group 1 (802.20), Group 2 (820.49), Group 3 (805.36). Following treatment, microbial counts decreased across all groups. (Table 1) Group 2 (259.60) and Group 3 (271.36) both achieved significantly greater bacterial reduction compared to Group 1 (492.97). There was no statistically significant difference between CHX and CPHE**.** This suggests that both CHX and CPHE were similarly effective and superior to saline in reducing microbial counts. The findings confirm that mechanical instrumentation, along with irrigation with chemical adjuncts such as CHX and CPHE, significantly enhances the antibacterial effect.

It aligns with the well-documented antibacterial efficacy of CHX, a gold standard irrigant. It is known for its broad-spectrum activity, substantivity, and ability to disrupt bacterial cell membranes and biofilms. It works primarily through its cationic (positively charged) nature, which allows it to bind to negatively charged bacterial cell walls, it acts as a bacteriostatic agent at low concentration it alters the osmotic equilibrium of the cell and causes leakage of low molecular weight substances such as potassium and phosphorous while at higher concentration it acts as a bactericidal agent by penetrating the cell and causing precipitation of cytoplasmic content of the cell.[11,12]

CPHE showed a reduction in bacterial count from 805.36 to 271.36, with a percentage reduction of 66.5% this antimicrobial activity of CPHE may be attributed to its rich phytochemical composition, including polyphenols, flavonoids, tannins, and alkaloids, which interfere with bacterial cell wall integrity, inhibit the enzymatic system, and suppress microbial adhesion and biofilm formation.[13] CPHE has shown inhibition in biofilm formation of E. faecalis bacteria in other studies.[14] These bioactive compounds likely act synergistically to produce a potent antibacterial effect similar to CHX. The comparable activity of CPHE to CHX suggests that it may act through multiple mechanisms similar to conventional chemical irrigants, but with the added advantage of being biocompatible and naturally derived.[15] The performance of CPHE, being nearly equivalent to CHX, suggests that CPHE may serve as a promising herbal alternative with comparable antimicrobial potential.

In contrast, saline (Group 1), though effective in mechanical flushing of debris and reducing the bacterial count by only 38.5%, lacks inherent antimicrobial activity. Its role in irrigation remains primarily physical rather than chemical, serving as a control in the present study to demonstrate the enhanced efficacy of bioactive irrigants.

The findings of the present *in-vivo* study are consistent with emerging research supporting plant-derived bioactive agents as sustainable alternatives to synthetic chemicals in endodontics. Cocoa Pod Husk Extract (CPHE) has the potential to be an eco-friendly adjunct in root canal disinfection.

5. Conclusion

Chlorhexidine (CHX) and Cocoa Pod Husk Extract (CPHE) achieved markedly greater microbial reduction, with no statistically significant difference between them. These findings highlight that CPHE exhibits antimicrobial efficacy comparable to CHX, suggesting its potential as a promising, herbal derived alternative irrigant in endodontic disinfection protocols.

6. Authors Contribution

1. **Supriya Ojha:** Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, writing – original draft.
2. **Gaurav Jain:** Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing – original draft, writing – review editing.
3. **Preeti Shukla:** Conceptualization, data curation, formal analysis, funding acquisition, investigation, writing – original draft.
4. **Pradyumna Misra:** Conceptualization, data curation, formal analysis.
5. **Sonali Verma:** Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, visualization, writing – original draft.

7. Source of Funding

None.

8. Conflict of Interest

None.

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