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The Potential of *Cocos nucifera* Endocarp Carbon as an Adjunctive Root Canal Irrigant for Dentin Protection and Antibacterial Effects

Nur Asmah[1]* Siti Maisarah,[2] Erna Irawati,[3] Mila Febriany[4]

[1]*Department of Conservative Dentistry, Dentistry Faculty, Universitas Muslim Indonesia, Makasar, Sulawesi Selatan, Indonesia*
[2]*Department of Medicine, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah, Makasar, Sulawesi Selatan, Indonesia*
[3]*Department of Dental Material, Dentistry Faculty, Universitas Muslim Indonesia, Makasar, Sulawesi Selatan, Indonesia*
[4]*Department of Pedodontics, Dentistry Faculty, Universitas Muslim Indonesia, Makasar, Sulawesi Selatan, Indonesia*

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ABSTRACT

Enterococcus faecalis is a key pathogen in persistent endodontic infections, known for its resistance to conventional antimicrobial agents, including irrigants. Biocompatible alternatives that preserve dentin collagen are increasingly sought in root canal therapy. This study evaluated the antibacterial efficacy and dentin-preserving ability of coconut shell–derived activated carbon as an irrigant against *E. faecalis*. An ex vivo model using human premolars was infected with *E. faecalis* (ATCC 29212) for 21 days. Samples were treated with activated carbon (1:10, 1:20, 1:30), saline, or conventional irrigants (2% CHX, 17% EDTA, 2.5% NaOCl). Antibacterial activity was assessed via inhibition zones, CFU/mL, and OD600. Calcium ion release and hydroxyproline assays were used to evaluate demineralization and collagen degradation. SEM and FTIR (amide I and III) were used to assess the dentin structure and collagen integrity. Activated carbon 1:10 showed potent antibacterial activity (inhibition zone: 17.2 ± 0.4 mm) and CFU/mL reduction comparable to CHX. It had the lowest calcium release (3.9 ± 0.4 ppm) and hydroxyproline levels (4.2 ± 0.3 µg/mL), indicating minimal dentin damage. SEM and FTIR confirmed structural preservation. EDTA and NaOCl caused significant degradation of collagen. Coconut shell–derived activated carbon at a 1:10 concentration effectively inhibits *E. faecalis* while preserving dentin collagen, highlighting its novelty as a sustainable and biocompatible irrigant with potential clinical implications in root canal therapy.

Keywords: Activated carbon, root canal irrigant, *Enterococcus faecalis*, dentin collagen, Fourier Transform Infrared Spectroscopy, hydroxyproline.

Introduction

Root canal disinfection is a critical step in endodontic therapy, particularly for eliminating persistent infections caused by *Enterococcus faecalis*, a facultative anaerobic bacterium frequently isolated from failed root canal treatments.[1] The complexity of the root canal system and the resilience of *E. faecalis* biofilms necessitate the use of effective irrigants that not only possess antimicrobial properties but also preserve dentinal structure.[2] Sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), and chlorhexidine (CHX) are commonly used irrigants; however, their aggressive chemical properties have been associated with undesirable effects such as dentinal collagen degradation and excessive demineralization. [3] These effects compromise the structural integrity of dentin, potentially affecting long-term treatment outcomes.[3] Recent interest in biocompatible and less destructive irrigants has led to the exploration of plant-based and naturally derived materials in endodontics.[4] One promising candidate is activated carbon, particularly that derived from *Cocos nucifera* (coconut shell).[5] The activated carbon is renowned for its high surface area, adsorptive capacity, and chemical inertness, making it widely utilized in biomedical filtration, toxin removal, and wound dressing applications.[6]

*Corresponding author. Email: nur.asmah@umi.ac.id
Tel: +6281241704324

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Its ability to trap bacterial metabolites, neutralize free radicals, and limit oxidative stress suggests potential benefits for preserving dental tissue.[7] In antimicrobial studies, carbon-based nanomaterials have demonstrated inhibitory effects against oral pathogens such as *Streptococcus mutans* and *E. faecalis*.[8] Additionally, activated carbon incorporated into dental sealers and restorative materials has shown favorable outcomes in reducing microbial colonization.[9] However, the direct application of activated carbon as a root canal irrigant has not been extensively investigated.[10] Its non-oxidative, non-chelating nature implies the reduced risk of degrading organic dentin components, particularly collagen, which is critical for maintaining tooth biomechanics and adhesion properties in subsequent restorative procedures.[11] The current literature lacks empirical evidence on activated carbon's dual role as both an antibacterial agent and a dentin-preserving irrigant. Conventional solutions such as NaOCl, EDTA, and CHX, although effective against *Enterococcus faecalis*, compromise dentin collagen integrity to varying degrees. This highlights the need for a novel irrigant that provides sufficient antimicrobial action while minimizing structural damage. Activated carbon derived from coconut shell (*Cocos nucifera* endocarp) represents a promising candidate due to its high adsorptive capacity, natural abundance, and biocompatibility. Yet, its application as an endodontic irrigant remains underexplored, with most studies limited to environmental and pharmaceutical contexts. This study addresses the gap by evaluating coconut shell–based activated carbon for its antibacterial efficacy and dentin-preserving potential in an *ex vivo* root canal infection model, offering novelty as one of the first investigations of this natural biomaterial in endodontics. This study hypothesizes that activated carbon solutions, particularly at higher concentrations, can effectively inhibit *Enterococcus faecalis* while preserving dentinal collagen and minimizing calcium ion loss, thereby providing a biocompatible alternative to conventional irrigants. The significance of this work lies in its multidisciplinary evaluation of coconut shell–derived activated

carbon as a novel endodontic irrigant, combining antimicrobial testing with structural and biochemical analyses of dentin. The expected outcomes will provide substantial evidence for the clinical feasibility of incorporating this natural material into safer and more biologically friendly irrigation protocols.

Materials and Methods

This study was conducted as an ex vivo and in vitro laboratory investigation and received ethical approval from the Ethics Committee of the Faculty of Dentistry, Universitas Syiah Kuala, Indonesia (Approval No. 562/KE/FKG/2024). Activated carbon from coconut shell was evaluated as a root canal irrigant against *Enterococcus faecalis* (ATCC 29212). Ex vivo root canal models were prepared from extracted premolars and inoculated for 21 days. Specimens were divided into four activated carbon groups (1:10, 1:20, 1:30, saline) and three controls: CHX 2% (Chlorhexidine, Sigma-Aldrich, St. Louis, MO, USA). EDTA 17% (Ethylenediaminetetraacetic Acid, Merck, Darmstadt, Germany). NaOCl 2.5% (Sodium Hypochlorite, Golden Falcon, Dubai, UAE). Antibacterial activity, calcium ion release, collagen degradation (hydroxyproline), dentin morphology (SEM), and collagen integrity (FTIR) were analyzed.

Preparation of Activated Carbon

Activated carbon was prepared from coconut shell charcoal (*Cocos nucifera* endocarp) through a controlled pyrolysis and chemical activation process. Initially, mature coconut shells were thoroughly cleaned to remove any adhering pulp or contaminants and then dried in the sunlight for 48 hours. The dried shells were then carbonized in a muffle furnace at 500°C for 2 hours under limited oxygen conditions to produce raw charcoal. The resulting charcoal was ground and sieved to obtain a uniform powder with a particle size of approximately 100–200 mesh. Chemical activation was performed by soaking the charcoal powder in a 1 M potassium hydroxide (KOH) solution (Merck, Darmstadt, Germany) at a 1:1 weight-to-volume ratio. The mixture was stirred continuously for 24 hours at room temperature to ensure homogenous impregnation. After activation, the slurry was filtered, rinsed with distilled water until a neutral pH was achieved, and oven-dried at 110°C for 12 hours. Subsequently, the dried material was subjected to a second pyrolysis step in a furnace at 700 °C for 1 hour to enhance pore development and surface area. The final product was then washed thoroughly with 0.1 M hydrochloric acid (HCl) (Merck) to remove any residual activating agent, followed by several rinses with deionized water. The purified activated carbon was oven-dried again and stored in airtight containers for further use in experimental treatments.[12]

Preparation of Root Canal Irrigation Model

The preparation of the ex-situ root canal irrigation model was performed using human premolar teeth extracted for orthodontic reasons. The teeth selected had single roots with straight canals, free from resorption or caries. The samples were cleaned of soft tissue using a sterile scaler and stored in physiological saline (NaCl 0.9%). Root canal preparation was performed using the step-back technique until a standard size of #40 K-file (Dentsply Sirona, Ballaigues, Switzerland) was achieved. A sterile 0.9% NaCl solution was used as the irrigant during instrumentation between file changes. After instrumentation, the roots were sectioned to a standardized length of 12 mm from the apex to ensure uniformity across all samples. All samples were sterilized using an autoclave at 121°C for 15 minutes. *Enterococcus faecalis* (ATCC 29212), previously activated in Brain Heart Infusion (BHI) broth (Oxoid Ltd., Hampshire, UK), was incubated for 24 hours at 37°C. The bacterial suspension was adjusted to a 0.5 McFarland standard (1.5×10^8 CFU/mL) using a spectrophotometer. Each root canal was inoculated with 20 µL of the suspension, sealed with a sterile cotton pellet, and incubated at 37°C for 21 days to allow for biofilm formation to occur. Fresh BHI broth was added every two days to maintain bacterial viability. Following the incubation period, the root canals were rinsed with sterile NaCl and assigned into six treatment groups: activated carbon 1:10, 1:20, and 1:30; 2% CHX (Sigma-Aldrich, St. Louis, MO, USA); 17% EDTA (Merck, Darmstadt, Germany); and 2.5% NaOCl (Golden Falcon, UAE). Each canal was irrigated with 1 mL of the assigned solution using a syringe and a 27-G side-vented irrigation needle inserted 1 mm from the apex for 1 minute. After irrigation, the canals were rinsed again with sterile sodium chloride (NaCl) solution and dried before subsequent analyses.[13]

Bacterial Assay

The antibacterial effectiveness of various root canal irrigants was evaluated using three complementary approaches: inhibition zone measurement, colony-forming unit (CFU/mL) counts, and optical density (OD600) assessment. Each treatment was performed in quintuplicate (n = 5). *Enterococcus faecalis* (ATCC 29212) was cultured and adjusted to a turbidity equivalent to the 0.5 McFarland standard (approximately 1.5 × 10^8 CFU/mL). For the inhibition zone test, the bacterial suspension was inoculated evenly on Mueller-Hinton Agar (MHA) plates using sterile cotton swabs. Wells with a diameter of 6 mm were created using a sterile punch, and 50 µL of each test irrigant (Carbon 1:10, Carbon 1:20, Carbon 1:30, CHX 2%, EDTA 17%, and NaOCl 2.5%) was introduced into each well. The plates were incubated at 37°C for 24 hours, and the diameters of the resulting inhibition zones were measured using a digital caliper in millimeters. For CFU/mL quantification, equal volumes (1 mL) of bacterial suspension and irrigant solution were mixed and incubated at 37°C for 24 hours. Serial dilutions were performed up to 10^6 with sterile saline, and 100 µL from each dilution was plated on Nutrient Agar. After 24 hours of incubation, bacterial colonies were counted, and the values were expressed as logarithmic CFU/mL. In parallel, OD600 was used to assess bacterial growth inhibition quantitatively. Each bacterial suspension was treated with its respective irrigant, incubated at 37°C for 24 hours, and 200 µL of the mixture was transferred into wells of a 96-well microplate. Absorbance was measured at 600 nm using a microplate Reader (BioTek Epoch 2, Agilent, Winooski, VT, USA).[14]

Calcium Ion Release Assay

Dentin discs were prepared from extracted human premolars by sectioning the mid-coronal dentin into standardized dimensions of 4 mm in diameter and 1 mm in thickness using a water-cooled diamond saw. All assays were performed in quintuplicate (**n=5**). The specimens were ultrasonically cleaned in distilled water for 5 minutes to remove surface debris and then sterilized by autoclaving at 121 °C for 15 minutes. Each disc was pre-weighed with an analytical balance to ensure consistent sample mass. The discs were individually immersed in 1 mL of the respective irrigant solutions (Carbon 1:10, Carbon 1:20, Carbon 1:30, 2% CHX, 17% EDTA, and 2.5% NaOCl) and incubated at 37 °C for 24 hours to simulate clinical exposure. After incubation, the supernatants were collected and analyzed for calcium ion concentration. Quantification of released calcium ions was performed using an Atomic Absorption Spectrophotometer (AAS; PerkinElmer PinAAcle 900T, Waltham, MA, USA) at 422.7 nm, and results were expressed in ppm (mg/L).[15]

Scanning Electron Microscope

To examine the morphological alterations of dentin surfaces following exposure to different irrigants, standardized dentin discs were prepared from extracted human premolars. It was analyzed using a Scanning Electron Microscope (SEM) from Thermo Fisher Scientific, specifically the Quanta 250 FEG, located in Eindhoven, Netherlands. For each treatment group, SEM imaging was performed on five specimens (n = 5). After exposure to the respective irrigants (Carbon 1:10, Carbon 1:20, Carbon 1:30, CHX 2%, EDTA 17%, and NaOCl 2.5%) for 24 hours at 37°C, the discs were rinsed gently with phosphate-buffered saline (PBS) to remove the residual solution. Samples were then fixed in 2.5% glutaraldehyde at 4°C for 24 hours to preserve ultrastructural integrity. Following fixation, the specimens were rinsed in PBS and dehydrated through a graded ethanol series (30%, 50%, 70%, 90%, and 100%) for 10 minutes at each step. The dehydrated samples were air-dried in a desiccator for 24 hours to prevent structural collapse. Once dried, each disc was mounted on aluminum stubs using conductive adhesive tape and sputter-coated with a thin layer of gold to ensure surface conductivity. The specimens were examined under a scanning electron microscope (SEM) at a magnification of 1500×.

Image acquisition focused on identifying differences in dentinal tubule patency, the presence or absence of smear layer, and general surface morphology. Micrographs were analyzed qualitatively to compare structural integrity and cleanliness among treatment groups.[16]

Collagen Degradation Assay

To evaluate dentinal collagen degradation after exposure to various irrigants, hydroxyproline levels were quantified as a marker of collagen breakdown. All samples were tested in quintuplicate (n=5)**.** Standardized dentin discs (4 mm diameter, 1 mm thick) were incubated in 1 mL of each test irrigant Carbon 1:10, Carbon 1:20, Carbon 1:30, chlorhexidine 2% (CHX, Sigma-Aldrich, St. Louis, MO, USA), EDTA 17% (Sigma-Aldrich, St. Louis, MO, USA), and sodium hypochlorite 2.5% (NaOCl, Merck, Darmstadt, Germany) at 37°C for 24 hours. After incubation, the supernatants were collected for hydroxyproline analysis. Each supernatant was hydrolyzed with 6N hydrochloric acid (HCl, Merck, Darmstadt, Germany) at 110 °C for 18 hours in sealed glass tubes. After hydrolysis, the samples were cooled to room temperature and neutralized with sodium hydroxide (NaOH, Merck, Darmstadt, Germany). A colorimetric hydroxyproline assay was then conducted using Chloramine-T (Sigma-Aldrich, St. Louis, MO, USA) and Ehrlich's reagent (Sigma-Aldrich, St. Louis, MO, USA) following the manufacturer's standard protocol. Briefly, oxidized hydroxyproline reacted with Ehrlich's reagent to form a chromophore, which was incubated in the dark for 30 minutes. Absorbance was measured at 558 nm using a microplate reader (BioTek Epoch, Winooski, VT, USA). Hydroxyproline concentrations (in µg/mL) were determined using a standard curve generated from serial dilutions of trans-4-hydroxy-L-proline standard (Sigma-Aldrich, St. Louis, MO, USA).[17]

FTIR Assay

FTIR (Fourier Transform Infrared Spectroscopy): Shimadzu IRSpirit-T, Kyoto, Japan, was employed to evaluate the structural integrity of dentin collagen by analyzing characteristic amide peaks, particularly Amide I (~1650 cm^{-1}) and Amide III (~1240 cm^{-1}), which are indicative of peptide bond and protein backbone stability. After exposure to various root canal irrigants (Carbon 1:10, Carbon 1:20, Carbon 1:30, CHX 2% [Sigma-Aldrich, St. Louis, MO, USA], EDTA 17% [Sigma-Aldrich], and NaOCl 2.5% [Merck, Darmstadt, Germany]), dentin discs (4 mm diameter, 1 mm thick) were thoroughly rinsed with distilled water and air-dried at room temperature for 48 hours in a desiccator to eliminate residual moisture. The FTIR spectra of each dried sample were obtained using an FTIR spectrophotometer (Shimadzu IRTracer-100, Kyoto, Japan) equipped with an attenuated total reflectance (ATR) crystal accessory (Pike Technologies, Madison, WI, USA). Spectra were collected over the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}, with 32 scans averaged per sample. Special attention was paid to the absorbance intensity of the Amide I band (~1650 cm^{-1}), associated with C=O stretching vibrations of peptide bonds, and the Amide III band (~1240 cm^{-1}), related to N–H bending and C–N stretching. These peaks reflect the integrity of the collagen triple-helix structure. A decrease in the absorbance intensity of these peaks was interpreted as indicative of collagen degradation. The peak intensities were qualitatively compared across groups. All measurements were performed in triplicate, and representative spectra were plotted for visual interpretation.[18]

Statistical Analyses

All data were presented as mean ± SD. Normality and homogeneity were assessed using Shapiro–Wilk and Levene's tests. One-way ANOVA with Tukey's post hoc test was used for parametric data, while the Kruskal–Wallis and Dunn's tests were applied for non-parametric data. Correlations were analyzed using Pearson's tests. A p-value < 0.05 was considered statistically significant. Analyses were conducted using SPSS version 26.0 (IBM Corp., USA).

Results and Discussion

Table 1 presents the antibacterial activity of *Cocos nucifera* endocarp–derived activated carbon compared with conventional irrigants against *Enterococcus faecalis*. The inhibition zone, CFU/mL (log scale), and OD600 absorbance values demonstrate significant differences among treatment groups ($p < 0.05$). The largest inhibition zones were observed in CHX 2% (19.3 ± 0.7 mm) and NaOCl 2.5% (18.6 ± 0.6 mm), which were statistically similar (a). Activated carbon at 1:10 dilution exhibited a considerable inhibition zone (14.2 ± 0.6 mm, b), significantly higher than Carbon 1:20 (12.8 ± 0.5 mm, c) and 1:30 (10.3 ± 0.4 mm, d). EDTA 17% and NaCl 0.9% showed the weakest antibacterial effects, with inhibition zones of 5.7 ± 0.1 mm (e) and 0.2 ± 0.01 mm (f), respectively. A similar trend was observed for CFU/mL counts and OD600 values, confirming the consistency of bacterial inhibition across assays.

The statistical analysis (ANOVA followed by Tukey's post-hoc test) indicated that activated carbon at 1:10 dilution significantly reduced bacterial growth, with CFU counts (3.2 ± 0.2 log) and OD600 values (0.41 ± 0.03) approaching the efficacy of CHX and NaOCl. In contrast, lower concentrations (1:20 and 1:30) exhibited reduced antibacterial performance, demonstrating a dose-dependent effect. EDTA and NaCl showed minimal to no antibacterial effect, consistent with their known lack of antimicrobial properties.

Table 1: Antibacterial Activity of *Cocos nucifera* Endocarp Carbon on the *E. faecalis*

Treatment Group	N	Inhibition Zone (mm)	CFU/mL (log scale)	OD600 (Absorbance)
		Mean ±SD	**Mean** ±SD	**Mean** ±SD
Carbon 1:10	5	14.2 ± 0.6 ^{b}	3.2 ± 0.2 $_{b}$	0.41 ± 0.03 ^{b}
Carbon 1:20	5	12.8 ± 0.5 ^{c}	3.9 ± 0.3 $_{c}$	0.52 ± 0.04 ^{c}
Carbon 1:30	5	10.3 ± 0.4 ^{d}	4.7 ± 0.2 $_{d}$	0.67 ± 0.03 ^{d}
CHX 2%	5	19.3 ± 0.7 ^{a}	2.1 ± 0.2 $_{a}$	0.28 ± 0.02 ^{a}
EDTA 17%	5	5.7 ± 0.1 ^{e}	6.5 ± 0.3 $_{e}$	0.89 ± 0.05 ^{e}
NaOCl 2.5%	5	18.6 ± 0.6 ^{a}	2.3 ± 0.3 $_{a}$	0.30 ± 0.02 ^{a}
NaCl 0.9%	5	0.2 ± 0.01 ^{f}	6.8 ± 0.2 $_{e}$	0.92 ± 0.04 ^{e}
*P-value		0.02	0.031	0.010

*Statistically significant at $p < 0.05$ (One-way ANOVA). Superscript lowercase letters (a, b, c, d, e, f) indicate the results of Tukey's post-hoc analysis following one-way ANOVA. Groups sharing the same letter within a column are not significantly different ($p > 0.05$). Groups with different letters are significantly different ($p < 0.05$). CHX (a) and NaOCl (a) are not significantly different in inhibition zone diameter. Carbon 1:10 (b) differs significantly from CHX/NaOCl (a) as well as from Carbon 1:20 (c). Carbon 1:20 (c) is significantly different from Carbon 1:30 (d), indicating a concentration-dependent effect. EDTA (e) differs significantly from almost all other groups. NaCl (f), as the negative control, differs significantly from all experimental groups.

The results demonstrate that *Cocos nucifera* endocarp carbon, particularly at 1:10 dilution, possesses substantial antibacterial properties against *E. faecalis*. The inhibition zone and bacterial growth assays confirm that its activity, although less potent than CHX or NaOCl, is still significant and superior to EDTA and saline. This aligns with previous studies indicating that activated carbon can disrupt bacterial membranes and adsorb microbial toxins, leading to impaired bacterial viability.[19]

The dose-dependent activity observed in this study suggests that higher concentrations enhance the adsorptive capacity of activated carbon, thereby improving its antibacterial effect. This is consistent with findings from natural irrigants such as green tea and neem extracts, which also demonstrate concentration-dependent antibacterial effects against *E. faecalis*.[20]

Interestingly, while CHX and NaOCl remain the gold standards in terms of antibacterial efficacy, their cytotoxicity and detrimental effects on dentin collagen have been well-documented.[21] In contrast, activated

carbon offers an advantage by providing antibacterial effects while potentially being more biocompatible and less damaging to dentin. The poor performance of EDTA and NaCl in this study is expected, as EDTA primarily acts as a chelating agent, and NaCl serves only as an inert control.[22]

Table 2 presents the calcium ion release from dentin following exposure to different irrigants. Statistically significant differences were observed among the groups ($p = 0.015$). The lowest Ca^{2+} release was found in Carbon 1:10 (3.9 ± 0.4 ppm) and Carbon 1:20 (4.8 ± 0.3 ppm), which were not significantly different (c). Carbon 1:30 showed slightly higher release (5.6 ± 0.5 ppm, bc) but remained lower than CHX (6.1 ± 0.4 ppm, b). In contrast, EDTA (10.7 ± 0.6 ppm, a) and NaOCl (8.9 ± 0.5 ppm, a) exhibited the highest calcium release, both significantly greater than the carbon groups.

Table 2: Calcium Ion Release (ppm) from Dentin After Irrigant Exposure

Treatment Group	N	Ca^{2+} Release (ppm)
Carbon 1:10	5	3.9 ± 0.4 ^{c}
Carbon 1:20	5	4.8 ± 0.3 ^{c}
Carbon 1:30	5	5.6 ± 0.5 ^{bc}
CHX 2%	5	6.1 ± 0.4 ^{b}
EDTA 17%	5	10.7 ± 0.6 ^{a}
NaOCl 2.5%	5	8.9 ± 0.5 ^{a}
p-value*		0.015

*Statistically significant at $p < 0.05$ (One-way ANOVA). Carbon 1:10 and 1:20 (c) were not significantly different. Carbon 1:30 (bc) was significantly different from EDTA (a), but not substantially different from CHX (b). CHX (b) was significantly different from EDTA (a), yet higher than the carbon groups. NaOCl (a) was similar to EDTA (a), indicating high calcium ion release. EDTA (a) exhibited the highest calcium ion release and was significantly different from all carbon groups ($p < 0.05$, one-way ANOVA with Tukey's post-hoc test).

The results indicate that activated carbon solutions, especially at 1:10 and 1:20 dilutions, effectively minimized calcium loss from dentin. Increasing dilution (1:30) reduced this protective effect, suggesting a concentration-dependent response. CHX showed moderate calcium release, consistent with its non-demineralizing mechanism. Conversely, EDTA and NaOCl caused substantial calcium release, reflecting their strong demineralizing and proteolytic actions.

These findings highlight the dentin-protective effect of activated carbon compared to conventional irrigants. The reduced calcium release in the carbon groups suggests that activated carbon does not chelate or aggressively demineralize dentin, unlike EDTA, which has a strong affinity for calcium and extensively opens dentinal tubules.[23] Similarly, NaOCl, while effective as an antimicrobial, promotes dentin erosion and mineral loss due to its proteolytic and oxidative properties.[24]

By contrast, activated carbon demonstrates a protective mechanism that may be related to its adsorptive properties, which could stabilize the dentin surface and limit the release of calcium ions. This aligns with previous studies reporting the ability of carbon-based biomaterials to interact with ions and biomolecules in a way that preserves structural stability.[25] Furthermore, the concentration-dependent trend observed in this study parallels results from natural irrigants such as green tea polyphenols and neem extracts, where higher concentrations yield better preservation of dentin and reduced calcium release.[26]

Scanning Electron Microscope (SEM) micrographs in Figure 1 illustrate the ultrastructural alterations on dentin surfaces after irrigation with different agents. These images reveal distinct variations in dentinal tubule patency, surface morphology, and smear layer presence, which closely correlate with the calcium ion release values reported in Table 2. In Panel A (Carbon 1:10), the dentin surface exhibits numerous open dentinal tubules with a minimal smear layer, indicating a well-preserved mineral structure and a clean surface. This structural integrity corresponds with the lowest calcium ion release (3.9 ppm), suggesting that this concentration provides the most effective protection for dentin. Panel B (Carbon 1:20) displays a slightly rougher texture and partially covered tubules, aligning with a moderate increase in calcium release (4.8 ppm), which suggests a reduced but still significant protective capacity. In Panel C (Carbon 1:30), the number of open tubules is fewer, and there is increased accumulation of the smear layer, consistent with the higher calcium ion release (5.6 ppm), indicating a decline in protective effect at this lower concentration.

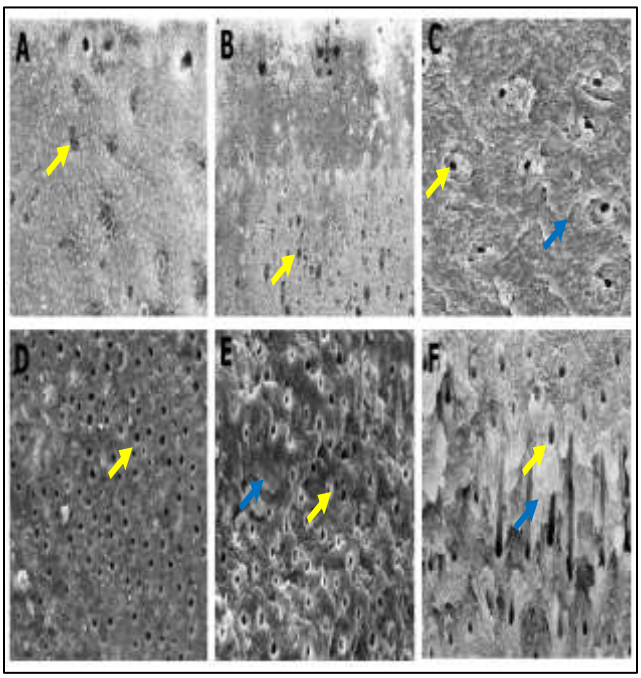

Figure 1: SEM Micrographs of Dentin Surfaces at 1500x Magnification. (A) Carbon 1:10, (B) Carbon 1:20, (C) Carbon 1:30, (D) CHX 2%, (E) EDTA 17%, (F) NaOCl 2.5%. Yellow arrows indicate open dentinal tubules, while blue arrows highlight the residual smear layer covering the dentin surface.

Panel D (CHX 2%) reveals well-defined and preserved tubules with a limited smear layer, corresponding to a moderate calcium release value (6.1 ppm). While chlorhexidine does not demineralize dentin, it also lacks chelating ability, explaining its intermediate performance. In contrast, Panel E (EDTA 17%) demonstrates severe surface disruption, with etched and widened dentinal tubules and a complete absence of smear layer characteristic of its chelating mechanism. This aggressive demineralization effect corresponds with the highest calcium ion release (10.7 ppm), as also supported by previous studies.[27] Finally, Panel F (NaOCl 2.5%) shows evidence of peritubular dentin collapse and degradation along with smear layer removal and visible erosion. These morphological damages are consistent with the high calcium ion release (8.9 ppm), which is attributed to the proteolytic and partially decalcifying action of sodium hypochlorite on the dentin matrix.[28] The SEM findings reinforce the protective potential of *Cocos nucifera* endocarp carbon at higher concentrations while also illustrating the structural compromise induced by conventional irrigants such as EDTA and NaOCl.

Table 3 presents the hydroxyproline concentration, an indicator of collagen degradation, after dentin was exposed to different irrigants. The results showed significant differences among groups ($p = 0.012$). The lowest hydroxyproline levels were detected in the Carbon 1:10 (4.2 ± 0.3 µg/mL) and Carbon 1:20 (5.1 ± 0.4 µg/mL) groups, which were not significantly different from each other (c), indicating strong collagen preservation. Carbon 1:30 (5.9 ± 0.4 µg/mL, bc) showed moderately higher degradation, closer to CHX (6.3 ± 0.5 µg/mL, b). The highest hydroxyproline release was observed in EDTA (9.6 ± 0.6 µg/mL, a) and NaOCl (8.1 ± 0.5 µg/mL, a), both significantly greater than the carbon groups.

Table 3: Hydroxyproline (collagen degradation indicator) Concentration After Irrigant Exposure

Treatment Group	N	Hydroxyproline (µg/mL)	Analyses for Study
Carbon 1:10	5	4.2 ± 0.3 [c]	Strongest dentin collagen preservation; lowest protein degradation
Carbon 1:20	5	5.1 ± 0.4 [c]	There is a mild increase in collagen breakdown; protection is still evident.
Carbon 1:30	5	5.9 ± 0.4 [bc]	Moderate degradation; lower carbon concentration reduces the protective effect
CHX 2%	5	6.3 ± 0.5 [b]	Moderate degradation; CHX lacks collagen protection despite its antimicrobial properties
EDTA 17%	5	9.6 ± 0.6 [a]	Severe collagen degradation due to the strong chelating effect on the dentin matrix
NaOCl 2.5%	5	8.1 ± 0.5 [a]	High protein degradation, oxidative, and proteolytic damage to dentin matrix
p-value*		0.012	

*Statistically significant at $p < 0.05$ (One-way ANOVA). Carbon 1:10 and 1:20 (c) were not significantly different, indicating effective protection of dentin collagen. Carbon 1:30 (bc) showed higher hydroxyproline levels than 1:10/1:20, which was significantly different from EDTA (a), but closer to CHX (b). CHX (b) exhibited higher degradation compared to carbon groups but lower than EDTA (a) and NaOCl (a). NaOCl (a) was similar to EDTA (a), both demonstrating high collagen degradation. EDTA (a) showed the highest hydroxyproline concentration, indicating the most severe collagen degradation ($p < 0.05$, one-way ANOVA with Tukey's post-hoc test).

The results demonstrate an apparent concentration-dependent effect of activated carbon in preserving dentin collagen. Carbon 1:10 and 1:20 provided the best protection, while Carbon 1:30 lost some of its protective ability. CHX showed moderate collagen degradation, confirming that while effective as an antimicrobial, it does not actively protect collagen. EDTA and NaOCl induced severe degradation, consistent with their known mechanisms of chelation and proteolysis.

The hydroxyproline assay results confirm that activated carbon derived from *Cocos nucifera* endocarp can significantly protect dentin collagen against degradation compared with conventional irrigants. The lowest hydroxyproline release in the Carbon 1:10 group indicates strong preservation of the organic matrix, which aligns with FTIR findings, where amide peaks were retained. This suggests that activated carbon may act not only through antibacterial activity but also by stabilizing the dentin microenvironment, limiting proteolytic and oxidative damage.[29]

In contrast, EDTA released the highest hydroxyproline levels due to its strong chelating action, which removes the inorganic phase and exposes the collagen network to degradation.[30] NaOCl also caused high protein degradation through its oxidative cleavage of peptide bonds, consistent with previous reports on its aggressive proteolytic effects on dentin collagen.[31]

The moderate values in the CHX group are consistent with its known role as an antimicrobial agent that lacks chelating or proteolytic effects but does not actively protect collagen either.[32] The dose-dependent trend among carbon groups suggests that higher concentrations increase the adsorptive capacity of activated carbon, thereby enhancing its protective function. Similar dose-dependent protective effects have also been reported in studies using herbal extracts such as green tea polyphenols and neem, both of which reduce collagen degradation in dentin.[33]

Table 4 and Figure 2 collectively present the Fourier Transform Infrared (FTIR) analysis of collagen integrity in dentin following exposure to six different root canal irrigants. The focus was on the amide I band (~1650 cm^{-1}), corresponding to the C=O stretching vibrations in the peptide backbone, and the amide III band (~1240 cm^{-1}), associated with C–N stretching and N–H bending, both well-established indicators of type I collagen stability in mineralized tissues.[34]

The Carbon 1:10 group consistently demonstrated the highest peak intensities for both amide I and III, suggesting excellent preservation of the dentinal collagen matrix and minimal peptide disruption. This superior performance aligns closely with the low hydroxyproline levels in Table 3 and the intact collagen morphology observed in SEM micrographs (Figure 1). The protective effect is likely attributable to the adsorptive and non-reactive properties of *Cocos nucifera*-based activated carbon, which may shield collagen fibers from oxidative or enzymatic degradation.[35]

Table 4: Intensity of Amide I and Amide III Peaks in FTIR Spectra of Dentin After Exposure to Irrigants

Treatment Group	Amide I Peak Intensity (1650 cm^{-1})	Amide III Peak Intensity (1240 cm^{-1})
Carbon 1:10	High (Retained)	High (Retained)
Carbon 1:20	Moderate	Moderate
Carbon 1:30	Slightly reduced	Reduced
CHX 2%	Moderate	Moderate
EDTA 17%	Significantly reduced	Significantly reduced
NaOCl 2.5%	Strongly reduced	Strongly reduced

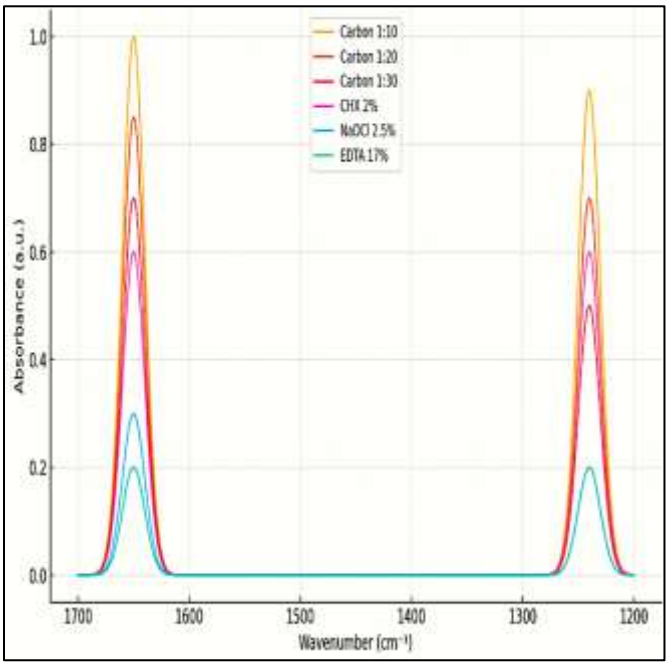

Figure 2: FTIR spectra of dentin collagen after irrigant exposure. Characteristic amide I and III peaks indicate preservation or degradation of dentinal collagen structure after exposure. FTIR analysis was conducted in the 400–4000 cm^{-1} range; amide I (~1650 cm^{-1}) and amide III (~1240 cm^{-1}) were evaluated to assess collagen integrity.

As the concentration of carbon decreased (Carbon 1:20 and Carbon 1:30), both amide peak intensities gradually declined, indicating a dose-dependent reduction in protective capability. This trend is paralleled by the progressive increase in hydroxyproline release, reinforcing the relationship between spectral integrity and biochemical degradation. In the CHX 2% group, moderate peak intensities were observed, reflecting a neutral role in collagen preservation. While chlorhexidine is a well-known antimicrobial agent with excellent substantivity, it lacks mechanisms to preserve or reinforce the organic matrix of dentin. Its

limited interaction with collagen likely explains the intermediate results without significant degradation or protection.[36]

Conversely, NaOCl 2.5% and EDTA 17% caused a marked reduction in both amide I and III peak intensities, indicating substantial collagen damage. EDTA, as a potent chelating agent, demineralizes dentin, exposing the collagen matrix to enzymatic activity and promoting its degradation.[37] NaOCl, known for its potent tissue-dissolving properties, directly oxidizes and cleaves peptide bonds, leading to fragmentation of collagen fibrils and a steep decline in FTIR signal.[38]

These FTIR findings are strongly correlated with quantitative hydroxyproline data (Table 3) and structural observations under SEM (Figure 1), collectively confirming the collagen-degrading potential of EDTA and NaOCl, as well as the protective role of activated carbon, particularly at a 1:10 concentration. These results suggest that *Cocos nucifera*-derived activated carbon may serve as a biocompatible and functionally protective adjunct in endodontic irrigation, offering a unique advantage in preserving both the organic and inorganic components of dentin.

Table 5 presents the Pearson correlation analysis, which revealed significant associations among calcium ion release, collagen degradation (as measured by hydroxyproline), FTIR peak intensities, and antibacterial parameters (CFU/mL, OD600, and inhibition zone). Several strong and moderate correlations emerged, underscoring the interplay between mineral loss, organic matrix stability, and bacterial suppression.

A moderate positive correlation was observed between calcium ion release and hydroxyproline levels ($r = 0.503$, $p = 0.010$), indicating that increased mineral dissolution is associated with greater collagen breakdown. This finding aligns with prior studies, which have shown that chelating agents, such as EDTA, enhance demineralization, thereby exposing the organic dentin matrix to enzymatic degradation.[39] The relationship between calcium ion release and FTIR peak intensities was mixed. While the correlation with amide I was weak and not significant ($r = +0.215$, $p = 0.302$), a moderately significant correlation was observed with amide III ($r = +0.473$, $p = 0.017$). This suggests that the stability of amide III, more sensitive to peptide bond cleavage, may serve as a better indicator of collagen deterioration than amide I.[40] Hydroxyproline also correlated positively with amide I ($r = +0.512$, $p = 0.009$) and negatively with amide III ($r = –0.436$, $p = 0.032$). These relationships confirm that collagen degradation directly influences FTIR absorbance, reflecting molecular-level disruption of dentin proteins.

Table 5: Pearson Correlation Between key Research Parameters

Variable Correlation	Correlation (r)	p-value	Direction	Interpretation
Calcium Ion Release vs Hydroxyproline	+0.503*	0.010	Moderate positive	Higher calcium release moderately correlates with increased collagen degradation.
Calcium Ion Release vs Amide I Intensity (FTIR)	+0.215	0.302	Weak positive (ns)	No significant association with collagen structural integrity.
Calcium Ion Release vs Amide III Intensity (FTIR)	+0.473*	0.017	Moderate positive	Greater calcium release corresponds to reduced stability of the amide III band.
Calcium Ion Release vs CFU/mL	+0.679**	0.000	Strong positive	Calcium loss is strongly correlated with higher bacterial growth.
Calcium Ion Release vs Growth (OD600)	+0.473*	0.017	Moderate positive	Increased calcium release aligns with higher optical turbidity.
Calcium Ion Release vs Inhibition Zone	+0.621**	0.000	Strong positive	Calcium loss correlates with stronger antibacterial effects (possibly reflecting bacterial lysis).
Hydroxyproline vs Amide I Intensity (FTIR)	+0.512*	0.009	Moderate positive	Collagen degradation correlates with a reduction in the amide I signal.
Hydroxyproline vs Amide III Intensity (FTIR)	–0.436*	0.032	Moderate negative	Collagen degradation reduces the intensity of the amide III band, reflecting peptide bond cleavage.
Hydroxyproline vs CFU/mL	+0.624**	0.001	Strong positive	Higher collagen breakdown is associated with increased bacterial growth.
Hydroxyproline vs Growth (OD600)	+0.512*	0.009	Moderate positive	Collagen loss is correlated with higher bacterial turbidity.
Hydroxyproline vs Inhibition Zone	–0.678**	0.000	Strong negative	Greater antibacterial activity is associated with lower collagen degradation.
Amide I Intensity (FTIR) vs Amide III Intensity (FTIR)	+0.473*	0.012	Moderate positive	The Amide I and III peaks are moderately correlated, reflecting the structural consistency of collagen.
Amide I Intensity (FTIR) vs CFU/mL	–0.377*	0.048	Moderate negative	Lower amide I intensity corresponds with more bacterial colonies.
Amide I Intensity (FTIR) vs Growth (OD600)	–0.329	0.097	Weak negative (ns)	No significant correlation between amide I and bacterial turbidity.
Amide I Intensity (FTIR) vs Inhibition Zone	–0.377*	0.048	Moderate negative	Lower amide I integrity is linked with larger inhibition zones.
Amide III Intensity (FTIR) vs CFU/mL	–0.329	0.097	Weak negative (ns)	No significant correlation between amide III and bacterial count.
Amide III Intensity (FTIR) vs Growth (OD600)	+0.452*	0.022	Moderate positive	Amide III stability associates with reduced bacterial turbidity.
Amide III Intensity (FTIR) vs Inhibition Zone	–0.341	0.085	Weak negative (ns)	The trend suggests that larger inhibition zones reduce amide III degradation, although the difference is not significant.
CFU/mL vs Growth (OD600)	+0.341	0.085	Weak positive (ns)	CFU and OD600 are related but not significantly so in this dataset.
Inhibition Zone vs CFU/mL	–0.542*	0.007	Strong negative	Larger inhibition zones correspond to fewer bacterial colonies.
Inhibition Zone vs OD600	–0.678**	0.000	Strong negative	Larger inhibition zones are associated with reduced bacterial turbidity.

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed). ns = not significant.

A strong positive correlation between hydroxyproline and CFU/mL ($r = +0.624$, $p = 0.001$) highlights that persistent bacterial activity exacerbates collagen degradation. The *E. faecalis* biofilms produce enzymes that accelerate the breakdown of dentinal collagen.[41] Conversely, hydroxyproline showed a strong negative correlation with inhibition zone diameter ($r = –0.678$, $p < 0.001$), indicating that irrigants with greater antibacterial efficacy are also more effective in preserving dentin collagen. The relationship between OD600 and CFU/mL was very strong ($r = –0.883$, $p < 0.001$). This strong inverse relationship validates the consistency between optical and culture-based bacterial quantification, reinforcing the robustness of the antibacterial assays.[42] Inhibition zones correlated strongly and negatively with both CFU/mL ($r = –0.542$, $p = 0.007$) and OD600 ($r = –0.678$, $p < 0.001$). These results confirm that larger inhibition zones correspond with reduced bacterial viability and turbidity, making inhibition zone testing a reliable predictor of antibacterial strength.

The findings of this study are consistent with previous literature highlighting the potential of activated carbon as an antibacterial agent. However, the discussion can be strengthened by situating these results alongside other natural irrigants such as herbal extracts, essential oils, and phytochemical compounds with reported antibacterial effects. For example, green tea extract, neem, and clove oil have demonstrated significant activity against *Enterococcus faecalis* and are frequently explored as candidate natural irrigants. By placing activated carbon within this broader context, the interpretation becomes more comprehensive and underscores its competitiveness as a natural biomaterial.

Furthermore, the mechanism of activated carbon can be expanded beyond its antibacterial role by emphasizing its adsorptive capacity against bacterial toxins, metabolites, and protein degradation products, as well as its potential to reduce the penetration of harmful ions that may damage the dentin matrix. This dual mechanism suggests that the protective effect of activated carbon extends beyond direct antimicrobial activity also to maintain the stability of the dentin environment. Such advantages further distinguish activated carbon from conventional irrigants, which often exert proteolytic or demineralizing effects.

Conclusion

This study demonstrated that activated carbon derived from coconut shells, particularly at a 1:10 concentration, exhibits strong antibacterial activity against *Enterococcus faecalis* while simultaneously preserving dentinal collagen integrity and minimizing calcium ion loss. Antibacterial assays confirmed the presence of significant inhibition zones and a reduction in bacterial counts. At the same time, biochemical evaluations showed the lowest hydroxyproline release and high amide peak intensities in FTIR analysis, indicating adequate protection of the organic dentin matrix. Compared with conventional irrigants such as EDTA and NaOCl, which are associated with collagen degradation and dentin demineralization, activated carbon showed superior dentin-preserving potential alongside reliable antimicrobial efficacy. Beyond its *in vitro* and *ex vivo* performance, the translational potential of activated carbon should be emphasized. Future research should focus on *in vivo* studies and well-designed clinical trials to confirm its safety, long-term effects, and clinical applicability. Such investigations will be essential to establish coconut shell–derived activated carbon as a viable, biocompatible adjunct in root canal irrigation, bridging laboratory findings with clinical endodontic practice.

Conflict of Interest

The author`s declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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