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A COMPARATIVE EVALUATION OF *OCIMUM SANCTUM* BASED INTRACANAL MEDICAMENT AGAINST ENTEROCOCCUS FAECALIS

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ABSTRACT

Aim

To evaluate and compare the antibacterial efficacy of *Ocimum sanctum* based intracanal medicament with calcium hydroxide against *Enterococcus faecalis*.

Material and methods

Thirty extracted single rooted anterior teeth were contaminated with *Enterococcus faecalis*. Afterwards, extracted human teeth were maintained in saline storage. The teeth were decorated to provide a standardized root length of 15 mm. The root canals were instrumented upto a size of 30k-file followed by instrumentation at 1mm short of the apical foramen upto a size of 35k-file.The root canals are filled with 17% EDTA for 3min and rinsed with 5 ml of saline. And then the apical foramina is sealed with light cured composite resin and then autoclaved at 121 °C for 15 min. Samples taken before and after procedures were cultured, and the colony-forming units (CFUs) were counted. The statistical analyses were performed with various tests.

Results

Calcium hydroxide intracanal medicament has proven effective in eliminating microorganisms present in the root canal system. The groups in which ocimum sanctum derived Ag-CuO based nanoparticle medicaments have no significant reduction in bacterial count compared to calcium hydroxide. CFU colony counts and one-way ANOVA statistical evaluation, with a post hoc Tukey statistically significant variation assessment applied.

Conclusion

Significant antibacterial properties against E. faecalis biofilm on root dentin was demonstrated by biosynthesized AgNPs. It can therefore be used as an antibacterial treatment to sterilize root canals. Biomechanical preparation with NaOCl considerably reduced the bacterial count in the root canal.

Key words:

Antimicrobial activity, *Enterococcus faecalis, Ocimum sanctum* herbal extracts, Ag CuO NPs, SEM

Introduction

Natural products are rare and valuable that contain safer, highly efficient antimicrobial agents. New advances in analytical science and technology, especially in phytotherapy, resulted in an innovative age of herbal products as well as anti-plaque treatments(Kingston 2011). *Ocimum sanctum* (Tulsi), a well-known culinary and medicinal fragrant herb native to the Indian subcontinent and belonging to the Lamiaceae family, has been used in Ayurvedic medicine for more than three thousand years (Khot et al. 2023). Tulsi is known as a "Elixir of Life" for its healing abilities and has been used to treat a number of conditions. Tulsi leaf extracts are used to treat bronchitis, rheumatism, and pyrexia(Deepika et al. 2022). Treatment of epilepsy, asthma or dyspnea, hiccups, cough, skin and hematological illnesses, parasite infections, neuralgia, headache, wounds, and inflammation, and oral problems are among the other known medicinal uses (Joshi 2017). Pharmacological and clinical studies showed that *O. sanctum* consists of terpenoids, iridoids, phenolic compounds, and flavonoids. These active components act as anti-microbial, anti-oxidative, anti-inflammatory, anti-arthritic, and anti-pyretic agents(Janani et al. 2020). Only a few studies use *O. sanctum* as an anti-oxidative agent (Chandrappa et al. 2015). Most of the phytochemical constituents of *O. sanctum* alter the microbial organism's structural and functional integrity(Choudhari et al. 2023).

Excellent biomaterials with distinctive physical, chemical, and biological characteristics have recently been developed thanks to the application of nanotechnology in dentistry (Jandt and Watts 2020). Because of their greater volume-to-surface area ratio and smaller particle size, which lead to a bigger response surface and more effective contact, the nanoparticles (NPs) exhibit superior antibacterial activities. They then enter the dentinal tubules and, at lower dosages, have a persistent antibacterial effect at the infection site. The discipline of nanotechnology has emerged more recently as a result of its dazzling applications in the healthcare area (Shaheen 2022). This draws a large number of researchers, who then develop various nanomaterials with particular purposes, such as curing a disease or improving and functioning in devices or items like domestic goods, cosmetics, medical supplies, etc. The need for these nanomaterials at the nanoscale in live cells is crucial in outcompeting viruses and organisms that cause illness. The microorganisms that cause sickness are becoming increasingly resistant to new antibiotic treatments these days (Maisch 2009). Researchers have reported using metallic nanoparticles, such as silver-copper oxide nanoparticles (Ag-CuNPs), as an efficient antibacterial agent in a number of studies to address this problem (Rizvi and Saleh 2018; Pellosi et al. 2023).

Ag-CuNPs are made of pure crystalline silver with dimensions ranging from 1–100 nm(Lakshmi and Dean -International Affairs, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences 2021) . They have recently gained popularity for a wide range of biomedical applications because of their enhanced and unique physicochemical properties, such as smaller particle size, higher surface area, and quantum confinement effects, among others, compared with bulk or powder material (Chitturi et al. 2018). In dentistry, Ag-CuNPs are used in the fields of endodontics, restorative dentistry, orthodontics, implantology, prosthodontics, and periodontics (Riad and Ibrahim 2021). They have been primarily used for disinfection, prophylaxis, and prevention of oral infections due to their favorable antimicrobial properties(Kamath et al. 2022). Microorganisms and their byproducts play a pivotal role in the development of pulpal and periapical lesions, with *Enterococcus faecalis* being frequently isolated in root canals associated with pulpal infections.

Enterococcus faecalis, a gram-positive facultative anaerobic bacterium, is an opportunistic pathogen that is commonly found in the human oral cavity and gastrointestinal tract(Stuart et al. 2006). Evidence has shown that *E. faecalis* is one of the most common pathogenic microorganisms in root canals with refractory apical periodontitis, as it is frequently isolated from root canals with endodontic failure. *E. faecalis* survives for a long time in root canals because it can tolerate a highly alkaline and oligotrophic environment to form biofilm deep into the dentin tubules and easily evade phagocytosis by host cells (Duggan(Duggan and Sedgley 2007). Moreover, *E. faecalis* can also be detectedine periradicular lesions and extraradicular biofilms in patients with refractory apical periodontitis. In this context, the present research paper focuses on the development of a novel *Ocimum sanctum* and silver nanoparticle based intracanal medicament against *E. faecalis*(Choudhari et al. 2023).

Materials and Methods

Collection of plant material and chemicals

Samples of *Ocimum sanctum* leaves were obtained at the location at 12.60° N, 77.85° E in Guttoor Village, Kelamangalam, Krishnagiri District, Tamil Nadu, India. The leaves were examined for authentication at the Department of Botany, Periyar University, Salem, Tamil Nadu, India. We purchased copper acetate ($Cu(CO_2CH_3)_2$) and silver nitrate ($AgNO_3$) from Sigma-Aldrich in India.

Green synthesis of bimetallic nanoparticles

The fresh leaves were properly cleaned with distilled water before being allowed to air dry at room temperature in the shade. An electric lab grinder was used to crush the dried leaves into a fine powder(Nasim et al. 2022). After mixing the resulting fine powder (10 g) with 100 mL of distilled water, the mixture was heated to 80 °C for 25 mins while being continuously agitated. The material was filtered using Whatman filtration paper No. 1. The extract was kept at 4 °C until it was needed.The precursor material used for the production of the nanoparticles was silver-copper (Ag-Cu). The bimetals nanoparticles were quantified, and 1000 mL of the plant extract was mixed with 2 mM of silver nitrate ($AgNO_3$-Sigma Aldrich) and Cupper acetate ($CuCO_2CH_3$-Sigma Aldrich). Subsequently, the metal solution received an addition of the aqueous extract (10 g of plant leaf powder combined with 100 mL of sterile distilled water). A 1:1 mixture of the metal solution and the aqueous extract was then combined. After adding 500 mL of *O. sanctum* leaf extract and mixing with magnetic stirring for one hour, the metal solution (Ag-Cu) was added. For an additional three hours, the mixture was vigorously stirred at 60 °C in a magnetic stirrer. The mixture was left to stand at room temperature for an entire night. Following this procedure, a yellow-brown color was observed. Following a 15-min centrifugation at 15,000 rpm, the liquid was diluted with distilled water to remove the sediment. The pellet and milli-Q-water were mixed at 15000 rpm for 15 mins. The sediment/pellet mixture was introduced into the Petri plate. after a hot air oven was used to dry the plate. The plate was then stored in a dryer for two days. Lastly, until they were needed again, the dried nanopellets were placed in an airtight container and stored at 4 °C (Al-Haddad et al. 2020).

Characterization of NPs

Various characterization methods were used to the bio-reduced Ag-CuO NPs. The UV-Vis spectrophotometer (Model T80+UV-Vis spectrometer) was used to measure the surface plasmon resonance (SPR) of the Ag-CuO nanoparticles in the 200–800 nm range. The diameters of the particles were assessed using scanning electron microscopy using a JEOLJEM-2100F SEM operating at a voltage of 200 kV (Moosavy et al. 2023).

Study design

Thirty human single-rooted teeth with single root canals were used. Teeth were debrided properly using saline and hydrogen peroxide and decoronated maintaining 15 mm length The root canals were instrumented upto a size of 30k-file followed by instrumentation at 1mm short of the apical Foramen upto a size of 35k-file. (Fig 1)

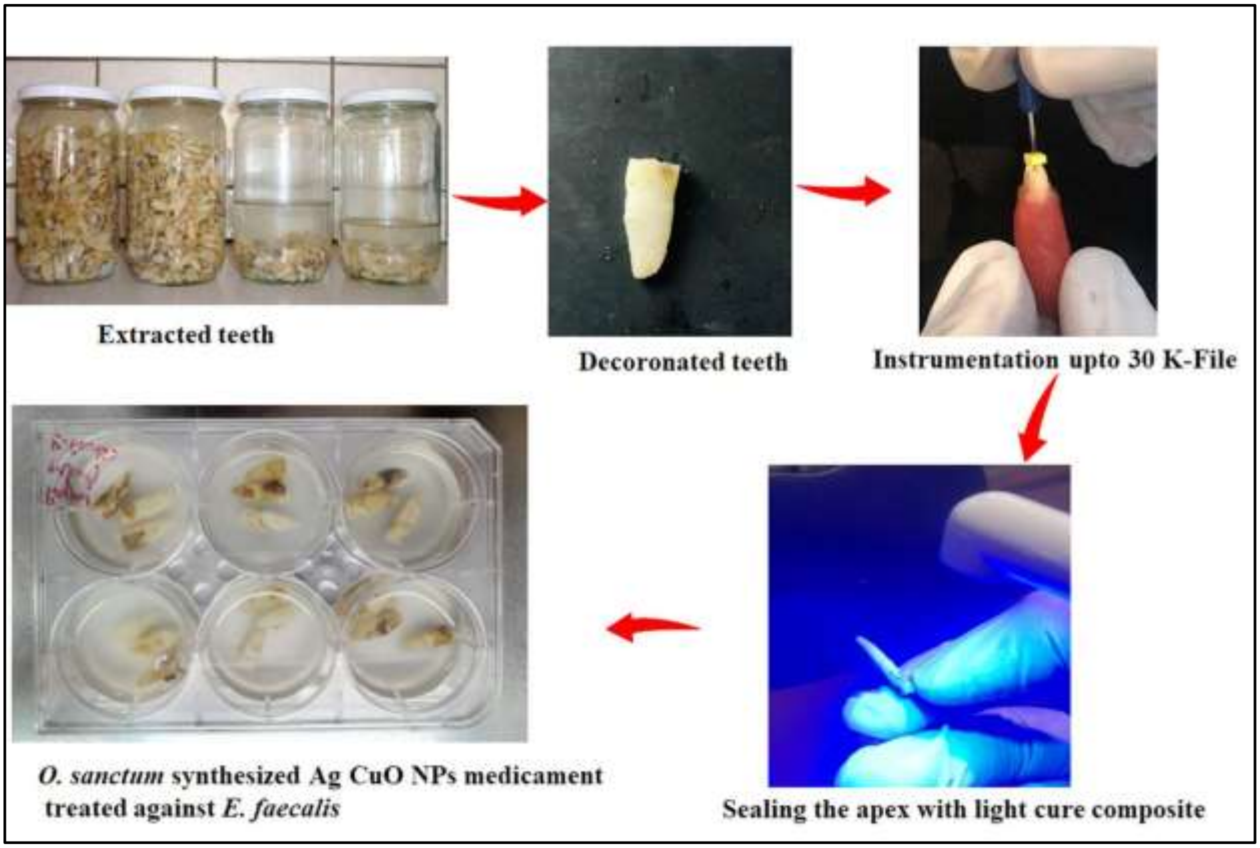

Fig.1 Overall methods for *O. sanctum* biosynthesized AgCuNPs medicament tested against *E. faecalis*

The root canals are filled with 17% EDTA for 3 min and rinsed with 5 ml of saline. And then the apical foramina is sealed with light cured composite resin and then autoclaved at 121 °C for 15 min. The canals were contaminated with *E. faecalis* and were then irrigated with 1% NaOCl. The roots were divided into 3 groups (n=10) according to the intracanal medication applied: i). Calcium hydroxide paste (group1) ii). *O. sanctum* aqueous extract (group 2), iii). *O. sanctum* mixed with Copper oxide based- silver nanoparticle (group 3).The following collections were made from the root canals: (IS): initial sample 21 days after contamination, a) S1 after instrumentation and irrigation with NaOCl , c) S2: 7 days after intracanal medication placement; . The results were analyzed statistically

Satatistical analysis

The statistical evaluation was performed using one-way analysis of variance (ANOVA). Furthermore, Graph Pad Prism software (Boston, MA 02110, USA) was used for data analysis.

Results

The progress of the formation of Ag-CuO NPs can be monitored by visual color changes or using UV-Vis. Spectroscopy, where a sharp peak due to surface plasmon resonance (SPR) of Ag-CuO NPs at around 430–450 nm is clearly observed. After successful synthesis of the Ag-CuO NPs the mixture is centrifuged at high rpm to separate the NPs followed by proper washing using solvents and dried in an oven at low temperature. (Fig 2)

Fig. 2 Green synthesis of Ag-Cu NPs from *Ocimum sanctum* leaf extract.

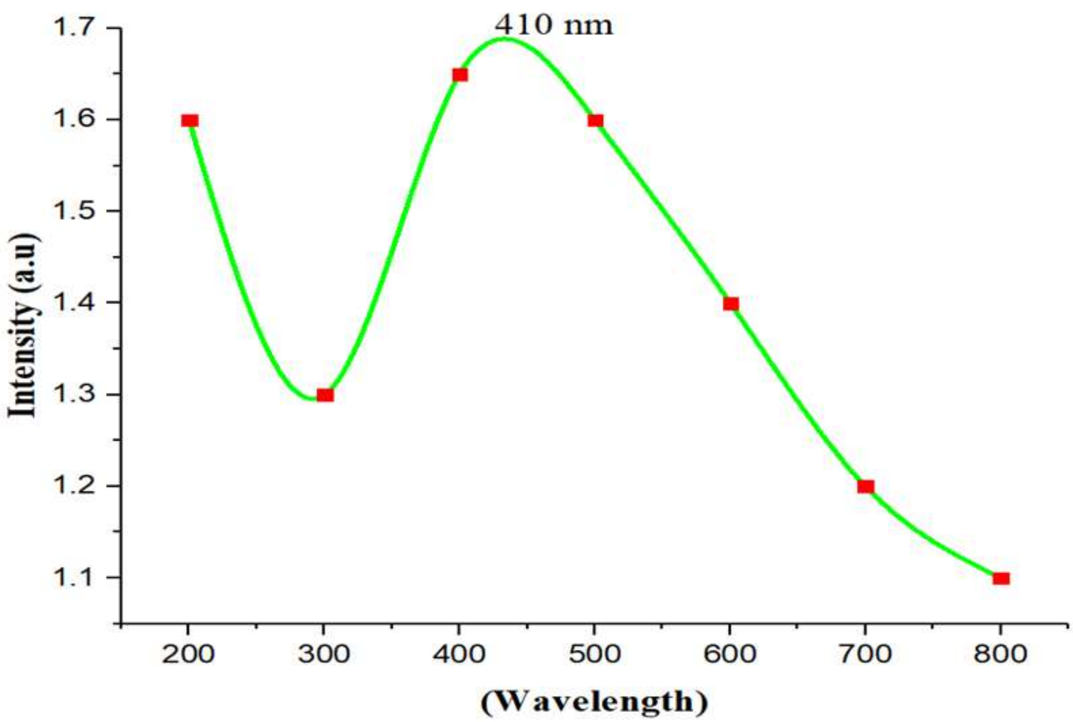

Fig.3. The absorption of UV spectrum of *O. santum* leaf extract at different wave lengths ranged from 200 to 800 nm revealed a peak at 410nm.

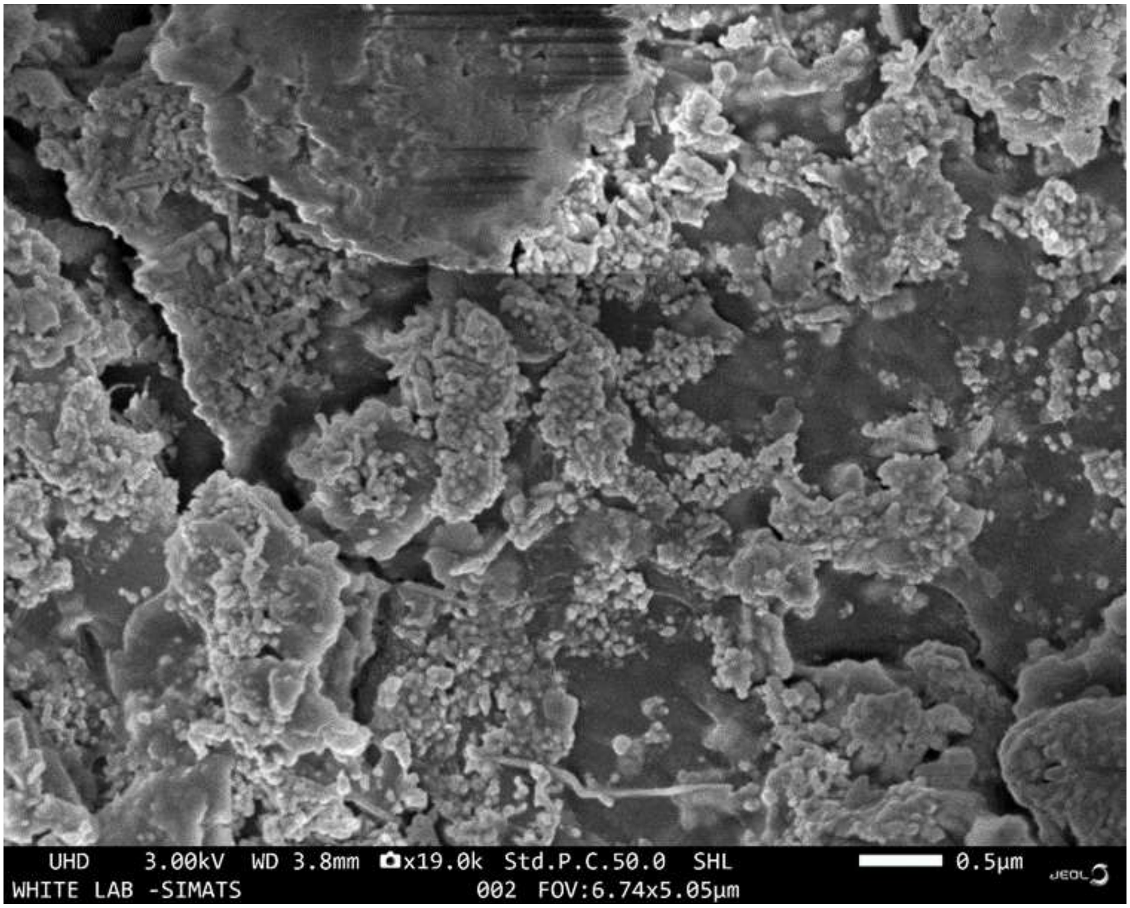

Fig.4. SEM images of *O. sanctum* biosynthesized Ag-CuO NPs.

The biosynthesized Ag-CuO NPs underwent scanning electron microscopy (SEM) to determine their morphology, size, and form. Ag-CuO NPs exhibit a range of sizes, morphologies, and characteristics. (Fig 2-3)

O. sanctum biosynthesized Ag-CuO NPs was exhibited antibacterial properties against *E. faecalis.* Ag ions with strong bactericidal properties are released by silver nanoparticles (Ag-CuO NPs). As an intracanal therapy, they have demonstrated encouraging outcomes in eliminating *E. faecalis*, a resilient bacteria linked to root canal failures. In SEM analysis, it was seen that green synthesized silver nanoparticles showed better results than calcium hydroxide, and according to literature review, it is seen that CFU of green synthesized Ag-Cu NPs was much less than those of calcium hydroxide used alone. (Fig 5 and 6)

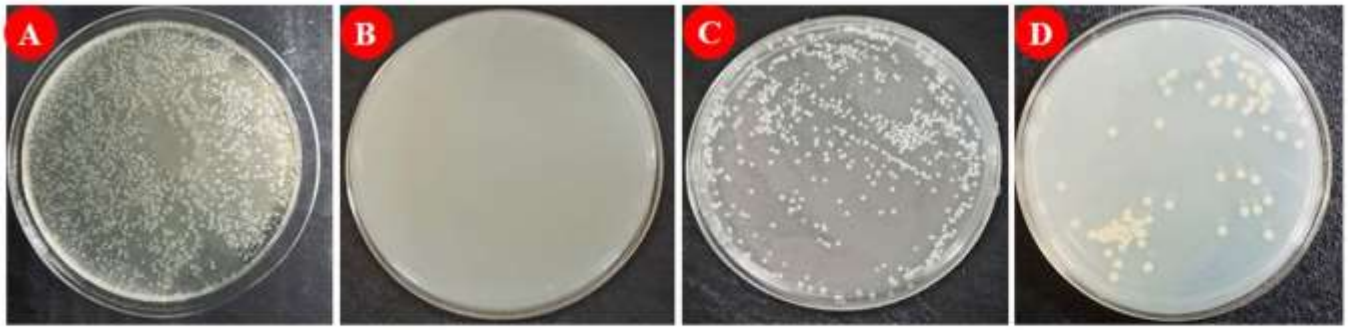

Fig.5. Colony count for *E. faecalis* treated with different intracanal medicament. **A**). Control (Without treatment), **B**). Calcium hydroxide Intracanal medicament, **C**). *O. sanctum* aqueous extract, **D**). *O. sanctum* leaf biosynthesized Ag CuO NPs.

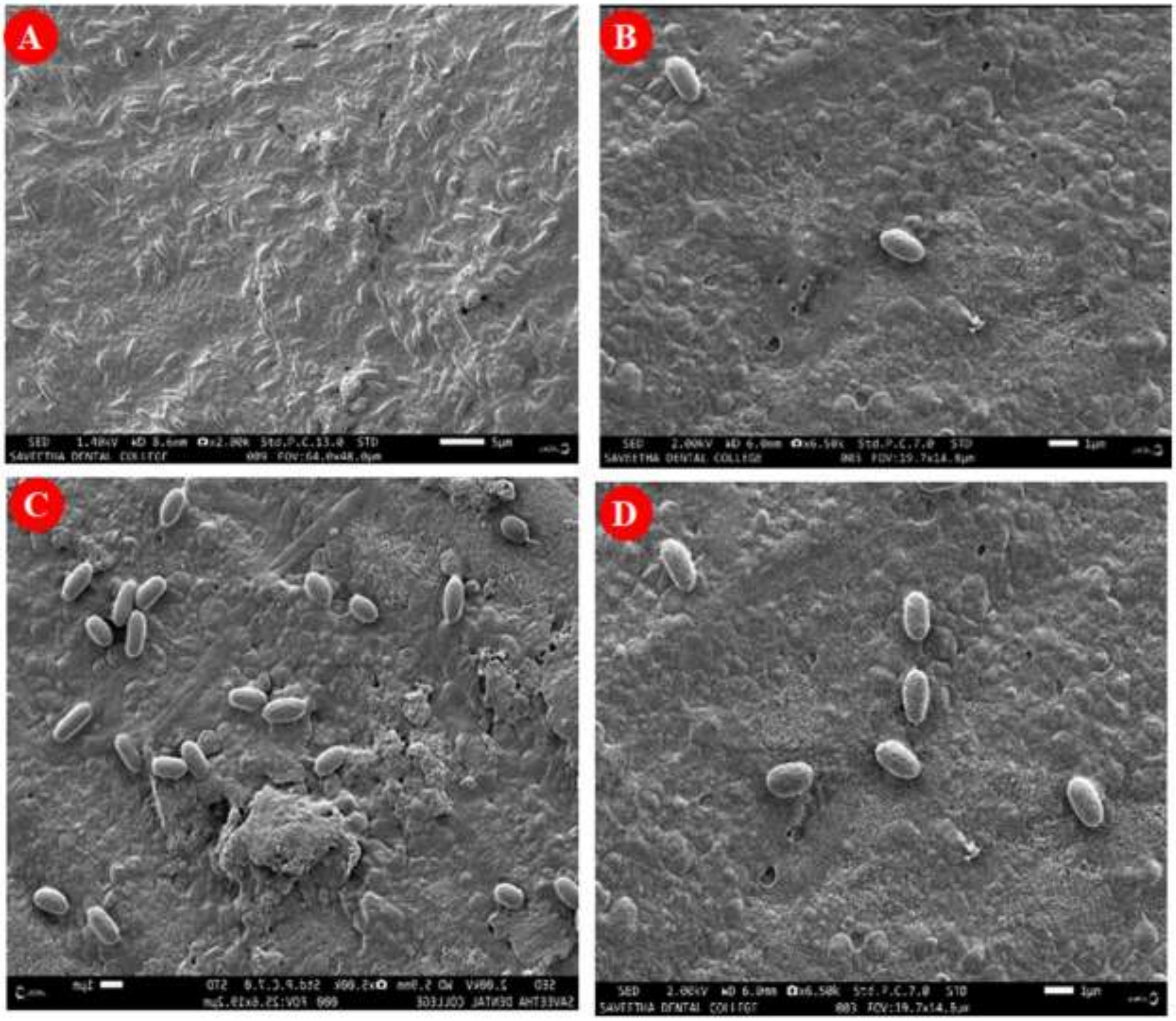

Fig.6. SEM analysis of *E. faecalis* treated with various intracanal medicament. **A**). Control (Without treatment), **B**). Calcium hydroxide Intracanal medicament, **C**). *O. sanctum* aqueous extract, **D**). *O. sanctum* leaf biosynthesized Ag CuO NPs.

Table 1. Colony forming unit of *E. faecalis* treated with biosynthesized Ag-Cu NPs.

Groups	I-S (Control) (CFU/mL)	1st collection S1(CFU/mL) after NaOCl irrigant	2nd collection S2 (CFU/mL) after 7days of intracanal medicament
Calcium hydroxide	362.08[b]	1.708[a]	0[c]
O. sanctum aqueous extract	377.6[a]	1.128[b]	0.856[a]
O. sanctum synthesized bimetallic silver-copper(Ag-CuO NPs)	342[c]	1.113[ab]	0.183[b]

[a]Mean values within the column followed by the same letter in superscript are not significantly different at $p < 0.05$ level. An analysis of variances (ANOVA) was used to assess the data using SPSS 20 (IBM) to find significant differences between means. (Table 1)

Discussion

Several studies in the field of dentistry documented the use of plant extracts in endodontics. Nanoparticles have been employed recently to reduce the bacterial load in endodontic infections. It is hypothesized that this is because of their nano size and structure, which increase surface area and can absorb pharmaceuticals and exert antimicrobial effect(Lakshmi and Nanobiomedicine Lab, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai 2021); on the other hand, calcium hydroxide and chlorhexidine have been used for a long time for eliminate *E. faecalis (Rodrigues et al. 2018)*. Metallic nanoparticles are frequently utilized because of their high bactericidal capability against Gram-positive, negative, and multidrug-resistant bacteria. It can interact with the cell wall of bacteria, causing structural alterations and tissue protein disruption. Therefore, the objective of the present research is to assess and compare the antibacterial effects of both silver-copper nanoparticles alone and calcium hydroxide.

According to the included investigations, intracanal medications containing Ag CuO NPs have strong antibacterial activity against *E. faecalis* that is similar to that of $Ca(OH)_2$. Ag CuO NPs produced by green biosynthesis utilizing plants were demonstrated in some studies to be as potent against *E. faecalis* (Ghasemi, Jamali-Sheini, and Zekavati 2017; Sacoto-Figueroa et al. 2021). Plant-derived Ag CuO NPs exhibited antibacterial activity comparable to that of 2% CHX (Bulut Kocabas et al. 2021). They produce materials with excellent monodispersity, size, and chemical composition, much like "nanofactories." Similarly, plants were used for their synthesis, including *Syzygium aromaticum* (Yadi et al. 2018; kadhem Salman and Hasson 2021).The antibacterial activity of *O. sanctum* Linn is attributed to the production of camphor, eucalyptol, eugenol, alpha-, beta-, and beta-bisabolene by its active component. They worked well against *Candida albicans* and *S. aureus* (Ramteke et al. 2013). Secondary molecules from these plants, such as flavonoids, phenolic acid, alkaloids, and terpenoids, function as reducing agents to produce Ag CuO NPs from a solution of silver nitrate and copper acetate. The use of silver copper nanoparticles (Group A) alone was found to have moderate antibacterial effect against *E. faecalis*. This is because the oxidation of the nanoparticles releases silver ions, which connect to the membrane of bacterial cells, penetrate the bacterium, and react with specific bacterial proteins, changing the bacterial metabolism and inhibiting essential enzymatic systems that lead to cell death. The results of our study agreed with those of (Zhang et al. 2009) et al.'s investigation.

Our research demonstrated that all three treatments worked in concert to provide a synergistic effect that allowed for the complete removal of *E. faecalis* from the root canal microbial community. The production of hydroxyl ions in the root canal kills bacteria by collapse the cytoplasmic membrane, triggering protein denaturation, and causing DNA damage (Abbas et al. 2019) .It is among the most affordable techniques that might prove to be highly beneficial for the production of nano-products in the biomedical field in the future.

Conclusion

In this study, the antimicrobial effectiveness of green synthesized Ag-Cu NPs as an irrigant has been evaluated against a single-species biofilm. Although *E. faecalis* can be found in cases of persistent endodontic infections, typically most endodontic infections are multi-species biofilms. Therefore, future studies should be evaluated against a polymicrobial biofilm and its disruption. To further strengthen the evidence, future animal studies and clinical trials are warranted. In this study, the antibacterial effect of green synthesized Ag-Cu NPs as an endodontic irrigant was evaluated against *E. faecalis* isolates from patients with failed root canal treatment. This is a first-of-its-kind project in which the effectiveness of Ag-Cu NPs against *E. faecalis* isolates from patients with failed root canal treatment was evaluated. However, further experiments in the future can be carried out to evaluate the effectiveness against a polymicrobial biofilm using animal studies. The aforementioned results also require *in vivo* testing.

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Conflicts of interest

The authors have nothing to declare.

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