RESEARCH PAPER

Assessment of antibacterial properties of green synthesized silver nanoparticles derived from Acacia nilotica

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Abstract

Aim: This study aims to analyze the Bio efficacy of Calcium hydroxide incorporated silver nanoparticles synthesized from *Acacia nilotica* as intracanal medicaments.

Background: The primary objective of any endodontic treatment is the removal of the infectious tissue from the root canal which also involves the removal of debris formed during the process of removal and to prevent the reinfection. This majorly involves killing if microbes, the source of infection. This study utilizes the properties of Calcium hydroxide incorporated silver nanoparticles synthesized from *Acacia nilotica* as intracanal medicament in endodontic treatment for the complete removal of microbes and prevention of recurrences.

Materials and method: 1% *Acacia nilotica* is made and kept in stirring condition for complete dissolution. Filtration was done using whatman no:1 filter paper and stored at 4°C.1 milli molar of silver nitrate was prepared and added to the filtered extract drop by drop under color change observed and the time period is noted. Centrifuge was done at 6000 rpm for 10 mins at room temperature at 4°C.Crystal violet assay was done.96 well plate was taken.100 ml of BHI medium was poured in 7 wells .1% CaOH in serial dilution was poured upto 6 wells.50 ml of enterococcus faecalis of OD600=0.1 incubated at 37 °C for 48 hours. Phosphate buffer saline (PBS) wash was done.100µL of 0.2% of crystal violet irrespective of control was added and incubated for 20 to 30 mins. Again PBS wash was done to remove excess and unbound stains.125 µL of 30% glacial acetic acid is mixed in all wells and ELISA plate reader was used to measure the observents at 570 nm.

Results: AgNps synthesized from *Acacia nilotica* incorporated $Ca(OH)_2$ has a high level of biofilm inhibition against the bacteria of about more than 50% at a concentration of 0.25% whereas the normal $Ca(OH)_2$ showed more than 50% biofilm inhibition at 1% and showed only inhibition of 40.86% at 0.25% concentration.

Conclusion: the study showed silver nanoparticles synthesized from *Acacia nilotica* incorporated $Ca(OH)_2$ had better action as intracanal medicament than Calcium hydroxide.

Key words: Acacia, Calcium Hydroxide, Enterococcus faecalis, Metal Nanoparticles, Enzyme-Linked Immunosorbent Assay

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Introduction

The principal objective of endodontic treatment is the complete elimination or substantial reduction of microorganisms from the infected root canal system to prevent or resolve apical periodontitis. Microbial invasion of the pulp and periradicular tissues predominantly by polymicrobial biofilms composed of anaerobic bacteria is the primary etiological factor in pulpal and periapical disease (Kakehashi et al., 1965; Sundqvist, 1992). Therefore, successful endodontic therapy relies on a combination of mechanical instrumentation, chemical irrigation, and intracanal medicaments to disrupt biofilms and eradicate residual bacteria within the complex anatomy of the root canal system, including lateral canals and dentinal tubules (Siqueira & Rôças, 2008; Nair, 2006). Sodium hypochlorite remains the gold-standard irrigant due to its broad antimicrobial activity and tissue-dissolving capacity, while adjunctive agents such as chlorhexidine and calcium hydroxide further enhance disinfection (Zehnder, 2006; Mohammadi & Abbott, 2009). Despite thorough chemomechanical preparation, complete sterility is difficult to achieve thus, the primary goal is to reduce microbial load to a level compatible with periapical healing (Byström & Sundqvist, 1981; Ricucci & Siqueira, 2010). Effective microbial control within the root canal system ultimately determines the long-term success and prognosis of endodontic treatment.[1, 2] Intracanal medicaments are considered a crucial step in eliminating residual microorganisms from the root canal system after mechanical instrumentation and irrigation (Siqueira & Rôças, 2008). They help reduce bacterial load in areas inaccessible to instruments, such as dentinal tubules and accessory canals (Nair, 2006). Calcium hydroxide has long been the most widely used intracanal medicament due to its antimicrobial properties and high alkalinity (Mohammadi & Dummer, 2011). However, persistent pathogens like *Enterococcus faecalis* have prompted the development of newer and more effective medicaments (Sathorn et al., 2007). Consequently, modern endodontics emphasizes the continuous improvement of intracanal medicaments to enhance root canal disinfection and treatment outcomes.[3]

An interappointment medicament used to significantly improve disinfection after chemomechanical procedures is called an intracanal medicament.[4, 5] The persistence of microorganisms within the root canal system is widely regarded as the primary cause of endodontic treatment failure (Nair, 2006). Residual bacteria can survive chemomechanical preparation and continue to induce periapical inflammation, leading to persistent or secondary apical periodontitis (Siqueira & Rôças, 2008). Therefore, long-term treatment success depends largely on effective microbial elimination and prevention of reinfection (Ricucci & Siqueira, 2010). The capacity of *E. Faecalis* to oppose bactericidal substances has been professed to be the justification for this organic entity to be the reason for persistent root canal infections.

Calcium hydroxide has a hard tissue initializing impact.[6, 7] It has exceptionally alkaline pH 12.5. Pulp tissue shows no or only a mild inflammatory response against it.[8] The alkaline pH, may aid in dissolving necrotic tissue remnants, bacteria and their byproducts.[9] It can be used for indirect and direct pulp capping, sealant and apical closure material.[10, 11] All calcium hydroxide has a restricted time span of usability as they in the end transform into calcium oxide.

The use of silver nanoparticles (AgNPs) has increased significantly in recent years due to their broad-spectrum antimicrobial properties and potential benefits for human health. Their ability to inhibit the growth of bacteria, fungi, and certain viruses has led to widespread incorporation into medical devices, wound dressings and dental materials.[12, 13] In healthcare, AgNPs are particularly valued for reducing infection rates and preventing biofilm formation, thereby improving patient outcomes. Additionally, their effectiveness at low concentrations and compatibility with various materials make them attractive for large-scale industrial applications. As concerns over antimicrobial resistance continue to rise, silver nanoparticles are increasingly viewed as an important adjunct in infection control and public health protection.[14, 15]

Acacia nilotica (commonly known as babul or gum arabic tree) is a medicinal plant widely distributed in tropical and subtropical regions and has been extensively used in traditional systems of medicine for its therapeutic properties. It is rich in bioactive phytoconstituents such as tannins, flavonoids, phenolic compounds, saponins, and alkaloids, which contribute to its well-documented antimicrobial, anti-inflammatory, antioxidant, and wound-healing activities. In dentistry, *Acacia nilotica* has gained attention due to its inhibitory effects against oral pathogens, including cariogenic and endodontic microorganisms. Its strong antibacterial potential, particularly attributed to its high tannin content, enables disruption of bacterial cell walls and interference with biofilm formation. Owing to these properties, *Acacia nilotica* is increasingly being explored as a natural alternative or adjunct in endodontic disinfection protocols and as a potential phytogenic agent for the green synthesis of nanoparticles aimed at enhancing antimicrobial efficacy. This study aims to evaluate the bioefficacy of Calcium hydroxide incorporated Silver nanoparticles as an intracanal medicament and to compare it with the bioefficacy of CaOH.

Materials and method:

Materials

Acacia nilotica was collected from Tamil nadu horticulture farm. All chemicals were of analytical grade and obtained from Merck, Mumbai, India and from Hi Media Laboratories, Mumbai, India. All aqueous solutions were prepared using ultrapure water produced by a Milli-Q water purification system.

Preparation of Plant extract

The bark of the plant was shade dried for 5-10 days, then powdered and stored for further experiment. 1% of $Ca(OH)2$ is made by mixing 1 g in 100 ml of distilled water.

1% *Acacia nilotica* was made and kept in stirring condition for complete dissolution. Filtration was done using whatman no:1 filter paper and stored at 4°C.1 milli molar of silver nitrate was prepared and added to the filtered extract drop by drop under color change observed and the time period was noted. Centrifugation was done at 6000 rpm for 10 mins at room temperature at 4°C. (Figure 1)

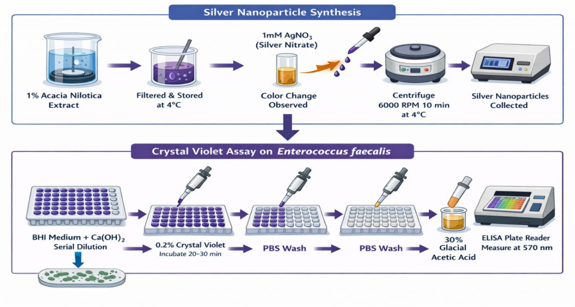

Figure 1. Schematic illustration of silver nanoparticle (AgNP) synthesis using 1% *Acacia nilotica* extract and their antibiofilm evaluation against *Enterococcus faecalis*. AgNP formation was confirmed by color change and collected by centrifugation. Antibiofilm activity was assessed using the crystal violet assay, and absorbance was measured at 570 nm.

Anti biofilm assay

The crystal violet assay was carried out to evaluate the antibiofilm activity using a sterile 96-well microtiter plate. Initially, 100 µL of Brain Heart Infusion (BHI) broth was dispensed into seven designated wells. A 1% calcium hydroxide [$Ca(OH)_2$] solution was prepared and serially diluted across six wells, while the seventh well containing BHI medium without $Ca(OH)_2$ served as the growth control. Subsequently, 50 µL of *Enterococcus faecalis* suspension adjusted to an optical density of 0.1 at 600 nm (OD_{600} = 0.1) was added to each well. The plate was then incubated at 37 °C for 48 hours under appropriate conditions to allow bacterial growth and biofilm formation.

After incubation, the planktonic (non-adherent) cells were carefully removed, and the wells were gently washed with phosphate-buffered saline (PBS) to eliminate loosely attached bacteria. Each well was then stained with 100 µL of 0.2% crystal violet solution, including the control well, and incubated for 20–30 minutes at room temperature to permit staining of the adherent biofilm.(Figure 2) Following staining, the wells were washed again with PBS to remove excess and unbound dye and allowed to air dry. To solubilize the bound crystal violet, 125 µL of 30% glacial acetic acid was added to each well. The intensity of the released stain, corresponding to the biofilm biomass, was quantified by measuring the absorbance at 570 nm using an ELISA plate reader.

Results

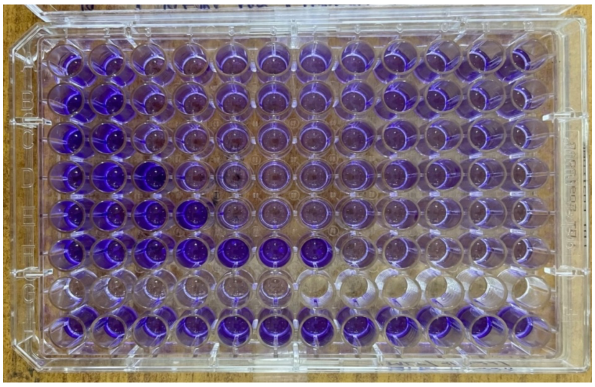

Figure 2 represents the results of serial concentration introduced into the wells of Ca(OH)2 and silver nanoparticles mediated Ca(OH)2.

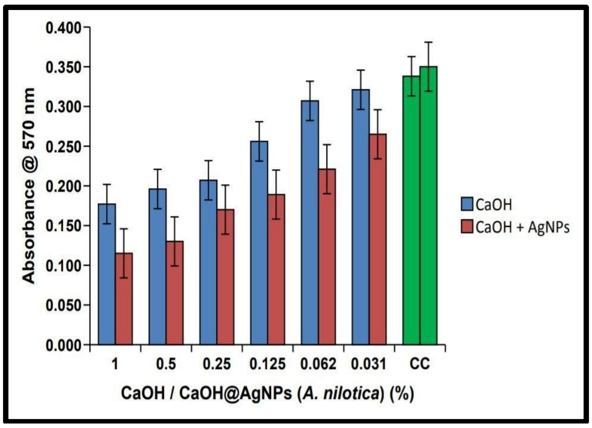

Figure 3: The graphical representation of comparative analysis on the biofilm inhibition between Ca(OH)2 and silver nanoparticles mediated Ca(OH)2.

Biofilm inhibition in %

Ca(OH)2	Ca(OH)2 + silver nanoparticles	Concentration %
49.43	67.14	1
44	62.59	0.5
40.86	51.43	0.25
26.86	46	0.125
12.29	36.86	0.0625
8.29	24.29	0.03125

The combination of calcium hydroxide mediated with silver nanoparticles showed a better result in inhibition of the biofilm. From the graphical data (Figure 3) it can be analysed that AgNPs incorporated Ca(OH) has a high level of biofilm inhibition against the bacteria of about more than 50% at a concentration of 0.25% whereas the normal Ca(OH)2 showed more than 50% biofilm inhibition at 1% and showed only inhibition of 40.86% at 0.25% concentration. Thus these values and representation has shown that naturally mediated silver nanoparticles incorporated Ca(OH)2 has a better result over Ca(OH)2. This proves it to be a better alternative by reducing the concentration and adverse effects but providing better results.

Discussion

Persistent endodontic infections are primarily associated with the survival of resistant microorganisms within the complex anatomy of the root canal system. Among these, *Enterococcus faecalis* is frequently implicated in post-treatment apical periodontitis due to its ability to penetrate dentinal tubules, form resilient biofilms, and tolerate harsh environmental conditions, including high alkalinity. Calcium hydroxide ($Ca(OH)_2$) has traditionally been the intracanal medicament of choice because of its strong alkaline pH, antimicrobial properties, and ability to induce hard tissue formation.[6] However, its limited effectiveness against established *E. faecalis* biofilms and reduced antibacterial activity over time has led researchers to explore adjunctive strategies to enhance its therapeutic efficacy.

Nanotechnology has emerged as a promising approach in endodontics, particularly through the use of silver nanoparticles (AgNPs), which possess broad-spectrum antimicrobial activity, high surface area to volume ratio, and the ability to disrupt bacterial cell walls and biofilms.[15] Green synthesis of silver nanoparticles using medicinal plant extracts such as *Andrographis paniculata* and *Ocimum sanctum* has gained attention due to its eco-friendly nature, cost-effectiveness, and improved biocompatibility. Incorporating silver nanoparticles (AgNPs) with calcium hydroxide [$Ca(OH)_2$] may offer a synergistic antimicrobial effect, improving penetration into dentinal tubules and enhancing biofilm disruption. In this context, this study investigates the combined use of $Ca(OH)_2$ and silver nanoparticles to evaluate their cytotoxicity, antimicrobial potential, and effectiveness against *E. faecalis* in both short- and long-term applications.

Nasim et al in his study have compared the cytotoxic activity and anti microbial activity of $Ca(OH)_2$ incorporated silver nanoparticles.[18] His study involves the green synthesis of silver nanoparticles using *Andrographis paniculata* and *Ocimum sanctum*. His study revealed that $Ca(OH)_2$ in corporated silver nanoparticles showed high antimicrobial and cytotoxic activity than $Ca(OH)_2$ individually. Similarly Rauf et al in his study has compared the efficacy of $Ca(OH)_2$ with or without a silver nanoparticle suspension to eliminate *Enterococcus faecalis* from root canals.[19] His study revealed that though there was no significant difference in the antimicrobial activity of $Ca(OH)_2$ in combined and individual form initially there was a significant increase in the antimicrobial activity of CaOH mediated nanoparticles in a period of 7 days. Supporting these findings Farzaneh et al in his investigations the antibacterial characteristic and *Enterococcus faecalis* (*E. faecalis*) biofilm suppression effect of of calcium hydroxide as intracanal medicaments in short and long-term using silver nanoparticles as vehicle. Cultures were made from each group after one week and one month, and the number of colonies was counted. Moreover, a sample of each group was examined under electron microscope. After one week, the mixture of calcium hydroxide and AgNPs was the most effective medicament against *E. faecalis* bacteria .Thus this study explains the potential of AgNPs to be used as an appropriate vehicle of calcium hydroxide in order to eliminate of *E. faecalis* biofilm from human dentine in short-term.

Therefore, the incorporation of silver nanoparticles into calcium hydroxide formulations represents a promising advancement in intracanal disinfection strategies. The synergistic interaction between the sustained alkaline environment provided by $Ca(OH)_2$ and the potent antimicrobial properties of AgNPs enhances bacterial eradication, particularly against resistant species such as *Enterococcus faecalis*.[20] Evidence from the discussed studies indicates improved antimicrobial performance over time, enhanced biofilm disruption, and favorable cytotoxic profiles when compared to calcium hydroxide used alone.[21] These findings support the growing interest in nanoparticle-assisted endodontic medicaments as a means of overcoming the limitations of conventional therapy.

Acacia nilotica has been extensively investigated for its antimicrobial and anti-inflammatory properties, largely attributed to its high concentration of condensed tannins, gallic acid, catechins, and other phenolic compounds. These phytoconstituents exert antibacterial effects by disrupting bacterial cell membranes, inactivating microbial enzymes, chelating metal ions essential for bacterial metabolism, and interfering with biofilm formation. In the context of oral and endodontic pathogens, several studies have highlighted its potential. For instance, Pai et al. (2010) reported that *Acacia nilotica* extract exhibited significant inhibitory activity against *Streptococcus mutans* and other oral bacteria, with results comparable to chlorhexidine in vitro.[22] Similarly, Bansal et al. (2011) demonstrated the antimicrobial efficacy of *Acacia nilotica* against a range of gram-positive and gram-negative bacteria, supporting its use as a natural antimicrobial agent in dental applications.[23] These findings substantiate the relevance of incorporating *Acacia nilotica* into intracanal medicament formulations.

Furthermore, the role of *Acacia nilotica* in nanoparticle synthesis enhances its clinical significance. Plant-mediated (green) synthesis of silver nanoparticles leverages phytochemicals as reducing and stabilizing agents, resulting in nanoparticles with improved biocompatibility and sustained antimicrobial action. Ibrahim (2015) reported that silver nanoparticles synthesized using plant extracts demonstrated superior antibacterial activity compared to plant extracts alone, indicating a synergistic effect.[24] In addition, Prabhu and Poulose (2012) reviewed the antimicrobial mechanisms of plant-mediated silver nanoparticles and emphasized their enhanced activity against resistant strains due to increased surface reactivity and ability to penetrate biofilms.[25] These comparative studies support the present rationale that *Acacia nilotica* mediated silver nanoparticles, when combined with calcium hydroxide, may offer improved antimicrobial efficacy against resistant organisms such as *Enterococcus faecalis*, thereby strengthening intracanal disinfection strategies.

The findings of the present study highlight the potential of green-synthesized *Acacia nilotica* silver nanoparticles in enhancing the antimicrobial efficacy of calcium hydroxide against resistant endodontic pathogens. The improved antibacterial activity observed with the combined formulation can be attributed to the synergistic interaction between the sustained high alkalinity of calcium hydroxide and the broad-spectrum antimicrobial action of silver nanoparticles. Silver nanoparticles are known to disrupt bacterial cell membranes, generate reactive oxygen species, interfere with DNA replication, and inhibit essential enzymatic pathways, while calcium hydroxide creates an unfavorable alkaline environment that denatures bacterial proteins and damages cytoplasmic membranes. Moreover, the phytochemical constituents of *Acacia nilotica*, including tannins and flavonoids, may act as both reducing agents during nanoparticle synthesis and as additional antimicrobial contributors, thereby potentiating the overall bactericidal effect. This multi-targeted mechanism is particularly advantageous against *Enterococcus faecalis*, which is capable of surviving harsh conditions and penetrating deeply into dentinal tubules.

Another important consideration is the role of green synthesis in improving the biocompatibility and sustainability of nanoparticle-based intracanal medicaments. Conventional chemical synthesis methods may involve hazardous reagents that can compromise biological safety, whereas plant-mediated synthesis offers a safer, eco-friendly alternative with inherent stabilizing properties. The nanoscale size of the particles enhances surface area and facilitates deeper penetration into complex root canal anatomy and biofilm matrices, potentially improving disinfection outcomes. Furthermore, the incorporation of *Acacia nilotica*-derived silver nanoparticles as a vehicle for calcium hydroxide may promote sustained antimicrobial release and improved contact with infected dentin surfaces. While these findings are promising, careful evaluation of optimal concentrations, long-term cytotoxicity, and clinical performance is essential before routine clinical implementation. Nonetheless, the present results support the growing paradigm shift toward phytogenic nanotechnology as an innovative and biologically favorable approach in contemporary endodontic therapy.

Conclusion

In conclusion, the integration of green-synthesized *Acacia nilotica* silver nanoparticles with calcium hydroxide represents a novel and promising approach in intracanal disinfection. As the demand for more potent and biologically compatible antimicrobial strategies increases, particularly against resistant pathogens such as *Enterococcus faecalis*, the development of phytogenic nanoparticle-based medicaments offers significant potential. The bioactive compounds present in *Acacia nilotica* not only facilitate eco-friendly synthesis of silver nanoparticles but may also contribute additional antimicrobial and antioxidant properties, thereby enhancing the overall therapeutic efficacy of the formulation.

The synergistic combination of calcium hydroxide [$Ca(OH)_2$] and *Acacia nilotica*-mediated silver nanoparticles may improve antibacterial action beyond that achieved by calcium hydroxide alone, promoting more effective elimination of *E. faecalis* and improved disruption of biofilms within the root canal system. Furthermore, green synthesis methods support better biocompatibility and reduced toxicity, aligning with the growing emphasis on sustainable and safe dental materials. Such advancements could play a crucial role in strengthening intracanal medicament protocols and improving the long-term success of endodontic therapy.

Limitations

Despite the promising findings, this study has certain limitations that must be considered while interpreting the results. First, the investigation was conducted under in vitro conditions, which may not accurately replicate the complex biological environment of the human root canal system. Factors such as host immune response, tissue buffering capacity, presence of organic matter, and variations in dentinal tubule anatomy can influence the antimicrobial efficacy of intracanal medicaments in clinical settings. Therefore, the results may not be directly extrapolated to in vivo conditions.

Second, the study primarily focused on *Enterococcus faecalis*, although polymicrobial infections are commonly associated with persistent endodontic lesions. Evaluating the formulation against a broader spectrum of endodontic pathogens would provide a more comprehensive understanding of its antimicrobial potential. Additionally, long-term cytotoxicity, optimal concentration, stability of green-synthesized *Acacia nilotica* silver nanoparticles, and potential effects on periapical tissues were not extensively assessed. Further in vivo studies and clinical trials with larger sample sizes are necessary to validate the safety, efficacy, and long-term outcomes of this nanoparticle enhanced calcium hydroxide formulation.

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