

Exploring the anti biofilm effect of patchouli oil coated with silver nanoparticles for improved anti bacterial effect

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Abstract:

Introduction: Biofilm forming from a variety of microbial pathogens can pose a serious health hazard that is difficult to combat. Nanotechnology, however, represents a new approach to fighting and eradicating biofilm forming microorganisms. Particularly, silver nanoparticles (AgNPs) have demonstrated exceptional bactericidal properties in a wide range of microorganisms, including some oral bacteria.

Aim: In the present study, the sustainable synthesis and characterization of biocompatible silver nanoparticles (AgNPs) from seed extracts of patchouli oil was explored.

Materials and method: Silver nanoparticles (AgNPs) doped with patchouli oil (*Pogostemon cablin*) were synthesized using a chemical reduction method. Briefly, an aqueous silver nitrate (AgNO₃) solution was used as the silver precursor, and patchouli oil was incorporated during the synthesis process to facilitate doping and enhance antimicrobial activity. The mixture was stirred under controlled temperature conditions until a visible color change indicated the formation of silver nanoparticles. The synthesized nanoparticles were then purified by centrifugation and washed with distilled water to remove unreacted components. The antibiofilm activity of the synthesized patchouli oil doped AgNPs was evaluated using standard biofilm inhibition assays against selected microbial strains. Biochemical analyses were conducted to assess microbial growth inhibition and biofilm formation. Additionally, the morphology and elemental composition of the synthesized nanoparticles were characterized using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Spectroscopy (EDX).

Conclusion: The present study demonstrates the potential of using plant-derived AgNPs to inhibit biofilm formation for therapeutic treatments that represent a new method of effectively treating a variety of infectious diseases caused by pathogenic microbes.

Keywords: Anti biofilm effect, Anti caries effect, Nanoparticles, Tooth decay

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Introduction:

Dental caries is an infectious oral disease originated by the presence of different microorganisms from well defined biofilms. Biofilms are complex communities of microorganisms that adhere to surfaces and are protected by a matrix of extracellular polymeric substances.¹ Biofilm forming from a variety of microbial pathogens can pose a serious health hazard that is difficult to combat. Some substances, like certain enzymes or antimicrobial agents, can exhibit anti-biofilm effects by disrupting or preventing biofilm formation.² The functional group modifications and decreased penetration of antimicrobials by biofilm components enhances the antibiotic resistance.

Nanotechnology offers promising approaches to combat biofilm formation. Nanomaterials, such as nanoparticles and nanocomposites, can be engineered to have antimicrobial properties, disrupting biofilm

formation and enhancing the effectiveness of antimicrobial agents.³ Additionally, nanoscale materials can be designed to penetrate the biofilm matrix, improving their ability to target and kill microorganisms within the biofilm. The unique properties of nanomaterials make them potential candidates for developing innovative strategies to prevent and treat biofilm-related issues in diverse applications.⁴

Silver nanoparticles have shown promise in the realm of dentistry, particularly in preventing and treating dental caries. The antimicrobial properties of silver nanoparticles can help inhibit the growth of bacteria.⁵ Silver nanoparticles exhibit potent antibacterial effects against a wide range of bacteria, including those responsible for dental caries. The small size of nanoparticles allows them to penetrate dental biofilms more effectively, reaching bacteria in hard to reach areas and disrupting their growth.⁶ Compared to

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traditional antimicrobial agents, there is evidence suggesting that bacteria may develop resistance less readily to silver nanoparticles, making them potentially more sustainable over time. When used in controlled amounts, silver nanoparticles have shown to be biocompatible with human cells, minimizing potential adverse effects.⁷

Patchouli oil, obtained from the leaves of *Pogostemon cablin*, contains a variety of bioactive compounds that contribute to its distinctive aroma and potential therapeutic properties. The major constituents of patchouli oil include patchouli alcohol, pogostone, α -bulnesene, and seychellene, which are known to exhibit antimicrobial, anti-inflammatory, and antioxidant activities.⁸ These compounds are responsible for the characteristic earthy fragrance of the oil and are widely utilized in perfumery, aromatherapy, and cosmetic formulations. In addition, patchouli oil has been incorporated into skincare products due to its reported ability to promote wound healing, reduce inflammation, and inhibit microbial growth on the skin.⁹ Several studies have demonstrated that essential oils containing patchouli alcohol can disrupt microbial cell membranes and inhibit the growth of certain Gram positive bacteria and fungi, which explains its growing interest in natural health and pharmaceutical applications.^{7,8}

Patchouli oil has attracted attention for its antimicrobial potential against microorganisms found in the oral cavity. Some in vitro studies have shown that extracts of *Pogostemon cablin* exhibit inhibitory activity against oral pathogens such as *Candida albicans* and *Enterococcus faecalis*, organisms commonly associated with oral infections and biofilm formation.⁹ Other research has explored the incorporation of patchouli oil into dental materials and nanoemulsion formulations to enhance antibacterial effects against periodontal pathogens.¹⁰ Despite these promising findings, current evidence remains limited and largely restricted to laboratory studies rather than clinical trials. Therefore, while patchouli oil shows antimicrobial properties that could theoretically contribute to the control of oral microorganisms, there is still insufficient scientific evidence to confirm its effectiveness specifically as an anti-caries agent. Further well designed experimental and clinical studies are necessary to evaluate its safety, optimal concentration, and potential role in preventive dentistry.^{11,12} The aim of this study was to synthesize silver nanoparticles using patchouli oil for exploring antibiofilm effect.

Materials and method:

Synthesis of silver nanoparticles (AgNps)

Silver nanoparticles (AgNPs) were synthesized using a chemical reduction method with gallic acid acting as the reducing and stabilizing agent. Briefly, 100 mL of 1×10^{-3} M silver nitrate (AgNO_3) solution was prepared and transferred into a 250 mL Erlenmeyer flask. The solution was placed on a magnetic stirrer and continuously stirred to ensure homogeneity. Subsequently, 10 mL of deionized Milli-Q water containing 0.01 g of gallic acid was prepared separately and added slowly to the AgNO_3 solution under constant stirring conditions. Gallic acid served as a natural reducing agent, facilitating the reduction of Ag^+ ions to metallic silver nanoparticles.

Following the addition of gallic acid, the reaction mixture was maintained under continuous stirring, and the pH of the solution was gradually adjusted to 11 by the dropwise addition of 1 M sodium hydroxide (NaOH). The alkaline environment promotes the reduction process and facilitates the nucleation and growth of silver nanoparticles. The formation of AgNPs was visually confirmed by a noticeable color change in the reaction mixture from colorless to yellowish-brown, indicating the reduction of silver ions and the formation of colloidal nanoparticles. (Figure 1) The reaction mixture was further stirred for a specific period to ensure complete reduction and stabilization of the nanoparticles. The synthesized AgNPs were then collected by centrifugation, washed with deionized water to remove unreacted components, and stored under appropriate conditions for further characterization and experimental analysis.

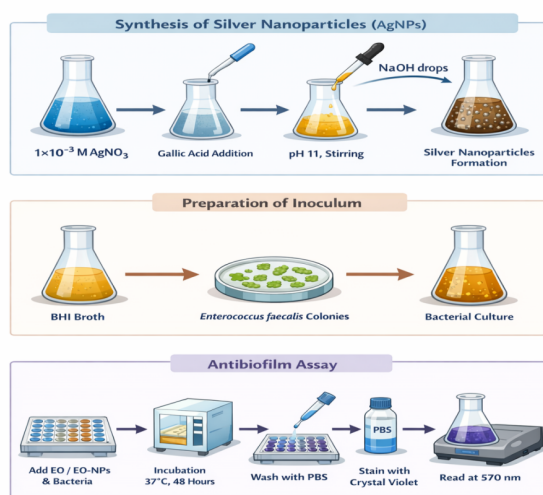


Figure 1: Schematic representation of the synthesis of patchouli oil-doped silver nanoparticles and evaluation of their antibiofilm activity against *Enterococcus faecalis*.

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Scanning Electron Microscopy (SEM)

The surface morphology and particle size of the synthesized patchouli oil-doped silver nanoparticles were examined using Scanning Electron Microscopy (SEM). The nanoparticle suspension was first centrifuged and washed with distilled water to remove impurities. A small amount of the purified sample was then drop-cast onto a clean conductive substrate and allowed to dry at room temperature. The dried samples were sputter-coated with a thin layer of gold to improve electrical conductivity and image quality. SEM analysis was performed under high vacuum conditions at an appropriate accelerating voltage to observe the morphology, shape, and distribution of the synthesized nanoparticles.

Energy Dispersive X-ray Analysis (EDX/EDAX)

The elemental composition of the synthesized nanoparticles was determined using Energy Dispersive X-ray Analysis (EDX) coupled with the SEM instrument. During SEM imaging, EDX spectra were collected from selected regions of the sample to identify the presence of constituent elements. The analysis was performed under standard operating conditions, and the characteristic X-ray peaks were recorded to confirm the presence of silver and other associated elements. The obtained spectra were used to verify the elemental composition and purity of the synthesized patchouli oil doped silver nanoparticles.

Biofilm assay

One hundred millilitre of Brain Heart Infusion (BHI) broth prepared in 250 mL

Erlenmeyer flask was inoculated with 4-5 Individual colonies of *Enterococcus faecalis*.

The samples (EO and EO-NPs) after diluting to desired concentrations (0.1 ml to 0.003 ml) were added to the MHI broth in the wells. Then the samples were inoculated with 50 μ L of the broth culture and incubated for 48 hours at 37°C. After the incubation, the broth was aspirated from the wells using a sterile pipette and washed with PBS solution. Then, 150 μ L of crystal violet (0.2%) was added to each well and allowed to stand for 15 - 20 minutes. This is followed by removal of the dye and washed with PBS to remove unbound and excess dye. Then the dye was dissolved by adding 150 μ L of glacial acetic acid (30%) in each well. Readings were taken using an ELISA Plate Reader at 570 nm and the absorbance value recorded.

Results

SEM and EDX

The SEM micrograph shows the surface morphology of patchouli oil coated silver colloidal nanoparticles.

The Figure 2(a) reveals the presence of irregular and quasi-spherical nanoparticles distributed across the surface with noticeable agglomeration. The particles appear clustered and attached to an underlying matrix, which is typical for nanoparticles stabilized with organic compounds. The aggregation observed may result from the interaction between silver nanoparticles and the organic constituents of patchouli oil, such as terpenoids and alcohol groups, which act as reducing and capping agents during nanoparticle formation. Based on the scale bar (1 μ m), the particles are within the nanometer range and exhibit non-uniform distribution, indicating successful formation of silver nanoparticles coated with patchouli oil components.

The EDS spectrum Figure (b) further confirms the elemental composition of the synthesized material. A strong characteristic peak observed at approximately 3 keV corresponds to the Ag L α emission line, confirming the presence of silver nanoparticles. The quantitative analysis indicates that silver (Ag) is the dominant element (\approx 57.1 wt%), demonstrating that the particles observed in the SEM image are primarily composed of silver. In addition to silver, oxygen (\approx 33.3 wt%) and carbon (\approx 9.6 wt%) are also detected. The presence of carbon and oxygen is attributed to the organic molecules present in patchouli oil, which serve as capping or stabilizing agents on the nanoparticle surface. Oxygen may also arise from functional groups in the oil or from slight surface oxidation of silver nanoparticles. Overall, the combined SEM and EDS results confirm the successful synthesis of patchouli oil-coated silver colloidal nanoparticles, where the essential oil components act as stabilizing agents while silver forms the core nanoparticle structure.

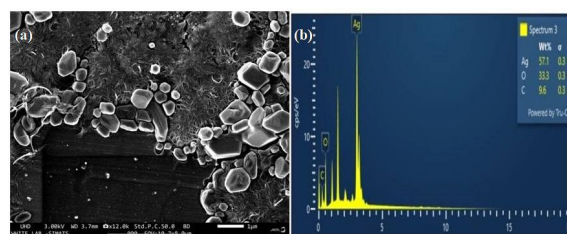


Figure 2: (a) This figure shows the The mean size of AgNPs which is coated with the patchouli oil particles, it shows the synthesized silver nano particles size range from 7 to 8 nm. (b) Energy-dispersive X-ray analysis (EDAX) is a technique used for the measurement of nanoparticles by SEM

Biofilm analysis

The results indicate the effect of patchouli oil and patchouli oil-coated silver nanoparticles on biofilm

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formation. Patchouli oil was used as the control, while silver nanoparticles coated with patchouli oil were used as the test sample. (Figure 3) The observations revealed that biofilm formation was significantly reduced in the presence of patchouli oil-coated silver nanoparticles compared to patchouli oil alone. Although patchouli oil possesses natural antimicrobial properties due to the presence of bioactive compounds such as terpenoids and patchoulol, the formation of biofilm was still noticeable when patchouli oil was used by itself.

However, when silver nanoparticles were incorporated and coated with patchouli oil, a stronger antibiofilm effect was observed. The reduced biofilm formation may be attributed to the synergistic antimicrobial activity between silver nanoparticles and the bioactive compounds of patchouli oil. Silver nanoparticles are known to disrupt microbial cell membranes, generate reactive oxygen species, and interfere with cellular metabolic processes, thereby inhibiting bacterial adhesion and biofilm development. In addition, the patchouli oil coating may enhance the stability and dispersion of the nanoparticles, allowing better interaction with microbial cells and biofilm matrices.

Therefore, the findings suggest that patchouli oil-coated silver nanoparticles exhibit enhanced antibiofilm activity compared to patchouli oil alone, indicating their potential application as an effective antimicrobial and antibiofilm agent in biomedical, pharmaceutical, or surface-coating applications. (Figure 4)

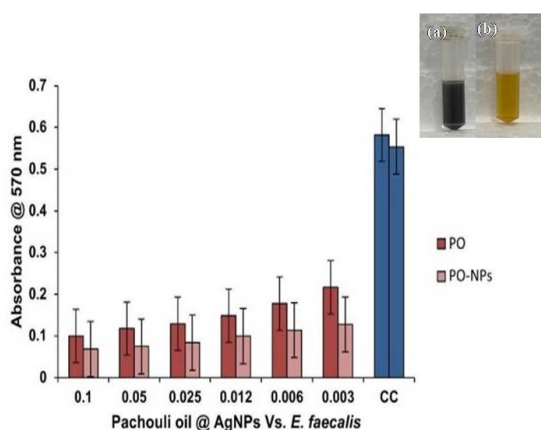


Figure 3: The graph indicates the concentration of inhibition of *E. faecalis* with patchouli oil and doped silver nano particles

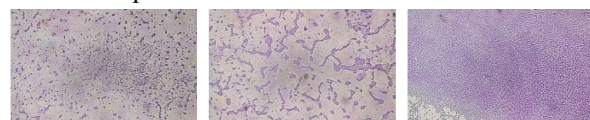


Figure 4: 40x magnification of *E. faecalis* biofilm before treating with patchouli oil and after treating with silver incorporated patchouli oil.

Discussion:

Enterococcus faecalis is a Gram-positive facultative anaerobic bacterium frequently associated with persistent endodontic infections and secondary dental caries due to its remarkable ability to survive harsh environmental conditions and form stable biofilms on dental surfaces.^{13,14} The biofilm-forming capacity of *E. faecalis* allows the bacteria to adhere to dentinal tubules and protect themselves from antimicrobial agents and host defense mechanisms, thereby contributing to treatment failure and recurrent infections. Therefore, strategies aimed at inhibiting *E. faecalis* biofilm formation are crucial for improving oral health outcomes.¹⁵ In the present study, the antibiofilm potential of patchouli oil-coated silver nanoparticles was evaluated against *E. faecalis*. The results demonstrated that biofilm formation was markedly reduced in the presence of patchouli oil-coated silver nanoparticles compared to patchouli oil alone.¹⁶ This enhanced inhibition may be attributed to the synergistic antimicrobial action of silver nanoparticles and the bioactive constituents of patchouli oil, which together may disrupt bacterial cell membranes, interfere with metabolic pathways, and inhibit bacterial adhesion, thereby preventing the establishment and maturation of *E. faecalis* biofilms.¹⁷ These findings suggest that patchouli oil-coated silver nanoparticles could serve as a promising antibiofilm agent for controlling *E. faecalis*-associated oral infections and improving anti-caries strategies.

Present study reported patchouli oil as an effective antimicrobial agent. The biosynthesized AgNPs were found to be stable for a long period (>90 days) of time. The long-term stability of the AgNPs solution may be due to the presence of small peptides and other proteins in the patchouli oil that are working as capping agents for AgNPs. Further, we observed the function of time for the synthesis of AgNPs and the results advocate that the intensity of this peak increases with an increase in the reaction time.¹⁷ As, the intensity of the surface plasmon peak is directly proportional to the density of the NPs synthesis in solution. The shape and structure of the nanoparticles have varying dimensions such as spherical, quasi-spherical, triangular and pentagonal as revealed by SEM. The reasons for this heterogeneous particle formation phenomenon may be due to fast utilization of capping molecules and particles formed later were

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left with less capping molecules and hence facing a condition of thermodynamic instability.

Although the cariostatic effect on dental tissues has not been clarified yet, the effectiveness in arresting cavities can be explained by the synergism of the components of their formulation (nanoparticles of silver, chitosan, and fluoride). Furthermore, silver ions can inhibit protein synthesis causing structural changes and cell death. Besides, the reduction in the size of silver nanoparticles implies an increase in the contact surface, which is an important condition for the antimicrobial effects of silver and which could prevent black stains on teeth.

In the review of Saviuc et al, it was shown that EOs are potent antibiofilm agents, acting by inhibition of the intercellular communication systems and by inducing changes in the substrate (referring to changing of redox potential, resistivity or pH). EOs could also kill the biofilm embedded cells by the alteration of the cytoplasmic membrane due to their hydrophobic constituents. The research performed by Selim and Burt groups revealed that the absence of the outer membrane in Gram-positive bacteria favors the direct interaction of the EOs with the cellular membrane, either affecting its permeability and causing the leakage of intracellular content or inactivating the bacterial enzymes.

EOs could also increase the oxidative stress in microbial cells, causing damages of intracellular macromolecules, leading to cellular apoptosis. Das et al. found that Chamomile EO induced the accumulation of reactive oxygen species (superoxide and peroxide) that could be responsible for the antimicrobial activity of this EO.

Many studies were devoted to finding new irrigants or interappointment to remove the microbial biofilms formed in the mouth, which prevent endodontic treatments. Therefore, there is a need for chemical substances as medications that have both antibacterial and antibiofilm activities.¹⁸ *E. faecalis* is commonly recovered from teeth with persistent endodontic infections, creating biofilms attached to the canal walls or located in isthmuses and ramifications from where are difficult to eliminate by current substances, such as sodium hypochlorite and chlorhexidine.¹⁹ Microbial biofilms and smear layer must be eradicated during endodontic treatment. Because the substances used as chemical irrigants are not bio-friendly with the dental and periradicular tissues, different natural substances have been studied as disinfectants of root canals.²⁰

Conclusion:

From the above study it can be concluded that the effect of AgNPs directly on the formation of biofilms was estimated by tube method by culturing patchouli oil in the presence of different concentrations of AgNPs. The results were indicated by the development of a thin layer of biofilms after staining with dye. In the case of patchouli oil it was observed that no visible biofilm formation was seen in culture tubes having AgNPs at a concentration of 100 g/ ml.

The use of the plant extracts has an advantage over chemical or physical synthesis of AgNPs due to their own antibacterial properties, their high level of efficacy, and their low toxicity. The present study demonstrates the potential of using plant-derived AgNPs to inhibit biofilm formation for therapeutic treatments that represent a new method of effectively treating a variety of infectious diseases caused by pathogenic microbes.

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