Bioactivity of HAp/Ag Mediated Composites Against Oral Pathogenic Microbes

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**Abstract:** Silver nanoparticles (Ag-NPs) are widely studied for their strong antibacterial activity, even at low concentrations. The synthesis of silver-doped hydroxyapatite (HAp/Ag) has garnered interest due to its potential biomedical applications, as hydroxyapatite, with the chemical formula Ca10(PO4)6(OH)2, is a major component of bone and dental enamel. HAp/Ag composites were prepared via simple adsorption, mixing hydroxyapatite powders with a silver nanoparticle solution under constant stirring at room temperature for 72 hours. The mixture was vacuum-filtered, dried at 100°C for 24 hours, and then at 200°C for 3 hours. The resulting HAp/Ag composite exhibited antibacterial and antifungal activity against *Staphylococcus aureus*, *Candida albicans*, and *Escherichia coli*. Maximum inhibition was observed at 100 µg/mL for all species. Morphological analysis revealed needle-like structures across the surface. In conclusion, HAp/Ag demonstrates promising antimicrobial properties, with significant potential for biomedical applications.

**Key words:** Silver nanoparticles (Ag-NPs), Hydroxyapatite (HAp), Antimicrobial activity, Composite synthesis, Zone of inhibition

# Introduction

Silver nanoparticles (Ag-NPs) have strong antibacterial properties, even at low concentrations, they have been the subject of extensive research. [(Harsha & Subramanian, 2022)](https://paperpile.com/c/i1NpAc/37jSd)[(Deepika et al., 2022)](https://paperpile.com/c/i1NpAc/yYopV)[(Solanki et al., 2022)](https://paperpile.com/c/i1NpAc/weF4E). Given its potential for use in biomedicine, the synthesis of silver-doped hydroxyapatite with antimicrobial activity—that is, antibacterial, antiviral, or antifungal activity—is highly desirable. Silver nanoparticles' (AgNPs') biological impact has been thoroughly investigated, and various production techniques have been tried. [(Aravind, 2021; Pham et al., 2020)](https://paperpile.com/c/i1NpAc/a9Rd+mKGR).Hydroxyapatite (HAp) corresponds to the basic chemical formula Ca10(PO4)6(OH)2 or Ca5(PO4)3(OH) with hexagonal crystallites as their structure [(Chidambaram et al., 2022)](https://paperpile.com/c/i1NpAc/Hs1ub).[(Ajay, Sasikala, et al., 2022)](https://paperpile.com/c/i1NpAc/MTDz2). Bone and dentin are particularly rich in this structure, and dental enamel also contains the single-crystal structure. [(Mehmood et al., 2016; Solati & Dorranian, 2015)](https://paperpile.com/c/i1NpAc/ti0T+7XMK). Numerous ceramic materials with uses in biomedicine were created. Because of its remarkable biocompatibility and bioactivity, hydroxyapatite (HAP) is one of them that is frequently utilised in dentistry and orthopaedics[(Ajay, Suma, et al., 2022)](https://paperpile.com/c/i1NpAc/OtPeL)[(Katyal et al., 2021)](https://paperpile.com/c/i1NpAc/OyR92). Because of its promising properties in terms of bioactivity and biological compatibility with cells and tissues, HAp has been used and studied in a variety of fields, most notably biomedical engineering, for a range of applications such as drug delivery, gene transfer, and biological analysis [(Solati & Dorranian, 2015)](https://paperpile.com/c/i1NpAc/7XMK). For instance, HAp has the ability to enhance the osteoblast adhesion to enhance the reformation process of bone even against the rejection of the body [(Duraisamy et al., 2021)](https://paperpile.com/c/i1NpAc/AqIL). Silver ions embedded in the HAP structure or silver nanoparticles adsorbed on the HAP surface can be found in nano HAP-silver composites [(Jabin et al., 2021)](https://paperpile.com/c/i1NpAc/b9Tz6)[(Balaji Ganesh S & Sugumar, 2021)](https://paperpile.com/c/i1NpAc/mVQsn) [(Govindaraj & Dinesh, 2021)](https://paperpile.com/c/i1NpAc/VJP2r). Because surface adsorbed AgNPs interact with bacterial cells, they have been shown to have superior antibacterial activity. Both elemental silver and its salts have long been known to have antimicrobial properties; they have been used to treat wounds and exhibit bactericidal and inhibitory effects against a variety of microorganisms, including viruses, fungi, and bacteria. [(Tiwari & Jain, 2023)](https://paperpile.com/c/i1NpAc/7kQ4j)[(Graf et al., 2023)](https://paperpile.com/c/i1NpAc/NRji5). Ag+ ions are highly toxic to microorganisms, but they are comparatively safe for animal cells [(Biju et al., 2024)](https://paperpile.com/c/i1NpAc/UtqN). The presence of acids produced by the bacterial metabolism that takes place on dental surfaces causes dental cavities, a demineralisation process that weakens and/or kills the tissues of the teeth [(Neha et al., 2021)](https://paperpile.com/c/i1NpAc/b6GAq)[(Maliael et al., 2021)](https://paperpile.com/c/i1NpAc/piEpy)[(Lakshmi, 2021)](https://paperpile.com/c/i1NpAc/wKq9f). The primary microorganisms linked to the pathophysiology of dental caries are Streptococcus mutants, Lactobacillus species, and Actinomyces species. Of these, Streptococcus mutans is the most significant because it can produce a variety of acids (lactic, propionic, acetic, and formic acids) from the metabolism of carbohydrates that, when present in the medium, dissociate and release hydrogen ions [(Ajay, Rakshagan, et al., 2022)](https://paperpile.com/c/i1NpAc/mNELF). By dissolving their minerals, these chemical species can cause demineralisation, which releases calcium and phosphorus from the enamel [(“Green Synthesis of Silver Nanoparticles Using Eucalyptus Leaf Extract,” 2015)](https://paperpile.com/c/i1NpAc/NWQv). Numerous microorganisms and potential infections in the respiratory and gastrointestinal systems can enter through the oral cavity, so it's important to take other microbes into account [(Sabarathinam & Madhulaxmi, 2021)](https://paperpile.com/c/i1NpAc/SSYNN)[(Sushanthi et al., 2021)](https://paperpile.com/c/i1NpAc/oInGK)[(Harsha et al., 2022)](https://paperpile.com/c/i1NpAc/UcvX7). One of the most prevalent species in the environment and a symbiotic partner is the Gram-negative bacterium Escherichia coli, which is a member of the intestinal microbiota [(Mittal et al., 2013)](https://paperpile.com/c/i1NpAc/ohHl). Nonetheless, certain pathogenic strains can result in infection. For instance, enteropathogenic E. coli is one of the most frequent causes of diarrhoea in children because it causes the intestinal microvilli to degenerate [(Dharman et al., 2021)](https://paperpile.com/c/i1NpAc/JAp2H). Staphylococcus aureus is a Gram-positive bacterium that causes a wide variety of infections, mainly infections in the bloodstream and food poisoning, its main impact is due to strains resistant to methicillin (MRSA). The aim of the present study is to study the bioactivity of HAp/Ag mediated composites against oral pathogenic microbes(Rafi et al., 2024).

# Materials and Methods

## Synthesis of HAp/Ag

The hydroxyapatite powders and silver nanoparticle solution were combined under continuous stirring at room temperature for 72 hours to create the composite of HAp-AgNPs. The mixture was then filtered under vacuum and dried at 100°C for 24 hours and then at 200°C for 3 hours [(Silva-Holguín & Reyes-López, 2020)](https://paperpile.com/c/i1NpAc/VhfF)

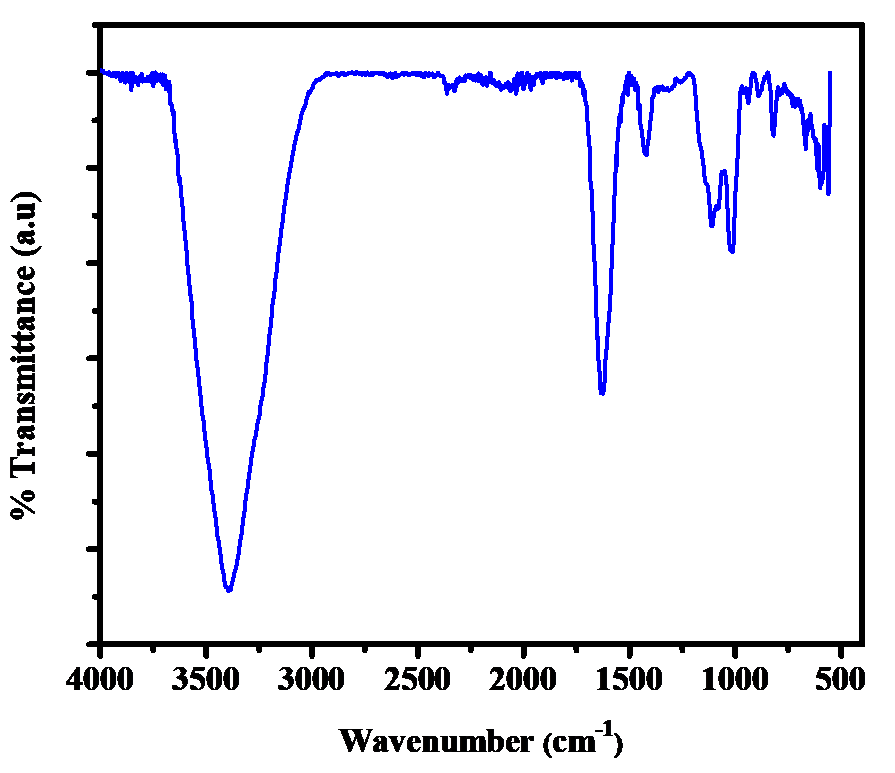
## Characterization

Using a Bruker-Germany powder diffractometer fitted with a CuKα radiation source (λ = 1.541 Å), the samples' X-ray diffraction (XRD) patterns were obtained at a scanning rate of 5°/min and a step time of 1 s, covering the range of 2θ = 10–70°. Scanning electron microscopy (SEM, Hitachi S-4800, Japan) with an energy-dispersive X-ray spectrometer (EDX) and transmission electron microscopy (TEM, Leica IEO 906E) running at 120 kV were used to examine the morphology and structure of the synthesised samples. Utilising KBr pellets, Fourier transform infrared spectra (FT-IR, Prestige-21, Shimadzu) were analysed within the wavenumber range of 4000–400 cm−1. A UV-2450 double-beam spectrophotometer (Shimadzu, Tokyo, Japan) operating in the 200–800 nm range was used for UV-vis spectroscopic investigations. The Ag-NPs' size distribution and average size were determined using a dynamic light scattering (DLS, Malvern Instruments Ltd., UK) Nano Zetasizer.

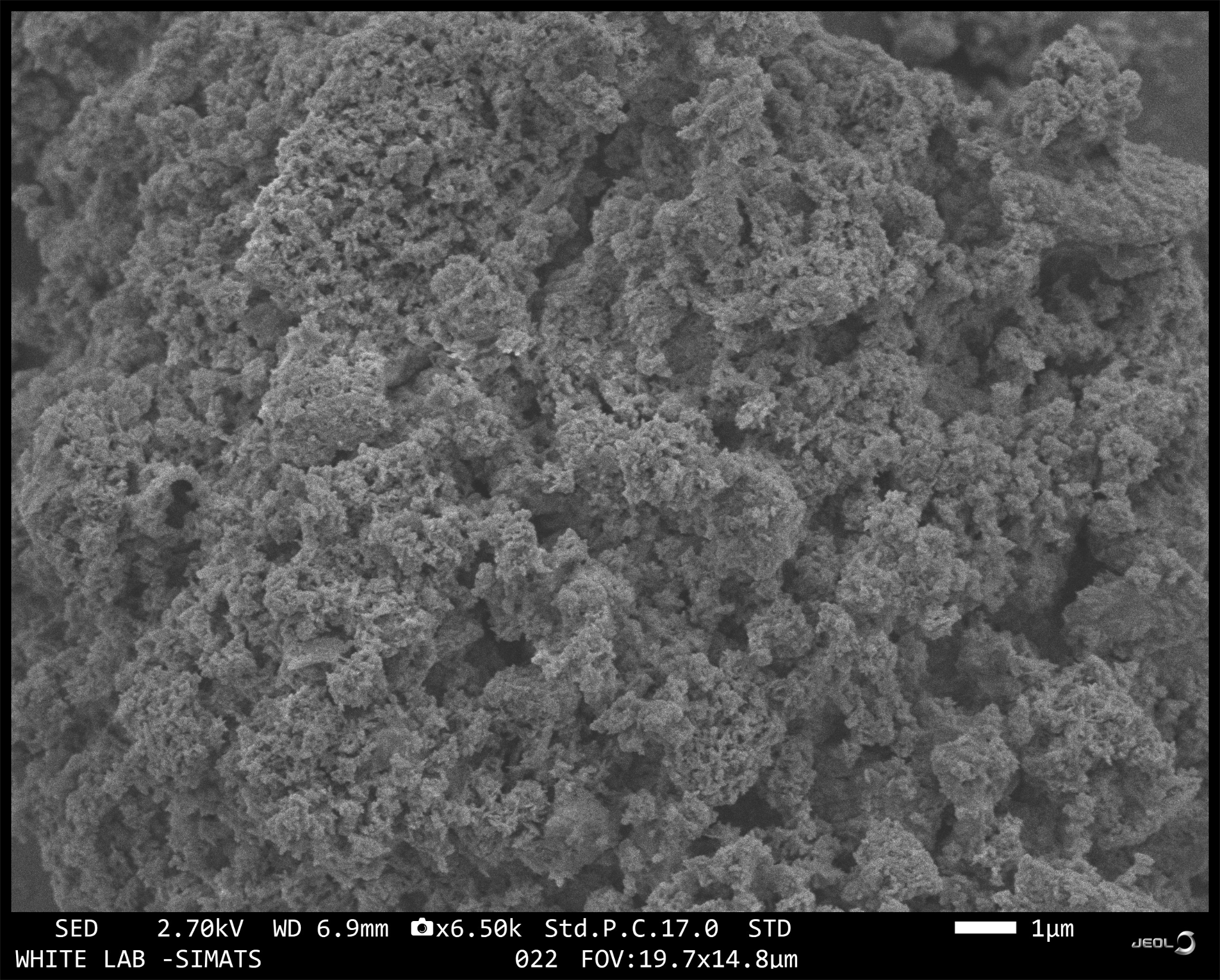
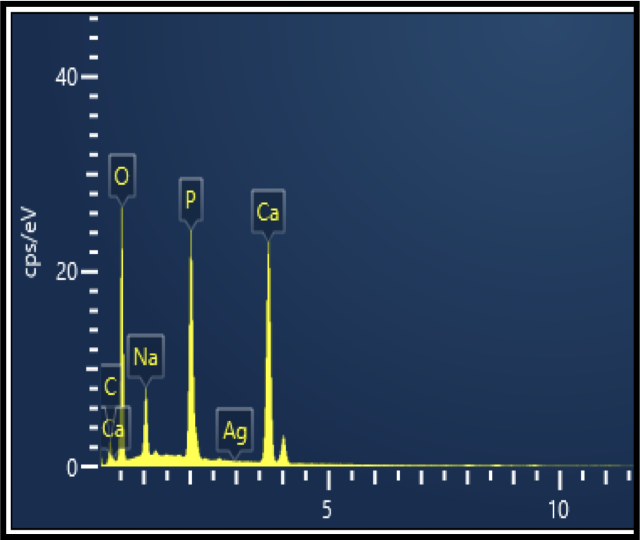
## Statistical analysis

Applying the Annova Two-way Factor method, statistical analysis was performed. When comparing the two groups with the control, the two-way factor method analysis of variance was used, and p-values ≤0.05 were deemed statistically significant.

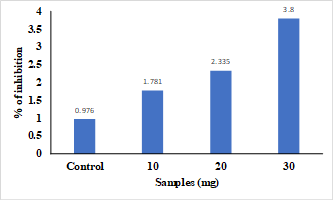
# RESULTS

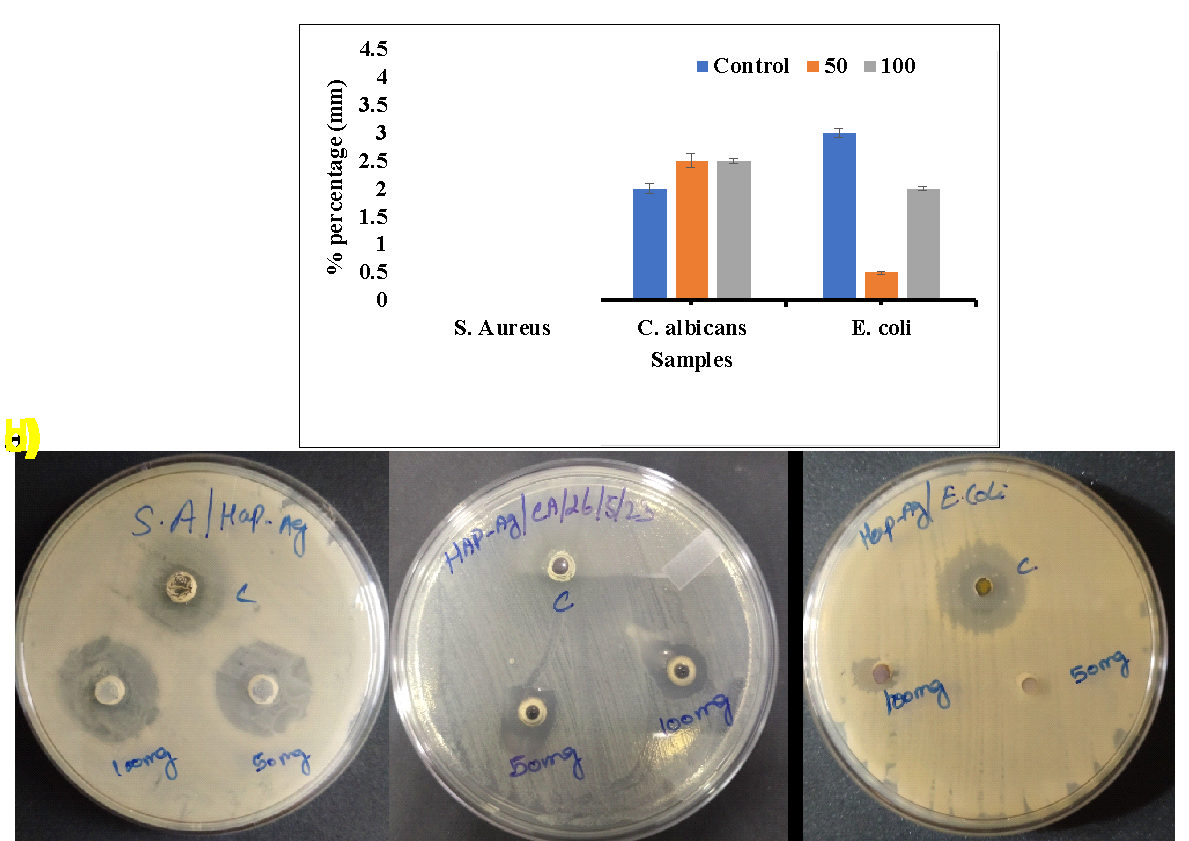


**Figure 1.** FT-IR of Hydroxyapatite loaded silver nanoparticles



**Figure 2:** SEM and EDX spectrum of HAp loaded Ag



**Figure 3.** Anti-oxidant activity of HAp loaded silver nanoparticles (DPPH Assay plotted linear values)

**Figure 4:** Zone of inhibition and Antibacterial activity of the sample with b) S. aureus, c) C. albicans, and d) E. coli HAp incorporated silver

The result showed that HAP/Ago has antibacterial activity against S. aureus, C. albicans, and E. coli at 50 and 100 micro gram/ml concentrations, with higher activity at 100 microgm/ml for both species. In surface morphology of HAp/Ag, small needle like shape were observed in the entire surface.

# Discussion

Ag-NPs have been synthesised by some researchers using plant extracts. The findings demonstrate that extracts of different plants with sizes ranging from 2 to 100 nm were used to create silver nanoparticles. The Ag-NPs produced in our study from the urban leaf extract of Centella asiatica (L.) range in size from 20 to 60 nm, with an average particle size of roughly 24.56 nm [(Du et al., 2009)](https://paperpile.com/c/i1NpAc/4F8t). Ag-NPs are made from wild Penicillium strains that have been isolated from the environment (Tuluwengjiang et al., 2024). The produced Ag-NPs showed inhibitory zones of 12.0–16.0 mm and were tested for their antibacterial activity against B. cereus, S. aureus, E. coli, and P. aeruginosa [(Du et al., 2009; Maliszewska & Sadowski, 2009)](https://paperpile.com/c/i1NpAc/4F8t+BQGc). Similarly, Mohammed et al. synthesised Ag-NPs using an aqueous extract of E. camaldulensis leaves. According to the test results, the inhibition zone for both Gram-positive (S. aureus and B. subtilis) and Gram-negative (P. aeruginosa and E. coli) bacteria was approximately 9.0–14.0 mm [(Morones et al., 2005)](https://paperpile.com/c/i1NpAc/HRcK)The antibacterial activity of the Ag-NPs was found to be higher in this study than in previous reports. Ag-NPs containing 0.003 weight percent Ag from Lysimachia foenum-graecum Hance extract exhibit an inhibition zone diameter of 15.58 ± 0.51 mm for E. coli. On the one hand, the location of NPs within bacterial membranes and cell walls may serve as evidence of the bactericidal mechanism of Ag-NPs. It can encourage the breakdown of cell walls and membranes and prevent enzyme activity [(Rai et al., 2009)](https://paperpile.com/c/i1NpAc/vz75). DNA lost its capacity to replicate when the cytoplasm membrane separated from the cell wall. Divergent views exist regarding the mechanism underlying Ag-NPs' antibacterial activity [(Tamboli & Lee, 2013)](https://paperpile.com/c/i1NpAc/Xgor). According to several studies, Ag-NPs have an antibacterial effect on the development of pits in the cell wall, which increases the permeability of the cell membrane and prevents bacterial cells from effectively controlling transport through the plasma membrane, ultimately leading to cell death. Lok et al. found that when microorganism cells were exposed to an active concentration of nanosilver right away, the outer membrane became unstable and the proton motive force dissipated [(Lok et al., 2006)](https://paperpile.com/c/i1NpAc/xchg). Under the same experimental conditions, the obtained results also demonstrate a greater antibacterial effect for Ag-NPs than for the HAp/Ag nanocomposite. The gradual release of Ag-NPs from the HAp/Ag nanocomposite, which interacts with bacterial proteins and enzymes, could be the cause of this.

# Conclusion

In conclusion, the HAp/Ag demonstrated antibacterial and antifungal activity against S. aureus, C. albicans, and E. coli when compared to the control. In both species, the maximum zone of inhibition was observed at a concentration of 100 microgrammes.

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