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ORIGINAL ARTICLE

Synthesis, Characterization and Antibacterial Activity of Silver Nanoparticles Prepared from Benzoin Gum (Loban) Extract

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ABSTRACT

Background: The rise of multi-drug resistance poses a significant challenge for treating infectious diseases. Nanotechnology-particularly the use of silver nanoparticles (AgNPs)-offers promising solutions.

Objective: To synthesize AgNPs using benzoin (reducing and stabilizing agent) as an eco-friendly (green) approach followed by the evaluation of their antibacterial activity against multi-drug-resistant pathogens.

Methodology: AgNPs were synthesized by reacting silver nitrate with benzoin extract. Formation of AgNPs was confirmed by UV–visible spectroscopy and Fourier-transform infrared (FTIR) spectroscopy. Antibacterial efficacy was tested against Gram-positive and Gram-negative bacterial strains.

Result: The benzoin-mediated AgNPs exhibited significant antibacterial activity against all tested strains, with the greatest inhibition against *Streptococcus faecalis*.

Conclusion: Benzoin-mediated green synthesis of AgNPs provides an eco-friendly method to produce potent antimicrobial agents, especially effective against drug-resistant pathogens.

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INTRODUCTION

The rise of multi-drug–resistant pathogens-driven by the overuse of broad-spectrum antibiotics-poses a critical challenge for infectious-disease treatment. Nanotechnology offers a powerful alternative: metal and metal-oxide nanoparticles (NPs) can be engineered to disrupt microbial membranes, inhibit biofilms, and circumvent conventional resistance mechanisms. In particular, silver nanoparticles (AgNPs) exhibit broad antibacterial activity against microbial strains[1,2].

NPs are traditionally synthesized by three routes: Physical methods demand high energy input. Chemical methods (e.g., $NaBH_4$ or sodium citrate reduction) often rely on toxic reagents and generate hazardous byproducts. Biological (green) methods use plant extracts or microorganisms as reducing and capping agents, proceeding under mild, eco-friendly conditions[3-5].

Plant-mediated green synthesis is especially attractive: secondary metabolites-sugars, ketones, aldehydes, carboxylic acids, and terpenoids-both reduce metal ions and stabilize the resulting NPs. This approach is rapid,

scalable, cost-effective, and yields biocompatible, stable NPs suitable for biomedical applications[6-11].

Inorganic NPs encompass metal-and metal-oxide–based particles (e.g., Co, Cu, Zn, Ag, Fe, CeO_2, Fe_2O_3, ZnO, TiO_2), whose properties vary with surface charge, size, morphology, and composition[12-14]. Chemical syntheses typically employ reducing agents ($NaBH_4$, ascorbic acid) and stabilizers (sodium citrate, polyethylene glycol) under controlled temperature (30–90 °C) and pH (adjusted with NaOH/KOH)[15]. Microbial methods trap metal ions on cell-surface enzymes, effecting reduction to NPs[16].

Styrax benzoin (benzoin resin) is an evergreen tree (family *Styracaceae*) native to Southeast Asia, valued for its aromatic resin. Its phytochemicals have not yet been applied to NP synthesis. Here, we report the green synthesis of AgNPs using *S. benzoin* extract and silver nitrate. The resulting AgNPs were characterized by UV–Vis spectroscopy and Fourier-transform infrared (FTIR) analysis. We also evaluated their antibacterial efficacy against representative human pathogens[17,18].

MATERIALS AND METHODS

Materials

The silver nitrate ($AgNO_3$), benzoin gum, (*Styrax benzoin*), Whatmann filter paper were utilized in this study.

Synthesis of NPs

Preparation of Benzoin Gum (Styrax Benzoin) Extract

Benzoin gum (20 g) was ground to a fine powder and boiled in 200 mL deionized water at 75 °C for 2 h followed by cooling. The mixture was filtered twice through Whatman No. 1 paper. The clear extract was stored at 4 °C until use.

Reduction of Silver Ions

A 0.1 M $AgNO_3$ solution was prepared by dissolving 2 g $AgNO_3$ in 200 mL deionized water and heating to 70 °C with magnetic stirring. The *S. benzoin* extract (200 mL) was added dropwise over 30 min at 65–70 °C under continuous stirring. The reaction mixture gradually turned from colorless to deep brown within 45 min and to black after 3 h, indicating AgNP formation. The colloidal suspension was centrifuged at 10,000 rpm, washed with water, and dried at 80 °C. The resulting AgNP powder was stored in a desiccator.

Characterization Studies

UV-Vis Spectrophotometer

The AgNP suspension (1 mg/mL) was scanned from 300 to 700 nm to confirm the surface-plasmon resonance peak at ~450 nm[19].

FTIR Spectroscopy

Dried AgNP powder was mixed with potassium bromide (KBr) and pressed into pellets. Spectra were recorded from 400–4,000 cm^{-1} (Nicolet iS10, Thermo Scientific) to identify capping phytochemicals[20].

Antibacterial Activity

The bacterial strains Gram-positive (*Bacillus subtilis, Streptococcus faecalis*) and Gram-negative (*Escherichia coli, Klebsiella pneumoniae*) were used in this study. The agar well diffusion method was used[21]. Agar plates were inoculated with each bacterial strain (0.5 McFarland) and incubated overnight. Wells (6 mm) were filled with 100 µL of AgNP suspensions at 5, 10, 20, and 40 mg/mL (wells 1–4). Well 5 contained 5 mg/mL levofloxacin as a positive control.

RESULT

Characterization Studies

UV-Vis Spectroscopy

The silver nanoparticles (AgNPs) synthesized using *Styrax benzoin* resin exhibited a prominent surface-plasmon resonance peak at 450 nm in the UV–Vis spectrum, confirming their formation (Figure **1** left).

FTIR Spectroscopy

FTIR analysis identified key functional groups from the resin capping the AgNP surface: a broad O–H stretch at 3402 cm^{-1}, C–H asymmetric stretches at 2931 and 2869 cm^{-1}, an aromatic C=C band at 1520 cm^{-1}, and a C–O–C vibration at 1039 cm^{-1} (Figure **1** right).

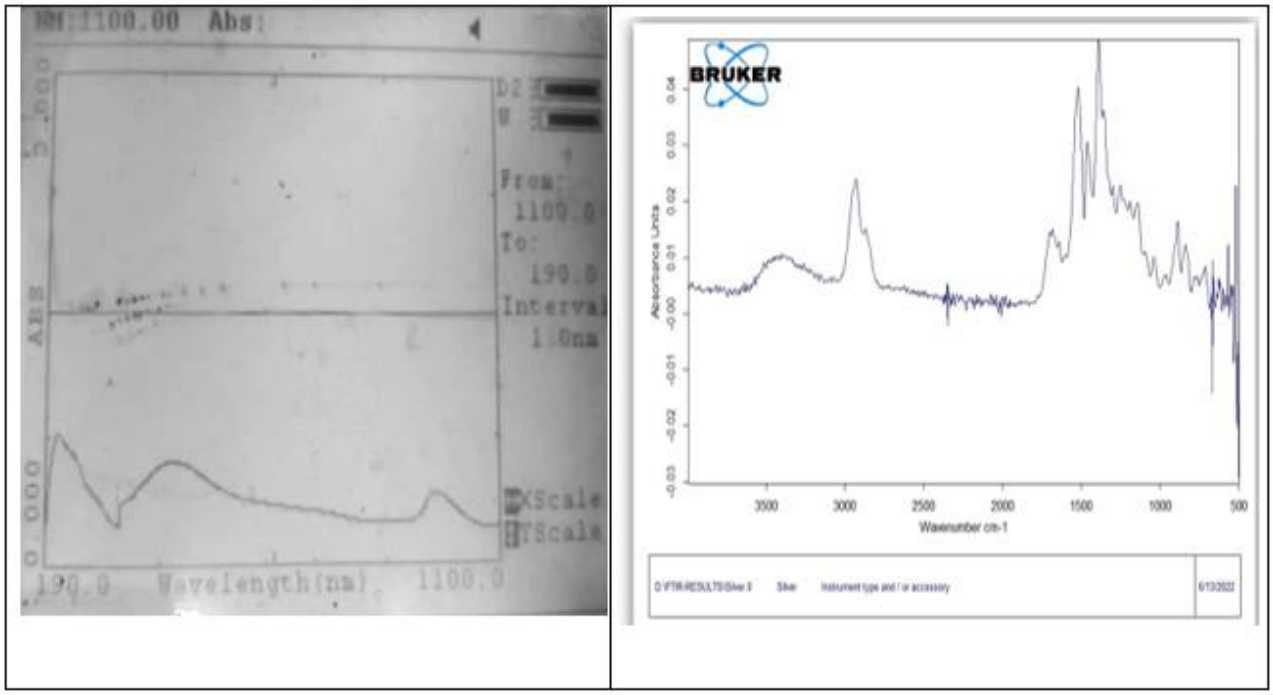

Figure 1. UV-vis spectrum (Left) and FTIR spectrum (Right) of AgNPs.

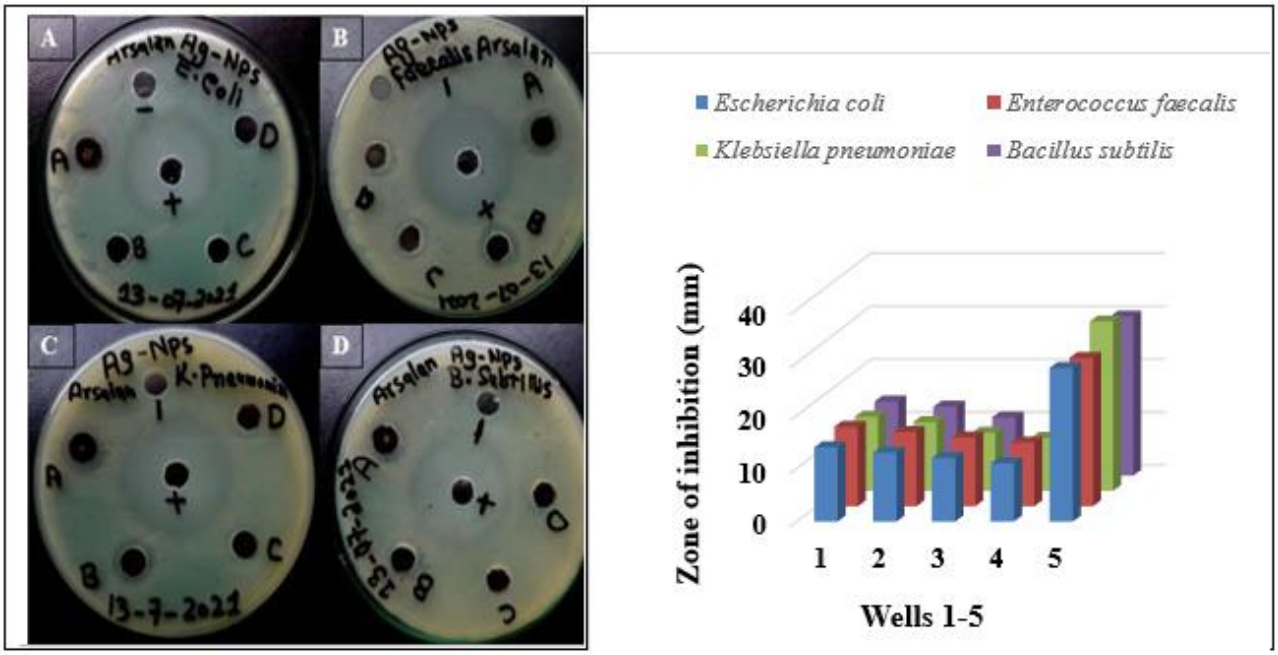

Figure 2: Antibacterial activity of AgNPs against four pathogens. Left panels (**A–D**) show agar-well diffusion inhibition zones for *E. coli* (**A**), *E. faecalis* (**B**), *K. pneumoniae* (**C**), and *B. subtilis* (**D**). Right panel (**E**) presents a comparative bar chart of the mean inhibition-zone diameters for each bacterial strain.

Antibacterial activity of AgNPs

In agar-well diffusion assays, nanoparticle dose correlated with antibacterial activity: the highest concentration produced inhibition zones of 30 ± 1 mm (*E. coli*), 32 ± 1.5 mm (*E. faecalis*), 36 ± 2 mm (*K. pneumoniae*), and 38 ± 2 mm (*B. subtilis*), whereas lower doses yielded zones of 10–20 mm across all strains (Figure. **2a-d** right -left).

DISSCUSSION

The UV–Vis peak at 450 nm is characteristic of spherical AgNPs and aligns with literature on biologically synthesized silver colloids[22]. FTIR spectra confirm that hydroxyl, methylene, and aromatic phytochemicals from *S. benzoin* serve dual roles as reducing and stabilizing agents, promoting nanoparticle stability and preventing aggregation[23].

The stronger inhibitory effects against Gram-positive bacteria (*B. subtilis*, *K. pneumoniae*) compared to Gram-negative (*E. coli*) likely reflect differences in cell-wall structure, as thicker peptidoglycan layers may facilitate more extensive nanoparticle interaction[24,25]. These findings corroborate previous reports of enhanced AgNP efficacy against Gram-positive pathogens and underscore the utility of plant-mediated synthesis for producing bio-capped nanoparticles with potent, broad-spectrum antimicrobial properties[26,27].

CONCLUSION

Green synthesis of AgNPs using *Styrax benzoin* resin is an eco-friendly, cost-effective method that yields stable nanoparticles with strong antibacterial activity. The bio-capped AgNPs demonstrate significant, dose-dependent inhibition of both Gram-positive and Gram-negative bacteria, highlighting their potential as next-generation antimicrobial agents. Further studies will focus on *in vivo* efficacy, cytotoxicity profiling, and scale-up production to advance these AgNPs toward clinical and commercial applications.

CONFLICT OF INTEREST

None.

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