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Green Synthesis of Silver Nanoparticles Using Hibiscus rosa-sinensis Leaf Extract and Evaluation of Antibacterial Activity

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Abstract: The synthesis of silver nanoparticles (AgNPs) using biological systems — particularly plant extracts — has emerged as a sustainable and environmentally benign alternative to conventional chemical reduction methods that involve toxic reducing and stabilising agents. This study reports the green synthesis of AgNPs using aqueous leaf extract of Hibiscus rosa-sinensis (shoe flower), a commonly available ornamental plant rich in polyphenols, flavonoids, and anthocyanins that serve as both reducing and capping agents for silver ions. The synthesis involved treating a 1 mM aqueous silver nitrate ($AgNO_3$) solution with varying volumes of Hibiscus rosa-sinensis leaf extract under controlled conditions of temperature (60°C) and extract concentration. Formation of AgNPs was monitored by visual colour change from colourless to brown and confirmed by UV-Vis spectroscopy showing surface plasmon resonance (SPR) at 432 nm. Nanoparticle characterisation by XRD confirmed a face-centred cubic (FCC) silver crystal structure with a mean particle size of 27.4 nm by Scherrer analysis. FTIR spectroscopy identified phytochemical functional groups responsible for silver ion reduction and surface capping. Antibacterial activity of the synthesised AgNPs was evaluated against four clinically significant pathogenic bacteria — Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923), Pseudomonas aeruginosa (ATCC 27853), and Klebsiella pneumoniae (ATCC 13883) — using the agar disc diffusion method and minimum inhibitory concentration (MIC) determination. All four bacterial strains showed significant susceptibility to AgNPs, with MIC values ranging from 6.25 to 12.5 μg/mL, demonstrating the strong antibacterial potential of Hibiscus rosa-sinensis-derived AgNPs.

Keywords: *Silver Nanoparticles, Green Synthesis, Hibiscus rosa-sinensis, Antibacterial Activity, SPR, Disc Diffusion*

1. Introduction

Nanotechnology has opened new frontiers in materials science, medicine, and environmental remediation through the development of nanoscale materials with unique and tunable physicochemical properties. Among the various metallic nanoparticles synthesised for research and application, silver nanoparticles (AgNPs) occupy a prominent position due to their well-documented broad-spectrum antimicrobial activity, catalytic properties, and applications in wound care, medical devices, water purification, food packaging, and diagnostic biosensors. The global market for silver nanoparticles is expanding rapidly, projected to reach USD 3.4 billion by 2027, driven predominantly by demand from the healthcare and consumer goods sectors.

Conventional chemical synthesis of AgNPs employs chemical reducing agents such as sodium borohydride, hydrazine hydrate, or citrate under controlled pH and temperature conditions. While these methods yield nanoparticles with well-controlled size and morphology, they use toxic chemicals that pose environmental and occupational health risks, generate hazardous byproducts, and require specialised handling facilities. As nanotechnology applications expand to include food contact materials, biomedical implants, and environmental remediation, the toxicological profile of synthesis routes becomes increasingly important.

Green synthesis of AgNPs — using biological entities including plant extracts, fungi, bacteria, and algae as sources of natural reducing and stabilising agents — offers a sustainable, non-toxic, and low-cost alternative. Plant extract-mediated synthesis is particularly attractive because it is a single-step, room-temperature-to-moderate-temperature process, produces stable nanoparticles without requiring additional capping agents, and can be performed without sophisticated laboratory infrastructure. The phytochemicals in plant extracts — polyphenols, flavonoids, terpenoids, alkaloids, and organic acids — act as multi-functional reducing and capping agents, simultaneously converting Ag^{+} ions to Ag^{0} and stabilising the resulting nanoparticles against aggregation.

Hibiscus rosa-sinensis (family Malvaceae), commonly known as the shoe flower or China rose, is widely cultivated across tropical and subtropical India and is traditionally used in Ayurvedic medicine for its anti-inflammatory, antifungal, and antioxidant properties. Its leaves are rich in phenolic compounds, flavonoids (particularly quercetin and kaempferol), anthocyanins, and polysaccharides — all of which are excellent candidates for silver ion reduction. However, the specific conditions for AgNP synthesis from Hibiscus rosa-sinensis leaf extract and the resulting nanoparticles' antibacterial spectrum have not been comprehensively characterised in the literature. This study addresses this gap.

2. Methodology

Fresh leaves of Hibiscus rosa-sinensis were collected from the campus of Sacred Heart College, Chalakudy, Kerala, washed thoroughly with deionised water to remove dust and surface contaminants, and shade-dried at room temperature for 5 days. Dried leaves were ground to a fine powder using a household electric grinder. Aqueous extract was prepared by boiling 10 g of leaf powder in 100 mL of deionised water at 80°C for 30 minutes under reflux conditions, then filtering through Whatman No. 1 filter paper and Millipore membrane (0.45 μm) to obtain a clear yellow-green extract. The extract was stored at 4°C until use. Preliminary phytochemical screening of the extract was performed for polyphenols (Folin-Ciocalteu method), flavonoids (aluminium chloride colorimetric method), and tannins (ferric chloride test).

Silver nanoparticles were synthesised by adding varying volumes of leaf extract (1, 3, 5, and 10 mL) to 90 mL of 1 mM aqueous $AgNO_3$ solution (prepared from 99.9% pure $AgNO_3$; SRL Chemicals, India) under constant magnetic stirring at 60°C. Colour change from colourless to brown, indicating AgNP formation, was monitored visually and by UV-Vis spectroscopy (Shimadzu UV-2450) at intervals of 15, 30, 60, and 120 minutes. The optimal extract volume (5 mL) was determined by maximising the SPR peak intensity. Final AgNPs were isolated by centrifugation (10,000 rpm, 20 min), washed twice with deionised water, redispersed in water, and lyophilised for solid-state characterisation. XRD was performed on a PANalytical X'Pert Pro diffractometer using Cu Kα radiation (2θ range: 20°–80°). FTIR was performed in KBr pellet mode (4000–400 cm^{-1}) on a Shimadzu IRPrestige-21 spectrometer.

Antibacterial activity was evaluated against four ATCC strains using the Kirby-Bauer agar disc diffusion method on Mueller-Hinton agar (HiMedia). AgNP stock solution (1 mg/mL in deionised water) was serially diluted to prepare test concentrations of 100, 50, 25, and 12.5 μg/mL. Sterile filter paper discs (6 mm diameter) were impregnated with 20 μL of each AgNP concentration and allowed to dry. Standard antibiotic discs (Ciprofloxacin

5 µg; HiMedia) and solvent controls were included. Plates were incubated at 37°C for 24 hours. Zone of inhibition (ZOI) diameters were measured in triplicate. MIC values were determined by the broth microdilution method following CLSI guidelines (CLSI M07-A11, 2018) using a 96-well plate format with AgNP concentrations ranging from 0.39 to 100 µg/mL.

3. Results and Findings

Phytochemical screening confirmed the presence of polyphenols (total phenolic content: 28.4 mg gallic acid equivalents/g dry extract), flavonoids (total flavonoid content: 12.7 mg quercetin equivalents/g dry extract), and tannins in the Hibiscus rosa-sinensis leaf extract. These phytochemicals provided the reducing capacity confirmed by the DPPH radical scavenging activity assay (IC_{50}: 0.24 mg/mL). Formation of AgNPs was confirmed within 30 minutes of adding extract to the $AgNO_3$ solution by the characteristic brown colouration. UV-Vis spectra showed a single, sharp SPR absorption peak at 432 nm, characteristic of spherical AgNPs and confirming successful nanoparticle formation. Peak absorbance was maximal at the 5 mL extract volume, which was used for all subsequent syntheses.

XRD analysis of lyophilised AgNPs revealed four characteristic diffraction peaks at 2θ values of 38.1°, 44.3°, 64.5°, and 77.4°, corresponding to the (111), (200), (220), and (311) crystallographic planes of face-centred cubic (FCC) silver (JCPDS No. 04-0783), confirming the formation of crystalline silver. No peaks attributable to silver oxide or silver chloride impurities were detected. Mean crystallite size calculated from the (111) peak using the Scherrer equation was 27.4 nm, consistent with the particle size inferred from the SPR peak position. FTIR spectrum of AgNPs showed a broad O-H stretching band (3200–3500 cm^{-1}) and C=O stretching bands (1620–1645 cm^{-1}) attributable to phenolic and carbonyl groups of adsorbed phytochemicals, confirming phytochemical-mediated capping and stabilisation.

Antibacterial disc diffusion assay results showed clear zones of inhibition around AgNP-impregnated discs for all four test bacterial strains. At 100 µg/mL AgNP loading, ZOI diameters were: E. coli (18.4 mm), S. aureus (21.2 mm), P. aeruginosa (16.8 mm), and K. pneumoniae (17.6 mm). S. aureus showed the highest sensitivity, consistent with reported higher susceptibility of Gram-positive bacteria to AgNP action. MIC values determined by broth microdilution were: E. coli (12.5 µg/mL), S. aureus (6.25 µg/mL), P. aeruginosa (12.5 µg/mL), and K. pneumoniae (12.5 µg/mL). All MIC values were markedly lower than the minimum inhibitory concentrations reported for comparable AgNPs synthesised by chemical reduction methods, suggesting that the phytochemical surface layer may contribute additional antimicrobial activity.

4. Discussion

The rapid synthesis of AgNPs (within 30 minutes) at 60°C using Hibiscus rosa-sinensis leaf extract confirms the reducing efficacy of the extract's polyphenolic and flavonoid content. The mechanism of silver ion reduction by plant polyphenols involves electron donation from hydroxyl groups on aromatic rings, resulting in Ag^{+} to Ag^{0} reduction and simultaneous oxidation of phenolic compounds to quinones. The adsorption of these phytochemicals onto freshly formed silver nuclei through carbonyl, hydroxyl, and amine functional groups (as evidenced by FTIR) provides steric stabilisation that prevents particle aggregation and accounts for the long-term colloidal stability (>30 days, observed during the study) of the synthesised AgNPs.

The strong antibacterial activity of the synthesised AgNPs against both Gram-positive (S. aureus) and Gram-negative (E. coli, P. aeruginosa, K. pneumoniae) organisms is consistent with published literature on green-synthesised AgNPs and reflects multiple concurrent antibacterial mechanisms: disruption of the bacterial cell membrane by electrostatic interaction with negatively charged phospholipid head groups, generation of reactive

oxygen species (ROS) including superoxide and hydrogen peroxide that cause oxidative damage to bacterial DNA, proteins, and cell membranes, and inhibition of key respiratory chain enzymes. The lower MIC values for S. aureus compared to Gram-negative organisms are likely attributable to the absence of an outer membrane lipopolysaccharide layer in Gram-positive bacteria, which provides an additional permeability barrier in Gram-negative species.

The use of Hibiscus rosa-sinensis as a reducing agent offers several practical advantages over other plant sources commonly used in AgNP green synthesis: the plant is widely available across India, the extraction procedure requires no organic solvents, the synthesis is performed at moderate temperature without high-pressure equipment, and the resulting nanoparticles have a favourable size and stability profile. These characteristics make this synthesis route accessible to researchers and applied chemists in resource-limited settings. However, standardisation of extract composition — which can vary with leaf age, season, and geographic origin — remains a challenge for reproducibility that future studies should systematically address.

5. Conclusion

This study demonstrates the successful green synthesis of crystalline, spherical silver nanoparticles (mean size 27.4 nm, FCC crystal structure, SPR at 432 nm) using aqueous Hibiscus rosa-sinensis leaf extract as a bioreducing and stabilising agent. The synthesised AgNPs exhibited potent antibacterial activity against four clinically significant pathogens, with MIC values in the range of 6.25–12.5 μg/mL, supporting their potential application as antibacterial agents in wound care, surface disinfection, or water treatment contexts.

Future research directions include: (i) isolation and identification of the specific phytochemical fraction responsible for silver reduction activity; (ii) cytotoxicity evaluation of the synthesised AgNPs against mammalian cell lines to establish an initial safety profile; (iii) investigation of synergistic effects of AgNPs in combination with conventional antibiotics, particularly for multidrug-resistant ESKAPE pathogens; (iv) scale-up synthesis optimisation for pilot-scale production; and (v) exploration of Hibiscus-derived AgNPs as photocatalytic agents for degradation of textile dye effluents, leveraging their UV-Vis absorption properties.

References

Ahamed, M., AlSalhi, M. S., & Siddiqui, M. K. J. (2010). Silver nanoparticle applications and human health. Clinica Chimica Acta, 411(23–24), 1841–1848.

Annamalai, A., Christina, V. L. P., Sudha, D., Kalpana, M., & Lakshmi, P. T. V. (2013). Green synthesis and characterisation of Ag NPs using aqueous extract of Phyllanthus maderaspatensis L. European Journal of Experimental Biology, 3(1), 417–422.

CLSI. (2018). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically (M07-A11). Wayne, PA: Clinical and Laboratory Standards Institute.

Gnanajobitha, G., Paulkumar, K., Vanaja, M., Rajeshkumar, S., Malarkodi, C., Annadurai, G., & Kannan, C. (2013). Fruit-mediated synthesis of silver nanoparticles using Vitis vinifera and evaluation of their antimicrobial efficacy. Journal of Nanostructure in Chemistry, 3(1), 67.

Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. Green Chemistry, 13(10), 2638–2650.

Rao, K. J., & Paria, S. (2013). Green synthesis of silver nanoparticles from aqueous Aegle marmelos leaf extract. Industrial and Engineering Chemistry Research, 52(46), 6164–6173.

Shankar, S. S., Rai, A., Ahmad, A., & Sastry, M. (2004). Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using neem (Azadirachta indica) leaf broth. Journal of Colloid and Interface Science, 275(2), 496–502.

Sharma, V. K., Yngard, R. A., & Lin, Y. (2009). Silver nanoparticles: Green synthesis and their antimicrobial activities. Advances in Colloid and Interface Science, 145(1–2), 83–96.

Singhal, G., Bhavesh, R., Kasariya, K., Sharma, A. R., & Singh, R. P. (2011). Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity. Journal of Nanoparticle Research, 13(7), 2981–2988.

Veeraputhiran, V. (2013). Bio-catalytic synthesis of silver nanoparticles. International Journal of ChemTech Research, 5(5), 2555–2562.