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STUDY OF ANTIMICROBIAL POTENTIAL OF SILVER NANOPARTICLES SYNTHESIZED FROM HIBISCUS ROSA - SINENSIS

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ABSTRACT

The rapid emergence of multidrug-resistant microorganisms has become one of the most significant global public health challenges, reducing the efficacy of conventional antimicrobial agents and necessitating the development of alternative therapeutic strategies. Green nanotechnology has emerged as a promising approach by combining the medicinal properties of plants with the unique physicochemical characteristics of metallic nanoparticles. The present study aimed to synthesize silver nanoparticles (AgNPs) using aqueous extracts of different parts of Hibiscus rosa-sinensis (leaves, petals, and stems) through an environmentally friendly green synthesis approach and to evaluate their antimicrobial activity against Escherichia coli and Aspergillus niger. The findings demonstrate that green-synthesized AgNPs possess excellent antimicrobial potential and may serve as eco-friendly alternatives for future biomedical, pharmaceutical, and antimicrobial applications.

Keywords: Green Synthesis, Biogenic Nanoparticles, Antimicrobial Resistance (AMR), Plant-mediated nanotechnology, Surface Plasmon Resonance (SPR), Antimicrobial Efficacy

INTRODUCTION

Microbial infections continue to be among the leading causes of diseases and mortality worldwide despite remarkable advances in medical science. Over the past few decades, the widespread and often indiscriminate use of antibiotics has accelerated the emergence of multidrug-resistant (MDR) microorganisms, posing a severe threat to global healthcare systems. According to the World Health Organization WHO (2023), antimicrobial resistance (AMR) is one of the top ten global public health threats, with resistant bacterial infections responsible for millions of deaths annually. Pathogenic microorganisms such as Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and various fungal species have developed resistance to several classes of antibiotics, significantly limiting treatment options and increasing healthcare costs. Consequently, there is an urgent need to identify safe, effective, sustainable, and environmentally friendly antimicrobial agents capable of overcoming microbial resistance. Medicinal plants have been utilized for centuries in traditional healthcare systems because they contain a diverse array of naturally occurring bioactive compounds with therapeutic properties. Scientific investigations have confirmed that medicinal plants possess antimicrobial, antioxidant, anti-inflammatory, anticancer, antiviral, and immunomodulatory activities, making them valuable sources for the discovery of novel pharmaceutical compounds. Among medicinal plants, Hibiscus rosa-sinensis commonly known as China rose and shoe flower, has attracted considerable scientific attention because of it's rich phytochemical composition and wide range of pharmacological activities. The antimicrobial properties of H. rosa-sinensis have been extensively investigated against both

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Gram-positive and Gram-negative bacteria as well as pathogenic fungi. Recent advances in nanotechnology have provided innovative solutions to enhance the biological effectiveness of medicinal plants. Nanotechnology involves the design, synthesis, and application of materials with dimensions ranging from 1 to 100 nm, which possess unique physicochemical, optical, catalytic, and biological properties compared with their bulk counterparts. Metallic nanoparticles, particularly silver nanoparticles (AgNPs), have gained tremendous attention because of their exceptional antimicrobial, antioxidant, anti-inflammatory, anticancer, antiviral, and woundhealing properties. Among various metallic nanoparticles, AgNPs are considered one of the most effective antimicrobial nanomaterials because they exhibit broadspectrum activity against bacteria, fungi, viruses, and even multidrug-resistant pathogens. During green synthesis, phytochemicals present in plant extracts reduce silver ions (Ag^+) from silver nitrate into metallic silver nanoparticles (Ag^0). Biomolecules such as flavonoids, phenolics, proteins, terpenoids, reducing sugars, and polysaccharides subsequently adsorb onto the nanoparticle surface, preventing aggregation and improving stability. Formation of AgNPs is commonly indicated by a visible colour change from pale yellow to brown due to the phenomenon of Surface Plasmon Resonance (SPR), while UV–Visible spectroscopy typically demonstrates characteristic absorption peaks between 420 and 450 nm. The synthesized nanoparticles were characterized by UV–Visible spectroscopy and evaluated for their antimicrobial activity against the bacterial pathogen Escherichia coli and the fungal pathogen Aspergillus niger using Agar Well Diffusion and Disc Diffusion methods. The study also aimed to compare the antimicrobial efficacy of crude plant extracts with their corresponding silver nanoparticles and to determine the most effective plant part for nanoparticle synthesis.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIALS

Fresh, healthy leaves, stems, and flower petals of Hibiscus rosa-sinensis were collected from a pesticide-free balcony garden located in Dombivli East, Maharashtra, India. Plant materials were selected carefully to ensure that they were free from disease, insect infestation, and physical damage. Collection from a pollution-free domestic garden minimized contamination from pesticides, heavy metals, and other environmental pollutants that could interfere with nanoparticle synthesis or antimicrobial activity. Immediately after collection, the plant materials were transferred to the laboratory in sterile polyethylene bags to maintain freshness and prevent microbial contamination. The collected samples were thoroughly washed under running tap water to remove adhering dust, soil particles, and other impurities. This was followed by repeated washing with distilled water to eliminate residual contaminants. Surface sterilization was then carried out using 70% ethanol for one minute to remove epiphytic microorganisms. The sterilized plant materials were subsequently rinsed three times with distilled water to remove traces of ethanol before further processing Nayak et al. (2015), Vanlalveni et al. (2021), Abada et al. (2024).

PREPARATION OF AQUEOUS PLANT EXTRACTS

The cleaned leaves, stems, and flower petals were dried separately in a hot air oven at 60°C for 24 hours until complete removal of moisture. Oven drying under controlled temperature prevents microbial spoilage while preserving heat-stable phytochemicals responsible for reduction and stabilization of silver nanoparticles. The dried samples were separately ground into coarse powder using a sterile electric grinder and stored in sterile airtight containers until extraction. For aqueous extraction, 10 g of powdered leaf, stem, and petal material was weighed separately and transferred into sterile 250 mL conical flasks. To each flask, 100 mL of distilled water was added to maintain a 1:10 (w/v) ratio, which is widely employed for extraction of water-soluble phytochemicals. The mixtures were heated in a water bath at 60°C for 30–45 minutes with intermittent stirring to facilitate extraction of biologically active compounds including flavonoids, phenolics, tannins, anthocyanins, proteins, glycosides, and reducing sugars. Following extraction, the mixtures were cooled to room temperature and filtered initially through sterile muslin cloth to remove coarse debris and subsequently through Whatman No.1 filter paper to obtain clear aqueous extracts. The filtrates were collected in sterile amber-coloured bottles and stored at 4°C until further use in nanoparticle synthesis to preserve phytochemical stability and minimize oxidation Ahmed et al. (2016), Vanlalveni et al. (2021), Abada et al. (2024).

PREPARATION OF SILVER NITRATE SOLUTION

Silver nitrate ($AgNO_3$) was used as the precursor salt for synthesis of silver nanoparticles. A 1 mM aqueous silver nitrate solution was prepared by dissolving 0.017 g of analytical-grade $AgNO_3$ in 100 mL of distilled water. Since silver nitrate is highly photosensitive, the solution was prepared in amber glassware and stored in the dark at room temperature until use to prevent photoreduction of silver ions.

GREEN SYNTHESIS OF SILVER NANOPARTICLES

Green synthesis of silver nanoparticles was carried out using aqueous extracts of leaves, petals, and stems of Hibiscus rosa-sinensis. For each synthesis reaction, 1 mL of the respective aqueous plant extract was mixed with 9 mL 1 mM silver nitrate solution, maintaining a 1:9 reaction ratio. The reaction mixtures were incubated at room temperature for 30–60 minutes without exposure to

direct sunlight. During incubation, a distinct colour change from pale yellow to dark brown was observed, indicating the reduction of silver ions (Ag^+) into metallic silver nanoparticles (Ag^0). This colour transformation is attributed to the excitation of surface plasmon resonance (SPR), a characteristic optical property of silver nanoparticles. The synthesized AgNP suspensions were subsequently used for spectroscopic characterization and antimicrobial evaluation.

CHARACTERIZATION OF SILVER NANOPARTICLES

UV–Visible Spectroscopic Analysis

The synthesized AgNP suspensions prepared from leaf, petal, and stem extracts were scanned using a UV–Visible spectrophotometer within the wavelength range of 300– 700 nm. Formation of silver nanoparticles was confirmed by the appearance of characteristic Surface Plasmon Resonance (SPR) absorption peaks between 420 and 430 nm, corresponding to the collective oscillation of conduction electrons on the nanoparticle surface when irradiated with visible light. Differences in SPR peak positions among nanoparticles synthesized from different plant parts were interpreted as variations in nanoparticle size, morphology, concentration, and phytochemicalmediated stabilization.

PREPARATION OF MICROBIAL CULTURES

The antimicrobial activity of the synthesized silver nanoparticles was evaluated against one bacterial strain (Escherichia coli) and one fungal strain (Aspergillus niger). Pure cultures were obtained from the Department of Botany laboratory and maintained under sterile laboratory conditions. The bacterial culture was inoculated into sterile nutrient broth and incubated at 37°C for 18–24 hours to obtain actively growing cells. Similarly, the fungal culture was inoculated into potato dextrose broth and incubated at 28°C for approximately 48 hours to obtain fresh fungal growth suitable for antimicrobial testing.

PREPARATION OF CULTURE MEDIA

Two different microbiological media were used depending upon the test organism. Nutrient Agar (NA) was prepared for cultivation of Escherichia coli, while Potato Dextrose Agar (PDA) was prepared for cultivation of Aspergillus niger. The prepared media were sterilized by autoclaving at 121°C under 15 psi pressure for 30 minutes. After sterilization, the molten media were allowed to cool to approximately 45–50°C before being aseptically poured into sterile Petriplates inside an aseptic antimicrobial setup and were kept undisturbed to solidify completely before inoculation.

INOCULATION OF TEST MICROORGANISMS

Fresh microbial suspensions were prepared from actively growing cultures. Sterile cotton swabs were immersed in the respective microbial suspensions, and the organisms were uniformly spread over the entire agar surface to obtain a confluent lawn culture. Separate sets of Nutrient Agar plates were prepared for Escherichia coli, while Potato Dextrose Agar plates were prepared for Aspergillus niger.

EVALUATION OF ANTIMICROBIAL ACTIVITY

The antimicrobial activity of aqueous plant extracts and their corresponding silver nanoparticles was evaluated using two standard microbiological techniques.

1) **Agar Well Diffusion Method:** A sterile cork borer was used to punch 6 mm diameter wells into the inoculated agar plates. Each well was filled aseptically with 50µL of the respective test sample, Leaf aqueous extract, Petal aqueous extract, Stem aqueous extract, Leaf AgNP suspension, Petal AgNP suspension, Stem AgNP suspension. The plates were allowed to stand undisturbed for 30 minutes at room temperature to permit diffusion of the samples into the agar before incubation. Disc
2) **Diffusion Method:** Sterile Whatman filter paper discs were immersed separately in aqueous plant extracts and AgNP suspensions. Each disc absorbed approximately 20–30 µL of the test solution. The impregnated discs were dried under sterile conditions to remove excess liquid before being placed gently on the inoculated agar surface using sterile forceps. The inoculated plates containing sample discs were left undisturbed for 30 minutes to facilitate diffusion before incubation.

INCUBATION OF PLATES

The inoculated plates were incubated under optimum growth conditions according to the microbial species. Bacterial plates (E. coli) were incubated at 37°C for 24 hours. Fungal plates (A. niger) were incubated at 28°C for 48–72 hours. Following incubation, plates were examined for development of clear inhibition zones surrounding the wells and discs.

MEASUREMENT OF ANTIMICROBIAL ACTIVITY

Antimicrobial activity was determined separately for aqueous plant extracts and AgNP suspensions by measuring the diameter of the Zone of Inhibition (ZOI) around each well and disc using a transparent ruler. Measurements were recorded in millimetres (mm).

DATA ANALYSIS

The experimental observations were compiled and compared to evaluate the antimicrobial performance of different treatments. Comparative analysis included Antimicrobial activity of aqueous plant extracts versus silver nanoparticles, comparative efficacy of leaf-, petal-, and stem-derived AgNPs, performance of Agar Well Diffusion and Disc Diffusion methods, comparative antibacterial and antifungal activities against the selected microorganisms The results were interpreted on the basis of inhibition zone diameter, allowing assessment of the influence of nanoparticle synthesis on antimicrobial efficacy and identification of the most effective plant part for biosynthesis of silver nanoparticles.

RESULTS AND DISCUSSION

PREPARATION OF AQUEOUS EXTRACTS OF HIBISCUS ROSA-SINENSIS

The aqueous extracts prepared from the leaves, petals, and stems of Hibiscus rosasinensis served as the primary source of phytochemicals for the biosynthesis of silver nanoparticles. Aqueous extraction was selected because water is a non-toxic, environmentally friendly, and economical solvent capable of extracting a wide range of hydrophilic bioactive compounds.

BIOSYNTHESIS OF SILVER NANOPARTICLES

Successful biosynthesis of silver nanoparticles was observed following the addition of aqueous plant extracts to 1 mM silver nitrate solution. Within 30–60 minutes of incubation at room temperature, a gradual colour change from pale yellow to dark brown was observed in all reaction mixtures. This visual transformation represented the first indication of silver nanoparticle formation. Among the three plant parts investigated, the petal extract exhibited the fastest and most intense colour development, followed by the leaf extract, whereas the stem extract showed comparatively slower colour formation. This observation suggests that flower petals possess a greater concentration of reducing phytochemicals, particularly anthocyanins and flavonoids, which accelerate nanoparticle formation.

UV–VISIBLE SPECTROSCOPIC CHARACTERIZATION

The biosynthesized silver nanoparticles were characterized using UV–Visible spectroscopy to confirm nanoparticle formation. The synthesized nanoparticles exhibited characteristic Surface Plasmon Resonance (SPR) absorption peaks between

420 and 430 nm, confirming the successful formation of silver nanoparticles.

Table 1

Table 1 UV–Visible SPR Peaks of Biosynthesized Silver Nanoparticles

Plant Part	SPR Peak (nm)
Leaf AgNPs	425
Petal AgNPs	430
Stem AgNPs	420

The slight variation observed among leaf, petal, and stem-derived nanoparticles can be attributed to differences in phytochemical composition. Flower petals contain abundant anthocyanins and flavonoids, which may produce nanoparticles differing slightly in size and capping characteristics from those synthesized using leaves or stems. Similarly, the relatively lower SPR wavelength observed for stem-derived nanoparticles may indicate differences in particle morphology or stabilization efficiency.

EVALUATION OF ANTIMICROBIAL ACTIVITY

The antimicrobial activity of aqueous plant extracts and their corresponding silver nanoparticles was evaluated against the bacterial pathogen Escherichia coli and the fungal pathogen Aspergillus niger using both Agar Well Diffusion and Disc Diffusion techniques. Clear zones of inhibition developed around wells and discs containing the synthesized AgNPs, indicating effective suppression of microbial growth. In contrast, the aqueous plant extracts produced comparatively smaller inhibition zones,

demonstrating lower antimicrobial efficacy than their nanoparticle counterparts. These observations indicate that the conversion of crude plant extracts into silver nanoparticles significantly enhanced antimicrobial activity. The increased effectiveness of AgNPs can be attributed to their nanoscale dimensions, larger surface-area-to-volume ratio, and improved interaction with microbial cells. Nanoparticles are capable of penetrating microbial cell walls more efficiently than phytochemicals present in crude extracts, thereby increasing intracellular accumulation and enhancing antimicrobial action.

COMPARATIVE ANTIMICROBIAL ACTIVITY OF DIFFERENT PLANT PARTS

A comparison of silver nanoparticles synthesized from different plant parts revealed considerable variation in antimicrobial efficacy. The observed order of antimicrobial activity was: Petal AgNPs > Leaf AgNPs > Stem AgNPs. Petal-derived silver nanoparticles consistently produced the largest inhibition zones against both Escherichia coli and Aspergillus niger, indicating superior antimicrobial performance. Leaf-derived nanoparticles exhibited moderate activity, whereas stem-derived nanoparticles showed comparatively smaller inhibition zones.

ANTIBACTERIAL ACTIVITY AGAINST ESCHERICHIA COLI

The antibacterial activity of the aqueous extracts and their corresponding silver nanoparticles synthesized from different parts of Hibiscus rosa-sinensis was evaluated against Escherichia coli using Nutrient Agar (NA) by both Agar Well Diffusion and Disc Diffusion methods. Distinct zones of inhibition were observed around the wells and discs containing AgNP suspensions, indicating effective inhibition of bacterial growth. Among all the treatments, silver nanoparticles exhibited significantly greater antibacterial activity than the corresponding aqueous plant extracts. The enhanced antibacterial activity of AgNPs demonstrates that the green synthesis process substantially improved the biological efficacy of the plant extracts. The nanoscale dimensions of AgNPs increase their surface-area-to-volume ratio, enabling greater interaction with bacterial cell walls and facilitating penetration into the cells.

Table 2

Sample	Agar well (mm)	Disc diffusion (mm)
Leaf Extract	5.5	3.0
Leaf AgNPs	9.0	6.0
Petal Extract	9.5	6.5
Petal AgNPs	12	8
Stem Extract	4.5	2.5
Stem AgNPs	7.5	4.5

ANTIFUNGAL ACTIVITY AGAINST ASPERGILLUS NIGER

The antifungal activity of the synthesized silver nanoparticles was evaluated against Aspergillus niger using Potato Dextrose Agar (PDA). Similar to the antibacterial assay, inhibition zones were observed around wells and discs containing AgNP suspensions, confirming their antifungal potential. Although all AgNP preparations inhibited fungal growth, the inhibition zones were comparatively smaller than those observed against E. coli. Dense fungal growth remained visible on several PDA plates, indicating that A. niger was relatively less susceptible to the synthesized nanoparticles than the bacterial strain. Fungal cell walls are thicker and more complex, consisting primarily of chitin, glucans, mannoproteins, and other polysaccharides that provide greater mechanical strength and act as barriers to nanoparticle penetration. Despite this reduced susceptibility, AgNP-treated plates consistently exhibited larger inhibition zones than plates treated with aqueous plant extracts. The results clearly indicate that nanoparticle synthesis enhanced the antifungal activity of Hibiscus rosasinensis extracts. As observed in the antibacterial assay, petal-derived AgNPs demonstrated the highest antifungal activity, followed by leaf-derived and stemderived nanoparticles. The superior performance of petal-derived nanoparticles further supports the role of phytochemical composition in determining nanoparticle synthesis efficiency and antimicrobial potential.

Table 3

Sample	Agar well (mm)	Disc diffusion (mm)
Leaf Extract	3.5	2.5
Leaf AgNPs	6.0	4.0
Petal Extract	7.5	5.0
Petal AgNPs	8.5	6.0

Stem Extract	3.5	2.0
Stem AgNPs	6.0	4.0

COMPARISON OF AGAR WELL DIFFUSION AND DISC DIFFUSION METHODS

The Agar Well Diffusion method consistently produced larger inhibition zones than the Disc Diffusion method for both bacterial and fungal cultures. This difference is primarily attributed to the larger volume of liquid sample introduced into the agar wells and the unrestricted diffusion of nanoparticles into the surrounding medium. In contrast, filter paper discs absorb only limited quantities of sample. Furthermore, adsorption of nanoparticles onto the filter paper may partially restrict their diffusion into the agar resulting in comparatively smaller inhibition zones.

The results clearly demonstrate that plant-mediated silver nanoparticles possess substantially greater antimicrobial activity than crude aqueous plant extracts. This enhanced activity results from the combined effects of nanoscale particle size, increased surface-area-to-volume ratio, efficient penetration of microbial cells, controlled release of silver ions, generation of reactive oxygen species, and synergistic interaction between silver nanoparticles and phytochemicals present on the nanoparticle surface. Among the three plant parts investigated, petals proved to be the most efficient source for nanoparticle synthesis and antimicrobial activity. The high concentration of flavonoids, anthocyanins, phenolic compounds, tannins, and antioxidant metabolites in flower petals appears to facilitate rapid reduction of silver ions and formation of highly stable nanoparticles with superior biological activity.

The comparative evaluation of bacterial and fungal pathogens further demonstrated that Escherichia coli was more susceptible than Aspergillus niger. This difference can be attributed to variations in cell wall architecture, permeability, and physiological characteristics between bacteria and fungi.

CONCLUSION

Comparative antimicrobial evaluation revealed that the synthesized silver nanoparticles exhibited significantly greater antibacterial and antifungal activity than the corresponding aqueous plant extracts. Among the three plant parts investigated, petal-derived silver nanoparticles produced the highest antimicrobial activity, followed by leaf-derived and stem-derived nanoparticles. These differences are attributed to variations in phytochemical composition, particularly the higher concentrations of flavonoids, anthocyanins, and phenolic compounds present in flower petals.

The antibacterial activity against Escherichia coli was greater than the antifungal activity against Aspergillus niger, indicating higher susceptibility of the bacterial strain to the synthesized nanoparticles. Furthermore, the Agar Well Diffusion method consistently produced larger inhibition zones than the Disc Diffusion method, demonstrating superior suitability for evaluating liquid nanoparticle suspensions.

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