

Research Article

Unlocking the Medicinal Potential of *Sarcococca saligna*: Green Synthesis of Silver and Gold Nanoparticles for Enhanced Antibacterial and Antifungal Applications

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Abstract | A notable advancement in nanotechnology is the eco-friendly and cost-efficient production of metal nanoparticles utilizing botanical extracts. Silver nanoparticles are particularly noteworthy due to their potent antibacterial properties and relevance in nanomedicine. This study presents a green synthesis method using *Sarcococca saligna* leaf extracts to successfully produce both silver and gold nanoparticles. The formation and stability of the nanoparticles were verified through a change in physical colour and characterized using Ultraviolet-Visible spectroscopy and Scanning Electron Microscopy. Antibacterial and antifungal activity assessments revealed moderate efficacy against *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli*, comparable to Amoxicillin and Fluconazole. Further discussion explores the antibacterial effects of Ag NPs, highlighting their potential applications in combating bacterial infections. These findings underscore the significance of green synthesis methods in nanoparticle production and their potential roles in various fields, including medicine and biotechnology.

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Keywords | Medicinal plants, *Sarcococca saligna*, Antibacterial assay, Antifungal assay, Silver nanoparticles, Gold nanoparticles



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Introduction

Medicinal plants hold a significant place in the natural world, serving as crucial resources that offer manifold benefits to humanity. They are

pivotal not only in providing sustenance but also in the treatment of diverse ailments in rural regions worldwide. The medicinal efficacy of plants can be attributed to the diverse array of phytochemicals they contain, with each phytochemical playing a crucial role

in combating various diseases. While the historical use of traditional remedies and raw botanical substances is well-documented, there remains a pressing need for systematic scientific examination of medicinal plants to validate their local applications, thereby enabling a more refined and effective utilization of these valuable natural resources (Rehman *et al.*, 2019).

The fast-evolving discipline of nanotechnology is centered on the production of innovative materials at the nanoscale. Nanotechnology has developed into an interdisciplinary field in the twenty-first century. The biosynthesis of metal nanoparticles is a good illustration. Biosynthetic nanotechnology is proving to be highly advantageous for several industries, including food and feed, healthcare, biomedical science, personal care products, chemical and pharmaceutical industries. A broad range of unique physicochemical features and a wide range of possible applications, including material science and biomedical applications, are produced by reduction in material size (Pillai *et al.*, 2020). The main advantage of gold nanoparticles over other materials is their ease of preparation through organic transformation under safer conditions (Patil *et al.*, 2023). The utilization of plant extract in the biosynthesis reaction is a significant area of nanoparticle biosynthesis. Green synthesis offers an advantage over chemical and physical processes since it is more economical, safe for the environment, can be efficiently synthesized for large scale production, and doesn't involve any dangerous chemicals, high pressure, energy, or any temperature risks (Veerasamy *et al.*, 2011).

Among the many nanomaterials that have been developed are silver nanoparticles (Ag NPs), which have the most powerful ability to fight against several pathogenic microbes. Other plant-based nanomaterials include copper, zinc, titanium, magnesium, gold nanoparticles. In the context of nanotechnology and nanomedicine, Ag NPs are particularly important (Logeswari *et al.*, 2015). Medicinal plants are rich in bioactive substances including tannins, phenolic compounds, and alkaloids etc., may be able to expedite the environmentally favorable conventional biosynthetic direction that converts metal ions into pharmacologically active nanoparticles (Habeeb *et al.*, 2022). In a research study, the purified compound apiin was obtained from henna leaves was employed at ordinary conditions applicable for synthesis of silver nanoparticles (Ag NPs, as

described by (Kasthuri *et al.*, 2009). Additionally, within an aqueous solution under similar ambient conditions, extracts of *Camellia sinensis* (Common green tea) were utilized which act as both reducing agent and a stabilizer to produce gold nanoparticles (Au NPs) as well as silver nanoparticles (Ag NPs) according to the findings of (Vilchis-Nestor *et al.*, 2008). Ag NP has also been synthesized using different plant extracts like lemongrass (*Cymbopogon citratus*), leaves of geranium (*Pelargonium graveolens*) and alfalfa (*Medicago sativa*) as well as many other plants (Gardea-Torresdey *et al.*, 2003; Shankar *et al.*, 2003).

Materials and Methods

Green synthesis of metallic nanoparticles

Preparation of samples: Silver and gold nanoparticles were synthesized from crude extracts of leaves of *Sarcococca saligna* (Christmas box or sweet box). The leaves were shade dried and powdered. 10 gm of fine powdered plant material was macerated in 50 ml of distilled water for about 6 h at 40°C of temperature. The extract was employed for filtration twice and concentrated using vacuum rotary evaporator at 45°C (Anwar *et al.*, 2019).

Synthesis of silver nanoparticles (Ag NPs)

The procedure followed with slight modifications for application of synthesis of Ag NPs which was given by (Veerasamy *et al.*, 2011). To produce Ag NPs, a 1mM solution of AgNO₃ was produced in deionized water. 95 ml of a 1 mM AgNO₃ solution was conjoined with 5 ml of aqueous solution of plant extracts in a beaker. It was heated for one hour at 75 °C in a water bath. The mixture's color changed from the plant extract solution's initial shade to a dark brown hue when silver nitrate was reduced to silver ions. Ag NPs were decreased in solution, and it was centrifuged for 15 minutes at 15,000 rpm. To get rid of any unattached particles, the solid nanoparticles were rinsed three times. The supernatant layer was carefully discarded and separated. By freeze-drying, the gold and Ag NPs were turned into a powder. The pelleted solid nanoparticles were re-dispersed in deionized water (Logeswari *et al.*, 2012; Rodríguez-León *et al.*, 2013; Veerasamy *et al.*, 2011).

Synthesis of gold nanoparticles (Au NPs)

To convert Au³⁺ ions to Au⁰, 5 ml of plant extract needed to be added to 95 ml of a 1 mM Gold (III)

chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) solution, that had to be constantly stirred for one minute at room temperature. The immediate colour changes in solution from the natural tint of the plant extracts to a deep ruby red indicated the initial successful synthesis of Au NPs. To concentrate the particles, the solution containing the Au NPs was centrifuged for 15 minutes at 15,000 revolutions per minute. After carefully removing the supernatant of liquid, the solid nanoparticles were rinsed three times to remove any loose particles. By freeze-drying, the gold and Ag NPs were turned into a powder. The pelleted solid nanoparticles were re-dispersed in deionized water (Chandran *et al.*, 2006; Philip, 2010).

Characterization

Using a UV-Visible Spectrophotometer (UV-1700 by Shimadzu) and samples in quartz cuvettes, the UV visible spectra were obtained. De-ionized water was used to dilute the colloidal solutions (1 ml) containing suspended silver and gold nanoparticles before they were observed at room temperature. UV-visible spectrophotometer was employed to study how silver ions are reduced. The spectrophotometer had a resolution of 1 nanometer and could detect light in the wavelength range of 200 to 800 nanometers. The scanning rate was 300 nm/min. The highest absorbance was found at 408.88 nm for silver and 737 nm for gold. Scanning Electron Microscopy (SEM) was used to evaluate the nanoparticles' further characterization. Images were captured using various resolution powers between 60000 and 100000 kv. (Banerjee *et al.*, 2014).

Anti-bacterial and anti-fungal activity

The "wells" method of agar diffusion was employed to examine the effects of synthesized silver and gold nanoparticles on bacterial and fungal pathogenic strains. Petri plates were filled with two layers of dense nutritional material (nutrient agar media). In the lower layer, unseeded media was used. Thin-walled stainless-steel cylinders were installed on the lowest layer. The standardization of "well" studies of the agar diffusion was ensured by utilizing a 6 mm "well" diameter and a 10 mm medium thickness. The top layer of the cylinders was filled with sterile, melted, and 40°C cooled agar material. This medium included the gram-positive bacterial strains of *Staphylococcus aureus* (ATCC 6538), and gram-negative bacteria *Escherichia coli* (ATCC 25922) as well as fungal strains of *Candida albicans* (ATCC 885-653), which

were the comparable standard of the daily test culture (fresh cultured microbes on daily basis to be used in the experiment). These bacterial and fungal strains were collected from Veterinary Research Institute (VRI) Abbottabad, Pakistan. Once the top layer had hardened, the cylinders were removed using sterile tweezers, and 60 μl of silver and gold nanoparticles solutions was added to the wells. Prior to incubation, the inoculated agar plates were allowed to sit at room temperature for 30 to 40 minutes to dry. Following this, they were placed in a thermostat-controlled incubator set at 37°C for 24 hours. To gauge the antibacterial activity of samples and standard drugs, the inhibition zone was measured in millimeters using a measuring scale from one edge of the disk to the other (Budniak *et al.*, 2021).

Results and Discussion

UV-visible spectroscopy

The UV-visible absorption spectra of metallic nanoparticles show a prominent surface plasmon resonance peak (SPR). The SPR peak is a result of the collective oscillation of free electrons within metallic nanoparticles when exposed to incoming light, which then propagates across the nanoparticles. The determination of the position and intensity of the SPR peak is dependent upon the specific configuration, composition, and size of the nanoparticles.

The current study involved the assessment of the UV-visible absorption spectra of both silver and gold nanoparticles. The spectral response of silver nanoparticles was observed at a wavelength of 408.88 nm, as represented in Figure 1. In contrast, the SPR peak of gold nanoparticles was observed at a wavelength of 737 nm, as shown in Figure 2.

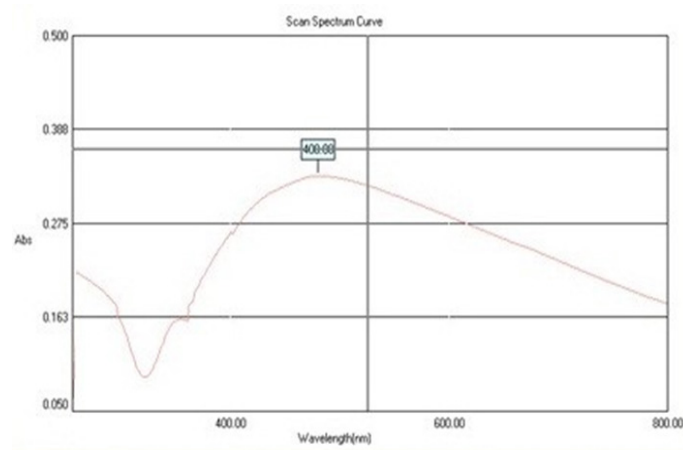


Figure 1: UV-Visible spectra of Ag NPs.

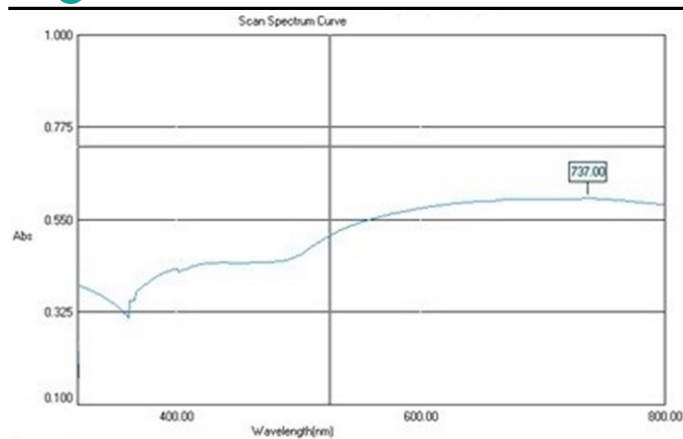


Figure 2: UV-Visible spectra of gold nanoparticles.

Scanning electron microscopy

One of the most important instruments for a thorough examination of metallic nanoparticles is scanning electron microscopy (SEM), which allows for an accurate assessment of the size and shape of the nanoparticles. Using SEM images, the size and shapes of the gold and silver nanoparticles were examined in the present study. SEM pictures (Figure 3) revealed the nanoparticles to be spherical and of varying sizes. The average size of silver nanoparticles was determined to be 10.6 nm at a resolution of 15 kV x 100,000, whereas at a resolution of 15 kV x 60,000, the average size was 32.8 nm, the smallest size was 21.6 nm, and the maximum size was 48.4 nm. The average size of the gold nanoparticles was also determined to be 12.8 nm at the highest resolution of 15 kV x 100,000, the minimum size was recorded to be 14.4 nm and maximum size is 28.8 nm. The average size of Au NPs at a resolution of (15 kv x 60, 000) was 18.1 nm, its minimum size is 16 nm and maximum size is 47.8 nm. Overall, the UV and SEM analyses confirm the successful harvesting of green silver and gold nanoparticles. The nanoparticles have a range of sizes and spherical in shape.

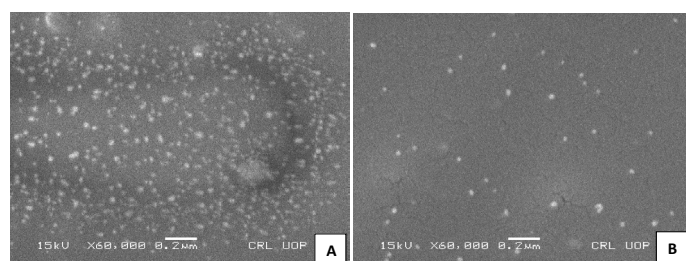


Figure 3: (A) SEM image of silver nanoparticles (B) SEM image of gold nanoparticles.

Antimicrobial assay

The effectiveness of silver and gold nanoparticles as antibacterial agents against gram-positive

(*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacterial strains was analyzed and presented in Table 1. The findings of the samples were compared with the results given by Amoxicillin being a standard antibiotic drug. The clear zones devoid of bacterial growth surrounding the antimicrobial disks, referred to as zones of inhibition (ZOI), were measured as follows: When it came to *E. coli* and *S. aureus*, Ag NPs showed ZOI values of 15.0±0.5 mm and 19.0±0.8 mm, respectively, whereas Au NPs showed ZOI values of 16±0.12 mm and 18±1 mm, correspondingly. Comparatively, Amoxicillin showed higher ZOI values of 29±0.2 mm against *S. aureus* and 25±0.9 mm against *E. coli*. The discernible disparity in ZOI values underscores that both silver and Au NPs possess a moderate antibacterial effect against the tested pathogenic microorganisms.

Table 1: Antibacterial activity of silver and gold nanoparticles.

Sr. No.	Bacterial strain	Zone of inhibition		
		Sample 1	Sample 2	Standard
1	<i>Escherichia coli</i>	15.0±0.5	16±0.12	25.0±0.9
2	<i>Staphylococcus aureus</i>	19.0±0.8	18±1	29.0±0.2

Sample 1=Silver nanoparticles, sample 2=gold nanoparticles, sample 3= Standard drug (Amoxicillin).

Table 2: Antifungal activity of silver and gold nanoparticles.

Sr. No.	Fungal strain	Zone of inhibition		
		Sample 1	Sample 2	Standard
1	<i>Candida albicans</i>	18.0±0.8	14±0.12	24.0±2.0

Sample 1=Silver nanoparticles, sample 2= gold nanoparticles, sample 3= Standard drug (Fluconazole).

Based on our research findings *Candida albicans* was subjected to testing against both Ag and Au NPs, with subsequent measurement of the respective zones of inhibition (Table 2). The findings showed that the ZOI for Ag NPs was 18.0±0.8 mm while the ZOI for Au NPs was 14±0.12 mm. These results were then contrasted with the ZOI produced by Fluconazole, the typical antifungal drug, which revealed a ZOI of 24.0±2.0 mm. These results open new possibilities for research into antifungal treatment by shedding light on the antifungal properties of silver and Au NPs in relation to a widely recognized pharmaceutical drug.

In the realm of research articles, the green synthesis of silver and gold nanoparticles is a promising way to

produce these materials using a green nano synthesis approach as highlighted in the study by Rizki and Klaypradit (2023). This approach involves employing plant extracts as both reducing and capping agents in lieu of potentially harmful chemicals, rendering the entire process more ecologically responsible. The literature has already documented instances of green synthesis for silver and gold nanoparticles, utilizing a variety of plant extracts (Vanlalveni *et al.*, 2021).

A UV-Vis spectrophotometer makes it simple to monitor the bio reduction of aqueous Ag⁺ and AuCl₄⁻ ions. The distinctive feature in the optical absorption spectra of metal nanoparticles is the surface plasmon resonance (SPR) band, which arises due to the collective vibration of electrons on the nanoparticles' surface when they interact with light (Nagajyothi *et al.*, 2012). In this study, we determined that the SPR peak for silver nanoparticles was observed at 408.88nm, while the corresponding peak for gold nanoparticles was identified at approximately 737nm. The green silver and gold nanoparticles created in the current work are compatible with the reported values in the published literature based on their UV-visible absorption spectra and SEM pictures. The SEM images vividly illustrate the spherical morphology and the diversity in nanoparticle sizes, as reported in prior studies (Maarebia *et al.*, 2019; Wei *et al.*, 2010). The average size of the silver nanoparticles was quantified at 10.6 nm, while the gold nanoparticles exhibited an average size of 12.8 nm. These findings align with the size parameters for silver and gold nanoparticles documented in the scientific literature using other green manufacturing techniques (Qu *et al.*, 2009; Sharma *et al.*, 2023).

In a research study that was carried out in 2010, Jones and Hoek investigated the antimicrobial properties of Ag NPs that were synthesized using a leaf extract from *Ocimum gratissimum* (also known as African basil) against a panel of five different bacterial strains, included two gram-negative species (*K. pneumoniae* and *E. coli*) and three gram-positive species (*S. aureus*, *B. subtilis* and *M. luteus*). According to the findings of this study, Ag NPs demonstrated dose dependent antibacterial response and results were more significant against gram-negative strains than gram-positive. The reason may be that gram-positive bacteria have stronger cell walls than gram-negative bacteria (Marambio-Jones and Hoek, 2010). It is believed that several mechanisms contribute to

nanoparticles' antibacterial effects. Because they are positively charged, Ag NPs can attach to negatively charged bacterial cell membranes. The binding of molecules to cell membranes can change the physical and chemical properties of the membranes, impairing the osmoregulation, permeability, and respiratory processes in the cell. Ag NPs can generate free radicals that harm bacterial cell membranes, allowing lipopolysaccharides and membrane proteins to leak out of the bacteria. One of the possible methods by which Ag NPs exert their bactericidal effect is this membrane damage (Samrot *et al.*, 2018). In addition to the fact that once within the cell, the nanoparticles would change the tyrosine phosphorylation of putative peptide substrates required for cell viability and division (Mubarak *et al.*, 2011). Because Ag NPs have a staggeringly huge surface region that considers better contact with microscopic organisms, i.e., bacteria, they have demonstrated effective antibacterial power when compared to other salts. After penetrating the bacterial cell wall, the nanoparticles adhere to it. Ag NPs interact with the bacterial membrane's phosphorus and contain sulfur proteins as well as the cell's DNA (Sondi and Salopek-Sondi, 2004). Ag NPs generate a zone characterized by low molecular weight within the bacterial core, serving as a protective barrier for the DNA against the infiltration of silver ions. As cell demise primarily arises from the process of cell division, the most effective target for these nanoparticles appears to be the respiratory chain. To enhance their bactericidal impact, these nanoparticles release silver ions, which subsequently amass within bacterial cells, as elucidated in a study conducted by (Logeswari *et al.*, 2015).

Numerous studies have provided evidence of nanoparticle synthesis facilitated by the utilization of plant extracts as catalytic agents. By employing a purified apiin compound, derived from henna (*Lawsonia inermis*) leaves under ambient conditions, silver nanoparticles with quasi-spherical morphology were synthesized. The *C. sinensis* extract, under normal conditions, facilitated the synthesis of both silver and gold nanostructures in aqueous solutions. Eco-friendly reactants sourced from living alfalfa, lemongrass broths, geranium leaves, and various other natural origins have been employed in the synthesis of AgNPs (Vilchis-Nestor *et al.*, 2008).

Conclusions and Recommendations

Using plant extracts to make silver and gold nanoparticles is a green and sustainable way to produce these valuable materials. nanoparticles present a promising avenue for the development of durable nanomaterials with potential antibacterial and antifungal attributes. Employing UV-visible spectroscopy and SEM, this study effectively characterized the nanoparticles, aligning with previously documented values in the literature. Furthermore, the noteworthy antimicrobial effects of these nanoparticles against diverse pathogens suggest their potential utility in advancing novel and more efficacious antimicrobial treatments.

The goal of future research will be to further investigate the possible uses of the nanoparticles and optimize the green synthesis process. Furthermore, the nanoparticles' toxicity and biocompatibility will be assessed.

Novelty Statement

This study is a unique combination of botany and nanotechnology. It introduces an innovative approach in nanotechnology by employing the leaves extracts of *Sarcococca saligna* for the synthesis of silver and gold nanoparticles. These nanoparticles possess potent antibacterial and antifungal properties against pathogens such as *Candida albicans*, *Staphylococcus aureus*, and *Escherichia coli*. This innovative approach not only contributes to the field of nanomedicine but also paves the way for sustainable solutions in combating microbial infections.

Author's Contribution

Sabi Ur Rehman: Collected plant material, worked on the synthesis of gold and silver nanoparticles as well as corresponding role.

Laiba Arshad: Worked on the extraction of plant material and wrote the introduction part.

Saman Ali: Worked on the antibacterial and antifungal assay and writing discussion part.

Shazma Massey: Methodology and overall management of the article.

Safeer Khan: Wrote the abstract and analyze the findings of the study.

Abdul Samad: Worked on data collection and data

analysis.

Fazal-Ur-Rehman: Conceived the idea and supervised the research work.

Conflict of interest

The authors have declared no conflict of interest.

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