Research Article

Synthesis, Characterization and Antibacterial Activity of Silver and Gold Nanopartical by Using *Isatis costatal* L. Flovoniod Extract

Nazim Ullah[1], Abdul Basit[1*], Alia Gul[2*], Irshad Ali Khan[3], Abdur Rehman[4], Noshin Shafqat[5], Attaullah[2] and Asma[2]

[1]Deportment of Botany, Islamia College, Peshawar, Khyber Pakhtunkhwa, Pakistan; [2]Department of Botany, Hazara University, Mansehra 21300, Pakistan; [3]Departmnet of Agriculture, The University of Swabi, Khyber Pakhtunkhwa, Pakistan; [4]Department of Entomology Agriculture University, Peshawar, Khyber Pakhtunkhwa, Pakistan; [5]Department of Agriculture, Hazara University, Mansehra 21300, Pakistan.

Abstract | Nanotechnology is a rapidly advancing field, with nanoparticles (1-100 nm) playing a crucial role in various disciplines such as medical chemistry, atomic physics, and material science. These nanoparticles exhibit unique properties due to their small size, which can enhance or alter the performance of materials they interact with. They can be synthesized using chemical, physical, or biological methods, with biological synthesis emerging as the most promising due to its eco-friendly, cost-effective, and efficient nature. This study explores the green synthesis of silver and gold nanoparticles using the crude flavonoid extract of Isatis costata. The plant extract serves as both a reducing and capping agent. The nanoparticles were characterized using UV-visible spectrophotometry and scanning electron microscopy (SEM). The formation of silver and gold nanoparticles was confirmed by a color change in the solution, with SEM analysis revealing uniformly dispersed, mostly spherical particles, slightly larger than expected. UV-visible spectra showed a surface plasmon resonance (SPR) peak between 200-250 nm. The antimicrobial activity of the nanoparticles was tested against several human pathogens, including *E. coli, S. aureus, P. aeruginosa, S. typhi*, and *B. subtilis.* Silver nanoparticles exhibited strong inhibitory effects, with the zone of inhibition increasing as the concentration of nanoparticles and flavonoid extract increased (3, 6, 9, and 12 µl). The results indicate that silver and gold nanoparticles synthesized with Isatis costata extract possess significant antibacterial properties. Based on these findings, these nanoparticles have potential applications in pharmaceuticals, particularly for combating bacterial infections.

Received | January 29, 2024; **Accepted** | May 23, 2025; **Published** | August 24, 2025
***Correspondence** | Abdul Basit and Alia Gul, Deportment of Botany, Islamia College, Peshawar, Khyber Pakhtunkhwa, Pakistan; Department of Botany, Hazara University, Mansehra 21300, Pakistan; **Email:** abdulbasiticp@gmail.com, aliagulbotanist@gmail.com
Citation | Ullah, N., A. Basit, A. Gul, I.A. Khan, A. Rehman, N. Shafqat, Attaullah and Asma. 2025. Synthesis, characterization and antibacterial activity of silver and gold nanopartical by using *Isatis costatal* L. flovoniod extract. *Sarhad Journal of Agriculture,* 41(3): 1255-1265.
DOI | https://dx.doi.org/10.17582/journal.sja/2025/41.3.1255.1265
Keywords | *Isatis*, Ecofriendly, Nanotechnology, Green synthesis NPs (nanoparticles)

Introduction

Nanoparticles have emerged as transformative entities in the realms of biomedicine and industry, captivating the attention of researchers and practitioners alike (Podutwar *et al*., 2024). The rapid advancement of nanotechnology has propelled nanoparticles to the forefront of scientific inquiry, driven by their unique properties and diverse applications. However, amidst the excitement surrounding their potential, concerns loom regarding the environmental and biological implications of nanoparticle interactions. This necessitates a comprehensive understanding of their behavior, toxicity profiles, and impact on living organisms (Carter *et al*., 2023). At the heart of nanotechnology lies the manipulation of matter at the Nanoscale, typically ranging from 1 to 100 nanometers. This scale introduces a plethora of unique phenomena and properties, distinct from those observed in bulk materials. Nanoparticles, characterized by their minuscule size and large surface area-to-volume ratio, exhibit heightened surface energy, spatial confinement, and reduced imperfections. These attributes underpin their remarkable functionalities and set the stage for groundbreaking applications across various disciplines (Ogochukwu *et al*., 2024).

In biomedical research, nanoparticles have emerged as indispensable tools, owing to their exclusive properties and compatibility with biological systems. They exhibit an innate affinity for biological molecules, facilitating controlled assembly processes for metal nanoparticle synthesis (Parashar *et al*., 2022). This controlled assembly not only ensures the reliability of synthesis methods but also aligns with eco-friendly practices, offering sustainable solutions for biomedical applications. Nanoparticles have revolutionized drug delivery systems, enabling controlled release of therapeutics and enhancing efficacy in treating various diseases (Feng *et al*., 2023). Gold and silver nanoparticles, in particular, have garnered significant attention for their therapeutic potential and diagnostic applications. Gold nanoparticles serve as carriers in thermotherapy, leveraging their unique properties for targeted delivery of therapeutic agents. Similarly, silver nanoparticles exhibit antimicrobial activity, making them invaluable in wound healing and combating infectious diseases (Paladini and Pollini, 2019). Their versatility extends to molecular imaging and diagnostics, where they serve as sensitive probes for targeting biological entities and imaging cellular structures (Shahalaei *et al*., 2024).

Despite their promise, the widespread adoption of nanoparticles necessitates a thorough understanding of their biocompatibility and toxicity profiles. Silver nanoparticles, for instance, exhibit increased antibacterial activities and potential wound treatment capabilities. However, their impact on biological systems requires comprehensive evaluation to mitigate potential risks (Chinnathambi *et al*., 2023). Moreover, silver nanoparticles hold promise as drug delivery systems, offering targeted delivery of therapeutics for various medical conditions, including cancer (Habeeb *et al*., 2022). Silver and gold nanoparticles hold immense significance in various fields, particularly in biomedicine and nanotechnology, owing to their unique properties and versatile applications. Silver nanoparticles, for instance, exhibit potent antimicrobial activity, making them invaluable in wound healing, infection control, and medical device coatings (Alsareii *et al*., 2022). Their ability to combat microbial contamination addresses a critical global health concern, offering promising solutions for mitigating infectious diseases and reducing healthcare-associated infections. Their biocompatibility and tunable surface properties make them ideal candidates for developing novel drug delivery platforms with enhanced therapeutic efficacy and reduced side effects. Additionally, silver nanoparticles possess optical characteristics that lend themselves to various imaging applications, facilitating precise localization and visualization of biological targets (Hang *et al*., 2024). Gold nanoparticles, on the other hand, serve as carriers in thermal therapy for biological targets, leveraging their unique photothermal properties to selectively destroy cancer cells while sparing healthy tissue (Sakhti *et al*., 2022). This targeted approach minimizes collateral damage and improves patient outcomes in cancer treatment. Furthermore, gold nanoparticles play a crucial role in molecular imaging and diagnostics, offering sensitive detection and visualization of biological molecules, cells, tissues, and tumors. Their high surface area-to-volume ratio and customizable surface chemistry enable precise tuning of optical properties for specific imaging modalities (Habeeb *et al*., 2022; Alsareii *et al*., 2022).

In the pursuit of environmentally sustainable nanoparticle synthesis methods, biological approaches have emerged as viable alternatives to conventional chemical methods. These methods, often referred to as green synthesis, harness the inherent capabilities of microorganisms, herbal extracts, and biopolymers for nanoparticle synthesis (Soni *et al*., 2024). Green synthesis offers distinct advantages, including reduced toxicity and environmental impact compared to chemical methods. Furthermore, it enables precise control over nanoparticle size, shape, and composition, facilitating tailored synthesis for specific applications (Harish *et al*., 2023). The biosynthesis of nanoparticles involves a diverse array of biological entities, including bacteria, fungi, and plants. Bacteria, such as *Pseudomonas stutzeri*, have demonstrated the ability to synthesize silver nanoparticles through enzymatic processes (Shankar *et al*., 2025). Fungi, on the other hand, excel in nanoparticle synthesis due to their high binding capacity with metal ions in intracellular regions (Ghosh *et al*., 2021). The extracellular production of nanoparticles by fungi offers advantages in downstream processing, facilitating the isolation of enzymes for subsequent applications (Alsareii *et al*., 2022).

Plants, however, provide a particularly promising platform for nanoparticle synthesis, owing to their accessibility, abundance, and diverse biochemical composition. The use of plant extracts in nanoparticle synthesis offers several advantages, including the absence of toxic chemicals and natural capping agents. This not only reduces the cost associated with traditional synthesis methods but also enhances the feasibility of large-scale production (Adeyemi *et al*., 2022). Medicinal plants, in particular, have attracted attention for their potential in nanoparticle synthesis and pharmaceutical applications (Alharbi *et al*., 2022). Among the secondary metabolites present in plants, flavonoids stand out as potent agents for nanoparticle production. Flavonoids such as quercetin and kaempferol have been shown to facilitate the green synthesis of silver and gold nanoparticles (Nadaf *et al*., 2022). Their ability to mediate nanoparticle synthesis underscores the diverse biochemical capabilities of plants and highlights the potential for sustainable, eco-friendly approaches in nanotechnology (Arif *et al*., 2022). The synthesis of nanoparticles using plant extracts represents a convergence of traditional knowledge and modern scientific advancements. Plants have long been revered for their medicinal properties, and the utilization of plant-derived compounds in nanoparticle synthesis builds upon this rich legacy. Furthermore, the exploration of novel plant sources for nanoparticle synthesis offers opportunities for biodiversity conservation and sustainable development (Gogoi *et al*., 2021).

In the context of nanoparticle synthesis, *Isatis costata*, a medicinal plant belonging to the Brassicaceae family, holds significant promise for producing silver and gold nanoparticles with biomedical applications. *Isatis costata* exhibits unique morphological and medicinal properties, making it an attractive candidate for nanoparticle synthesis studies (Ashiq *et al*., 2021). The plant's annual or biennial herbaceous nature, coupled with its height, branching pattern, and leaf morphology, provides a suitable platform for extracting bioactive compounds conducive to nanoparticle synthesis (Kirillov *et al*., 2023).

The utilization of *Isatis costata* in nanoparticle synthesis aligns with the principles of green nanotechnology, emphasizing sustainable and eco-friendly approaches to material synthesis (Aswathi *et al*., 2023). By harnessing the plant's natural capping agents and bioactive constituents, researchers can develop cost-effective and environmentally benign methods for producing silver and gold nanoparticles (Adeyemi *et al*., 2022). This not only addresses the growing demand for environmentally clean synthesis protocols but also enhances the feasibility of large-scale nanoparticle production for biomedical applications. Furthermore, *Isatis costata*'s medicinal importance in treating gout (arthritis) underscores its therapeutic potential beyond nanoparticle synthesis (Ashiq *et al*., 2021). The plant's bioactive compounds, including flavonoids and secondary metabolites, contribute to its anti-inflammatory and analgesic properties, offering relief to individuals suffering from arthritic conditions (Nadaf *et al*., 2022). By leveraging *Isatis costata*'s medicinal properties in nanoparticle synthesis, researchers can develop multifunctional nanomaterials with enhanced therapeutic efficacy for treating inflammatory disorders and other medical conditions.

In summary, silver and gold nanoparticles represent versatile materials with diverse applications in biomedicine, nanotechnology, and materials science. *Isatis costata*, with its unique morphological and medicinal properties, serves as a promising candidate for producing nanoparticles with biomedical applications. By harnessing the plant's natural constituents and green synthesis methods, researchers can develop sustainable and cost-effective approaches to nanoparticle synthesis, paving the way for innovative solutions in healthcare and materials engineering.

Materials and Methods

Collection of plant materials and extract preparation

Fresh, mature leaves of Isatis costata were collected from the upper Dir district of Khyber Pakhtunkhwa (KPK), Pakistan, in May. The plant was identified as Isatis costata Linn. and a dried specimen was preserved in the herbarium of the Botany Department at Islamia College, Peshawar. The leaves were washed several times with tap water to remove dust and then air-dried under the sun to remove residual moisture. The dried leaves were ground into powder using a grinder machine. For the extraction, 1% of the powdered leaf material was mixed with deionized water in a 250 mL conical flask. The mixture was incubated for 30 minutes, followed by centrifugation at 5000 rpm for 30 minutes at room temperature. The supernatant was separated and filtered using filter paper. This solution was used for reducing silver ions (Ag^{+}) to silver nanoparticles (Ag^{0}) (Bao *et al*., 2011). A flavonoid stock solution for nanoparticle synthesis was prepared by dissolving 0.5 grams of the extract in 20 mL of water and mixing it on a stirring device until a dark brownish solution formed (Feng *et al*., 2023).

Alkali reagent test for identification and confirmation of flavonoids

The presence of flavonoids in the extract was confirmed using the alkali reagent test. A molar solution of NaOH was prepared, and a small amount of the extract was mixed with water and NaOH in a test tube. The solution was shaken well until a yellow color appeared, indicating the presence of flavonoids (Alharbi *et al*., 2022).

Silver nitrate stock solution ($AgNO_3$)

A molar solution of silver nitrate was prepared by dissolving 169.87 grams of $AgNO_3$ in 1000 mL of water. However, to reduce the cost, the quantity was adjusted by dissolving 1 gram of $AgNO_3$ in 5.7 mL of water to form a molar solution. A milli-molar solution was prepared by taking 31 µL of $AgNO_3$ solution and dissolving it in 969 µL of water using a micropipette (Kusumaningrum *et al*., 2018).

Synthesis of silver nanoparticles

Ten different concentrations of plant extract and $AgNO_3$ solutions were mixed in variable ratios (1:1, 1:2, 1:10) to synthesize silver nanoparticles. A color change from blue to pink was observed at a concentration ratio of 1:3, indicating the formation of silver nanoparticles (Habeeb *et al*., 2022). The nanoparticles were confirmed using UV-visible spectroscopy.

Gold chloride stock solution ($AuCl_3$)

A gold chloride stock solution was prepared by dissolving 1 gram of $AuCl_3$ in 3.3 mL of water. A milli-molar solution of $AuCl_3$ was prepared by mixing 31 µL of the molar solution with 969 µL of water using a micropipette (Ezeuko *et al*., 2022).

Synthesis of gold nanoparticles

Ten different concentrations of plant extract and $AuCl_3$ solutions were mixed in variable ratios (1:1, 1:2, 1:10). A color change from yellow to gold was observed at ratios of 1:3 and 1:4, indicating the formation of gold nanoparticles (Arif *et al*., 2022). Confirmation of the nanoparticles was done through UV-visible spectroscopy.

Characterization of silver and gold nanoparticles

UV-visible spectroscopy: The optical properties of the silver and gold nanoparticles were determined using a UV-visible spectrophotometer (MRL, Department of Physics, University of Peshawar) following the method prescribed earlier (Leyu *et al*., 2023).

SEM analysis

The morphological characteristics of the synthesized nanoparticles were analyzed utilizing a Scanning Electron Microscope (SEM) at the CRL, Department of Physics, University of Peshawar. The SEM analysis was performed according to the methodology outlined by Sher *et al*., (2022).

Antibacterial activity

Bacterial culture: The antibacterial activity was evaluated using 24-hours old broth cultures of five bacterial strains: *Escherichia coli*, *Salmonella enterica Typhi*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*. The bacterial strains were isolated from the laboratory at the Agricultural University of Peshawar. Bacteria were cultured in nutrient broth (E-media) at 37°C for 24 hours (Arjyal *et al*., 2020). The nutrient agar medium was prepared by dissolving 28 g of agar in 1000 mL of distilled water, followed by autoclaving the media and petri dishes at 120°C for 45 minutes.

Antibacterial test procedure

Bacterial strains were uniformly distributed on nutrient agar plates with a sterile spreader. Four wells were created in each petri dish, and varying concentrations of synthesized nanoparticles and flavonoid extract were applied to assess their antibacterial effects. The plates were incubated at 37°C for 24 hours, and the zone of inhibition was assessed post-incubation (Arya *et al*., 2019).

Experimental design and statistical analysis

The leaf samples were randomly collected from different plants in the study area to minimize bias. All experiments were conducted in triplicate (biological replications), and the results were presented as the mean±standard deviation (SD). Statistical analysis was performed using ANOVA followed by Tukey's post-hoc test for multiple comparisons, and a significance level of $p \leq 0.05$ was considered statistically significant.

Results

Reduction of silver and gold oxide into nanoparticles

The present study explores the eco-friendly and simple synthesis of silver and gold nanoparticles at room temperature, utilizing aqueous crude extracts (flavonoids) from *Isatis costata* aerial parts as reducing and stabilizing agents. Upon adding the aqueous flavonoid extract to a milli-molar solution of $AgNO_3$ and $AuCl_3$, color changes were observed: from yellow-brown to dark brown for silver nanoparticles (AgNPs) and from pink to reddish-brown or yellowish for gold nanoparticles. The color change occurred within minutes to hours, indicating the successful formation of nanoparticles, as depicted in Figure 1. Literature reports suggest that dark brown color signifies the synthesis of nanoparticles.

Figure 1: *Color changes were observed: from yellow-brown to dark brown for silver nanoparticles (AgNPs) and from pink to reddish-brown or yellowish for gold nanoparticles.*

UV-visible spectrophotometer analysis

The reduction of silver ions to silver nanoparticles, upon exposure to plant extracts, was confirmed by the color change, which is attributed to the surface plasmon resonance (SPR) phenomenon. The free electrons in metal nanoparticles interact with light waves, causing the SPR absorption bands. For silver nanoparticles, a sharp SPR peak was observed around 420 nm. UV-visible spectrophotometer analysis of the silver and gold nanoparticles synthesized using *Isatis costata* plant extract revealed that the SPR peaks for the reduced nanoparticles appeared in the range of 200-250 nm (Figure 2). The reduction of metal ions was rapid, with more than 90% of Ag^+ ions being reduced within four hours of adding the metal ions to the plant extract. Notably, the metal nanoparticles remained stable in solution for over a month post-synthesis.

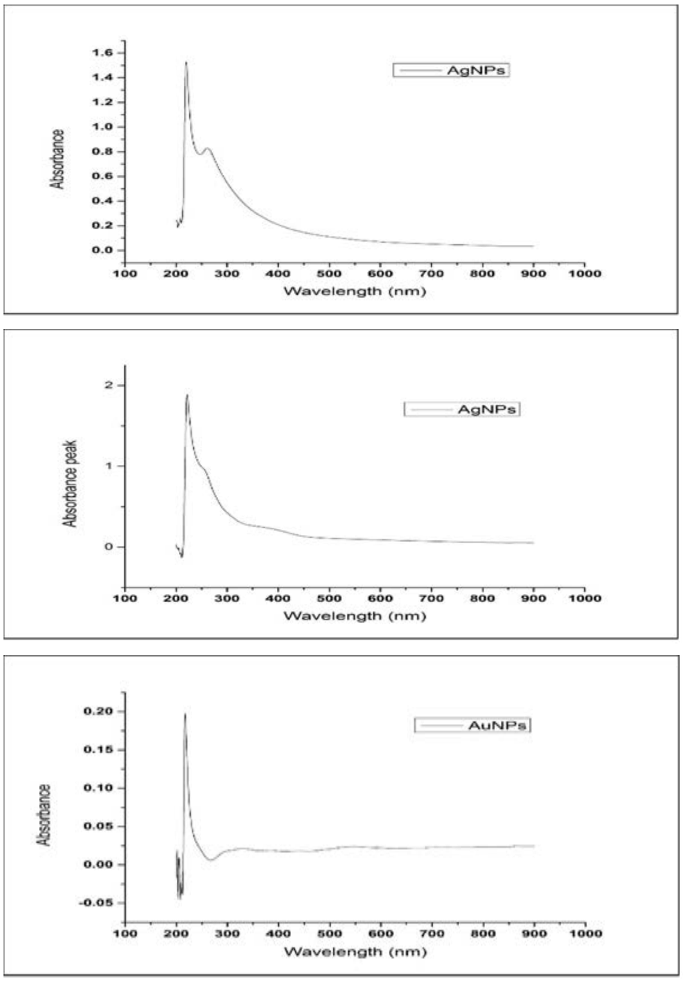

Figure 2: *UV-spectral analysis of gold and silver nanoparticles in visible region.*

Scaninining electronic microscopy (SEM) analysis

Scanning Electron Microscopy (SEM) was used to analyze the morphology and size distribution of the synthesized silver and gold nanoparticles. The SEM images showed that the nanoparticles were uniformly dispersed within the flavonoid extract, with a predominantly spherical morphology. The particles exhibited a fairly narrow size distribution, though some variation in size was observed. The measured size of the nanoparticles exceeded the expected range of 1-100 nm, as they ranged from 0.2 µm to 1 µm, indicating that the nanoparticles formed through the green synthesis method were slightly larger than anticipated. The spherical shape was consistent across most particles, suggesting that the flavonoids played an important role in controlling the nanoparticle formation, although some irregularities in shape were also present. The particle size and shape could be influenced by factors such as the concentration of the extract, the type of metal ion used, and the reaction time. These results are consistent with previous studies that reported nanoparticles formed through plant extract-mediated synthesis often show some degree of size variation and larger dimensions than those produced by conventional chemical methods (Figures 3 and 4).

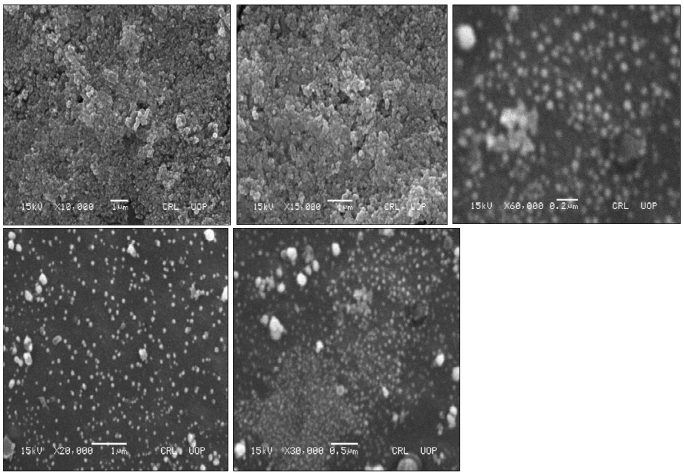

Figure 3: *SEM analysis of Silver nanoparticles.*

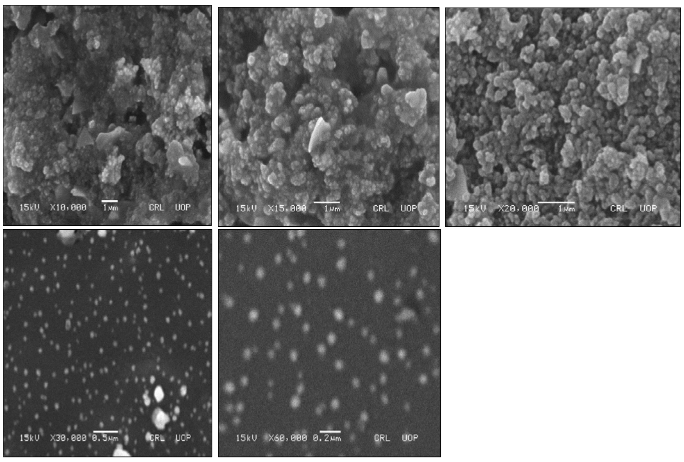

Figure 4: *SEM analysis of gold nanoparticles.*

Antibacterial potential of flavonoids and nanoparticles
Pure flavonoids and green synthesized nanoparticles of *Isatis costata* was demonstrated for their antibacterial activity against the following five different strains of bacteria, such as *Salmonella typhi, Escherichia coli, Pseudomonas aurogenosa, Bacillus subtilis, and S. aurous.* The antibacterial activity of flavonoids and nanoparticle were checked by using different concentration *i.e.* 3µl, 6µl, 9µl and 12µl of flavonoids and nanoparticles, and measured their inhibition zones. The following table showed the inhibition zones of silver and gold nanoparticles in mm. It was observed that the pure flavonoid do not show any effect against bacterial strains. However inhibition zone increased by increasing the concentration of silver nanoparticles. The highest value of zone of inhibition were showed by *Bacillus subtalis* (9mm) under 3µl of silver nanoparticles followed by 35mm under 12µl solution of silver nanoparticles. However in *Salmonella typhi* the highest value of zone of inhibition were 26mm under 3µl while no inhibition zone were found at 12µl solution of silver nanoparticles showing irregularity of the increasing order of zone of inhibition with increasing concentration (Table 1). Similarly the lowest value of zone of inhibition were found in *Staphylococcus aureus* (5mm) under 3µl and 15mm at 12µl solution of silver nanoparticles respectively (Table 1). Under gold nanoparticles the highest value of zone of inhibition were found in *E. coli* (15mm) at 3µl and 17mm at 12µl solution of gold nanoparticles followed by *S. aureus* and *S. typhi*. No inhibition zone were found for both *P, aeruginosa* and *B, subtilis* respectively (Table 2). For crude flavonoid at different concentration no inhibition zone were found, respectively.

Table 1: *Zone of inhibition of different bacterial strains under silver nanoparticle in millimeter (mm).*

S. No	Name of bacteria	Conc. Of AgNPs and ZOI in mm				Incubation period
		3µl	6 µl	9 µl	12 µl	
1	*Escherichia coli*	20	16	16	25	24 hours
2	*Bacillus subtilis*	9	15	24	35	24 hours
3	*Pseudomonas aeruginosa*	11	11	15	11	24 hours
4	*Salmonella typhi*	26	13	22	-	24 hours
5	*Staphylococcus aureus*	5	8	10	15	24 hours

Table 2: *Zone of inhibition of different bacterial strains under gold nanoparticle in millimeter (mm).*

S. No	Name of bacteria	Conc. of AuNPs and ZOI in mm				Incubation period
		3µl	6 µl	9 µl	12 µl	
1	*Escherichia coli*	15	15	9	17	24 hours
2	*Bacillus subtalis*	-	-	-	-	24 hours
3	*Pseudomonas aeruginosa*	-	-	-	-	24 hours
4	*Salmonella typhi*	10	9	13	14	24 hours
5	*Staphylococcus aureus*	12	10	11	15	24 hours

Table 3: *Mild result of crude flavonoids at different concentration.*

S/ No	Name of bacteria	Conc. of crude flava-noids and ZOI in mm				Incu-bation period
		3µl	6 µl	9 µl	12 µl	
1	*Escherichia coli*	-	-	-	-	24 hours
2	*Bacillus subtalis*	-	-	-	-	24 hours
3	*Pseudomonas aeruginosa*	-	-	-	-	24 hours
4	*Salmonella typhi*	-	-	-	-	24 hours
5	*Staphylococcus aureus*	-	-	-	-	24 hours

Discussion

The green synthesis of metallic nanoparticles (NPs) has received considerable attention in recent years, mainly because of its environmentally friendly characteristics, simplicity, and cost efficiency. The synthesis of silver and gold nanoparticles (AgNPs and AuNPs) through the reduction of metal salts in solution is affected by multiple factors, such as temperature, concentration, molar ratios of metallic salt and reducing agent, and the incorporation of different reagents (Sawer *et al*., 2022). In our study, we utilized *Isatis costata* aerial part extract, rich in flavonoids, to reduce silver and gold ions and stabilize the nanoparticles, offering a promising green approach to nanoparticle synthesis. Flavonoids, being secondary metabolites, are well known for their antioxidant properties and potential for synthesizing nanoparticles (Elshafie *et al*., 2023).

The synthesis of silver and gold nanoparticles was confirmed visually by the color change in the reaction mixture. The solution of silver nitrate turned from yellow-brown to dark brown, while the gold chloride solution changed from yellow to pink or reddish-brown, signifying the formation of AgNPs and AuNPs, respectively. These color changes have been reported in other studies as well, where the reduction of metal ions by plant extracts leads to similar visible color transitions, indicating successful nanoparticle formation (Parashar *et al*., 2022; Hosnedlova *et al*., 2022). These findings support the idea that plant-derived compounds, especially flavonoids, play a crucial role in the reduction and stabilization of nanoparticles in aqueous solutions (Nadaf *et al*., 2021).

UV-Vis spectroscopy serves as a reliable method for confirming nanoparticle formation. This study presents the UV-Vis spectra of silver and gold nanoparticles, which display characteristic surface plasmon resonance (SPR) peaks at approximately 420 nm and 520 nm, respectively. The observed SPR peaks are characteristic of silver and gold nanoparticles, attributed to the collective oscillation of free electrons on the nanoparticle surface in resonance with incident light (Leyu *et al*., 2023; Arif *et al*., 2022). Our results are consistent with recent studies, which have shown that the SPR absorption bands for AgNPs and AuNPs typically appear in the 400-550 nm range, depending on the size and shape of the nanoparticles (Jain and Mehata, 2020). Additionally, the rapid reduction of metal ions in the plant extract, with more than 90% of Ag^+ ions reduced within 4 hours, is in line with previous findings (Ezeuko *et al*., 2022), suggesting the efficiency of *Isatis costata* flavonoids as reducing agents.

Analysis via scanning electron microscopy (SEM) indicated that the synthesized nanoparticles were primarily spherical, exhibiting sizes between 0.2 µm and 1 µm. The particle size distribution exceeds the typical range of 1-100 nm observed in numerous plant-mediated nanoparticle synthesis studies; however, it remains within the acceptable limits for biomedical applications (Sher *et al*., 2022). The particle size observed in this study may be influenced by various factors, including the concentration of the extract, metal ion concentration, and reaction time. This variation in nanoparticle size has been reported by other researchers as well, highlighting the complex and multifaceted nature of plant-mediated synthesis (Carter *et al*., 2023; Hosnedlova *et al*., 2022). Despite the variability in particle size, the uniform dispersion of nanoparticles observed in the SEM images indicates the effective stabilizing role of flavonoid compounds in preventing agglomeration.

The antibacterial properties of the synthesized nanoparticles were evaluated using an agar well diffusion method against five pathogenic bacterial strains: *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*. The silver nanoparticles exhibited remarkable antibacterial activity, with inhibition zones ranging from 5 mm to 35 mm depending on the bacterial strain and concentration of nanoparticles. These findings are consistent with recent studies, where silver nanoparticles demonstrated significant antibacterial effects against a broad range of bacterial pathogens, including both Gram-negative and Gram-positive bacteria (Alahmad *et al*., 2022). The mechanism of action of silver nanoparticles is multifaceted and includes the release of silver ions, disruption of cell membrane integrity, and interference with bacterial enzymatic functions, ultimately leading to bacterial cell death (Do *et al*., 2025). The observed inhibition across all five bacterial strains in our study further supports the broad-spectrum antimicrobial activity of silver nanoparticles.

In contrast, gold nanoparticles displayed moderate antibacterial activity, with inhibition zones ranging from 15 mm to 17 mm for select bacterial strains. Previous studies have also shown that gold nanoparticles exhibit variable antibacterial activity depending on factors such as size, shape, and concentration (Jain and Mehata, 2017; Hussein *et al*., 2021). The antibacterial mechanism of gold nanoparticles is less well understood compared to silver nanoparticles, and it is likely that the observed activity in this study is due to a combination of physicochemical properties of the nanoparticles and interactions with bacterial cell walls. Further research is needed to elucidate the exact antibacterial mechanisms of gold nanoparticles and optimize their activity against different pathogens.

The synthesized nanoparticles demonstrated notable antibacterial activity, especially silver nanoparticles, which exhibited considerable potential for antimicrobial applications. This study's results correspond with recent literature, emphasizing the efficacy of plant-derived compounds in nanoparticle synthesis and their potential applications in biomedical fields, including infection control and wound healing. Future research should concentrate on refining synthesis conditions to improve nanoparticle characteristics and investigate the mechanisms underlying the antibacterial activity of both silver and gold nanoparticles.

Conclusions and Recommendations

The biological synthesis of silver and gold nanoparticles utilizing aerial parts from the Isatis costata plant presents an environmentally sustainable, straightforward, and effective method for nanoparticle production. The synthesized nanoparticles displayed both spherical and tubular morphologies, with estimated dimensions between 0.2 and 0.5 µm. The observed sizes were increased as a result of nanoparticles being encased in a thin layer of proteins and metabolites, including terpenoids, which possess functional groups such as amines, alcohols, ketones, and aldehydes. The findings were confirmed through characterization utilizing UV-vis spectrophotometry and SEM techniques. The concentration of plant extract to metal ion ratio is critical in influencing the morphology of the nanoparticles. Increased concentrations produced sheet-shaped nanoparticles, whereas decreased concentrations led to spherical forms. The sizes of the nanoparticles varied with concentration, influenced by the reduction of metal ions. From a technological perspective, silver nanoparticles exhibit potential applications in the biomedical domain. This procedure presents multiple advantages, such as cost-effectiveness, compatibility with medical and pharmaceutical applications, and appropriateness for large-scale commercial production.

Based on the findings from this study, further research is recommended to investigate the specific antibacterial mechanisms of silver and gold nanoparticles, including their interactions with bacterial membranes, intracellular targets, and biofilms. Optimizing the synthesis parameters, such as plant extract concentration, reaction time, and temperature, could enhance the yield, size, and stability of the nanoparticles, improving their antimicrobial properties. Additionally, it is crucial to assess the biocompatibility of the nanoparticles through in vitro and in vivo studies to evaluate their cytotoxic effects and safety for biomedical applications. Scaling up the synthesis process in a cost-effective manner to meet large-scale production demands is also necessary, with a focus on ensuring compliance with regulatory requirements. Finally, collaboration with industry partners will be essential for commercializing nanoparticle-based antimicrobial products for clinical use.

Acknowledgements

We are highly thankful to department of Botany Islamia College Peshawar for providing healthful and friendly environment to conduct the research work,, especially thankful to Dr. barkatullah for corporating with present work

Novelty Statement

This study introduces a novel, eco-friendly method for synthesizing silver and gold nanoparticles using Isatis costata L. flavonoid extract as a natural reducing and stabilizing agent. The biosynthesized nanoparticles exhibit significant antibacterial activity, highlighting their potential for biomedical applications.

Author's Contribution

Nazim Ullah: Conduct the research
Abdul Basit and Alia Gul: Write the manuscripts and review
Irshad Ali Khan, Abdur Rehman and Attaullah: Write the methodology
Noshin Shafqat and Asma: Improved the Script

Conflict of interest
The authors have declared no conflict of interest.

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