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# Green synthesis of gold nanoparticles from *Sophora flavescens* extract and their antibacterial effect against some pathogenic bacteria

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## ABSTRACT

**Aims:** The current study was aimed to evaluate the antibacterial activity of biogenic synthesized golden nanoparticles from *Sophora flavescens* Aiton roots aqueous extract against multidrug-resistant (MDR) clinical bacterial isolates.
**Methodology and results:** The green synthesis of gold nanoparticles (AuNPs) was accomplished using *S. flavescens* roots aqueous extract and examined using many accepted techniques. The antibacterial activity of *S. flavescens* extract and the aqueous AuNPs at concentrations (7% and 9%) ppm were investigated against two clinical MDR bacteria, including Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Pseudomonas aeruginosa*). The findings demonstrate inhibitory activity against the selected MDR bacterial isolates for the aqueous extract of *S. flavescens* and the aqueous AuNPs noted by the significant decrease in the number of bacteria after treatment with highly significant differences (P≤0.01) compared to the untreated control.
**Conclusion, significance and impact of study:** *Sophora flavescens* root extracts and their biosynthesized AuNPs with antibacterial activity may find broad applications in fighting MDR pathogenic bacteria and therapeutic manufacturing.

*Keywords:* Antibacterial activity, gold nanoparticles, green synthesis, multi-drug resistance pathogenic bacteria, *Sophora flavescens*

## INTRODUCTION

One of the biggest threats to public health today is the rise of antibiotic resistance, particularly given the paucity of new, effective antimicrobial drugs being discovered (El-Hamid *et al*., 2020; Zaboon and Al-Hayanni, 2021). Several strategies have been used to update the antimicrobial chemotherapeutic alternatives that are now accessible (Casciaro *et al*., 2017; Abbas and Hegazy, 2020; Thapa *et al*., 2020). Currently, nanotechnology shows promise as cutting-edge technology for creating novel antimicrobial medicines with a wide range of features, including effective targeting, enhanced pharmacokinetic profile and decreased toxicity (Tao, 2018; Thapa *et al*., 2020).

Numerous nanoscience and nanotechnology sectors use metallic nanoparticles (NPs) that display antibacterial properties. These nanoparticles potentially replace the functions of commonly used medications (Rao *et al*., 2017).

Emerging materials known as gold nanoparticles (AuNPs) have optical and electrical properties that are different from those of conventional materials and have a bright future in the field of medicine (Cabuzu *et al*., 2015; Tao, 2018). These characteristics include stability, surface plasmon resonance, surface chemistry, multifunctionalization and a high surface area-to-volume ratio. By carefully adjusting the components and concentrations, GNPs may advantageously be readily manufactured in various forms and sizes (Wiley *et al*., 2007). The adjustable size of AuNPs makes it simpler for them to pass through cellular membranes and impacts cellular permeability, protein synthesis and metabolism, which causes bacterial cell death (Tao, 2018; Yougbare *et al*., 2019).

Beyond their use as carriers of antibodies, antibiotics, vaccines and medicines, several recent papers have highlighted AuNPs' potential antibacterial activity (Arvizo *et al*., 2012; Zhang, 2015; Casini *et al*., 2018; Gurunathan *et al*., 2020). The antibacterial effects of AuNPs have numerous potential mechanisms: the capacity to penetrate microbial cells, the enhancement of cell membrane damage, the assistance in bacterial DNA destruction and the release of reactive oxygen species (ROS) (Cabuzu *et al*., 2015; Zhang *et al*., 2015; Yougbare *et al*., 2019).

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The need to find environmentally benign solutions to prevent the use of harmful chemicals, especially for medical purposes, is growing despite the availability of flexible chemical and physical processes for the production of nanoparticles (Salem and Fouda, 2021). The utilization of natural resources to create metallic nanoparticles (NPs) has drawn increasing interest from scientists, particularly since Cu, Ag and Au NPs are considered to be biocompatible nanomaterials (Rao *et al*., 2017). The ecologically beneficial approach of creating metallic nanoparticles by plant-mediated biosynthesis has gained popularity, replacing the need for harmful or organic compounds (Nayantara and Kaur, 2018). Due to the vast range of health applications, the synthesis of gold nanoparticles using plant extracts has attracted significant interest in the field of biomedicine (Al Saqr *et al*., 2021).

*Sophora flavescens* is a member of the Fabaceae family and is widely cultivated in China. The root of *S. flavescens*, also known as "Kushen" in traditional Chinese medicine, is said to be a potent antibacterial and anti-inflammatory herb that has been used to treat a variety of bacterial and viral infections, including eczema, psoriasis, chronic proctitis and bacillary dysentery (Xiong *et al.*, 2016). Additionally, *S. flavescens* alkaloids are widely employed in agriculture as green antibacterial agents with an insecticidal action (Zhao *et al*., 2021).

Therefore, the purpose of this work was to investigate AuNPs' antibacterial efficacy after green synthesizing them. In order to create AuNPs, *S. flavescens'* aqueous root extract has been employed. Against two Gram-positive and Gram-negative bacterial strains, the antibacterial activity of AuNPs was examined.

## MATERIALS AND METHODS

### Bacterial isolates

The Department of Biology, College of Science for Girls, University of Baghdad's laboratories provided two pathogenic bacterial isolates (Gram-positive and Gram-negative). They were originally isolated from clinical specimens and were initially diagnosed as *S. aureus* and *P. aeruginosa*, and their confirmatory diagnosis was performed using the Vitek-2 system, which confirmed that they were *S. aureus* and *P. aeruginosa*, in addition to determining their antibiotics susceptibility pattern and these two isolates were used in the current study.

### Antibiotics susceptibility test

Various classes of antibiotics were used in the antibiotic susceptibility test, which was carried out on bacterial isolates by the disk diffusion method (Kirby-Bauer method) as described by Bauer *et al*. (1966) and Clinical Laboratory Standard Institute recommendations (CLSI, 2020). The sensitivity of antibiotics was determined by measuring the inhibition zone diameter. In order to identify whether the bacteria were susceptible (S), intermediate (I) or resistant (R) to the antibiotics, reference tables were employed.

### Preparation of *S. flavescens* root aqueous extract

*Sophora flavescens* root samples purchased from local markets in Baghdad city were identified by a botanist (specialist in plant taxonomy in the Department of Biology/College of Science for women. Aqueous extracts of *S. flavescens* root were prepared according to Al-Hayanni and El-Shora (2021). Soak the dried root powder (60 g) with 600 mL distilled water (DW) for 3 h. To create an aqueous extract, the extraction solutions were filtrated with filter paper (Whatman No. 1) and evaporated under decreased pressure using a rotary evaporator (N-1200B, EYELA) to obtain an aqueous extract (4 g). Two concentrations of aqueous plant extracts which include 7% and 9% ppm were prepared by dissolving 700 and 900 mg, respectively, of each dried extract in 10 mL distilled water and then sterilizing with a Millipore filter unit (0.22 µm).

### Gold chloride ($HAuCl_4$) solution preparation

In 100 mL of deionized distilled water (DW), 1 g of gold chloride ($HAuCl_4$) was dissolved. Then, 4 mL of the prepared $HAuCl_4$ solution was added to another 96 mL of DW to make a 1 mM solution (Al Saqr *et al*., 2021).

### Preparation of gold nanoparticles by green synthesis using *S. flavescens* extract

At room temperature, a reaction mixture containing 4 mL of root extract and 16 mL of aqueous gold chloride solution (1 mM) was constantly stirred while being watched for color changes. Gold chloride was completely bio-reduced to gold nanoparticles from plant extract in the reaction mixture over the course of 24 h. The first indication that gold nanoparticles or AuNPs had formed came from a change in color (Al Saqr *et al*., 2021).

### Characterization and examination of gold nanoparticles

After getting a stable color change, the solution was centrifuged for 10 min at 5000 rpm to remove any precipitate. The obtained supernatant was centrifuged for another 30 min at 15000 rpm. To eliminate any residual plant extract, the pellet was then washed three times with deionized distilled water. The final pellet was resuspended in deionized DW before being used for characterization.

### UV-Visible spectroscopic analysis

UV-Visible spectroscopy, a powerful method for the characterization of colloidal particles, was used to track the signature of AuNPs. Synthesized AuNPs were characterized by UV-Vis analysis in the wavelength range 300 to 800 nm (The UV-Vis spectra were recorded in a Shimadzu, Japan). UV-Vis spectrophotometer operating at a resolution of 1 nm for the confirmation of complete bioreduction of gold ions to gold nanoparticles in aqueous

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plant root extract. Deionized water was utilized as a blank solution.

**Fourier transform infrared spectroscopy (FTIR)**

To identify the functional groups involved in the synthesis, a Fourier transform infrared spectroscopy study was done. In order to examine the freeze-dried samples of plant root extract and AuNPs, FTIR (Shimadzu, Japan) was used in transmittance mode with a 4/cm resolution. The spectrum that resulted was captured between 400 and 4000/cm.

**Determination of zeta (ζ) potential**

Zeta potential was analyzed (Shimadzu, Japan) by adding a solution of gold nanoparticles in dip cells using a syringe and measured by droplet electrophoretic light scattering at 25 °C.

**Atomic force microscopy (AFM)**

An atomic force microscope (Shimadzu, Japan) was used to analyze the samples in order to determine the precise particle size. This instrument detects the atomic range of particles in tapping mode.

**Atomic absorption spectroscopy (AAS)**

To measure the number of gold nanoparticles (Shimadzu, Japan). The samples were taken out and centrifuged. Centrifugation at a speed of around 15000 rpm may readily separate the zero-valent metallic forms of Au nanoparticles.

**Antibacterial activity assay of *S. flavescens* root aqueous extract and the aqueous AuNPs**

The antibacterial activity of the *S. flavescens* root aqueous extract and the aqueous AuNPs against antibiotic-resistant *S. aureus* and *P. aeruginosa* was carried out (Balouiri *et al*., 2016). 0.1 mL of standardized bacterial inoculum ($1.5 \times 10^8$ CFU/mL, 0.5 McFarland's standard) of each bacterial isolate and 0.1 mL of each concentration (7% and 9% ppm) prepared from the extract and from the aqueous AuNPs were transferred to a sterile test tube, then incubated at 37 °C for 15 min and the mixture was poured onto sterile Petri dishes containing Muller-Hinton agar (MHA) and spreading it by the spreader. And all the dishes were incubated at 37 °C for 24 h and the antibacterial activity was observed by counting bacterial colonies and compared with the control. Three dishes (replicates) were used for each concentration to reduce the errors that resulted from conducting the experiment**.**

**Statistics analysis**

The Statistical Analysis System (SAS, 2018) application was employed to identify the impact of various factors on research parameters. In this study, the least significant difference (LSD) test (ANOVA) was used to compare the means significantly.

**RESULTS AND DISCUSSION**

**Visual confirmation**

AuNPs production was initially seen and visually verified. The plant extract was added to a 1 mM gold chloride solution, which initially caused the reaction mixture to become reddish pink. The solution's color then abruptly shifted to pink as the incubation period was extended to 24 h. Figure 1 illustrates the whole bioreduction and the production of more AuNPs. The excitations of surface plasmon resonance in the nanoparticles caused the color to emerge in the reaction mixture (Khan *et al*., 2018). In the work of Folorunso *et al*. (2019), the process of creating gold nanoparticles for Annona muricata is quickly initiated and is finished after 22 h of incubation.

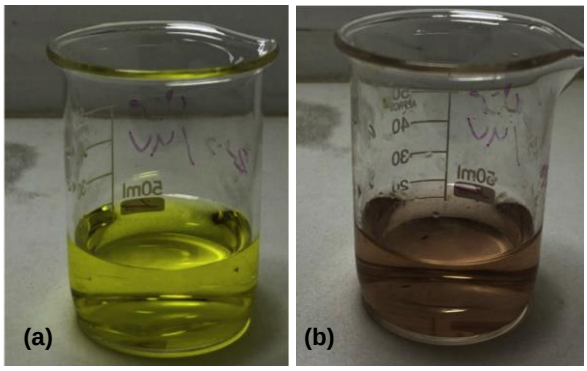


**Figure 1:** Visualization of the green synthesis of gold nanoparticles. (a) a 1 mM gold chloride solution; (b) a solution containing gold nanoparticles.

**UV-Visible spectroscopic analysis**

By using UV-VIS spectrophotometry, the combination of *S. flavescens* roots extract solution and 1 mM gold chloride solution was examined. UV-Visible range surface plasmon resonance (SPR) bands with unique appearances can be seen in Figure 2 and the highest absorption range was 540 nm. Because of the oscillation of electrons on the surface of AuNPs, the surface plasmon resonance of these nanoparticles produces different shades depending on their size and shape (Sett *et al*., 2016). According to Kaykhaii *et al*. (2018), the maximum wavelength of produced gold nanoparticles as measured by spectrophotometry in the visible range is 535 nm.

**Fourier transform infrared spectroscopic analysis (FTIR)**

The potential biomolecules in the *S. flavescens* root extract that may be responsible for capping and the

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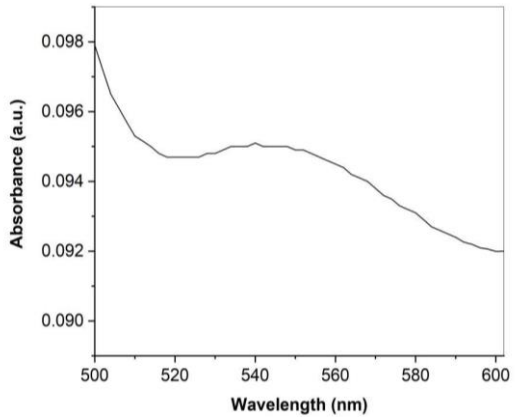


**Figure 2:** UV-spectral analysis for gold nanoparticles.

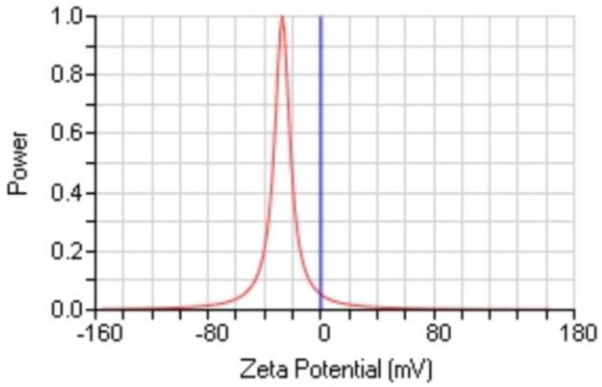


**Figure 4:** Zeta (ζ) potential of Au nanoparticles synthesized by extract of roots of *S. flavescens.*

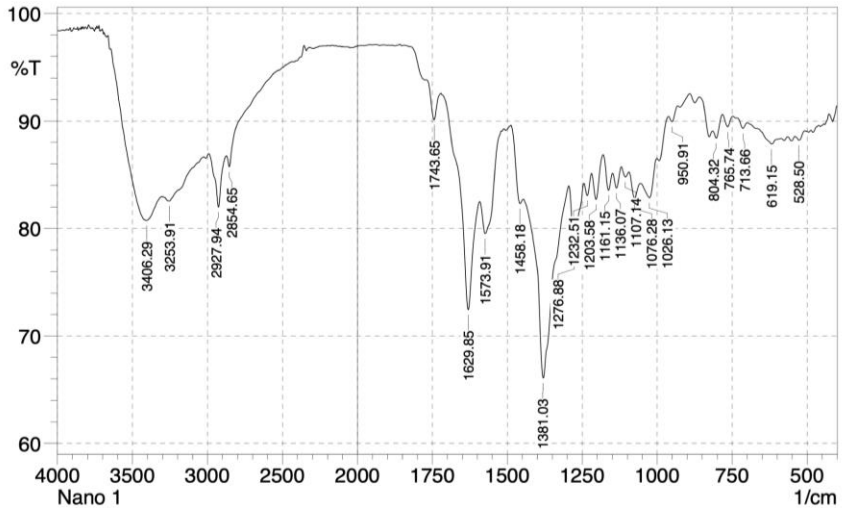


**Figure 3:** FTIR spectrum of gold nanoparticles made from *S. flavescens* root extract.

effective stability of the gold nanoparticles were determined using FTIR measurements. Using an FTIR spectrophotometer, an Au nanoparticle was used to measure the infrared radiation spectrum (IR) at wavelengths between between 4000/cm and 500/cm. Figure 3 may be seen at 3406.29, 2927.94, 2854.65, 1743.65, 1573.91, 1458.81, 1276.88, 1232.51, 1161.15 and 1026.13/cm. The best bands at 3406.29/cm (N-H stretching amide), 2927.94 and 2854.65/cm (C-H stretching alkane) and 1743.65/cm (C-H stretching aldehyde) were caused by the reduction of Au3+ to Au0. The NH bent corresponds to the abandon at 1573.91 and 1458.81/cm. C-N stretching mode might be the cause of the absorption bands at 1276.88/cm. The stretching mode of -C-O-C might be responsible for the absorption bands at 1232.51 and 1161.15/cm. Sharp bands at 1026.13/cm in wavelength correspond to -C-N functional groups. The water-soluble chemicals like flavonoids, terpenoids and protein contained in the extract of the roots of the *S. flavescens* plant correlate to vibrational bands for bonds like N-H, C=C, C-O and C-N. The reduction of gold ions, synthesis and stability of the biosynthesized nanoparticles may be caused by the presence of these biomolecules in the extract (Kaykhaii *et al*., 2018).

**Zeta (ζ) potential**

The colloidal metal nanoparticles' stability is gauged by their zeta potential. The stability of colloids was also influenced by the zeta potential, which is shown by the surface charge of nanoparticles. Greater zeta potential values that inhibit aggregate formation between nanoparticles give nanoparticles their increased stability (Noruzi, 2015). Zeta potential was measured at -27.40 mV. Figure 4 showed that the produced *S. flavescens* root extract gold nanoparticles were stable, as indicated by the larger negative zeta potential value (Ahmad *et al*., 2018).

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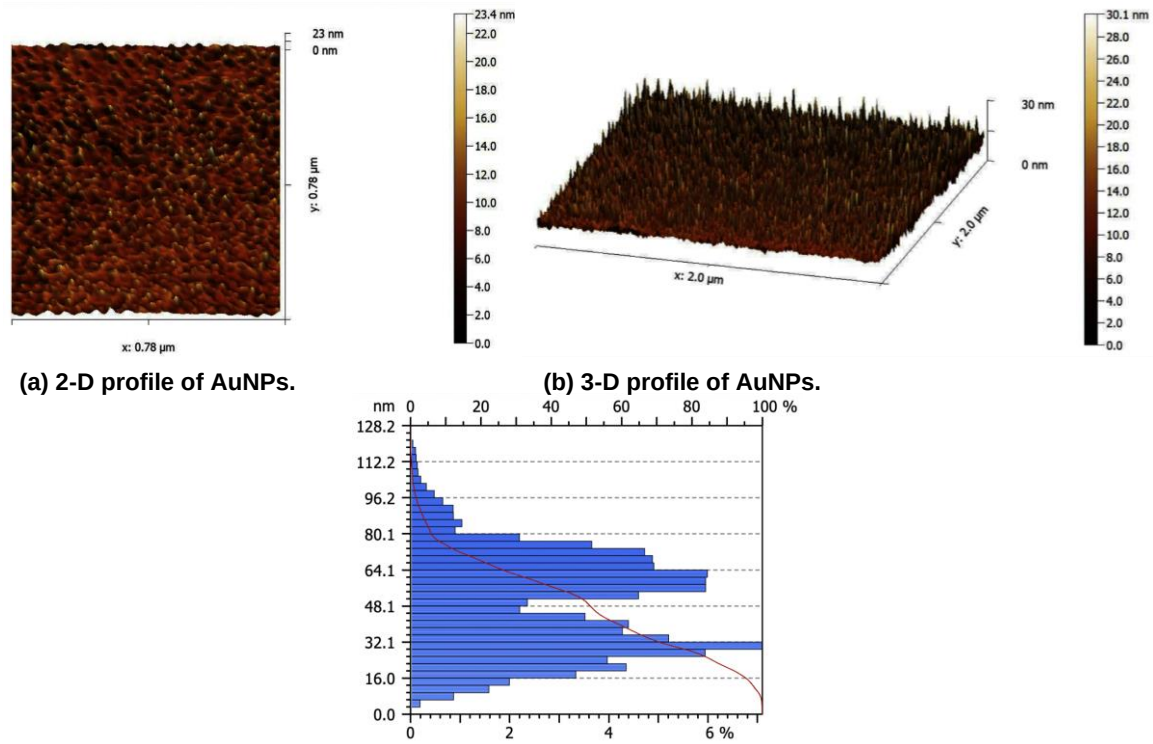


**(a) 2-D profile of AuNPs.** **(b) 3-D profile of AuNPs.**

**(c) AuNPs average size range.**

**Figure 5:** AFM photos of AuNPs in solution displaying their surface structure and size distribution. (a) The AFM picture depicted AuNPs in two dimensions; (b) The AFM picture depicted AuNPs in three dimensions; (c) Displayed a column AFM diagram showing the size distribution of AuNPs.

**Table 1:** The antibiotics susceptibility test results of *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates by Kirby Bauer method.

| Antibiotics | *S. aureus* | Antibiotics | *P. aeruginosa* |
|---|---|---|---|
| Ciprofloxacin | R | Amikacin | R |
| Clindamycin | R | Cefepime | R |
| Erythromycin | R | Ceftazidime | R |
| Gentamicin | S | Gentamicin | R |
| Tetracycline | R | Levofloxacin | R |
| Trimethoprim | S | Meropenem | R |
| Vancomycin | R | Tobramycin | R |

R, resistant; S, susceptible.

**Atomic force microscopy (AFM)**

The produced AuNPs' existence and size distribution were assessed by AFM analysis. Figure 5 shows the generation of both two-dimensional (2D) and three-dimensional (3D) pictures from a scanning area that was 11 m in a tapping mode. Given that the majority of the particles had a spherical topology and a diameter of about 51.69 nm, the photos demonstrate that AuNPs are distributed uniformly.

**Atomic absorption spectroscopy (AAS)**

AAS analysis of the gold ion concentration revealed the transformation of Au ions into GNPs. Initial HAuCl standard solution preparation and AAS analysis was placed at 0 min. After adding leaf extract, the reaction solution's Au ion concentration was measured to assess the amount of GNPs present (Singh and Kundu, 2014). GNP concentrations were between 956 and 1321 ppm.

**Antibiotics susceptibility test**

According to the results of the antibiotic susceptibility test, and as shown in Table 1, the two bacterial isolates exhibited a multi-drug resistant (MDR) pattern. The isolate of *S. aureus* was sensitive to only two of the chosen antibiotics (Gentamicin and Trimethoprim). While the isolate of *P. aeruginosa* was resistant to all the selected antibiotics.

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**Table 2:** The antibacterial activity of the *Sophora flavescens* roots aqueous extract and the aqueous gold nanoparticles against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

| The antibacterial activity of the *Sophora flavescens* roots aqueous extract | | |
|---|---|---|
| The concentration of extract (%) | *Staphylococcus aureus* (CFU/mL) | *Pseudomonas aeruginosa* (CFU/mL) |
| Control | $1.5 \times 10^{8a}$ | $1.5 \times 10^{8a}$ |
| 7% | $1.3 \times 10^{6b}$ | $1.9 \times 10^{6b}$ |
| 9% | $4.7 \times 10^{2c}$ | $6.\ 6 \times 10^{2c}$ |
| LSD value | 27.79* | 32.66* |
| The antibacterial activity of the aqueous gold nanoparticles | | |
| The concentration of AuNPs (%) | *Staphylococcus aureus* (CFU/mL) | *Pseudomonas aeruginosa* (CFU/mL) |
| Control | $1.5 \times 10^{8a}$ | $1.5 \times 10^{8a}$ |
| 7% | Non$^{b}$ | Non$^{b}$ |
| 9% | Non$^{b}$ | Non$^{b}$ |
| LSD value | 19.47* | 19.47* |

Means with different letters in the same column differed significantly. *(P≤0.05).

**The antibacterial activity**

The present study showed that *S. flavescens* root aqueous extract and gold nanoparticles had antibacterial effects against MDR *S. aureus* and *P. aeruginosa* isolates, with highly significant differences (P≤0.01) in comparison with the control. Whereas the concentrations of 7% and 9% ppm of the aqueous extract reduced the number of *S. aureus* to $1.3 \times 10^6$ CFU/mL and $4.7 \times 10^2$ CFU/mL, respectively, while the number decreased to $1.9 \times 10^6$ CFU/mL and $6.6 \times 10^2$ CFU/mL for *P. aeruginosa*, respectively, compared to the bacterial number in control ($1.5 \times 10^8$ CFU/mL). As for the nanoparticles, the growth disappeared completely for the two concentrations used. In addition, the gold nanoparticles have the highest antibacterial activity than aqueous extract as shown in Table 2 and Figures 6 and 7.

The current results showed that *S. flavescens* root aqueous extract exhibited more antibacterial activity against Gram-positive bacteria than Gram-negative, and this is compatible with the study of Yang *et al.* (2015) that revealed aqueous extract from *S. flavescens* roots had an inhibitory effect on *S. aureus* but had no effect on *P. aeruginosa*, and in an earlier study, the crude *S. flavescens* extract also displayed remarkable inhibitory activity against the methicillin-resistant *S. aureus* and methicillin-sensitive *S. epidermidis*, displaying especially potent antibacterial activity against *S. epidermidis* (Yang *et al*., 2007). Maybe due to the structure of the bacterial cell wall, because lacking Gram-positive bacteria for a layer of outer membranes., which makes the permeability of materials entering the cell greater compared to Gram-negative bacteria, whose inner wall has an internal barrier represented by a common lipopolysaccharide with multiple proteins that has the ability to prevent the passage of a lot of killer substances into the cell (Nader *et al*., 2010; Al-Mossawei *et al*., 2014) and also may be due to the high resistance to antibiotics of *P. aeruginosa* more than *S. aureus* in the present study.

Both conventional and modern medicine have regarded *S. flavescens* wide range of biological activity, as a significant resource. More than 200 chemicals have been extracted from *S. flavescens*, a potential traditional medicine, with flavonoids and alkaloids being the main constituents. At least 50 pure compounds and crude extracts from *S. flavescens* have been shown to have extensive anticancer, antibacterial, antipyretic, antinociceptive and anti-inflammatory pharmacological properties in recent *in vitro* and in animal research (He *et al*., 2015).

Since ancient times, people have valued using gold as the preferred inorganic antibacterial agent to prevent infections and spoilage. GNPs inhibited both Gram-positive and Gram-negative isolates in the current investigation; this finding is consistent with that of Al-Sager *et al*. (2021) obtained. There are various mechanisms responsible for this inhibiting impact. GNPs target bacterial cell membranes, cell walls, DNA and proteins in addition to their great penetrating capacity (Arvizo *et al*., 2012; Patel *et al*., 2013; Singh *et al*., 2018). GNPs have been shown time and time again to cause pits to form in bacterial cell walls and membranes.

It has been demonstrated that GNPs may target the subcellular compartments of cell membranes, causing pits and cellular death. Additionally, GNPs bind the peptide surface to the glycan ports of the cell wall and break down the N-acetylglucosamine and N-acetylmuramic acid linkage in glycans, which causes pit formation in glycans (Li *et al*., 2018). As stated by earlier research, Gram-ve bacteria are extra susceptible to metal nanoparticles. Gram-ve bacteria have a thinner cell wall than Gram+ve bacteria. The thickset cell wall may decline the permeation of nanoparticles within cells (Alfahad *et al*., 2022).

GNPs have been shown to target new bacterial targets, such as respiratory chain dehydrogenases and bacterial chromosomes, in addition to cidal targeting of cell walls and membranes (Yougbare *et al*., 2019). Additionally, metallic nanoparticles have an extra biocidal action due to their capacity to generate reactive oxygen species (ROS), which impede the oxidation of freed gold ions (Iswarya *et al*., 2016; Yougbare *et al*., 2019).

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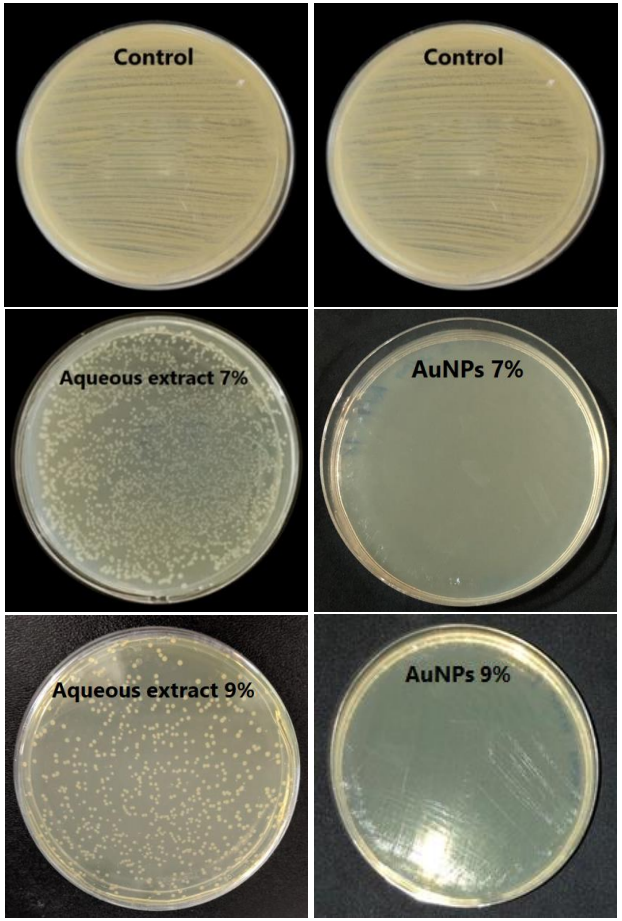


**Figure 6:** (a) The *Sophora flavescens* root aqueous extract's antibacterial efficacy against *Staphylococcus aureus* at doses of 7% and 9%; (b) The aqueous gold nanoparticles' antibacterial efficacy against *S. aureus*.

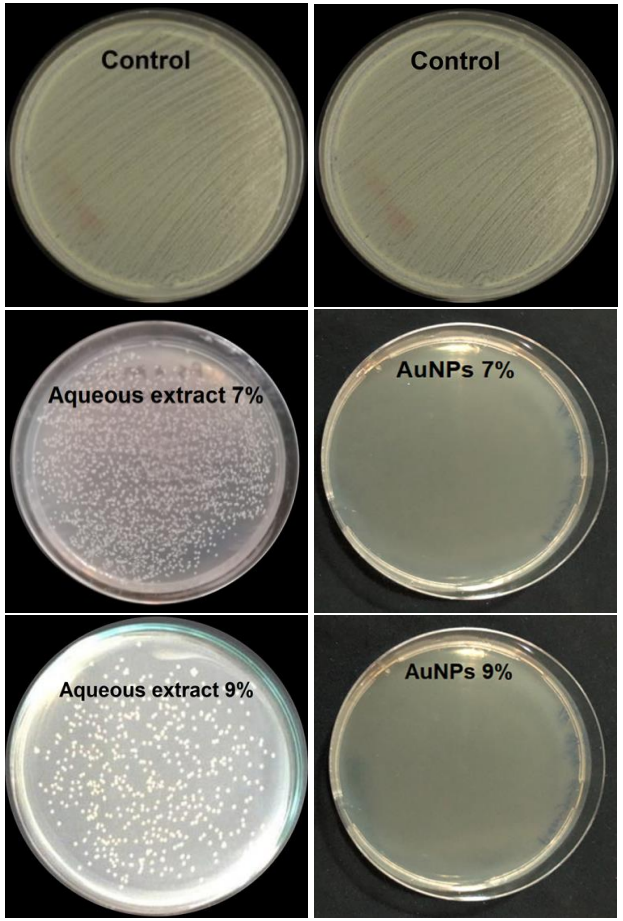


**Figure 7:** (a) The *Sophora flavescens* root aqueous extract's antibacterial activity against *Pseudomonas aeruginosa* at concentrations of 7% and 9%; (b) The aqueous gold nanoparticles' antibacterial activity against *P. aeruginosa*.


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## CONCLUSION

Gold nanoparticles can be synthesized by the green method from *S. flavescens* plant extracts successfully. Research findings suggest that gold nanoparticles have higher antibacterial activity than aqueous extract. Further research is needed to explore the potential of *S. flavescens.*

## REFERENCES

**Abbas, H. A. and Hegazy, W. A. H. (2020).** Repurposing anti-diabetic drug "Sitagliptin" as a novel virulence attenuating agent *in Serratia marcescens*. *PLoS ONE* **15(4), e0231625.**

**Ahmad, T., Bustam, M. A., Irfan, M., Moniruzzaman, M., Asghar, H. M. A. A. and Bhattacharjee, S. (2018).** Green synthesis of stabilized spherical shaped gold nanoparticles using novel aqueous *Elaeis guineensis* (oil palm) leaves extract. *Journal of Molecular Structure* **1159, 167-173.**

**Al Saqr, A., Khafagy, E., Alalaiwe, A., Aldawsari, M. F., Alshahrani, S. M., Anwer, M. K., Khan, S., Lila, A. S. A., Arab, H. H. and Hegazy, W. A. H. (2021).** Synthesis of gold nanoparticles by using green machinery: Characterization and *in vitro* toxicity. *Nanomaterials* **11(3), 808.**

**Al-Hayanni, H. S. A. and El-Shora, H. (2021).** Various extracts of some medicinal plants as inhibitors for beta-lactamase activity. *Baghdad Science Journal* **18(1), 47-53.**

**Al-Mossawei, M. T., Ali, A. H., Abid, H. S. and Murad, R. H. (2014).** Antimicrobial activity of ethanolic extracts of *Raphanus sativus* and *Cyperus rotundus* against some pathogenic bacteria and *Candida albicans*. *Baghdad Science Journal* **11(2), 748-756.**

**Alfahad, M. A., Al-Hayanni, H. S. A. and Alnuaimi, M. T. (2022).** Antibacterial activity of biologically synthesized silver nanoparticles using seeds extract of *Nigella sativa* coated on antibiotics against antibiotic-resistant *Salmonella typhi*. *Indian Journal of Ecology* **49 (Special Issue 19), 396-402.**

**Arvizo, R. R., Bhattacharyya, S., Kudgus, R. A., Giri, K., Bhattacharya, R. and Mukherjee, P. (2012).** Intrinsic therapeutic applications of noble metal nanoparticles: Past, present and future. *Chemical Society Reviews* **41, 2943-2970.**

**Balouiri, M., Sadiki, M. and Ibnsouda, S. K. (2016).** Methods for *in vitro* evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis* **6, 71-79.**

**Bauer, A. W., Kirby, W. M., Sherris, J. C. and Turck, M. (1966).** Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology* **45(4), 493-496.**

**Cabuzu, D., Cirja, A., Puiu, R. and Grumezescu, A. M. (2015).** Biomedical applications of gold nanoparticles. *Current Topics in Medicinal Chemistry* **15(16), 1605-1613.**

**Casciaro, B., Moros, M., Rivera-Fernández, S., Bellelli, A., de la Fuente, J. M. and Mangoni, M. L. (2017).** Gold-nanoparticles coated with the antimicrobial peptide esculentin-1a(1-21)$NH_2$ as a reliable strategy for antipseudomonal drugs. *Acta Biomaterialia* **47, 170-181.**

**Casini, A., Sun, R. W. and Ott, I. (2018).** Medicinal chemistry of gold anticancer metallodrugs. *Metal Ions in Life Sciences* **18, 13.**

**CLSI, Clinical and Laboratory Standards Institute. (2020).** Performance Standards for Antimicrobial Susceptibility Testing. 30th edition. CLSI supplement M100. CLSI, Wayne, PA.

**El-Hamid, M. I. A., El-Sayed, Y. E. N., Toka, M. K., Hegazy, W. A. H., Mosbah, R. A., Nassar, M. S., Bakhrebah, M. A., Abdulaal, W. H., Alhakamy, N. A. and Bendary, M. M. (2020).** Promising antibiofilm agents: Recent breakthrough against biofilm producing methicillin-resistant *Staphylococcus aureus*. *Antibiotics* **9(10), 667.**

**Folorunso, A., Akintelu, S., Oyebamiji, A. K., Ajayi, S., Abiola, B., Abdusalam, I. and Morakinyo, A. (2019).** Biosynthesis, characterization and antimicrobial activity of gold nanoparticles from leaf extracts of *Annona muricata*. *Journal of Nanostructure in Chemistry* **9, 111-117.**

**Gurunathan, S., Qasim, M., Choi, Y., Do, J. T., Park, C., Hong, K., Kim, J. H. and Song, H. (2020).** Antiviral potential of nanoparticles—Can nanoparticles fight against coronaviruses? *Nanomaterials* **10(9), 1645.**

**He, X., Fang, J., Huang, L., Wang, J. and Huang, X. (2015).** *Sophora flavescens* Ait.: Traditional usage, phytochemistry and pharmacology of an important traditional Chinese medicine. *Journal of Ethnopharmacology* **172, 10-29.**

**Iswarya, V., Manivannan, J., De, A., Paul, S., Roy, R., Johnson, J. B., Kundu, R., Chandrasekaran, N., Mukherjee, A. and Mukherjee, A. (2016).** Surface capping and size-dependent toxicity of gold nanoparticles on different trophic levels. *Environmental Science and Pollution Research* **23, 4844-4858.**

**Kaykhaii, M., Haghpazir, N. and Walisadeh, J. (2018).** Biosynthesis of gold nanoparticles using aqueous extract of stem of *Periploca aphylla* plant. *Journal of Nanostructures* **8(2), 152-158.**

**Khan, S., Bakht, J. and Syed, F. (2018).** Green synthesis of gold nanoparticles using *Acer pentapomicum* leaves extract its characterization, antibacterial, antifungal and antioxidant bioassay. *Digest Journal of Nanomaterials and Biostructures* **13(2), 579-589.**

**Li, X., Hu, Z., Ma, J., Wang, X., Zhang, Y., Wang, W. and Yuan, Z. (2018).** The systematic evaluation of size-dependent toxicity and multi-time biodistribution of gold nanoparticles. *Colloids and Surfaces B: Biointerfaces* **167, 260-266.**

**Nader, M. I., Ghanima, K. K., Ali, S. A. and Azhar, D. A. (2010).** Antibacterial activity of ginger extracts and its essential oil on some of pathogenic bacteria. *Baghdad Science Journal* **7(3), 1159-1165.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

**Nayantara and Kaur, P. (2018).** Biosynthesis of nanoparticles using eco-friendly factories and their role in plant pathogenicity: A review. *Biotechnology Research and Innovation* **2(1), 63-73.**

**Noruzi, M. (2015).** Biosynthesis of gold nanoparticles using plant extracts. *Bioprocess and Biosystems Engineering* **38(1), 1-14.**

**Patel, M. N., Bhatt, B. S. and Dosi, P. A. (2013).** Synthesis and evaluation of gold(III) complexes as efficient DNA binders and cytotoxic agents. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **110, 20-27.**

**Rao, Y., Inwati, G. K. and Singh, M. (2017).** Green synthesis of capped gold nanoparticles and their effect on Gram-positive and Gram-negative bacteria. *Future Science OA* **3(4), FSO239.**

**Salem, S. S. and Fouda, A. (2021).** Green synthesis of metallic nanoparticles and their prospective biotechnological applications: An overview. *Biological Trace Element Research* **199, 344-370.**

**SAS (2018).** Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS Institute Inc., Cary, North Carolina, USA.

**Sett, A., Gadewar, M., Sharma, P., Deka, M. and Bora, U. (2016).** Green synthesis of gold nanoparticles using aqueous extract of *Dillenia indica*. *Advances in Natural Sciences: Nanoscience and Nanotechnology* **7(2), 025005.**

**Singh, P., Pandit, S., Mokkapati, V. R. S. S., Garg, A., Ravikumar, V. and Mijakovic, I. (2018).** Gold nanoparticles in diagnostics and therapeutics for human cancer. *International Journal of Molecular Sciences* **19(7), 1979.**

**Singh, P. K. and Kundu, S. (2014).** Biosynthesis of Gold Nanoparticles Using Bacteria. *Proceedings of the National Academy of Sciences, India, Section B: Biological Sciences* **84, 331-336.**

**Tao, C. (2018).** Antimicrobial activity and toxicity of gold nanoparticles: Research progress, challenges and prospects. *Letters in Applied Microbiology* **67, 537-543.**

**Thapa, R. K., Diep, D. B. and Tønnesen, H. H. (2020).** Topical antimicrobial peptide formulations for wound healing: Current developments and future prospects. *Acta Biomaterialia* **103, 52-67.**

**Wiley, B., Sun, Y. and Xia, Y. (2007).** Synthesis of silver nanostructures with controlled shapes and properties. *Accounts of Chemical Research* **40, 1067-1076.**

**Xiong, X., Yao, M., Fu, L., Ma, X. and Zhang, X. (2016).** The botanical pesticide derived from *Sophora flavescens* for controlling insect pests can also improve growth and development of tomato plants. *Industrial Crops and Products* **92, 13-18.**

**Yang, J., Liu, P. and Wu, X. Y. (2007).** *In vitro* antibacterial activity of extractum *Sophora flavescens* against *Staphylococcus epidermidis*. *Chinese Journal of Nosocomiology* **17, 1357-1358.**

**Yang, J. F., Yang, C. H., Wu, C. C. and Chuang L. Y. (2015).** Antioxidant and antimicrobial activities of the extracts from *Sophora flavescens*. *Journal of Pharmacognosy and Phytochemistry* **3(6), 26-31.**

**Yougbare, S., Chang, T. K., Tan, S. H., Kuo, J. C., Hsu, P. H., Su, C. Y. and Kuo, T. R. (2019).** Antimicrobial gold nanoclusters: Recent developments and future perspectives. *International Journal of Molecular Sciences* **20(12), 2924.**

**Zaboon, S. M. and Al-Hayanni, H. A. S. (2021).** Detection of virulence factor genes and antibiotic resistance of Enteropathogenic *Escherichia coli* (EPEC) isolated from children with diarrhea. *Biochemical and Cellular Archives* **21(1), 791-796.**

**Zhang, X. (2015).** Gold nanoparticles: Recent advances in the biomedical applications. *Cell Biochemistry and Biophysics* **72, 771-775.**

**Zhang, Y., Dasari, T. P. S., Deng, H. and Yu, H. (2015).** Antimicrobial activity of gold nanoparticles and ionic gold. *Journal of Environmental Science and Health, Part C* **33, 286-327.**

**Zhao, W. Y., Xiao, W., Hao, H. Q., Zheng, K., Zhang, D. D., Jin, Q. and Zhu, Q. S. (2021).** Comparison of activity and components of *Sophora flavescens* root and seed. *IOP Conference Series: Earth and Environmental Science* **705, 012021.**