

## Original Research Article

# Green Synthesis and Characterization of Silver Nanoparticles Using *Codonopsis pilosula* Extract and Evaluation of Its Antimicrobial Activity

Suha M.A. Al-Mudhafar<sup>1\*</sup>, Nadia Jaffer Kadhum<sup>1</sup>, Sahar M. A. Al-Mothafer<sup>1</sup><sup>1</sup>General Directorate of Education Basrah Governorate, Basrah, Iraq**\*Corresponding Author:** Suha M.A. Al-Mudhafar

General Directorate of Education Basrah Governorate, Basrah, Iraq

**Article History****Received:** 04.03.2026**Accepted:** 30.04.2026**Published:** 07.05.2026

**Abstract:** **Background:** Nanotechnology, particularly the use of nanoparticles (NPs), is rapidly evolving and has the potential to transform various scientific and technological sectors. **Aim:** Preparation of the green synthesis and characterization of silver nanoparticles using an extract of the plant *Codonopsis pilosula*, and the evaluation of its antimicrobial agents. **Methodology:** This paper used a green synthesis approach to synthesize silver nanoparticles (AgNPs) using an aqueous extract of *Codonopsis pilosula* roots. It was done by taking the plant extract and mixing it with 2 mM AgNO<sub>3</sub> solution and letting the mixture stay at 30 °C for four hours. A change of color proved the formation of AgNPs and was characterized by Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), and UV-Vis spectroscopy. The FTIR analysis of the plant extract identified important functional groups used in the plant. **Results:** Successful preparation of spherical sized AgNPs with SEM indicating size ranges of 33.74-81.63 nm. XRD patterns were used to confirm the presence of crystalline silver compounds and a characteristic absorbance peak of 340 nm was exhibited by the UV-Vis spectroscopy. More importantly, these biosynthesized AgNPs demonstrated high levels of antibacterial activity against different pathogenic bacteria such as *Acinetobacter baumannii*, *Escherichia coli*, and most importantly, *Staphylococcus aureus*, by disrupting cell membranes and reactive oxygen species generation. **Conclusion:** This study demonstrates a greener, more affordable and biocompatible method of making metallic nanoparticles, providing a greener alternative to traditional chemical techniques of making more complex biomedical and pharmaceutical applications.

**Keywords:** AgNPs, *Codonopsis pilosula*, *Staphylococcus Aureus*, Green Synthesis.

## INTRODUCTION

Nanotechnology, particularly the use of nanoparticles (NPs), is rapidly evolving and has the potential to transform various scientific and technological sectors. NPs are used in biomedicine, agriculture, pharmaceuticals, textiles, food technology, catalysis, sensors, aerospace, automotive, and electronics. Their high surface area-to-volume ratio makes them ideal for surface interactions [1, 2]. Metal nanoparticles can be produced using chemical, physical, and biological techniques [3, 4]. Silver nanoparticles are recognized for their distinctive biological functions, including food preservation, wound healing, cosmetics, biomarking, water purification, sensing, and drug delivery. Furthermore, it possesses atypical applications in paints, electronics, textiles, and catalysts. Medicinal herbs are particularly significant due to their secondary metabolites, which function as reducing and stabilizing agents for nanoparticles at the nanoscale [5]. The rising prevalence of bacterial infectious diseases, coupled with the increasing problem of antibiotic resistance, has prompted the need for novel substances and innovative methodologies to combat bacterial infections effectively [6]. AgNO<sub>3</sub> is a chemical utilized in nanoparticles manufacturing. It is recognized for its properties as an antibacterial agent and growth inhibitor and is applicable in green synthesis production [7].

Recent studies indicate that nanoparticles influence all living creatures, from bacteria to people, and the metals utilized in their manufacture have a detrimental impact on the environment [8, 9]. Biosynthesis is crucial for preventing the generation of detrimental by-products using an eco-friendly and sustainable method. The biosynthesis of metal and

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**Citation:** Suha M.A. Al-Mudhafar, Nadia Jaffer Kadhum, Sahar M. A. Al-Mothafer (2026). Green Synthesis and Characterization of Silver Nanoparticles Using *Codonopsis pilosula* Extract and Evaluation of Its Antimicrobial Activity. *South Asian Res J App Med Sci*, 8(3), 74-82. 74

metal oxide nanoparticles has incorporated several living entities, including plant extract, bacteria, and algae. Among the current eco-friendly methods for synthesizing metal and metal oxide nanoparticles, using plant is a swift, straightforward, and uncomplicated procedure for large-scale nanoparticle production, in contrast to nanoparticles derived from algae, fungi, and bacteria. The synthesized green nanomaterial have significant applications in the pharmaceutical industry, including formulating innovative medications and drug delivery system and synthesizing functional nanodevices [10]. Metal nanoparticles can be produced using chemical, physical, and biological techniques [3, 4]. The biological technique (green synthesis) is preferred for nanoparticle synthesis due to its safety, eco-friendliness, non-toxicity, efficiency, cost-effectiveness, and biocompatibility. Green chemistry is a sophisticated alternative for synthesizing metal nanoparticles using biological agents, including bacteria, fungi, algae, plants, and human cells [11]. Plants are favored for nanoparticles synthesis because they contain phytochemicals, including proteins, sugars, terpenoids, organic acids, vitamins, and polyphenols. These serve as reducing and stabilizing agents for composite nanoparticles tailored to specific shaped and sizes. Silver is favored over other elements in nanoparticle production due to its potent anti-inflammatory, anti-vascular, antifungal, and antibacterial properties [12].

Plants synthesize secondary metabolites to safeguard against environmental stressors [13]. The application of natural goods in addressing global public health issues is significant. These products have anti-inflammatory, anticancer, antioxidant, antidepressant, and antibacterial properties [14, 15]. Medical plants offer valuable resources for novel therapeutic compounds, gaining prominence particularly when conventional synthetic medications prove ineffective [16]. *Condonopsis pilosula* (Franch.) Nannf. (CP), a perennial herb of the Campanulaceae family, natively inhabits the mountainous regions of Vietnam, China, and India, characterized by its bell-shaped flowers and wiry stems. Traditionally, preparation from CP roots have been utilized as a medical remedy for health enhancement, disease prevention, and treatment [17]. The primary chemical components of CP roots comprise phytosterols, sesquiterpenes, alkaloids, triterpenes, phenylpropanoid glycosides, polysaccharides, alkyl alcohol glycosides, and polyacetylene glycosides, which may serve as a superior source for the synthesis of AgNPs and AuNPs [18, 19]. *C. pilosula*, also called Dangshen, is a perennial blooming plant belonging to the bellflower family. It is indigenous to Aisa, thriving in woods, meadows, and scrublands. *C. pilosula* is a significant therapeutic herb in traditional Chinese medicine [20]. *Codonopsis radix* is the desiccated root of *C. pilosula*. It forms a plant medicine with deep historical proportion. It includes a range of bioactive ingredients, including polyacetylene, polysaccharides, phenylpropanoids, and alkaloids triterpenoids, providing significant medical and culinary benefits. Consequently, it has garnered significant attention from numerous scientist [21]. Therefore, the present study aims to achieve the green synthesis and characterization of silver nanoparticles using an extract of the plant *Codonopsis pilosula*, and the evaluation of their biological activity as antimicrobial agents.

## MATERIALS AND METHODS

### Plant Collection

The roots of the *C. pilosula* plant, which were collected in early summer 2024 from local markets in Basra, were collected in August 2025. The roots were ground mechanically into powder and stored at a temperature of -4 °C in the refrigerator until use.

### Materials

Bacterial strains for microbiological testing were obtained from several hospitals in Iraq: *Acinetobacter baumannii*, *Staphylococcus aureus*, *Enterobacter cloaca*, *Klebsiella pneumoniae*, and *Escherichia coli*. Additional fundamental chemicals, such as Mueller Hinton agar, nutrient agar, and silver nitrate, Whatman paper, were procured from a local supplier.

### Preparation of the Aqueous Extract

To produce the aqueous extract, the roots of *C. pilosula* were rinsed with water, air dried in the absence of sunlight to eliminate contaminants, and ground using an electric grinder. Then 5 g of this roots were weighed with a digital balance with an accuracy of 0.001 g (Model HT500, AND, Japan). Manufacturing a root extract entail boiling pulverized roots in distilled deionised water, subjecting it to a water bath for 10 minutes, and filtering it using Whatman filter paper for 1 hour, repeated three times at 25°C. The filtered extract is contained in a sealed tube, encased in aluminium foil, and stored in the refrigerator at 4–7 °C to maintain its stability and efficacy [22, 23].

### Synthesis of Green Silver Nanoparticles

The synthesis of silver nanoparticles (AgNPs) involves the reduction of silver ions using a reducing agent present in an aqueous extract. A 2 mM solution of AgNO<sub>3</sub> was prepared by dissolving 0.39 g of AgNO<sub>3</sub> in 1 liter of deionized distilled water. This solution serves as a source of silver ions (Ag<sup>+</sup>). Ten ml of the aqueous extract was combined with 90 ml of the two mM AgNO<sub>3</sub> solution. This results in a mixture where the reducing compounds in the extract can react with the silver ions. The mixture was maintained at around 30°C, slightly above room temperature, for 4 hours. This temperature provides the energy needed to facilitate the reduction process without denaturing any active compounds in the extract. During this time, the extract reduces the Ag<sup>+</sup> ions to Ag<sup>0</sup>, forming silver nanoparticles. The color change from clear to

brown is a characteristic indicator of silver nanoparticle formation. This is due to surface plasmon resonance (SPR), a property of nanoparticles that leads to distinctive optical properties as the size and shape of the nanoparticles affect the absorption of visible light [23].

### Physical Analysis

#### Scanning Electron Microscope (SEM)

We employed a high-quality Scanning Electron Microscope (TESCAN MIRA model, Czech Republic) to enhance the investigation and examination of the synthesized nanoparticles' morphological properties. The material was thoroughly dried to achieve high-quality SEM pictures. The drying procedure involved: Dissolving green nanoparticles (0.01 g) in acetone, submitting the sample to an ultrasonic bath, and keeping it in an oven at 40 °C for 8 hours. Upon structural analysis using SEM, the nanoparticle powder is coated with a thin layer of gold to enhance surface conductivity. Furthermore, AgNPs powder must be applied onto an aluminum substrate [24].

#### X-Ray Diffraction (XRD)

The dimensions of silver nanoparticle crystals were determined via X-ray diffraction (Philips *et al.*, model, Netherlands) at the angles of 78.2°, 64.4°, 44.3°, and 38.1° (2θ), corresponding to the peaks (111), (200), (220), and (311). The peak width at half maximum intensity (FWHM) was calculated using the formula provided by Basaluis *et al.*, [25]:

$$D = (K\lambda) / (\beta \cos \theta)$$

D represents the crystallite size, β denotes the peak width at half maximum intensity, θ signifies the Bragg angle of the peak, λ indicates the X-ray wavelength, and K is a constant value equal to 0.9.

#### UV–Vis Spectrum

The UV-vis spectrum was recorded using a UV-1800 model from Shimadzu Company, Japan. Analysis was conducted using continuous scanning at a wavelength of 200–800 nm, and the spectra of green silver nanoparticles were compared with the control [26].

#### Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) was conducted in the 4000-500 cm<sup>-1</sup> range using Bruker Tensor 26 (Germany) to identify covalent bonds of potential functional groups in the dried Cp extract and powdered Ag NPs.

### Antibacterial Properties

#### Activation and Purification of Bacterial Cultures

Bacteria were incubated in a nutrient agar culture medium at 36 °C for 24 h for activation.

#### Disc Diffusion Assay

Disc diffusion was performed according to conventional methodology, and dilutions of the aqueous extract and synthesized silver nanoparticles were prepared. Subsequently, a solution of uniform bacteria strains was seeded into Muller-Hinton agar, and the plant extracts, in diameter. The cultured samples were incubated for 24 hours at 27 °C, and the diameter of the growth inhibition zone was measured [27, 28].

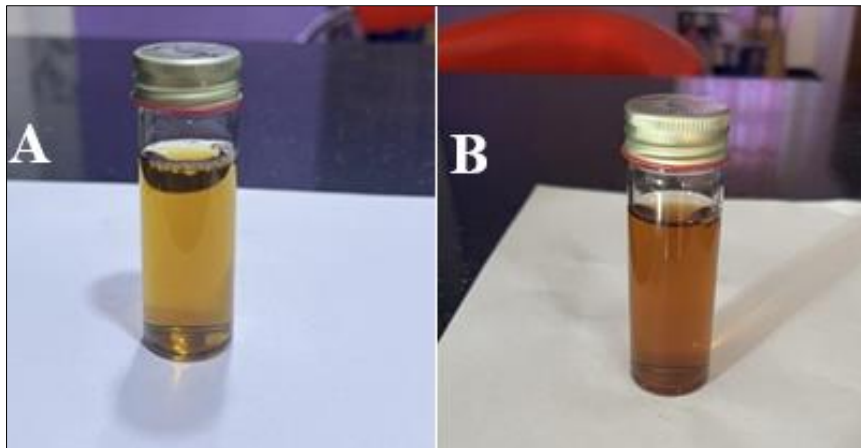
#### Statistical Analysis SPSS

The Statistical Package for the Social Sciences (SPSS) application (2019) was utilized to identify the impact of various factors on study parameters. Least Significant Difference (LSD) was employed to compare means and standard deviations significantly (ANOVA/one-way: probability 0.05) in this investigation, SPSS (2019).

## RESULTS AND DISCUSSION

### Synthesis of AgNPs

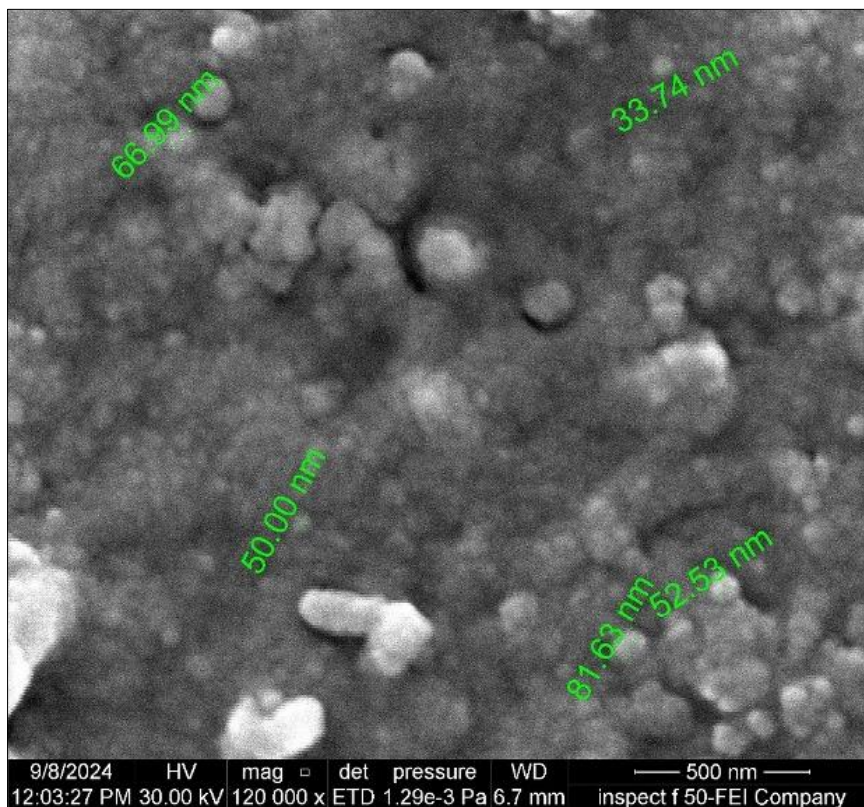
Incorporating *C. pilosula* leaf extract into Ag nitrate solution induces a colour transformation ranging from light beige to deep brown within ten minutes of the reaction. The synthesis of AgNPs in the reaction solution induces a color shift owing to their distinct optical characteristic. The color alteration results from activation surface plasmon resonance (SPR) vibration in silver nanoparticles produced within the reaction media. Suggesting the reduction of silver nitrate to silver nanoparticles (Fig. 1). This investigation indicates that the alteration in solution color post-reaction serves as visual indicator successful synthesis, as illustrated in (Fig. 1), corroborating the findings of Doan *et al.*, [29].



**Fig. 1: Formation of AgNPs after: A) 1 hour. B) 4 hours**

### **Structural and Physical Characteristics of Silver Nanoparticles Scanning Electron Microscope (SEM)**

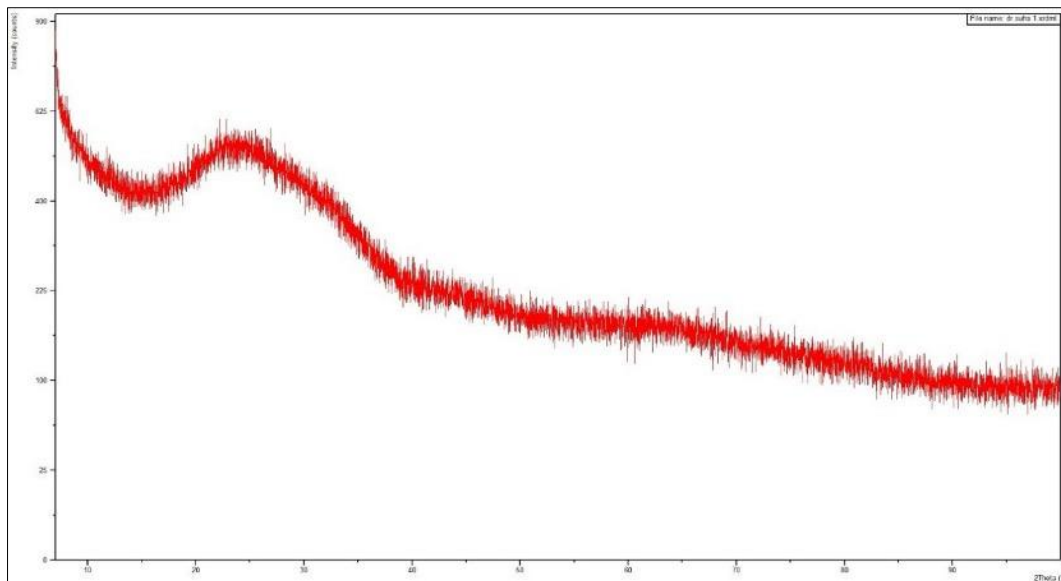
SEM microscopy was utilized to analyze a dried solid CP-AgNPs sample. To do this, the colloiddally stable CP-AgNPs solution was subjected to centrifugation to isolate the solids. The SEM pictures of CP-AgNPs solution was subjected to centrifugation to isolate the solids. The SEM pictures of CP-AgNPs (Fig. 2) reveal that the MNPs are spherical, with sized ranging from 33.74 to 81.63 nm. Doan *et al.*, findings indicated that SEM microscopy was utilized for dried solid AgNPs samples [29]. SEM pictures of CP-AgNPs reveal that the MNPs are spherical and somewhat homogeneous in size, consistent with current research findings.



**Fig. 2: SEM of AgNPs using *C. Pilosula* root extract**

### **XRD**

The XRD pattern exhibits a pronounced peak across the spectrum of 20 values form 20 o to 40o, confirming the presence of silver compounds in the synthesized spherical nanoparticles (Fig. 3).

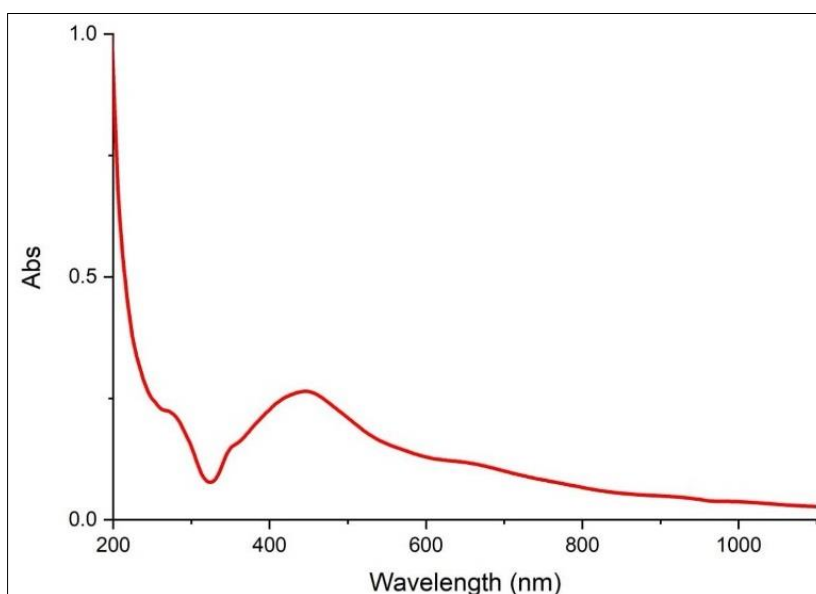


**Fig. 3: XRD of CP-AgNPs**

The study’s results align with those of Doan *et al.*, which determined the crystalline structure characteristic of CP-AgNPs by XRD patterns [29]. Analogous research has documented the occurrence of Cl<sup>-</sup> in aqueous plant extracts [30, 31]. Some investigations contradict the current research findings, indicating that the XRD pattern of CP-AgNPs exhibits four distinctive peaks at 2θ angles, which are indicative of the face-centered cubic structure of Ag nanoparticles [32, 33].

**UV-Vis Spectrum**

UV-Vis spectral analysis was conducted utilizing a UV-Vis spectrophotometer. Silver nanoparticles are widely recognized for their yellowish-brown coloration in aqueous solutions, attributed to the activation of surface plasmon vibrations within the nanoparticles. Incorporating *C. pilosula* extract into the aqueous solution of the silver ion complex resulted in a color transition from clear to yellowish-brown, indicative of silver ion reduction and the creation of silver nanoparticles. UV-Vis’s spectroscopy is widely acknowledged as a method for analyzing size- and shape-controlled nanoparticles in aqueous solutions. (Fig. 4) illustrates the UV-Vis spectra obtained from the reaction media following 24 hours. The absorption spectra of silver nanoparticles generated in the reaction medium exhibit an absorbance peak at 340 nm, with peak broadening indicating polydispersity of the particles. Ahmed *et al.*, delineates a cost-effective and environmentally benign method for the green production of silver nanoparticles from AgNO<sub>3</sub> solution utilizing *Skimmia laureola* extract as both a reducing and capping agent [34]. Nanoparticles were analyzed with UV-VIS absorption spectroscopy.

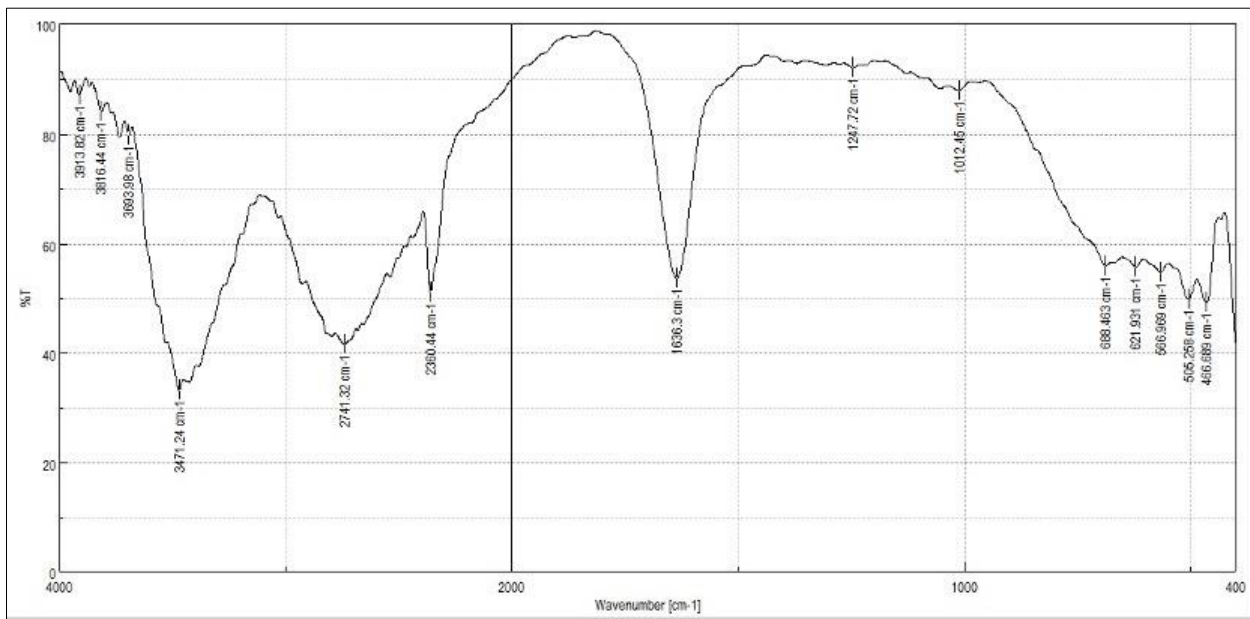


**Fig. 4: UV-vis of CP-Ag NPs**

**Fourier Transform Infra-Red (FTIR)**

The FTIR spectra of CP-AgNPs, in (Fig. 5) show the appearance of major bands at 3441.24, 2741.32, 1636.3, 1247.72, and 1012.45  $\text{cm}^{-1}$ . The peak value in the range of 1000-1300  $\text{cm}^{-1}$  was the characteristic of carbohydrate, while the 3300- 3500  $\text{cm}^{-1}$  wide adsorption band corresponds to carbohydrates (cellulose, hemicellulose, starch, monosaccharide, etc.)

The broad band at 3471  $\text{cm}^{-1}$  is attributed to the O-H stretching vibration of alkaloids, phytosterols, phenylpropanoid glycosides, alkyl alcohol glycosides, polyacetylene glycosides, and polysaccharides found in the plant extract [35, 36]. The water-soluble polyol molecules primarily facilitate the reduction and stabilization of biosynthesized CP-AgNPs [37, 38]. Furthermore, the absorption bands at 2741 and 1636  $\text{cm}^{-1}$  indicate C-H and C=O groups, respectively. The 1247 and 1012  $\text{cm}^{-1}$  peaks correspond to C=O groups in aromatic molecules [39]. Thus, the FTIR spectra demonstrate that the organic components of the plant extract functioned as an efficient reducing agent and stabilizer for CP-AgNPs, corroborating the findings of Doan *et al.*, [29].



**Fig. 5: FTIR of *C. pilosula* extract**

**Antibacterial Effects**

The antibacterial efficacy of silver nanoparticles produced from the natural extract of *C. pilosula* against pathogenic microorganisms was assessed using the disc diffusion technique on Mueller-Hinton agar plates. The diameter of the inhibitory zones (in mm) surrounding each whole containing the plant extract and nanoparticles was measured (Table 1).

**Table 1: The standard antibiotic ampicillin (0.1  $\mu\text{g}/\text{mL}$ ) was positive control, while aqueous CP extract acted as negative control**

Type of Bacteria	Mean $\pm$ SD		
	Bacteria source	CP-AgNPs	Ampicillin (0.1 $\mu\text{g}/\text{mL}$ )
<i>Acinetobacter baumannii</i>	Urine	19 $\pm$ 0.81 bc	9 $\pm$ 0.58 ab
<i>Staphylococcus aureus</i>	Wound	22 $\pm$ 1.08 a	8 $\pm$ 0.42 b
<i>Enterobacter cloacae</i>	Blood	20 $\pm$ 0.92 ab	10 $\pm$ 0.63 a
<i>Klebsiella pneumonia</i>	Sputum	18 $\pm$ 0.75 bc	10 $\pm$ 0.63 a
<i>Escherichia coli</i>	Sputum	17 $\pm$ 0.57 c	8 $\pm$ 0.42 b
LSD value	---	2.063 *	1.679 *

\* Means having with the different letters in same column differed significantly. ( $p \leq 0.05$ ).

The mechanism behind the antibacterial action of silver nanoparticle (AgNPs) remains ambiguous to this day. Silver nanoparticles (Ag NPs) can generate reactive oxygen species (ROS) and hydroxyl ions, leading to rapid microbial mortality. The action involves breaking the cell membrane, generating reactive oxygen species (ROS), penetration of the membrane, and subsequent binding to DNA and proteins. The antibacterial action of Ag NPs likely arises from their surface charge, which interacts with the negatively charged bacteria [40]. The finding indicated that the silver nanoparticles produced by CP-AgNPs had the most excellent antibacterial efficacy against *S. aureus*. The minimal antibacterial efficacy

of the silver nanoparticles produced by CP-AgNPs was observed against *E. coli*. The plant extract functioned as the negative control, whereas ciprofloxacin (1 g/ml) acted as the positive control. The research conducted by Doan *et al.*, strongly corresponds with the present findings, demonstrating the targeted antibacterial efficacy of biosynthesized silver nanoparticles [29]. Both results underscore the efficacy of these nanoparticles against several bacterial species, specifically: Gram-positive bacteria: *S. aureus* (associated with skin infections, pneumonia, and other illnesses), *B. subtilis* (often responsible for food deterioration). Gram-negative bacterium: *Escherichia coli* (*E. coli*, a prevalent bacterium capable of inducing gastrointestinal infections). The selectivity in antibacterial efficacy noted in both investigations indicates that the biosynthesized silver nanoparticles can specifically target certain bacterial species, likely attributable to variations in the structure of bacterial cell walls.

## CONCLUSION

This study was able to show how silver nanoparticles (AgNPs) could be greenly synthesized in aqueous root extract of *Codonopsis pilosula*. AgNPs synthesized demonstrated a pronounced antimicrobial effect against a number of pathogenic bacteria, such as *Acinetobacter baumannii* and *Escherichia coli*, and the antimicrobial effect was the most effective on *Staphylococcus aureus*. The disruption of cell membranes and the production of reactive oxygen species are considered to be the main mechanisms of action. In the end, this research demonstrates a greener, more affordable and biocompatible method of making metallic nanoparticles, providing a greener alternative to traditional chemical techniques of making more complex biomedical and pharmaceutical applications.

**Conflict of Interest:** All authors acknowledge that there is no conflict of interest in this article.

**Funding:** No funding.

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