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Green synthesis, characterization, and antimicrobial activity of silver nanoparticles synthesized using *Annona squamosa* L. leaf extract

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Abstract

A green synthesis of silver nanoparticles (AgNPs) has drawn a lot of interest because it is scalable, economical, and environmentally benign. In this work, silver nanoparticles were quickly biosynthesised using leaf extract from *Annona squamosa* L. Fourier Transform Infrared (FTIR) spectroscopy, X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and UV-Vis spectroscopy were used for characterization. At 435 nm, surface plasmon resonance was detected by UV-Vis examination. SEM showed that the nanoparticles were spherical and ranged in size from 35 to 90 nm. While FTIR investigation revealed functional groups involved in reduction and capping, XRD verified the crystalline nature. *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Escherichia coli* were all significantly inhibited by the produced AgNPs. These results show the potential of AgNPs mediated by *A. squamosa* in antibacterial applications.

Key words : *Annona squamosa,* Green synthesis, Silver nanoparticles, Antimicrobial activity, Characterization, FTIR, SEM, XRD.

Alternative antimicrobial techniques must be developed in light of the major threat to world health posed by the fast emergence of antibiotic-resistant bacteria. Because of their broad-spectrum antibacterial activity and several mechanisms of action, including as membrane rupture, ROS production, and interference with DNA replication, silver nanoparticles (AgNPs) have attracted a lot of attention among these[8,9,21]. While traditional

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nanoparticle synthesis techniques frequently employ hazardous chemicals and a lot of energy, green synthesis with plant extracts provides a more economical and environmentally responsible option that is in line with green chemistry principles[1,3]. The stability and biological efficiency of nanoparticles are improved by phytochemicals such flavonoids and phenolics, which function as natural reducing and stabilizing agents[19]. The medicinal plant *Annona squamosa* L., sometimes known as the sugar apple, is full of bioactive substances that have antioxidant and antibacterial qualities[25]. Its leaves, which are especially rich in polyphenols, have demonstrated potential for aiding in the production of biocompatible AgNPs with significant antibacterial properties[27,30]. This study emphasizes sustainable methods for biomedical applications by concentrting on the green production and characterisation of AgNPs using *A. squamosa* leaf extract and assessing their antibacterial effectiveness against certain clinical pathogens[18].

Preparation of Leaf extract :

Fresh leaves of *Annona squamosa* were cleaned, dried in the shade, and ground into a powder. After 25 to 30 minutes of boiling in 100 milliliters of distilled water, five grams of the powder were filtered using Whatman No. 1 filter paper.

Precursor solution preparation :

In 100 milliliters of distilled water, 0.169 grams of $AgNO_3$ were dissolved to create a 10 mM $AgNO_3$ solution.

Production of Nanoparticles :

After heating 45 milliliters of $AgNO_3$ solution in a beaker, 5 milliliters of *A. squamosa* extract was added dropwise. Surface plasmon resonance-induced AgNP production was evidenced by a color shift from pale yellow to dark brown. For additional analysis, the colloidal solution was kept at 4°C.

Characterization of AgNPs :

Several analytical methods were used to characterize the silver nanoparticles (AgNPs) made from *Annona squamosa* leaf extract. To verify the creation of nanoparticles by detecting surface plasmon resonance, UV-visible spectroscopy was carried out in the 300–700 nm range. SEM-EDX, or scanning electron microscopy combined with energy dispersive X-ray analysis, was used to ascertain the nanoparticles' dimensions, form, and elemental makeup. The crystalline structure of the AgNPs was evaluated by X-ray diffraction (XRD) examination employing Cu Kα radiation (40 kV, 30 mA) over a 2θ range of 10°–80°. The functional groups in charge of stabilizing and reducing silver ions during nanoparticle production were identified using Fourier Transform Infrared Spectroscopy (FTIR) in the 4000–400 cm^{-1} range.

Antimicrobial Activity :

The antimicrobial activity of biosynthesized silver nanoparticles (AgNPs) was evaluated in vitro against *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, obtained from the MTCC, Chandigarh. Mueller-Hinton agar plates that had been inoculated with the bacterial cultures were used in the agar disc diffusion procedure. On the plates were sterile discs impregnated with 25 µg/ml of AgNPs, as well as untreated sterile discs for the negative control and standard antibiotic discs (ciprofloxacin and linezolid) for the positive control. The antibacterial effectiveness was evaluated by measuring the zones of inhibition following incubation. This technique successfully illustrated how green-synthesised AgNPs might suppress the test pathogens.

Characterization of Silver Nanoparticles :

A UV-visible absorption peak at 435 nm, which indicates surface plasmon resonance and nanoparticle stability, verified the successful manufacture of silver nanoparticles (AgNPs) using *Annona squamosa* leaf extract (Figure 1)[10,14,20]. In line with earlier research on green synthesis, SEM analysis showed primarily spherical particles with sizes ranging from 35 to 90 nm (Figure 2)[15,29]. The face-centered cubic crystalline structure of silver was confirmed by XRD patterns, which showed distinctive peaks at 38°, 44°, 64°, 78°, and 81° (Figure 3). The size of the crystallites matched the results of SEM [6,23]. The significance of plant polyphenols and proteins in reduction and stabilization was demonstrated by FTIR analysis, which revealed important functional groups at 3348, 2903, and 1441 cm^{-1} (–OH and –CH stretching) and at 1083 and 1046 cm^{-1} (C=O and C–N) (Figure 4)[7]. These findings validate the eco-friendly synthesis of stable and biologically active AgNPs.

Antibacterial activity of Annona squamosa-mediated Silver Nanoparticles :

The agar disc diffusion method was used to evaluate the antibacterial activity of biosynthesized silver nanoparticles (AgNPs) against *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Escherichia coli.* According

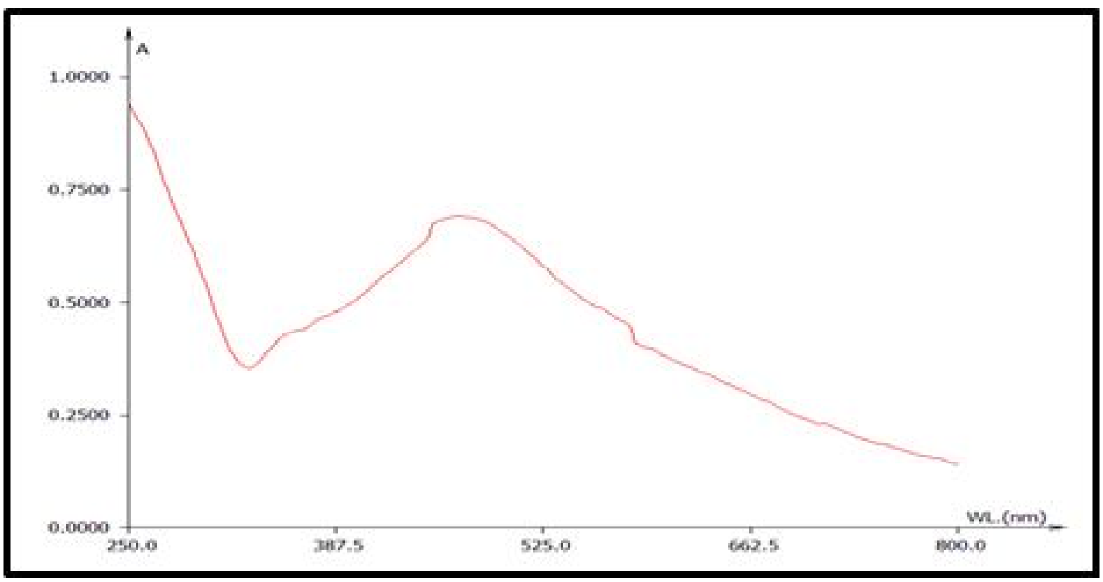
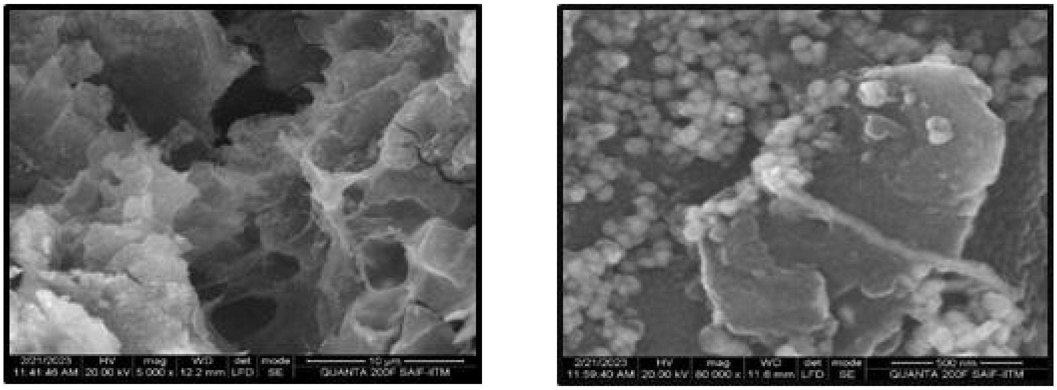


Figure 1. UV- spectrum -Green silver nanoparticles



2A **2B**

Figure 2. Shows the SEM images of *Annona squamosa* mediated AgNPs

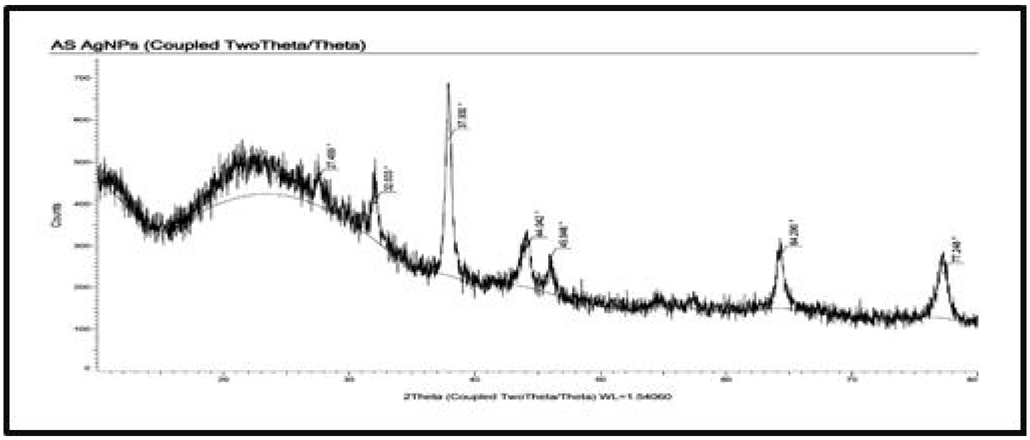


Figure 3. XRD patterns of *Annona squamosa* mediated AgNPs

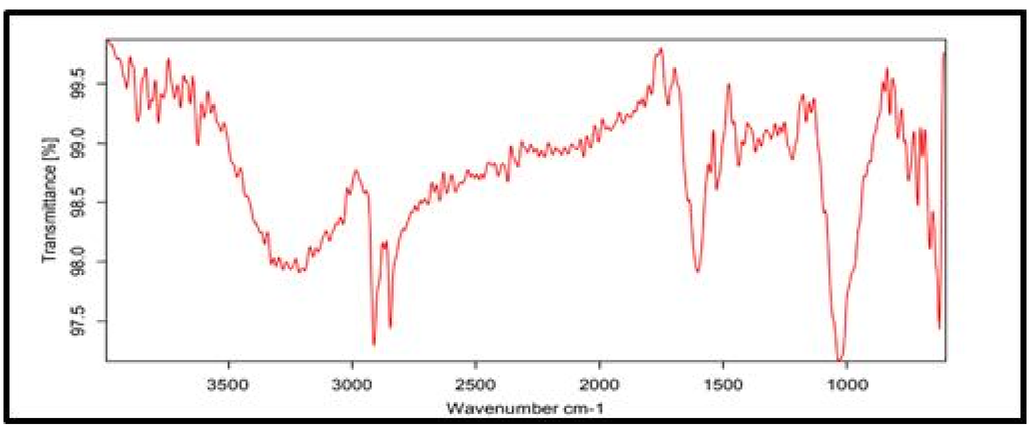


Figure 4. FTIR graph of *Annona squamosa* mediated AgNPs

to Table-1 and Figure 5, several zones of inhibition were seen, with *E. coli* exhibiting the maximum sensitivity (12 mm), followed by *K. pneumoniae* (9 mm) and *P. aeruginosa* (8 mm). In line with previous findings, the AgNPs showed significant inhibitory zones for E. coli (12 mm), *K. pneumoniae* (9 mm), and *P. aeruginosa* (8 mm)[2,5,22]. Given their natural source and absence of chemical enhancers, the green-synthesised AgNPs exhibited strong antibacterial activity even if these inhibitory zones were smaller than those generated by conventional antibiotics (20–22 mm).

Table-1. Antibacterial activity of *Annona squamosa*-mediated AgNPs

Bacteria	AS-AgNPs	+ve Control	–ve Control
Escherichia coli	12 mm	20 mm	NZ
Klebsiella pneumoniae	9 mm	20 mm	NZ
Pseudomonas aeruginosa	8 mm	22 mm	NZ

NZ – No zone of inhibition

The negative control (sterile disc) showed no inhibitory zones, confirming that the AgNPs were the only source of the antibacterial activity. AgNPs' capacity to cling to bacterial membranes, produce reactive oxygen species (ROS), and interfere with cellular processes is probably connected to the antibacterial mechanism[11,16,24]. Despite the possibility of reduced inhibition zones due to variables like particle size, dosage, or the lack of synergistic agents, the biosynthesized AgNPs present a viable environmentally friendly substitute for traditional antimicrobials[4,28]. *E. coli's* increased susceptibility is probably caused by its thinner peptidoglycan layer, which makes it easier for nanoparticles to penetrate[17]. These results are in line with other observations on plant-derived AgNPs and their action against Gram-negative bacteria[12,13,26]. Notably, the inhibition of *P. aeruginosa*, a multidrug-resistant pathogen, highlights the potential of *A. squamosa*-mediated AgNPs in combating resistant bacterial strains.

Figure 5. Shows the antibacterial activity of *Annona squamosa* mediated AgNPs

Using leaf extract from *Annona squamosa,* this study successfully illustrates the green synthesis of silver nanoparticles, offering a practical and affordable substitute for chemical processes. The creation of stable, spherical, and crystalline AgNPs was validated by characterization. The produced nanoparticles exhibited significant antibacterial activity, especially against *E. coli*. Given the rise in antibiotic resistance, these results point to their potential as natural antibacterial agents. To evaluate their therapeutic significance, adjust formulations, and gauge cytotoxicity, more research is required.

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