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# Synthesis and Characterization of Silver Nanoparticles using aqueous extract of *Andrographis paniculata* and their Antimicrobial Activities

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**Abstract:**
Silver nanoparticles (Ag-NPs) have been successfully prepared with simple and green synthesis method by reducing Ag+ ions in aqueous extract as a reducing agent. In this study, aqueous extract of *A. paniculata* was used as a reducing and stabilizing agent. The structural and antimicrobial properties of the synthesized nanoparticles were studied. X-ray diffraction patter (XRD) pattern showed the crystalline nature of particles. The average diameter of the nanoparticles was calculated by TEM and XRD was around 83nm. Energy dispersive X-ray spectroscopy (EDS) spectrum and XRD pattern suggested that prepared nanoparticles were highly pure. The nanoparticles showed excellent antimicrobial activity against various bacterial. Moreover, *P. aeruginosa* exhibited the highest sensitivity to nanoparticles while *B. Subtilis* was the least sensitive.

**Key words:** *Andrographis paniculata***,** aqueous extract, nanoparticles, SEM, XRD, antimicrobial activity.

## 1. Introduction:

Silver nanoparticles have drawn the attention of researchers because of their suitable applications in the fields of electronic, material science, and medicine [1, 2]. In recent years, green synthesis of metal nanoparticles is an interesting issue of the nano-science and nano-biotechnology. There is a growing attention to biosynthesis the metal nanoparticles using organisms. Among these organisms, plants seem to be the best candidate and they are suitable for large-scale biosynthesis of nanoparticles. Nanoparticles produced by plants are more stable, and the rate of synthesis is faster than that in the case of other organisms. Moreover, the nanoparticles are more various in shape and size in comparison with those produced by other organisms [3, 4].

Antimicrobial properties of silver nanoparticles caused the use of these nano-metals in different fields of medicine, various industries, animal husbandry, packaging, accessories, cosmetics, health, and military. Silver nanoparticles show potential antimicrobial effects against infectious organisms such as *Escherichia coli*, *Bacillus subtilis*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Syphilis typhus*, and *Staphylococcus aureus* [5, 6]. Moreover, these nanoparticles have drawn the attention of researchers because of their extensive applications in areas such as mechanics, optics, biomedical sciences, chemical industry, electronics, space industries, drug-gene delivery, energy science, catalysis [7, 8], optoelectronic devices [9, 10], photo-electrochemical applications [11], and nonlinear optical devices [12, 13].

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*Andrographis paniculata* (Burm. F) Nees, commonly known as the "king of bitters," is an herbaceous plant belonging to the Acanthaceae and is found throughout tropical and subtropical Asia, Southeast Asia, and India.

Extracts of this plant and andrographolide exhibit pharmacological activities such as those that are immunostimulatory [12, 14], antiviral [15], and antibacterial [16]. As major active constituent, andrographolide exhibits a broad range of biological activities, such as anti-inflammatory, antibacterial, antitumor, antidiabetic, antimalarial, and hepatoprotective [17].

**2 Materials and Methods:**

*Andrographis paniculata* plant collected from botanical garden at Dr N R S Ayurvedic Medical College, Bandar Road, Vijayawada. The plant parts were separated cleaned with sterile distilled water and then they were air dried for 7 days. The plants were ground to a fine powder. The powder obtained was extracted with distilled water. To 5g of powdered sample, 100ml of distilled water was added and boiled to 60-70$^{o}$C for about 10mins. Then the resulting crude extracts filtered through 0.25µ filter and stored in refrigerator. 10 ml of plant extract was added into 90 ml of prepared aqueous solution of 1mM silver nitrate for reduction into Ag+ ions and kept in magnetic stirrer for 1 hour at room temperature.

**2.1 Characterization of synthesized nanoparticles:**

**2.1.1 UV-VIS Spectra analysis:**

The reduction of pure Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 3hours after diluting a 1ml of the sample into 4ml of distilled water. UV-Vis spectral analysis was done by using UV-VIS spectrophotometer.

**2.1.2 SEM analysis of silver nanoparticles:**

Scanning Electron Microscopic (SEM) analysis was done using instrument LEO 1420 VP Scanning Electron Microscopic. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5min.

**2.1.3 XRD analysis of silver nanoparticles:**

The particle size and nature of the silver nanoparticle were determined using XRD. This was carried out using Bruker- D4 ENDEAVOR XRD-6000/6100 model with 30kv, 30mA with Cu kα radians at 2Θangle. X-ray powder diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground, and average bulk composition is determined. The particle or grain size of the particles on the silver nanoparticles was determined using Debye Sherrer's equation.

$$D = \frac{0.94\lambda}{B\cos\Theta}$$

**2.1.4 EDX analysis of silver nanoparticles:**

To gain further insight in to the features of the silver nanoparticles, analysis of the sample was performed using Energy Dispersive microanalysis techniques. EDX analysis was carried to confirm the presence of silver in the particles as well as to detect other elementary compositions of the particles.

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**2.2 Antimicrobial activity:**

The antimicrobial activity of the synthesized nanoparticles was tested against four different pathogenic organism *B. Subtilis, E.coli, P. aeruginosa, S. aureus, A.niger*. Bacterial sensitivity to nanopaticles was commonly tested using a disc diffusion assay, utilizing antibiotics or NPs impregnated disks. After the incubation period, the growth inhibition zone was measured and the results of the inhibition were measured in milli meters.

**3. Results and Discussion:**

It is well known that silver nanoparticles exhibit a yellowish-brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [19]. Reduction of silver ions to silver nanoparticles could be followed by a color change and UV-Vis spectroscopy. The technique outlined above has proven to be very useful for the analysis of nanoparticles [20, 21, and 22]. Therefore, the progress in conversion reaction of silver ions to silver nanoparticles was followed by a color change and spectroscopic techniques. Figure 1 shows the photographs of sample solutions containing silver nitrate and silver nitrate in the presence of optimized amounts of plant extract solutions after completion of the reaction. The appearance of yellowish-brown color confirms the formation of silver nanoparticles in the solution.

The silver nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization of silver nanoparticles (Sun et al., 2001). The absorption spectrum (Figure 2) of the yellowish-brown silver nanoparticle solution prepared with the proposed method showed a surface Plasmon absorption band with a maximum of 424 nm, indicating the presence of Ag nanoparticles. The dry powders of the silver nanoparticles were used for XRD analysis. The diffracted intensities were recorded from 20◦ to 80◦ at 2 theta angles. The diffraction pattern in Figure 3 corresponds to pure silver metal powder. The XRD pattern indicates that the nanoparticles had a spherical structure. No peaks of the XRD pattern of $Ag_2O$ and other substances appear in Figure 3, and it can be stated that the obtained silver nanoparticles had a high purity. The observed peak broadening and noise were probably related to the effect of nano-sized particles and the presence of various crystalline biological macromolecules in the plant extracts. The obtained results illustrate that silver ions had indeed been reduced to AgO by *Andrographis paniculata* plant extract under reaction conditions.

Scanning electron microscopy provided further insight into the morphology and size details of the silver nanoparticles. Comparison of experimental results showed that the diameter of prepared nanoparticles in the solution was about 83-667 nm. Figure 4 shows the scanning electron micrograph of silver nanoparticles obtained from the proposed bio-reduction method. TEM micrograph was employed to visualize the size distribution, shape and average diameter of nano-particles synthesized using the selected plant extracts.

A typical TEM image [Figure 5] of biologically synthesized AgNPs suggests that the particles are uneven in shape. Some are spherical, rod, and triangular shaped particles with a varying size of 83-667nm. A size variation of 667, 333 and 83 nanometers size nano particles were formed during synthesis with *A. paniculata* plant.

Results of the growth inhibition study of the synthesized nanoparticles demonstrated excellent antimicrobial activity against a range of bacteria. The diameter of inhibition zone reflects magnitude of susceptibility of microbes. The strains susceptible to silver nanoparticles exhibited larger zone of inhibition, whereas resistant strains exhibit smaller zone of inhibition. The synthesized nanoparticles show high resistance against the growth of *P. aeruginosa*. At a concentration of 1000µg/ml, the nanoparticles show resistance against the growth of all the microbes in the study

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except *B. Subtilis.* At a very low concentration also the particles show effective growth inhibition of the microbes. The results of the zone inhibition were given in table 1.

**4. Conclusion**:

A critical need in the field of nanotechnology is the development of reliable and eco-friendly processes for synthesis of metallic nanoparticles. Here, we have reported a simple biological and low-cost approach for preparation of stable silver nanoparticles by reduction of silver nitrate solution with a bioreduction method using manna of *Andrographis paniculata* aqueous extract of the whole plant as the reducing agent for their efficient antibacterial and antimicrobial properties. The characteristics of the obtained silver nanoparticles were studied using UV-Vis, XRD, EDX, and SEM techniques. The results conformed the reduction of silver nitrate to silver nanoparticles with high stability and without any impurity. Comparison of experimental results showed that the average size of synthesized silver nanoparticles was about 83nm. The synthesized particles show effective growth inhibition on various pathogenic microbes. This study indicates that AgNPs can be used as effective antibacterial materials against various microorganisms which can endanger human beings.

**Figures and Tables:**


**Figure 1: color change of the silver nitrate solution by the addition of the aqueous extract of *A. paniculata***

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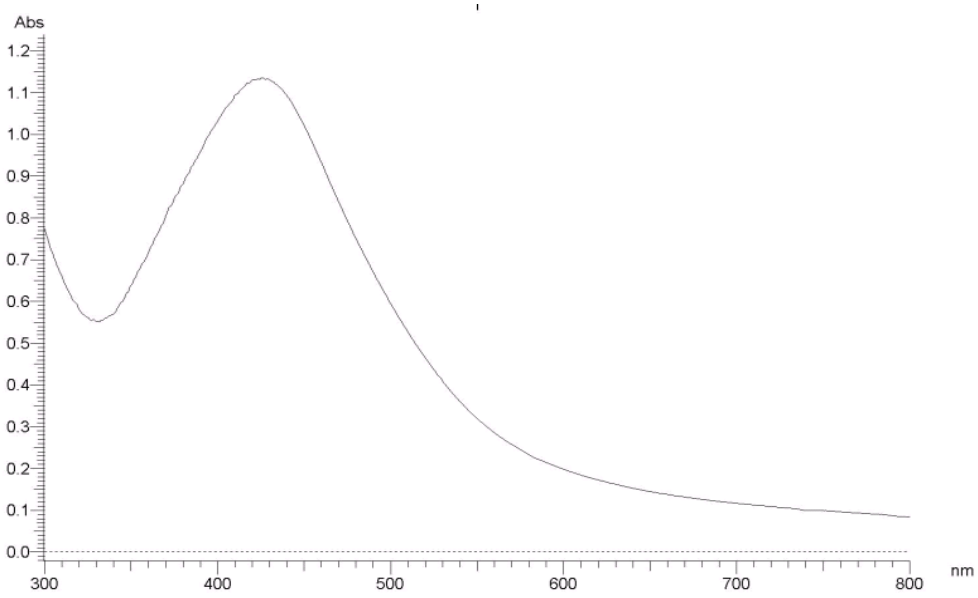


**Figure 2: UV-Vis absorption spectra of silver nanoparticles prepared with *A. paniculata* extract**

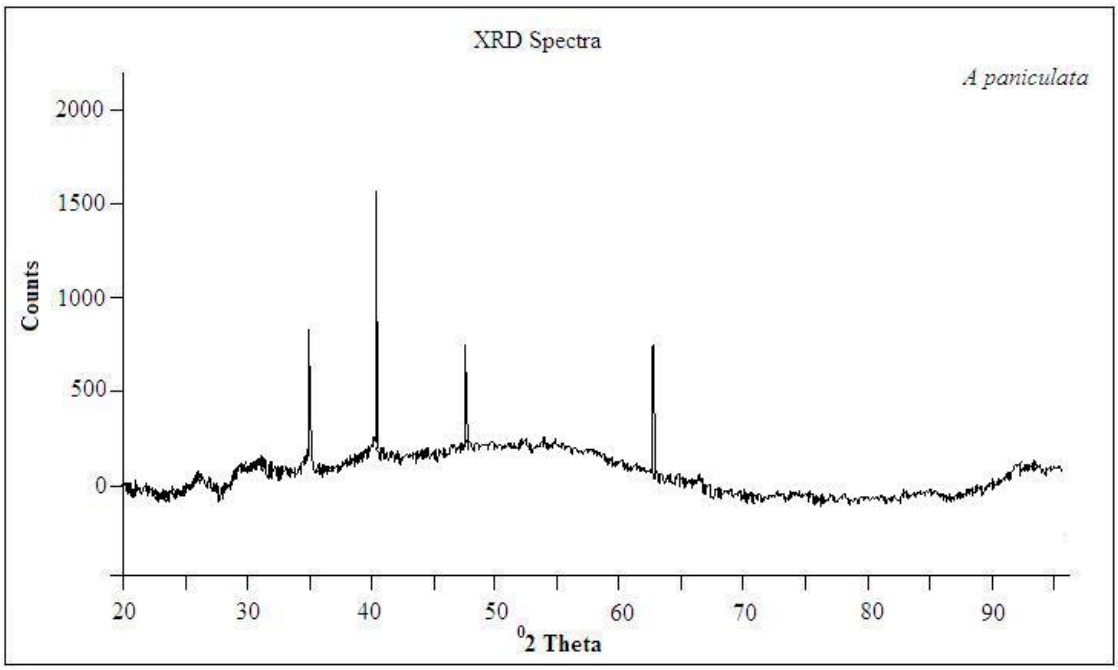


**Figure 3: XRD spectra for the synthesized nanoparticles**

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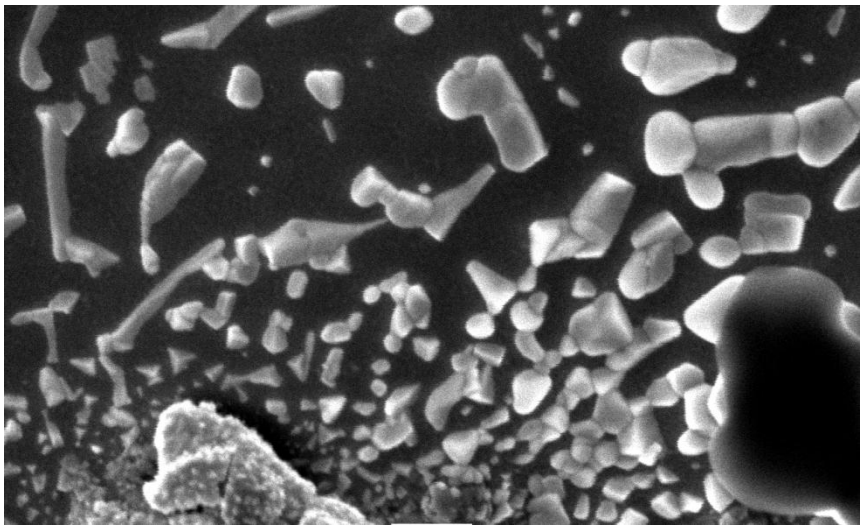

**Figure 4: SEM image for the synthesized nanoparticles**


**Figures 5: TEM images for the synthesized nano-particles of *A. paniculata***

| Nano particles | Concentration | B. Subtilis (le24) | E.coli (ts22) | P. aeruginosa (GF) | s.aureus (SSB1) | A.niger |
|---|---|---|---|---|---|---|
| *A. paniculata* | 100µg/ml | -ve | -- | 2mm | -ve | 1mm |
| | 500µg/ml | -ve | 2mm | 4mm | -ve | 2mm |
| | 1000µg/ml | -ve | 7mm | 10mm | -ve | 8mm |

**Table 1: Inhibition zone for the silver nanoparticle against microorganism under the study**

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