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BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANO PARTICLE FROM *ACANTHOSPERMUM HISPIDUM* ROOT EXTRACT

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ABSTRACT

Plant extracts are very cost effective and eco-friendly, thus, can be an economic and efficient alternative for the large-scale synthesis of nanoparticles. The preparation of silver nanoparticles by reduction of silver ions with *Acanthospermum hispidum* root extract isolated from ethyl acetate/ethanol as a solvent characterized by UV-visible absorption spectrum, X-Ray Diffraction (XRD), EDX, Fourier Transform Infrared (FTIR) and Transmission Electron Microscopy (TEM). Analysis of TEM showed that the synthesized silver nanoparticles are in spherical shape with an average size of 29 nm. Further the XRD analysis confirms the nano-crystalline phase of silver with face centred cubic (FCC) crystal structure. The UV/Vis spectra absorption peak confirms their production. Pioneering of reliable and eco-friendly process for synthesis of metallic nanoparticles biologically is an important step in the field of application of nano medicine. Thus, these silver nanoparticles (Ag-NPs) may prove as a better candidate for drugs and can potentially eliminate the problem of chemical agents because of their biogenic nature.

Key words: *Acanthospermum hispidum* root extract-Silver nano particle-Characterization

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1.0 INTRODUCTION

Nanotechnology provides the tools and technology platform for the investigation and transformation of biological systems, and biology offers inspiration models and bio-assembled components to nanotechnology. Among the different living organisms used for nanoparticles synthesis, plants are of particular interest in metal nanoparticles synthesis because of its advantage over other environmentally benign biological process as it eliminates the elaborate process of maintaining cell cultures. Plant mediated synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliest. Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process[5]. The most important application of silver nanoparticles in medical industry is topical ointments to prevent infection against burn and open wounds[3]. The reduction of Ag+ ions by combinations of bio molecules found in the extracts such as vitamins, enzymes/proteins, organic acids such as citrates, amino acids, and polysaccharides[4].

Acanthospermum hispidum (member of the Asteraceae family) is considered as an important medicinal plant of India. It is found as a weed along the roads and in moist habitats throughout India. The common name of this medicinal plant in Kannada is known as Kandlemullu. . The species is easily identifiable and grows abundantly during the rainy seasons in NE Brazil; is amenable to cultivation without loss of its

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phytochemical profile, and toxicological studies have showed its safety as a medicine (although more studies will be required in that direction). As such, the compilation of the accumulated knowledge concerning this species will aid in evaluating its pharmacological value, guaranting quality control of the final product, and in preparing recommendations for usages and dosages that offer both safety and efficiency to the user. *Acanthospermum hispidum* plant is important for its medicinal properties. It possesses antibacterial and antifungal properties. The crushed herb is used in the form of a paste to treat skin ailments and the leaf juice is reportedly used to relieve fevers[1].

Acanthospermum Hispidum (DC) is a medicinal plant (figure 1). *A. hispidum* is used in traditional medicine for the treatment of jaundice, malaria, vomiting, cephalgias, head-ache, abdominal pain, convulsions, stomach ache, constipation, eruptive fever, snake bite, epilepsy, blennorrhoea, hepato-biliary disorders,malaria, microbial infection and viral infections. *A. hispidum* appears to contain phytoconstituents that may be useful adjuvant for antibiotic formulations. It is used for the treatment of skin aliments and to treat cough and bronchitis. It is also used as an antifeedant[2].

In view of the importance of *A. hispidum* and silver nanoparticles, the present work has been planned to synthesize and characterize the silver nanoparticles synthesized from the root extract (EtAc/EtOH) of *A. hispidum* using $AgNO_3$using greener techniques and characterise them with different techniques with the aim of development of Nano medicine.

Figure 1: Schematic representation of *Acanthospermum hispidum* plant, roots and powder

2. Materials and Method

All chemicals and reagents had analytical grade. Silver nitrate, n-hexane with high purity purchased from Sd Fine/Merck India Chemicals, India.

2.1 Apparatus and Instruments: The conventional Soxhlet extraction apparatus was used, which consists of a condenser, a Soxhlet chamber, and an extraction flask. The extractor thimble was permeable one with 44 mm internal diameter and 200 mm external length. The rotary evaporator was used for evaporation of solvent of extracted material.

2.2 Sampling and extraction

***Plant Material*:** Fresh roots of *A. hispidum* plant roots in bulk collected in the month of May 2012 from agricultural fields of local area of Tenali revenue subdivision, Andhra Pradesh. 30x10 cm roots were collected cut in to small pieces (figure 1), washed and dried in sunlight for one month completely to eliminate surface moisture. Then roots packed into envelop and kept in oven at 55°C temperature for further dryness. Dried material was grinded separately in a mortar obtained fine powder and sieved; which was then kept in plastic bags for further use.

Preparation of plant extract: The dry root powder material of *A. hispidum* passed through sieve (100□). The coarse powdered drug (200grams) was extracted in Soxhlet apparatus for 48 h with ethyl acetate and ethyl alcohol (60:40) combination, the extract obtained was concentrated under reduced pressure in rotatory

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evaporator below 75°C temperature to get Then the filtered extract was stored in refrigerator at 4°C for further use in synthesis of silver nanoparticles.

2.3 Synthesis of AgNPs (SNPs): The synthesis of silver nanoparticles was done by mixing *A. hispidum* root extract and 1 mM of aqueous silver nitrate solution ($AgNO_3$) in the ratio 1:20 added to plant extract ethanolic solution and heated at 80 ± 2°C until the colour of the solution was changed from colour less to light brown (Figure 2). Resulted solutions were settled for 24 hours in dark to avoid any further photochemical reactions, after that the solution was centrifuged at 2000 rpm for 20 minutes with magnetic shaker. The supernatant was discarded and the pellet was air dried in the incubator.

The bioreduction of Ag^+ ions was monitored by periodic sampling by the UV spectrophotometer. The AgNPs in the freeze-drying bottle were suspended in ultrahigh purity water for all characterization methods and antibacterial assays. During biosynthesis of silver nanoparticles when stem extract was added to 100 ml of 1 mM $AgNO_3$ salt, the ionization took place as follows:

$AgNO_3(aq) \leftrightarrow Ag^+(aq) + NO_3^-(aq)$

$e^- + Ag^+ \rightarrow Ag^o$

It is assumed that the silver ions enter inside the plant cell via the H^+ATPase protein embedded in the thylakoid membrane by an electro genic pump. Synthesis of silver nanoparticles is a photochemical reduction reaction.

2.4 Characterization techniques

- UV–visible spectroscopy: The formation of dark brown color during the synthesis was confirmed as the formation of AgNPs. The reduction of the pure AgNPs was recorded under UV–visible spectroscopy using ELico model UV–visible spectrophotometer between 300 nm and 700 nm. The UV–visible spectra of the plant leaf extract and silver nitrate solution were also recorded.
- FTIR analysis was done using Perkin Elemer Spectrum-1, and was used to identify the chemical constituents in the region of 400-4000 cm^{-1} of the Ag-NPs
- XRD measurement: XRD measurements of Ag-NPs were cast into glass slides were done by Phillips PW 1830 instrument. The operating voltage of 40 kV and current of 30 mA with Cu kα radiation of 0.1541 nm wavelength, in the 2θ range 10- 80º, step size 0.02/θ.
- The morphology of the Ag-NPs was analyzed using an SEM. The powdered Ag-NPs were uniformly spread and sputter coated with platinum in an ion coater for 120 seconds, then observed by SEM JEOL-JSM 6360 MODEL, JAPAN). The size distribution of the nanoparticle was obtained by counting 150 particles from an enlarged SEM image.32 Elemental analysis of the powdered Ag-NPs was conducted using an EDX detector (EDS, EDAX Inc., Mahwah, NJ, USA) attached to the SEM machine.
- TEM analysis of Ag-NPs: Sample for TEM analysis was prepared, as mentioned in IR sample preparations. The sample was first sonicated (Vibronics VS 80) for 5 minutes. Ag-NPs were loaded on carbon coated copper grids, and solvent was allowed to evaporate under Infra light for 30 minutes. TEM measurements were performed on Phillips model CM 20 instrument, operated at an accelerating voltage at 200 kV.

3.0 Results and discussion

3.1 UV-Visible Spectroscopy Characterization AgNPs

In the present work, aqueous leaf extract of *Acanthospermum hispidum* root extract has been used for the synthesis of silver nanoparticles by reducing silver ions. It is well known that silver nanoparticles attain light brown color in aqueous solution (6). When the aqueous solution of silver ions is mixed with aqueous leaf extract, its color started to change from light yellow to dark yellow, indicating the initial synthesis of silver nanoparticles (Fig. 2). This change in color arose due to excitation of surface plasmon resonance (SPR) with the silver nanoparticles. The exact mechanism of biosynthesis of silver nanoparticles is not well understood (7). Color change was followed by taking UV–visible spectroscopy of colloidal solution after 24 h reaction. It is proved to be a best technique for monitoring the formation of silver nanoparticles in the colloidal solution.

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Due to excitation in SPR, silver nanoparticles showed absorption peak at 439 nm (Fig. 2). Other investigators also observed the maximum absorption of colloidal silver solution in the range of 400–450 nm. Our results were in good agreement with the results of Sarkar et al. (8) where the silver nanoparticles using *Parthenium* leaf extract at room temperature showed absorption spectrum at 440nm.

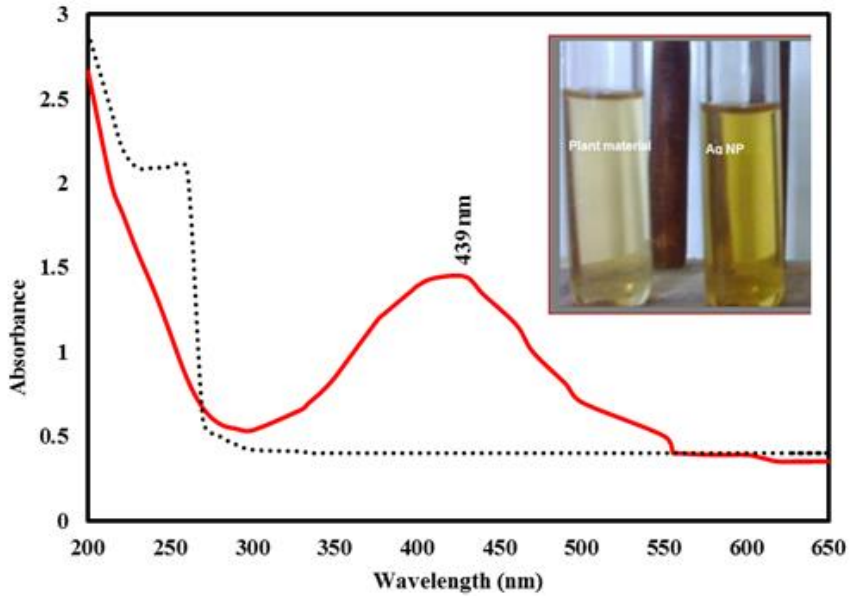

Figure 2: UV-Visible spectra of *Acanthospermum hispidum* root extract and visual observation in colour change

3.2 SEM-EDX Analysis of Silver Nanoparticles

The SEM –EDX images of silver nanoparticles from leaf extract of *Acanthospermum hispidum* root extract are shown in Fig. 3. It can be seen from Fig. 3 that the obtained silver nanoparticles were high density pre-dominantly spherical which was similar to previous work reported (9). The size varies from 15 to 55 nm. The micrograph showed individual particles as well as number of aggregates. The variable size particles may be due to different compounds which are involved in the capping of nanoparticles. Energy dispersive X-ray was used to determine the weight percentage of silver present in material. Silver usually showed a strong peak at 3 keV(10). EDX graph is shown in Fig. 3, which demonstrates a peak at 3 keV. It confirms the presence of silver in the respective material. The peak for C was also observed which may be due to the presence of precipitate in plant material.

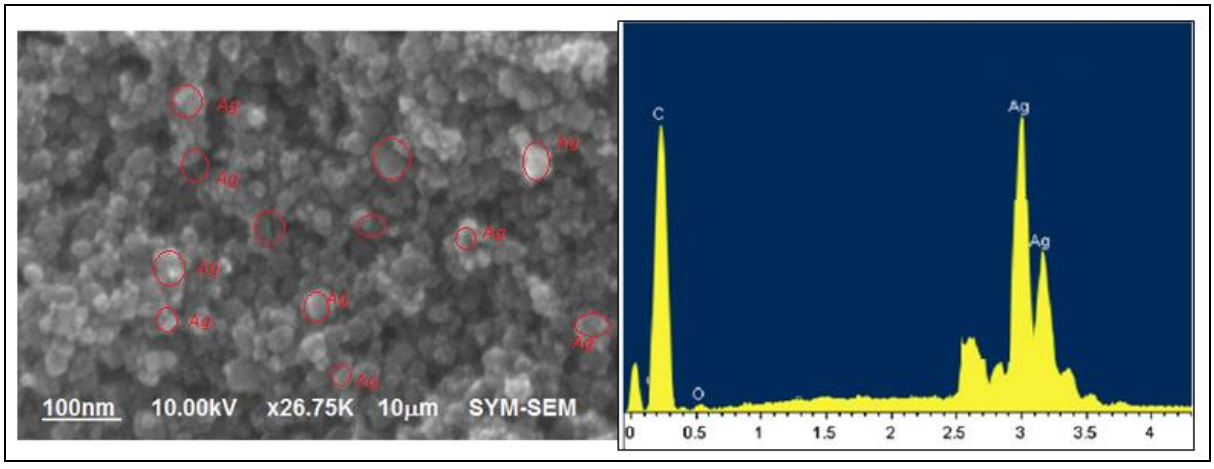

Figure 3: SEM-EDX Analysis of *Acanthospermum hispidum* root extract

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3.3 X-ray diffraction (XRD) Studies

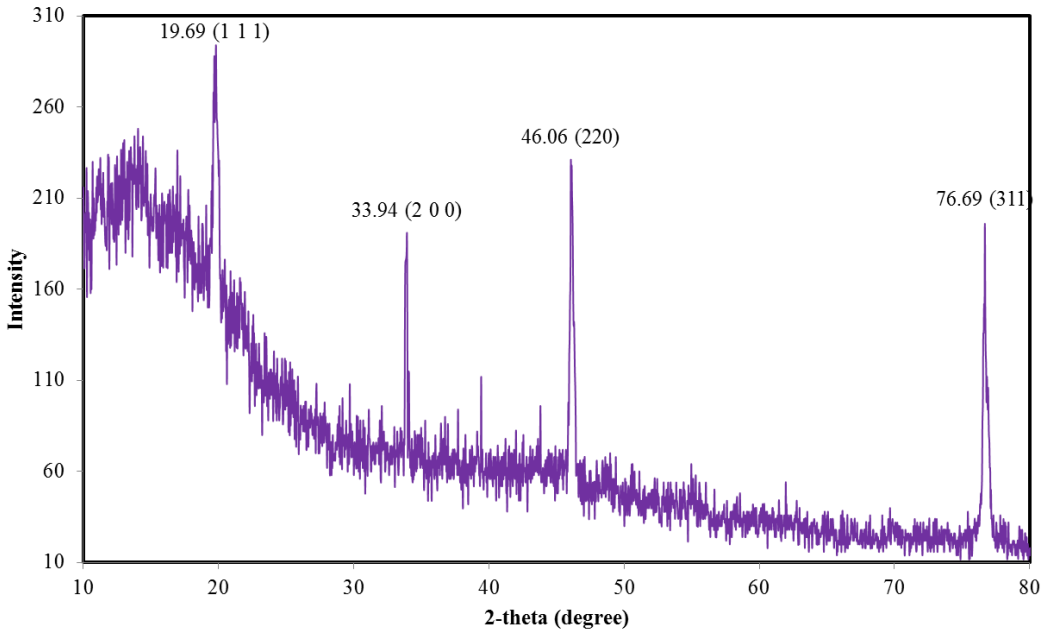

Figure 4: XRD spectra of *Acanthospermum hispidum* root extract

Analysis through X-ray diffraction was carried out to confirm the crystalline nature of the particles, and the XRD pattern showed numbers of Braggs reflections that may be indexed on the basis of the face cantered cubic structure of silver. A comparison of our XRD spectrum with the standard confirmed that the silver particles formed in our experiments were in the form of nanocrystals, as evidenced by the peaks at 2θ values of 19.69 33.94, and 46.06, and 76.69 corresponding to (111), (200), (220) and (311), respectively Bragg reflections of silver. The X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag+ ions by the mulberry leaves extract are crystalline in nature. As mentioned in the method section, the silver nanoparticles once formed were repeatedly centrifuged and redispersed in sterile distilled water prior to XRD analysis, thus ruling out the presence of any free biological material that might independently crystallize and giving rise to Bragg reflections. It was found that the average size from XRD data and using Debye-Scherer equation was 29± 2 nm. The XRD pattern of the dried AgNPs obtained *Acanthospermum hispidum* root extract is shown in Fig. 4. The XRD patterns thus clearly illustrates that the AgNPs synthesize by the present green method are crystalline in nature. The average particle size of silver nanoparticles synthesized by the present green method can be calculated using Debye-Scherrer equation (13).

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

Where D = the crystallite size of AgNPs particles

λ = the wavelength of x-ray source (0.1541 nm) used in XRD

β = the full width at half maximum of the diffraction peak.

K = the Scherrer constant with value from 0.9 to 1.

θ = the Bragg angle.

3.4 Characterization of silver nanoparticles by FTIR

The FT-IR spectrum of synthesized silver nanoparticles was shown in fig. 5. The spectrum showing a band at 3424 cm^{-1} corresponds to-OH and-H bonded alcoholic and phenolic groups. The band at 1769 cm^{-1}, 1075 cm^{-1} signifies the-C=O stretch in acids, esters, ethers etc. The carbonyl group from amino acid residues and peptides of proteins has the stronger ability to bind to metal (11). The peaks correspond to 2859 cm^{-1} reveals C-H stretching vibration, indicating the presence of alkanes.

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The proteins could be most possibly from a coat covering the metal nanoparticles to prevent agglomeration of the nanoparticles and stabilizing them in the medium. This evidence suggests that the biological molecules could possibly perform the function for the formation and stabilization of the silver nanoparticles in aqueous medium (12).

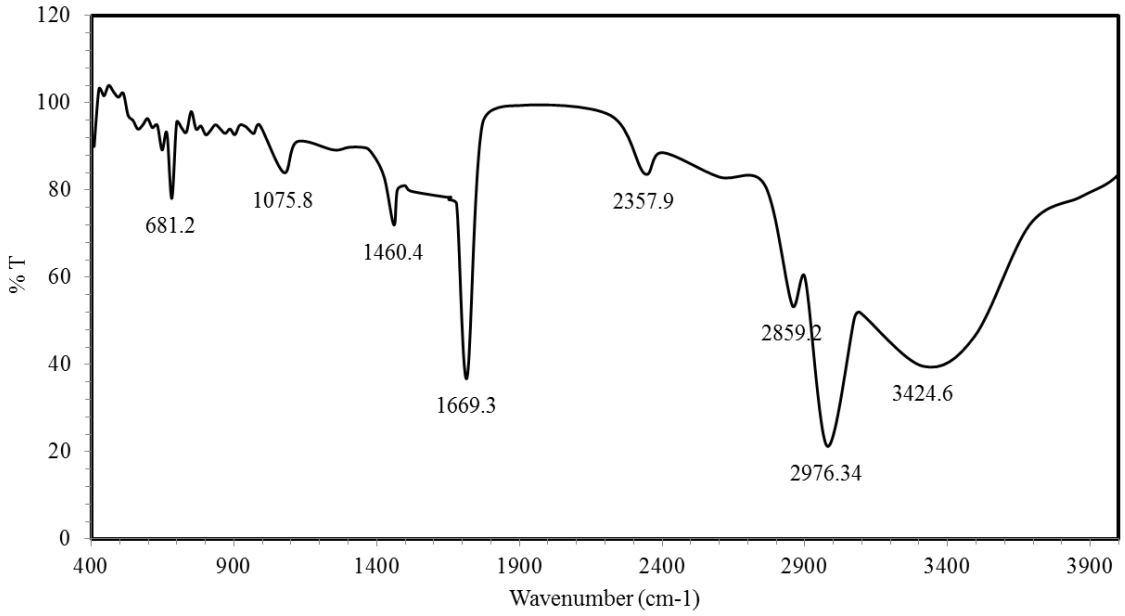

Figure 5: FT-IR spectra of *Acanthospermum hispidum* root extract

3.5 TEM-SAED-silver nanoparticle distribution

TEM image of silver nanoparticles derived from papaya leaf extract was shown in fig. 6. The morphology of our silver nanoparticles was very different from other studies that reported which is showing spherical, oval and pentagonal. The particle size of silver nano particles are found to be in the range of 10–50 nm. The size of the particles extended from 5 to 70 nm, and the mean particle size was around 29 nm (Fig. 6). The Selected-Area Electron Diffraction (SAED) patterns given reveal bright dots (figure 6), indicating that the nanoparticles are crystalline in nature. The data obtained corroborates other studies on green synthesis of metal nanoparticles

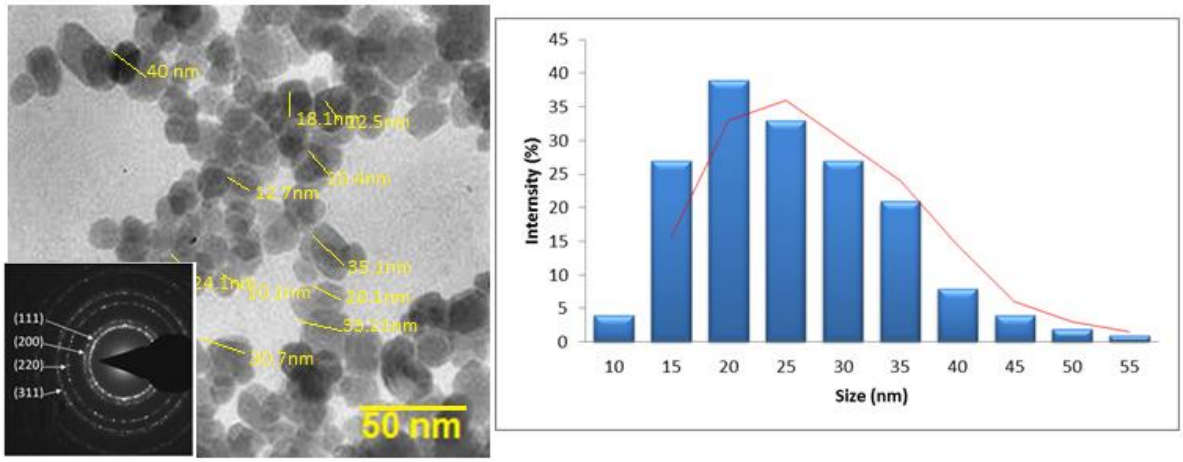

Figure 6: TEM-SAED (inset) and particle size histogram of *Acanthospermum hispidum* root extract

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. The Selected-Area Electron Diffraction (SAED) patterns given reveal bright dots (figure 6), indicating that the nanoparticles are crystalline in nature. The data obtained corroborates other studies on microwave assisted synthesis of metal nanoparticles

4.0 Conclusion

In conclusion, there has been an exponentially increasing interest in biological synthesis of AgNPs. In this study, AgNPs were synthesized by an ecofriendly and rapid method using *Acanthospermum hispidum* root extract extract. *A. hispidum* root extract has been used as a reducing agent for the synthesis of silver nitrate into silver nanoparticles. Green synthesized silver nanoparticles are confirmed by color change which was characterized by UV-Vis spectroscopy at 439nm. Further characterization with SEM and TEM analysis shows the spherical AgNPs of particle size ranging from 29 to 33 nm. FTIR showed the structure, the respective bands of the synthesized nanoparticles, and the stretch of bonds. EDX showed the elemental composition of synthesized silver nanoparticles.

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