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Production of AgNPs in unprocessed leaf extract and their identification by UV-Vis spectroscopy, Diffraction, and transmission electron microscopy

Rishabh Bhardwaj[1*], Dr. Pankaj Gupta[2]

[1] Research Scholar, Sunrise University, Alwar, Rajasthan

[2] Professor (Dept. of Science), Sunrise University, Alwar, Rajasthan

Abstract - There is a low-cost and environmentally friendly alternative to conventional nanoparticle synthesis that can be obtained from plant extracts. The present paper reports the reduction of silver ions with extracts from Emblica officinalis, and Eucalyptus hybrida, resulting in stable, homogeneous silver nanoparticles. Silver nanoparticles can be obtained by this straightforward method in a short amount of time, making it of interest to scientists around the world. Various techniques, including UV-Vis spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), and energy diffraction X-ray (EDX) analysis, were used to determine that the silver nanoparticles range in size from 20 nm to 80 nm and have a variety of shapes and sizes. Using X-ray diffraction, researchers found that the nanostructures had a crystal structure characteristic of a face-centered cubic. The presence of an absorption peak in UV/Vis spectra is evidence that they are manufactured. The development of a safe and environmentally benign method for the biological synthesis of metallic nanoparticles is a major step forward in the realm of nanobiotechnology's practical applications. Because of their biogenic nature, silver nanoparticles (Ag-NPs) may show to be a more effective medication option. Antibiotic resistance has spread at a frightening rate due to careless usage of the drugs. Ag-NPs have the potential to provide a one-size-fits-all answer to this pressing issue.

Keywords - UV-Vis spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), and energy diffraction X- ray (EDX) analysis, , silver nanoparticles

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INTRODUCTION

It is common practise to investigate the plasmonic resonance of nanoparticles using UV Vis spectroscopy. UV-VIs spectroscopy absorbance data analysis provides evidence for the formation of silver nano-particles and plasmonic resonance. Particle sizes can be roughly estimated as well. UV-vis spectroscopy is used to monitor the synthesis and stability of AgNPs and is a very useful and reliable technique for primary characterization of synthesised nanoparticles. Because of their one-of-a-kind optical properties, AgNPs have been shown to have pronounced interactions with only certain wavelengths of light. The synthesis of nanoparticles and nanomaterials for use in applications such as electrochemistry, catalysis, sensors, biomedicines, pharmaceutics, health care, cosmetics, food technology, textile industry, mechanics, optics, electronics, space industry, energy science, optical devices, etc. has given rise to nanotechnology, an interdisciplinary field combining Chemistry, Life sciences, Physics, and Material Science [1-7]. Diagnostics, imaging, and the delivery of genes and drugs are just a few examples of how nanotechnology is being used to advance medical care. During a talk titled "There's Plenty of Room at the Bottom" given at a conference of the American Physical Society on December 29, 1959 at Caltech, Richard Feynman initially proposed the concept of nanotechnology. Engines of Creation, Drexler's 1986 book, is credited with popularising the term nanotechnology .The first book to propose making gadgets out of Nanoscale materials was Eric

Drexler's "Engines of Creation: The Coming Era of Nanotechnology".

In order to extend the useful lifetime of metal nanoparticles, it is essential to choose stabilising agents and pathways that are safe for the environment and can be easily implemented. So, researchers are coming up with new, more ecologically friendly ways to synthesise NPs that meet all of the criteria for an ideal synthesis: formation at room temperature, neutral pH, cheap cost. Different nano-material synthesis strategies have been developed with these objectives in mind. Plants and plant extracts appear to be the most promising biological alternatives. We present herein a green approach for the room-temperature production of silver nanoparticles from plant extracts of Emblica officinalis, Terminalia catappa, and Eucalyptus hybrida. Multiple techniques, including UV-Vis spectroscopy, XRD, and TEM, were used to learn more about these nanoparticles. In addition, we plan to employ these Nanoparticles to test the efficacy of the nanosilver-phytochemical composite formulation in vitro.

MATERIALS AND METHODS

Extracts from the leaves of three different plants were used in the synthesis of silver nanoparticles.

The leaves of the plants used were freshly picked and rinsed in distilled water. It's estimated that roughly 50 g of leaves were chopped up. The leaves were finely chopped and dipped into distilled water. Then, for the next 10-12 minutes, the concoction was brought to a boil. Using Whatmann filter paper, the extract was filtered, and the resulting filtrate was collected. This leaf extract was used in a 1:4 ratio with an aqueous solution of 1 mM Silver nitrate (99.99%) in a conical flask, under sterile circumstances, to synthesise silver nanoparticles. We brought the pH level up to 8.0. The conical flasks were then stirred at 100 rpm in dark at 25°C on shaker for 1 to 2 h. The solution was seen to change colour. There was also a parallel run of a control set (with just the extract and no AgNO3). A further negative control with only 1 mM AgNO3 was kept in the identical circumstances. Several techniques were employed to determine the characteristics of silver nanobioconjugates.

Synthesized Silver Nanoparticles: Characterization

Ultraviolet/Visible Spectroscopy

Visual observation of a colour change in the cell filtrate following silver nitrate treatment was the primary method used for detecting Ag-NPs (1 mmol -l). Scanning the absorbance spectra in the region of 250 to 800 nm on a UV-Visible spectrophotometer allowed for further characterisation of the produced Ag-NPs. It was seen that the aqueous silver nitrate solution turned brown after 2 minutes after the extract was added to the flask. This indicated the formation of silver nanoparticles. In order to dry the AgNPs, the solution was put into Petri plates and baked at 50 degrees Celsius for 24 hours to achieve the signature dark brown colour. The XRD method was used to analyse the compounds' production and purity. An X-ray diffractometer (PANalytical-XPERT PRO diffractometer equipment) was used to record XRD patterns of a drop-coated film of AgNPs on a glass substrate at a scanning rate of 2° min1 over a broad range of Bragg angles using CuK radiation (1.5405) at a voltage of 40 kV and a current of 30 mA. Using a TECHNAIG20-STWIN (200 KV) equipment, HRTEM was performed with a line resolution of 2.32 nm (in angstrom). Drop coating AgNPs on a carbon-coated copper grid allowed for the capture of these photos. The HRTEM instrument was used to take pictures of AgNPs using energy dispersive spectroscopy.

Transmission Electron Microscopy

TEM images were captured by a scanning electron microscope. Overnight at room temperature, samples were fixed in 2.5% glutaraldehyde. To remove moisture from the fixed sample, we used a gradient of alcohol (from 10% to 95%), incubating it for 20 minutes at each concentration before finally dipping it in absolute alcohol for 2 to 5 minutes. Dehydrated material was then placed in a drop on a glass slide and coated with monolayer platinum to make the surface conduct.

Data from X-Ray Diffraction

An XRD Diffractometer was used to take X-ray diffraction measurements of silver nanoparticle powders with varying interaction times (PANalytical-XPERT PRO diffractometer system). The wavelength of 1.54060 A° was chosen because it corresponds to Cu K. The current running through the generator was 30 mA, and the voltage was 40 kV. It was decided that a temperature range of 10–100 degrees Celsius would be used for the scanning process. Debye Scherrer equation was also used to calculate the crystallite size.

Microscopy with Transmitted Electron Beams (TEM)

In order to prepare samples for TEM analysis, a Silver Nano Bio Conjugates solution was drop-cast onto a carbon-coated copper TEM grid. The Silver Nano Bio Conjugates solution was centrifuged at 10000 rpm for 5 minutes, and the isolated silver Nano Bio Conjugates were dissolved in 100 l double-distilled water and sonicated in a bath sonicator for 15 minutes before being cast onto the grid. The TEM images were recorded on a high resolution electron microscope (HRTEM: JEOL JEM 2010) operating at an accelerating voltage of 200 kV. The HRTEM: JEOL JEM 2010 instrument in NPL, New Delhi was used to capture the FFT images using the instrument's in-built software for the FFT algorithm for image processing.

Infrared spectroscopy with a Fourier transform

After centrifuging the silver nano bioconjugates at 15,000 rpm for 15 minutes to eliminate any free proteins or other chemicals in the solution, the samples were ready for FTIR spectral analysis. To concentrate the Ag-NPs, the filtrate remaining after the Ag+ ions had been completely removed was centrifuged at 15,000 rpm for 15 minutes, with the supernatant being replaced by distilled water at regular intervals. The silver nano bioconjugates were centrifuged three times, then resuspended in double-distilled water and analysed using Fourier transform infrared spectroscopy. A white precipitate forms when sodium chloride is added to a solution containing unreacted silver ions. However, the addition of sodium chloride did not result in the formation of a precipitate, proving that there was no unreacted silver present in the nanoparticle solution. The biomolecules that may have been involved for the bioreduction of the Ag^{+} ions and capping of the biosynthesized silver nanoparticles by the leaf broth were identified by FTIR spectroscopy. The sample's FTIR spectrum, acquired with 4 cm^{-1} resolution using a spectrometer from Perkin-Elmer, spans from 450 to 4000 cm^{-1}.

RESULTS AND DISCUSSION

Extracts from the leaves of three different plants were used in the synthesis of silver nanoparticles.

Extracts from the leaves of three different plants (E. officinalis, E. hybrida, and T. catappa) were used in the synthesis of silver nanobioconjugates. The EDX spectrum of drop-coated films of silver nanoparticles was recorded after they were synthesised by Emblica crude emblica by reducing Ag ions. The resulting nanoplates ranged in size from 80 to 300 nm (Figure 1). Extracts of E. hybrida leaves and aqueous silver nitrate were analysed using UV-vis spectroscopy. When reacting with silver, a surface plasmon resonance band appears at 440 nm and steadily grows in strength as a function of time without a corresponding change in peak wavelength (Figure 2). Size and shape of metal nanoparticles, as well as the dielectric constant of the metal and the surrounding medium, determine the frequency and width of the surface plasmon absorption. Using an extract from E. hybrida leaves and 1 mM aqueous $AgNO_3$, we reduced Ag+ to silver nanoparticles in solution, as seen in these scanning electron microscopy (SEM) pictures taken at 26°C.

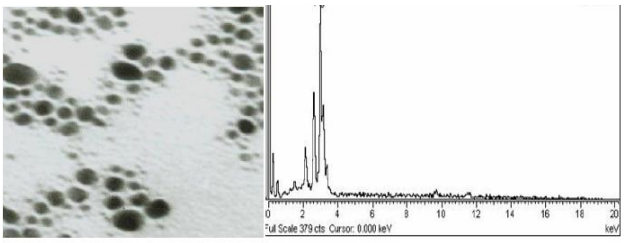

Figure 1. Synthesis of nanoplates by crude *Emblica*, (80 to 300 nanometers) of silver by reducing Ag ions EDX spectrum recorded from drop-coated films of silver nanoparticles.

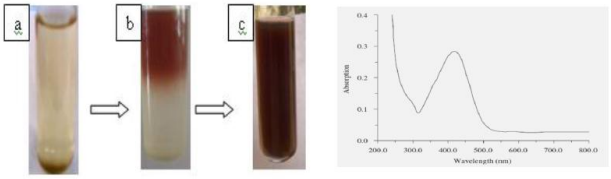

Figure 2. Pictures showing the color changes before (a), during (b) and after the process of reduction of Ag+ to Ag nanoparticles (c).

Silver nanobioconjugates: a characterization

Light spectroscopy in the ultraviolet-visible spectrum.

The yellowish-brown hue of silver nanoparticles in water is well known. These hues result from the excitation of surface plasmon vibrations in the metal nanoparticles, which happen between 380 and 440 nm in an aqueous solution. Figure 4 inset demonstrates that after 1 to 3 hours of treatment with silver nitrate, the biomass of E. officinalis, E. hybrida, and T. catappa turned a dark reddish-brown colour due to the creation of silver nanoparticles outside of the cells. This demonstrates that the procedure was relatively swift. The SPR signal produced by silver nano bio conjugates in the UV-visible spectrum between 340 and 450 nm is responsible for this hue. Synthesis of Silver Nano Bio Conjugates was evidenced by an increase in absorbance in the 340–450 nm wavelength range over time. However, in untreated sets, no noticeable colour shift was seen.

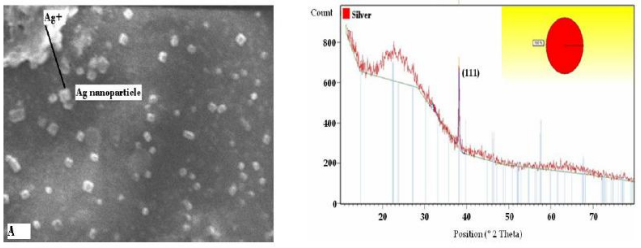

Figure 3. SEM images of reduction of Ag+ to Silver nanoparticles.

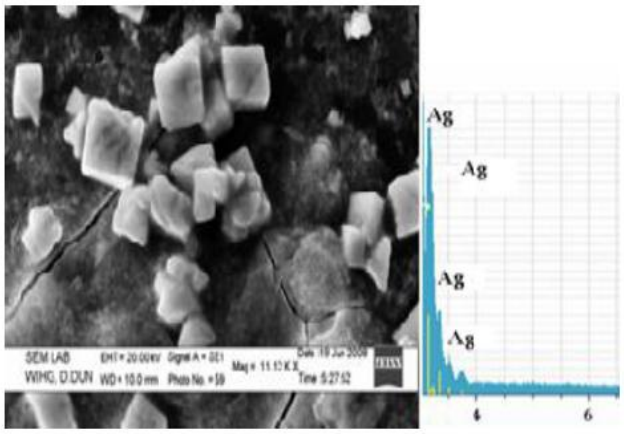

Figure 4. SEM image of the silver nanoparticles synthesized by treating *Eucalyptus hybrida* leaf extract with $AgNO_3$ aqueous solutions. Silver nanoparticles formed were predominantly cubical with uniform size, EDX spectrum recorded from a film, after formation of silver nanoparticles. Different X-ray emission peaks are labelled.

After 2 h of response time, the surface plasmon band's intensity is seen to reach a maximum.

Data from X-Ray Diffraction

In Figure 5, we see the XRD pattern for Silver Nano Bio Conjugates made with E. hybrida, T. catappa, and E. officinalis extract. As shown in Table 1, the silver nanoparticles generated using the T. catappa and E. officinalis extracts exhibit a number of Bragg reflections that can be indexed using the fcc structure of silver. Crystalline silver nanoparticles were generated when Ag+ ions were reduced by the T. catappa, E. hybrida, and Emblica officinalis extract. This was demonstrated by the XRD pattern. According to the predicted d spacing values for each 2 value, the (101), (111), (200), and (220) lattice spacing are widened in comparison to the bulk form, with the effect being most noticeable for the (111) planes. In addition to the Bragg peaks typical of face-centered cubic (fcc) silver nanocrystals (JCPDS—International Center for Diffraction Data, PCPDFWIN v. 1.30, 31-1238) that correspond to the lattice planes (101), (111), (200), and (220), respectively. Peaks at 2 = 7.9, 11.4, 17.8, 30, 32, 38, and 44° have been observed in XRD patterns of pure silver ions. The calculation provided further evidence that particles do indeed exist in a single crystal structure. Peak sharpening is a hallmark of particles operating in the nanoregime. Therefore, it may be concluded from the XRD pattern that silver nanoparticles are crystalline.

Table 1. Lattice spacing values calculated from the 2θ values obtained from the XRD pattern of silver nanoparticles synthesized using *Terminalia catappa* leaf, Eucalyptus hybrid and *Emblica officinalis*.

Pos. [°2Th.]	Lattice Planes (hkl)	Standard Ag (A°)	*Terminalia catappa* -Ag (A°)	*Emblica officinalis* Ag (A°)	*Eucalyptus* Ag (A°)
32	(101)	2.815	-	2.81446	-
38	(111)	2.359	2.35291	2.35567	2.2111
45	(200)	2.044	2.03897	-	-
65	(220)	1.445	1.44446	-	-

Microscopy using Transmitted Electron Beams (TEM)

Drop-coated films of the Silver Nano Bio Conjugates produced with T. catappa leaf extract were used to record the TEM images in Figure 8. The silver nanoparticles are highly polydisperse, ranging in size from 10 to 40 nm, with an average size of ca. 20 nm, and can be detected at low magnification (Figure 6). The shape of the silver nanoparticles becomes more discernible under a microscope (Figures 6B and C). Most of the particles are round, whereas only a fraction are lengthy. The presence of the bioorganic molecules may have acted as a driving force in the observed assembly of the silver particles. TEM images don't prove bioorganic material is attached to silver nanoparticles, but it's intriguing to see that most particles aren't touching and instead have a very consistent spacing between them.

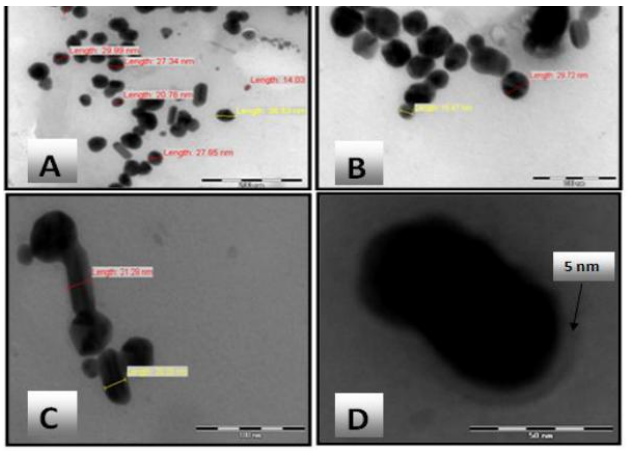

Figure 8. TEM images of silver nano bio conjugates synthesized by *Terminalia catappa* extract.

The FTIR spectrometer uses the Fourier transform to analyse infrared light.

Results from testing components of T. catappa leaf revealed that alkaloids,

The leaf contains tannin, flavonoid, and glycosides. The chemical shift of the functional groups involved in bioreduction is depicted in Figure .7 which displays the FTIR absorption spectra of the dried biomass of T. catappa leaf before and after bioreduction. Within the range of 1000–1800 cm^{-1},

we found a number of absorbance bands with their centres at 1109, 1244, 1317, 1384, 1446, 1517, 1631, and 1726 cm1. The stretch vibrations of -C-O, -C=C, and RHC=O were all linked to the corresponding absorbance bands at 1109, 1631, and 1726 cm^{-1}. The -C-O groups of polyols like flavones and polysaccharides in the biomass may contribute significantly to the band at 1109 $^{cm-1}$. By comparing (a) and (b), we can deduce that the polyols are primarily responsible for the reduction of silver ions because the band at 1109 cm^{-1} disappears after bioreduction. There was a significant increase in the silver nanoparticle absorption about 1384 cm^{-1} (a) due to the presence of NO_3. The heterocyclic compounds that are water-soluble components in biomass are largely responsible for the bonds or functional groups like -C-O-C-, -C-O-, -C=C-, and -C=O. This suggests that the capping ligands of the nanoparticles are the water-soluble heterocyclic compounds such alkaloids and flavones.

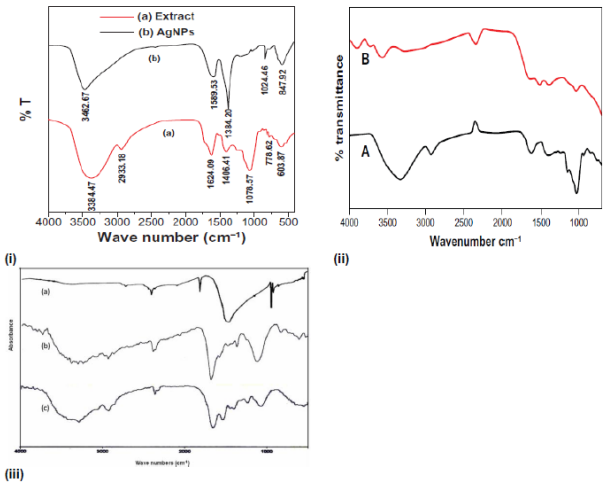

Figure 13. Typical FTIR absorption spectra of the (i) *Terminalia catappa* leaf biomass before bioreduction (a), after bioreduction of silver ions (b). (*ii) Emblica officinalis* biomass before bioreduction (a), after bioreduction of silver ions (b). (iii) Fourier transform infrared absorption spectra of Eucalyptus hybrida extract (A) $AgNO_3$, (b) before bioreduction and (c) after complete bioreduction of Ag+ ions at 50°C.

ONCLUSIONS

This research looked into the possibility of using plant extracts in the synthesis of Ag-NPs. We now know that a straightforward biological process can be used to produce Ag-NPs. Capping of Ag-NPs has been verified by FTIR analysis. By preventing Ag-NPs from aggregating in a colloidal solution, these capped particles will improve the stability of Ag-NPs. The fact that they are topped off with biomolecules makes them a potentially strong candidate for use as drug delivery systems. Synthesis of nanoparticles using plant extracts, however, may solve this issue with chemical agents, reducing the risk of negative side effects from their use and making nanoparticles more biocompatible.

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Corresponding Author

Rishabh Bhardwaj*

Research Scholar, Sunrise University, Alwar, Rajasthan