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Phytofabrication of Silver Nanoparticles from Plant Sources: Synthesis and Characterization

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Abstract

The necessity to create ecologically friendly nanoparticles without the use of hazardous chemicals is expanding as worldwide competitions intensify. One of the possible research topics is the biosynthesis of silver nanoparticles (AgNPs) utilizing plant extracts. Metal ion bioreduction is a very quick process that can be easily scaled up and carried out at room temperature. Using Eclipta prostrata, & Punica Granatum plant extract using diffraction and transmission electron microscopy, the current study reports a quick and environmentally friendly production of AgNPs. Temperature, time, and redundant concentration are among the process factors that were examined for their effectiveness and impact on the biosynthesis of AgNPs. Aqueous leaf extract of Eclipta prostrata, & Punica Granatum was used to quickly manufacture AgNPs, which were detected when the medium changed brown upon the addition of silver ions. UV-visible spectroscopy was used to analyze the stability of biosynthesized AgNPs, and transmission electron microscopy, dynamic light scattering, Fourier transform infrared spectroscopy, and X-ray diffraction were used to examine physicochemical properties.

Key words : Silver nanoparticles, Extract, Morphology, Anti-bacterial, synthesized.

Nanoparticles, generally considered as particles with a size of up to 100nm, exhibit completely new or improved properties as compared to the larger particles of the bulk material that they are composed of based on specific characteristics such as size, distribution, and morphology.[16] In recent years, the biosynthetic method using plant extracts has received more attention than chemical and physical methods, and even than the use of microbes, for the nano-scale metal synthesis due to the absence of any requirement to maintain an aseptic environment. Nanoparticles have attracted considerable attention owing to their various applications. The silver nanoparticles are reported to possess anti-bacterial,[17] antiviral,[4] anti-fungal activity.[2] Synthesis of nanoparticles using plants or microorganisms can potentially eliminate this problem by making the nanoparticles more bio-compatible. Indeed, over the past several years, plants, algae, fungi, bacteria, and viruses have been used for low-cost, energy-ef cient, and nontoxic production of metallic nanoparticles.[3] Use of plant extract for the synthesis of nanoparticles could be advantageous over other environmentally benign biological processes by eliminating the elaborate process of maintaining cell cultures. Recently green silver nanoparticles have been synthesized using various natural products like Nelumbo nucifera,[13,15] Ocimum sanctum,[14] Pongamia pinnata[7] and Cinnamon zeylanicum.

The plant, *Eclipta prostrata* L., a member of the Asteraceae plant family and commonly known as False Daisy, has been used as a traditional medicine to treat hyperlipidaemia, atherosclerosis, hepatic disorders, spleen enlargement, and skin diseases in Asia.[7,11] Earlier literature surveyed on the plant revealed that the major compounds isolated from the E. prostrata were volatile components like, flavonoid, iso flavonoids and coumestan.[4,6,11] *E. prostrata* and Eclipta alba are rich in avonoids broadly belonging to the class of phenolic compounds. In the present study, slow reduction of the aqueous silver ions along with the shape directing effects of the constituents of the *E. prostrata* extract play a key role in the formation of the silver nano circular in shape with smooth edges nanoparticles tested against the larvicidal activity.[1]

The pomegranate, or Punica granatum, is a tiny tree that grows to a height of five to eight meters. It is mostly found in Iran, the Himalayas in northern India, China, the United States, and the Mediterranean region. Because it can adapt to harsh ecological circumstances, Pg is one of Iran's most significant indigenous plants, growing in most of the country's arid and semiarid regions. During a germplasm collection, more than 764 cultivars of Punica granatum (Pg) were gathered and cultivated in the Iranian cities of Saveh and Yazd. Each cultivar has unique fruit traits such as size, color, flavor, ripening time, and resistance to disease.[5]

Preparation of Plant Extract : The crude extract from the plant was obtained through several techniques, such as soxhlet

Table-1. Silver nitrate solution and aqueous plant extract at various concentrations

Factores		**Levels**		
		1st	**2nd**	**3rd**
A	Concentration of AgNo3 (mM/ml)	2	5	10
B	Concentration of Plant Extract (ml)	25	50	75
C	pH	5	7	9
D	Incubation Time Period (Min)	10	30	60
E	Incubation Temperature (°C)	25	35	50

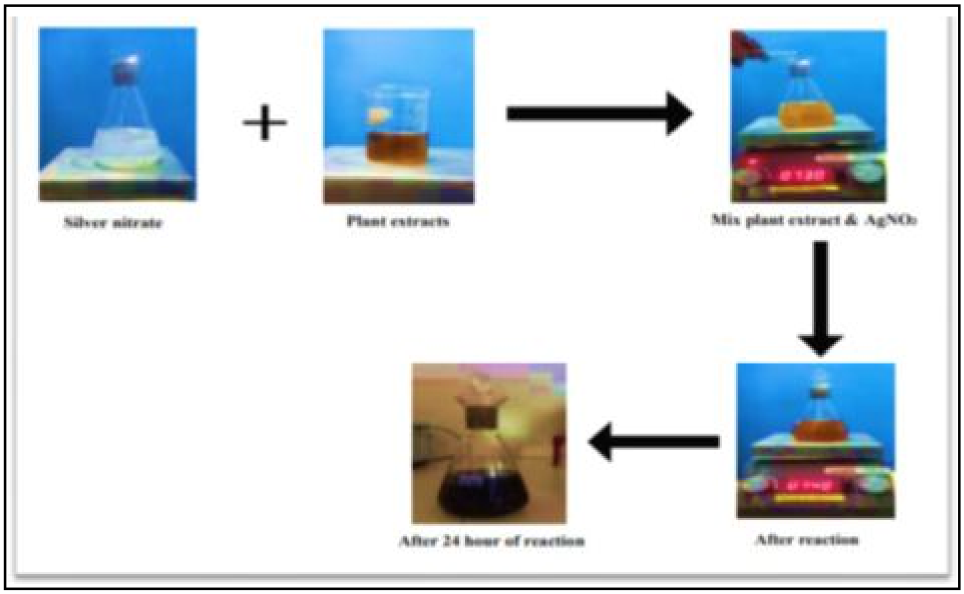

Figure 1. Synthesis of Silver Nanoparticles

extraction, employing ethanol and methanol as solvents, and decoction extraction using an Erlenmeyer flask. Approximately 20g of the dried powder of respective plant sp. were boiled in 100ml of double-distilled water for 10-15min. The aqueous solution was filtered and stored at 400 C until further use.

Green Synthesis of Silver Nanoparticles : The silver nanoparticles were synthesized by adding both silver nitrate solution and aqueous plant extract at various concentrations (Table-1) and heated at 70ºC with continuous stirring for 1-3 hrs. These reaction mixtures were incubated at different time intervals and different temperatures and pH represented in figure 1.

Percentage yield of synthesized silver nanoparticles:- A 100 ml mixture of plant extracts and silver nitrate solutions were centrifuged at 15000 rpm and washed in distilled water and dried at room temperature for 24 hours. The resultant AgNPs powder weights were noted. The percentage yields were calculated by :

$$\% \text{ yield} = \frac{\text{Mass of } Ag^0}{\text{Mass of } Ag^+ \text{ ion}} \times 100$$

When 10ml $AgNO_3$-plant extract solution were centrifuged, the mass of silver nanoparticles produced were 18.5 mg. The percent yield of AgNPs synthesized was 80.7 ± 0. 3%, implying that 80.7 ± 0.3 % of the silver ions were converted to atomic state hence forming silver nanoparticles.

Characterization of silver nanoparticles

1. UV-visible spectroscopy:- Spectra were

recorded using a UV-Vis spectrophotometer within the wavelength range of 200 nm to 1000 nm. Measurements were conducted at different time points, spanning up to 24 hours from the initiation of the reaction by adding $AgNO_3$ to the plant extract. Additional spectra were recorded 24 hours after the addition of $AgNO_3$. Notably, within the initial hour of the reaction, silver ions underwent reduction, leading to the formation of silver nanoparticles. $AgNO_3$ was utilized to maintain reaction control throughout the process.

2. Physico-chemical characterizations of optimized AgNPs:- Optimized AgNPs (Opt-AgNPs) were characterized physicochemically by the UV-Vis and DLS methods as described in the design of the experiment. In addition, AgNPs were characterized by atomic absorption (AAS), zeta potential, and transmission electron microscopy (TEM).
3. Atomic absorption spectroscopy (AAS):- The silver concentration in each synthesis was determined with the flame method using the AAS technique using a Thermo Scientific ICE 3000, USA. A sample of the colloidal solution of the undiluted nanoparticles was nebulized and disseminated as an aerosol to measure the parts per million Ag.
4. Zeta potential and Particle Size Distribution:- The Zeta potential was determined by Laser Doppler Electrophoresis using a Zetasizer Nano ZS and the Zetasizer software. The nanoparticles were diluted in water type 1 at controlled temperature (23 °C) and three measurements were made, each of them with 30 s of equilibrium and 15 runs of 10 s in length.
5. Transmission electron microscopy (TEM):- The size and morphology of the samples were confirmed by transmission electron microscopy (TEM) using a Tecnai F20 Super Twin TMP, FEI. The samples were prepared using a drop of approximately 60 nm thickness of each suspension and deposited on a carbon membrane.
6. Refractive index:- The optical properties of AgNPs can also be affected by the refractive index of the surrounding medium, which is utilized during their production and stabilization processes. The absorption spectrum tends to shift towards higher wavelengths (red shift) as the refractive index of the medium increases, and vice versa. To calculate the refractive index of the silver nanoparticles, an Abbe or Bausch refractometer can be used. At a constant temperature of 25°C, a single, undiluted droplet of silver nanoparticle solution is placed in the test chamber of the Abbe refractometer. The refractive index (RI) of the silver nanoparticles is then measured and recorded.
7. Fourier Transform Infrared Spectroscopy (FTIR):- For FTIR measurements, the synthesized silver nanoparticle solution underwent centrifugation at 10,000 rpm for 30 minutes as a preparatory step. The resulting pellet was then rinsed three times with 5 mL of deionized water to eliminate any enzymes or free proteins that were not encapsulating the silver nanoparticles. Subsequently, the pellet was dried using a vacuum dryer. The dried AgNPs were analyzed via FTIR using the potassium bromide (KBr) pellet method, with a ratio of 1:100. The spectrum was obtained using a Jasco FT/IR-6300 Fourier transform

infrared spectrometer equipped with a JASCO IRT-7000 Intron Infrared Microscope operating in transmittance mode, with a resolution of 4 cm^{-1}.

8. X-Ray Diffractometry:- The graphical data obtained from X-ray diffraction (XRD) analysis is utilized to investigate the size and composition of silver nanoparticles. Scherrer's formulas are commonly employed in interpreting XRD findings. This fine powder is then utilized in the XRD experiment, where X-rays are directed at the sample, causing diffraction patterns that provide information about the crystalline structure, size, and composition of the nanoparticles.
9. Stability of the synthesized Ag-NPs:- The stability of synthesized Ag-NPs was assessed using a zeta potentiometer at various pH levels post-synthesis. It was observed that the synthesized Ag-NPs remained stable across a broad pH range spanning from 6 to 12.

Visible observation:- Based on recent studies, the silver nanoparticle solution is dark brown or dark reddish in hue. Prior the addition of $AgNO_3$, the color of E. prostrata and P. granatum was red, but following the addition of $AgNO_3$, the color changed to dark brown, indicating the synthesis of AgNPs (Figure 2). This color shift is caused by the quantum confinement characteristic, known to be a size dependent feature of nanoparticles that influences their optical properties.

Figure 2. Visible observation of silver nanoparticles with different Time

The particles were validated for the presence of a maximum absorbance peak at 400 nm in the UV-Visspectroscopy. Optimized reaction parameters for synthesis of stable AgNPs were addition of 5 mL of AI-extract to 45 mLof $AgNO_3$ (1 mM) at pH 7 and incubation at 25æ%C in dark at 200 rpm. AgNPs were stable in all the five solutions tested shown in Figure 3 & 4.

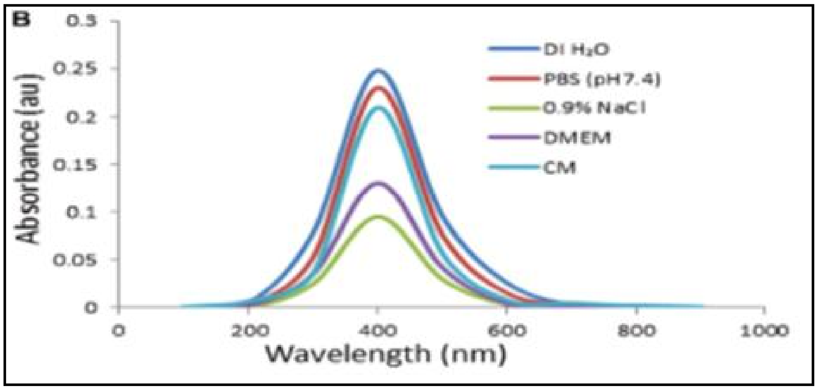

Figure 3. UV Absorption spectra in different test solutions.

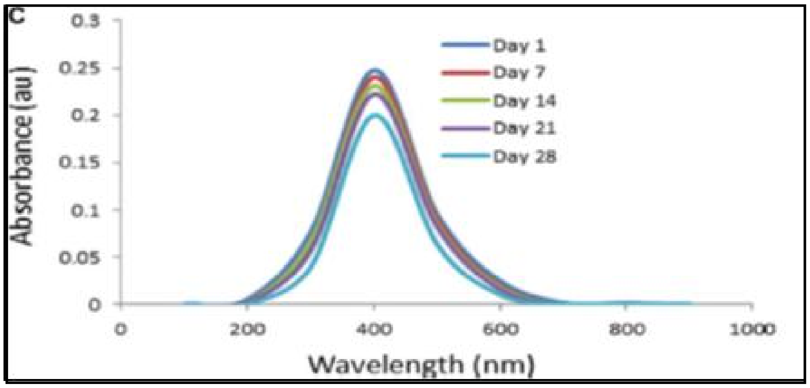

Figure 4. On-shelf stability at various time intervals in UV-Vis spectroscopy analysis

The AgNPs exhibited relatively narrow particle size distribution (Figure 5) (z average = 1239 d.nm), as the relatively low polydispersity index (PDI = 14.61) values of DLS measurements. The results of the measurement of the zeta potential (Figure 6) value showed the average zeta potential value was -0.876 mV.

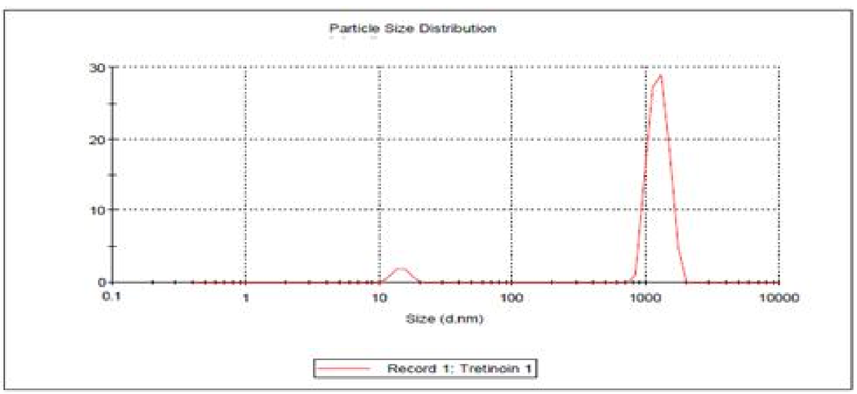

Figure 5. Partical size distribution of Silver Nanoparticles

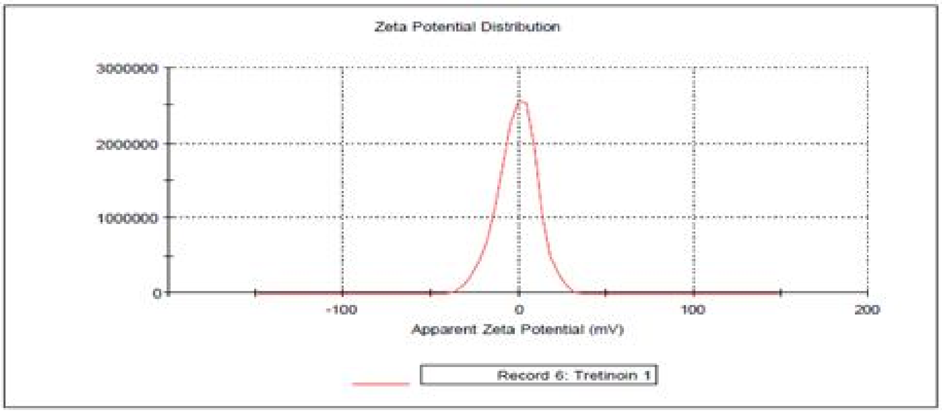

Figure 6. Zeta Potential of Silver Nanoparticles

Transmission electron microscopy (TEM):- The analysis provides the morphology and size details of the nanoparticles show in figure 7

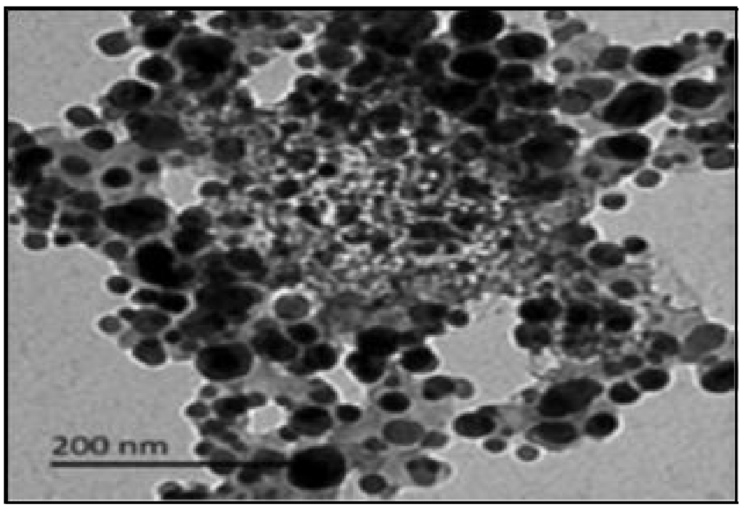

Figure 7 :- Transmission electron microscopy (TEM) of Silver Nanoparticles

The refractive index of the plant leaf extract was found to be 1.36, which is slightly higher than that of water. In colloidal solution, a layer of plant extract with active functional molecules is formed around the AgNPs, so the observed peak is slightly shifted towards the red wavelength of light.

The potential molecules in the plant extracts were determined using FTIR analysis represented ion figure 8. The phytochemical examination of Eclipta Prostrata and Punica granatum indicates the presence of flavonoids, alkaloids, steroids, saponins, and proteins.

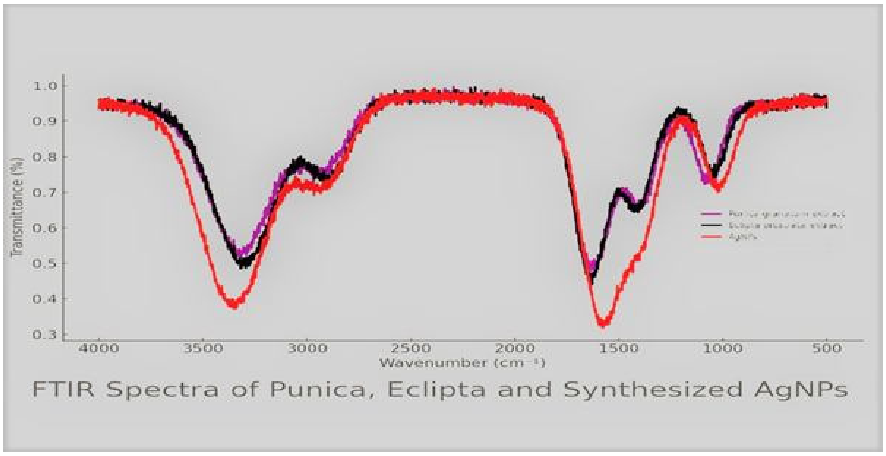

Figure 8. FTIR spectral analysis of Silver Nanoparticles

X-ray diffraction was used to confirm the crystalline nature of the particles. The biosynthesized silver nanoparticle by employing Eclipta prostrata and Punica granatum extract was further demonstrated and confirmed by the characteristic peaks observed in figure 9.

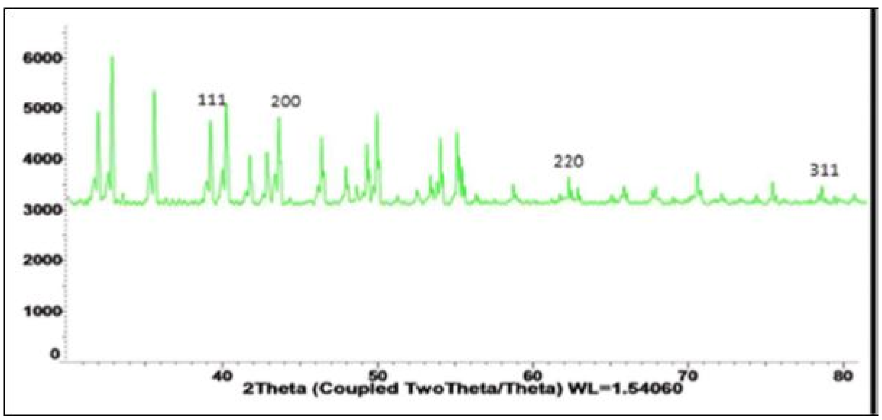

Figure 9. XRD Measurement of extract and Silver Nanoparticles

The stability of AgNPs was studied by UV-Vis spectroscopy after 30 days. Green synthesized AgNPs showed a characteristic SPR peak at the same λmax with the same absorption intensity. This clearly indicated the strong stability of AgNPs for 30 days, which was very supportive and convenient for the synthesis of nanoparticles.

Using Eclipta prostrata and Punica Granatum plant extract using diffraction and transmission electron microscopy, the current study reports a quick and environmentally friendly production of AgNPs. Temperature, time, and redundant concentration are among the process factors that were examined for their effectiveness and impact on the biosynthesis of AgNPs. Aqueous leaf extract of Eclipta prostrata, & Punica Granatumwas used to quickly manufacture AgNPs, which were detected when the medium changed brown upon the addition of silver ions. UV-visible spectroscopy was used to analyze the stability of biosynthesized AgNPs, and transmission electron microscopy, dynamic light scattering, Fourier transform infrared spectroscopy, and X-ray diffraction were used to examine physicochemical properties.

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