Green synthesis and characterisation of Silver nanoparticles from the medicinal plant *Pithecellobium dulce*

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Abstract

Objective: To introduce a cost effective and eco-friendly technique of preparing silver nanoparticles (Ag NP) from the medicinal plant, *Pithecellobium dulce*.
Methods: Ag NP of different concentration was prepared and characterized by using UV-Vis Spectrophotometer, Transmission Electron Microscopy (TEM), Scanning Electron Microscopy, X-Ray Diffraction (XRD), and Fourier Transform Infra Red (FTIR).
Results: Studies by UV-Vis spectroscopy showed absorption spectra of silver nanoparticles at an absorbance peak at 475nm, in all the three different concentrations (1mM, 3mM and 5mM). The size of Ag NP ranged 50-70nm and appeared to be dominantly spherical (and occasionally triangular). The XRD pattern revealed that these silver nanoparticles have a crystalline nature.
Conclusion: These nanoparticles produced by green synthesis proved stable in solution and can have promising role in nano-medicine.

***Keywords*:** *Pithecellobium dulce,* Bioreduction, Silver nanoparticles.

1. Introduction

Green synthesis of nanoparticles is an emerging branch of nanotechnology [1]. The use of environmentally benign materials like plant leaf extract, bacteria and fungi for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and biomedical applications as they do not use toxic chemicals in the synthesis protocols [2]. Bio-inspired synthesis of nanoparticles provides advancement over chemical and physical methods as it is cost effective and no need to use high pressure, energy, temperature and toxic chemicals [3].

In this study silver nanoparticles were prepared with different concentrations as these particles are fast and specific in their target towards the applications where they are evaluated for their antimicrobial activity. Medicinal plants are used in large proportions by the developing nations. This is because of the long lasting improvement against diseases after herbal treatment. Hence this investigation was focused in searching for new drugs from medicinal plants.

2. Materials and methods

Homogenate was prepared by weighing 20grams of fresh leaves of *Pithecellobium dulce* collected from Chennai, India; Washed thoroughly (thrice) in distilled water and homogenized using a mortar and pestle. The homogenate was filtered using a sterile gauze cloth and then transferred to a sterile container for further study.

2.1. Preparation of silver nitrate solution: Commercially purchased silver nitrate was used to prepare 1mM, 3mM, and 5mM concentrations using distilled water.

3. Preparation and characterisation of silver nanoparticles

To 75ml of each millimolar concentration of silver nitrate, 7.5ml of the plant homogenate was added into a clean conical flask. The conical flasks were then exposed to the sunlight (while being continuously shaken) for the synthesis of the nanoparticles to begin. The colour of the mixture turns from green to brown when exposed to sunlight and once it turns to colourless, the particles were settled at the bottom of the flasks.

3.1. UV-Vis Spectra analysis for the crude extract

Aliquots are made from the above mixture at different time interval to monitor the formation of nanoparticles for the maximum of 5 h at 1 h interval. The reduction of pure silver ions were observed by measuring the UV-Vis spectrum of the reaction at different time intervals taking 1ml of the samples against distilled water as blank. UV-Vis spectral analysis has been done by using an Elico spectrophotometer at a resolution of 1 nm from 200 to 1100 nm using a one-centimetre quartz cuvette.

Once the particles settle down, they were then centrifuged (6000 rpm) and the supernatant was removed. To the particles now settled at the bottom of the centrifuge tubes, about 1ml acetone was added for the removal of the moisture content from the nanoparticles. The nanoparticle suspension were transferred to a watch glass, air dried, collected, weighed and stored in a sterile container.

3.2 TEM analysis

Samples were suspended in double distilled water. A drop of thin suspension is placed on a “staining mat”. Carbon coated grid is inserted into the drop with the coated side upwards. After ten minutes at an accelerating voltage of 80kV, the removed grid was air-dried and screened in JEOL JEM 100SX Transmission Electron Microscope.

3.3 FTIR analysis

Mixing of samples with KCl were carried out as per the procedure procured from Sigma. The samples were made into thin disc with the help of machine and placed in FTIR for the analysis of nanoparticles.

3.4.XRD analysis

A coated film was prepared with a drop of synthesized Ag NP’s for determining the formation of silver nanoparticles by an X’ Pert Pro X-ray diffractometer operated at a voltage of 40kV and a current of 30mA with Cu K-α_1 radiation.

3.5. SEM analysis

The synthesized Ag NPs were fabricated onto a clean electric stubs and allowed for the water to completely evaporate. SEM observations were carried out on a ZEISS EVO 40 EP.

4. Result

Figure 1 shows the maximum absorbance at 440nm, indicating that the formation of spherical Ag NPs synthesized from crude extract in majority or anisotropic particles whose appearance and the ration increases with time in this UV-Vis spectrographs.

Figure 1: UV-Vis Absorption spectrum of nanoparticle synthesized from crude extract of *P. dulce* extract at different time intervals

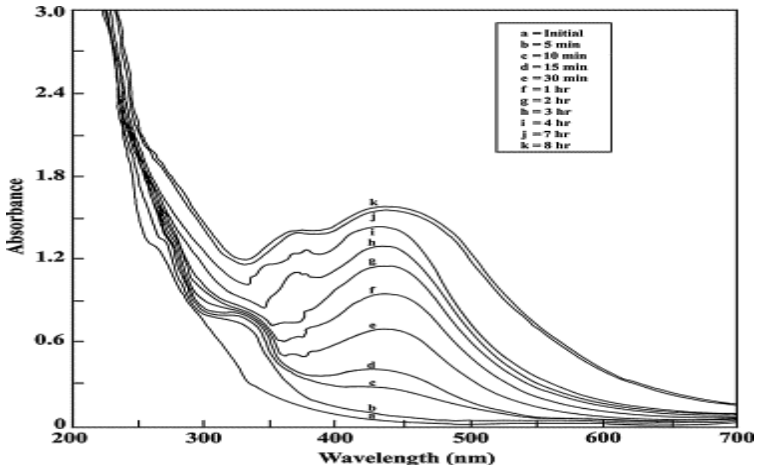


Figure 2: TEM Micrograph of nanoparticles synthesized from crude extract of *P. dulce*

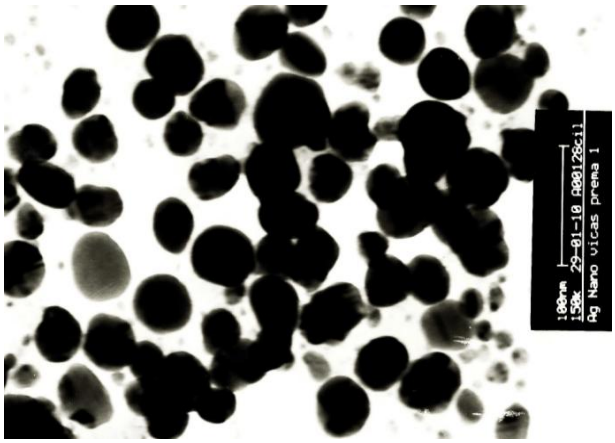


Figure 2 reveals the average mean size of the AG NPs synthesized with *P. dulce* extract scanned using TEM was 100nm and seems to be spherical in morphology.

Figure 3: SEM images of nanoparticles synthesized from crude extract of *P. dulce*

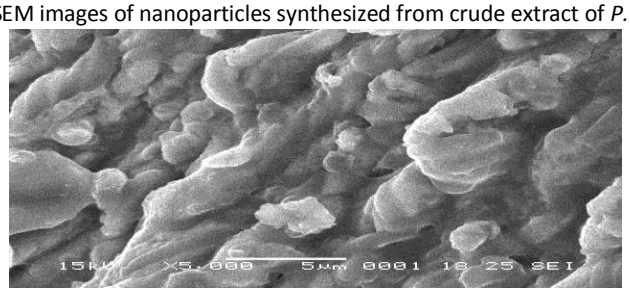
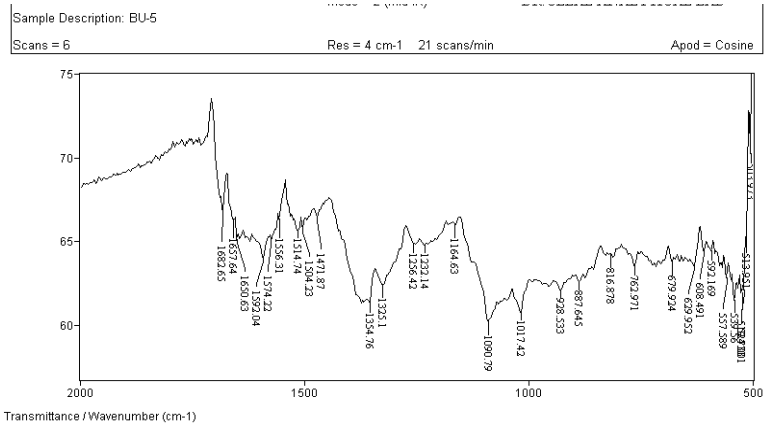


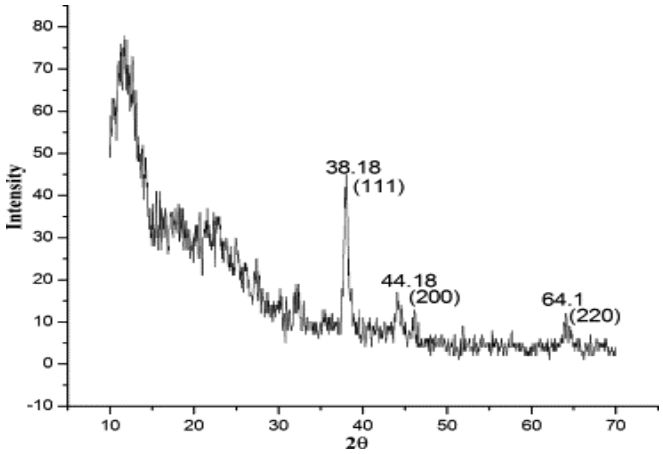
Figure 3 of SEM micrographs of Ag NPs obtained from the filtrate synthesized with the *P. dulce* shows high density of Ag NPs are spherical shaped, well distributed without aggregation in solution.

Figure 4: FTIR Spectra of nanoparticles synthesized from crude extract of *P. dulce*



FTIR analysis represented absorbance bands from 501 to 1560cm^{-1} thus helping in the characterization of the extract and the resulting nanoparticles (Fig.4).

Figure 5 **:** XRD patterns recorded for the nanoparticles synthesized from crude extract of *P.dulce*



The silver nanostructure biosynthesized from *P. dulce* extract was confirmed by the peaks observed in XRD image at θ =64.1^0 marked with (220) respectively. Based on the face-centred crystal structure of silver, a number of Bragg reflections corresponding to the (220) respectively sets of lattice plans. In Fig. 5, the XRD pattern shows clearly that Ag-NPs are crystalline in nature.

5. Discussion

Amongst the three different concentrations of silver nitrate, 3mM concentration of silver nitrate produced more silver nanoparticles. Hence, further studies of analyzing the silver nanoparticles and their application in antibacterial activity was carried out with the nanoparticles produced with 3mM concentration of silver nitrate. Recent studies have demonstrated that specially formulated metal oxide

nanoparticles have good antimicrobial activity [4]. The antibacterial and antiviral actions of silver, silver ion and silver compounds have been thoroughly investigated [5,6]. Microbes are unlikely to develop resistance against silver, as they do against conventional and narrow target antibiotics because the metal attacks a broad range of targets in the organisms, which means that they would have to develop host mutations simultaneously to protect themselves. Thus silver ions have been used in dental resin composites [7], in synthetic zeolites [8] and in coatings of medical devices found that silver nanoparticles undergo size dependent interaction with HIV-I. [9] have also reported the size dependent interaction of silver nanoparticles with Gram-negative bacteria. Report from [10] states that upon addition of silver ions into the filtered cell free filtrate in the dark samples changes its color from almost colourless to brown with intensity increasing during the period of incubation. [11] reported the conversion of 3mM silver nitrate solution to nanosilver by *Fusarium oxysporum* in an aqueous medium due to the change in color of the reaction mixture from pale yellow to dark brown. It is generally recognized that UV–Vis spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous suspensions [12].

[13, 14, 15] reported that the UV-VIS spectrum of the solution of *Coriolus versicolor* showed the maximum absorption at 440nm. A long tailing on the larger wavelength side may be due to the small amount of aggregated particles. Apart from this, the absorption peak at 210 nm was assigned to the strong absorption of peptide bonds in the filtrate. The absorption at 280 nm indicated the presence of tryptophan, tyrosine or phenylalanine residues in the protein. This observation indicates the release of proteins into filtrate that suggests a possible mechanism for the reduction of metal ions present in the solution. Observation of the strong but broad surface Plasmon peak has been well known in the case of various metal nanoparticles over a wide size range of 2-100nm [12&13] suggested that the shoulder at 370nm corresponded to the transverse plasmon vibration in silver nanoparticles, whereas the peak at 440nm was due to excitation of longitudinal plasmon vibrations. In the present study, the peak value was observed at 381nm.

6. Conclusion

A promising production of silver nanoparticles which are quite stable in solution was obtained through the bio-reduction of aqueous Ag+ ions by the homogenate extract of *Pithecellobium dulce.* This green chemistry achievement in synthesizing silver nanoparticles has many advantages such as ease with which the process can be scaled up, economic viability, *etc.*

7. Reference

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