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Research Article

GREEN SYNTHESIS AND CHARACTERIZATION OF NANO SILVER USING LEAF EXTRACT OF *MORINDA PUBESCENS*

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ABSTRACT

Wide application of nanoparticles stimulates the need for synthesizing them. But, the conventional methods are usually hazardous and energy consuming. This leads to focus on "Green synthesis" of nanoparticles which seems to be an easy, efficient and eco-friendly approach. In this study, the green synthesis of silver nanoparticles was carried out using leaf extract of *Morinda pubescens* as reducing agent. The nano synthesis was monitored under different range of temperatures, radiations, time periods and concentrations of the leaf extract. The optimized nano silver thus obtained was quantified and characterized using UV-Visible spectroscopy, Scanning Electron Microscopy, Transmission Electron Microscopy, X-ray Diffraction and Fourier Transform Infrared spectroscopy. The results emphasized the potent application of *Morinda pubescens* leaves in the synthesis of nano silver with economic viability and ease in scaling up for mass production.

Key words: Nanoparticles, Green synthesis, *Morinda pubescens,* UV-Visible spectroscopy, Scanning Electron Microscopy, Transmission Electron Microscopy, X-ray Diffraction, Fourier Transform Infrared spectroscopy.

INTRODUCTION

Synthesis of nanoparticles to have a better control over particle size, distribution, morphology, purity, quantity and quality, by employing environment friendly economical processes has always been a challenge for the researchers[1]. Chemical reduction is the most frequently applied method for the preparation of silver nanoparticles as stable, colloidal dispersions in water or organic solvents. Commonly used reductants are borohydride, citrate, ascorbate, and elemental hydrogen[2]. The synthesis of nanoparticles by chemical reduction methods are often performed in the presence of stabilizers in order to prevent unwanted agglomeration of the colloidal nanoparticle solution. Also, some chemical methods cannot avoid the use of toxic chemicals in the synthesis protocol. Since noble metal nanoparticles such as silver nanoparticles are widely applied to human contacting areas, there is a growing need to develop environmentally friendly processes for their synthesis that do not use toxic chemicals. Biological methods of nanoparticle synthesis using microorganism[3], enzyme[4] and plant or plant extract[5] have been suggested as possible ecofriendly alternatives to chemical and physical methods. Using plant for nanoparticle synthesis can be advantageous over other biological processes as it eliminates the elaborate process of maintaining cell cultures. It can also be suitably scaled up for large-scale synthesis of nanoparticles.

Silver nanoparticles have more applications in many areas, including biomedical, materials science, and catalysis. A single silver nanoparticle interacts with light more efficiently than a particle of the same dimension composed of any known organic or inorganic chromophore[6]. Silver is also the only material whose plasmon resonance can be tuned to any wavelength in the visible spectrum. Fundamental studies carried out in the last three decades show that silver nanoparticles exhibit a rare combination of valuable properties, namely, unique optical properties associated with the surface Plasmon resonance (SPR), well-developed surfaces, catalytic activity, high electrical double layer capacitance, etc[7]. Nanosilver has been used extensively as anti-bacterial agent in the health industry, food storage, textile coatings and a number of environmental applications. It is important to note that despite of decades of use, the evidence of toxicity of silver is still not clear. Products made with nanosilver have also been approved by a range of accredited bodies[8].

Morinda pubescens commonly known as Indian mulberry or Noni or Bartondi belongs to family Rubiaceae. The plants are found as weeds in the dried region of Maharashtra[9]. The ethnobotanical uses of *M. pubescens* have been reported[10]. The bark of *M. pubescens* is useful in eczema, fever due to primary complex, ulcer and glandular swellings while leaves are useful in digestive disorders especially in children and in venereal diseases.

The present study deals with the successful synthesis of silver nanoparticles from silver nitrate through a simple green route using the leaf extract of *Morinda pubescens* as the reducing agent.

MATERIALS

Silver nitrate solution was purchased from HiMedia Laboratories Pvt. Limited, Mumbai, India. The Leaves of *Morinda pubescens* Linn. were collected from the forest region of IIT Campus, Chennai and identified by the Department of Plant Biology and Plant Biotechnology, Meenakshi College, Chennai.

METHODS

Preparation of aqueous silver nitrate

1 mM Silver nitrate solution was prepared and stored in amber coloured bottle.

Preparation of extract by conventional method

The *Morinda pubescens* leaves were washed several times with deionised water. 100gm of finely cut *Morinda pubescens* leaves was taken and boiled in 300ml of double distilled water for 3mins and filtered. After centrifugation at 10,000rpm for 15mins, the supernatant was collected and stored at 4°C.

Preparation of extract by homogenization method

The *Morinda pubescens* leaves were washed several times with deionised water. 100g of the leaves was homogenized in 300ml water with the help of mortar and pestle and filtered. Then the filtrate was centrifuged for 15min at 10,000rpm, supernatant was collected and stored at 4°C.

Optimization and synthesis of silver nanoparticles

Different concentrations of leaf extracts (1ml, 3ml and 5ml) were taken separately and to this 10ml of 1mM Siver nitrate solution was added with constant stirring and exposed to different conditions like sunlight radiation,UV radiation, several short burst of microwave irradiation at a frequency of 2.45GHz in a domestic microwave oven (National Model N N-GD 576M) , at a power output of about 100W in a cyclic mode (on 15s, off 15s) to prevent overheating as well as aggregation of metals, Room temperature and Direct boiling. The colour change of the solution was checked periodically. The colour change of the leaf extract from yellow to dark brown indicated the silver nanoparticles were synthesized from the leaves. Bioreduction of silver ions in the solution was monitored using Genesys 10 UV-VIS spectrophotometer. Then the conical flasks were incubated at room temperature for 48 hours. The contents were centrifuged at 10,000rpm for 15 minutes. The supernatant was used for the characterisation of the silver nanoparticles. The same was performed for both conventional and homogenization method of extracts.

Production and Recovery of silver nanoparticles by centrifugation

Among various concentrations and methods used, sunlight irradiation method was very effective and 1ml of homogenized leaf extract had shown more synthesis of nanoparticles. Further it was chosen for bulk production as 10ml leaf extract in 100ml of 1mM Siver nitrate. After bioreduction, the solution consisting of hydrosols of silver nanoparticles was subjected to centrifugation at 10,000rpm for 15 minutes. The pellet formed was dissolved in 0.1 ml of toluene water and air dried.

Characterization of silver nanoparticles by UV-Visible spectroscopy

UV-Visible spectroscopy is used for monitoring the signature of silver nanoparticles. UV-Visible spectroscopy is a powerful tool for the characterization of colloidal particles. Noble metal particles are ideal candidates for study with UV- Vis spectroscopy, since they exhibit strong surface plasmon resonance absorption in the visible region and are highly sensitive to the surface modification.

Characterization of silver nanoparticles by Scanning Electron Microscopy (SEM)

This study was undertaken to know the size and shape of the silver nanoparticles biosynthesized using *Morinda pubescens.* SEM analysis was done using FEI Quanta 200 SEM machine. 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. Then the film on the SEM grid was allowed to dry and the images of nanoparticles were taken.

Characterization of silver nanoparticles by Transmission Electron Microscopy (TEM)

TEM is a method of producing images of a sample by illuminating the sample with electronic radiation (under vacuum), and detecting the electrons that are transmitted through the sample.

Using Philips CM12 Transmission Electron Microscope, electrons in the electron gun were accelerated across a potential difference of the order of 100,000 volts between the cathode and anode. The condenser lens was used to focus the electron beam emerging from the electron gun onto the specimen to permit optimal illuminating conditions for visualizing and recording the image. The specimen image generated by the objective lens was subsequently magnified in one or two more magnification stages by the intermediate and projector lens and projected onto a photographic plate.

Characterization of silver nanoparticles by X-Ray Diffraction (XRD)

The air dried nanoparticles were coated onto XRD grid and analyzed for the formation of silver nanoparticle by Philips X-Ray Diffractometer with Philips PW 1830 X-Ray Generator operated at a voltage of 40kV and a current of 30mA with Copper Potassium alpha radiation. The diffracted intensities were recorded from 10' to 80' of 20 angles.

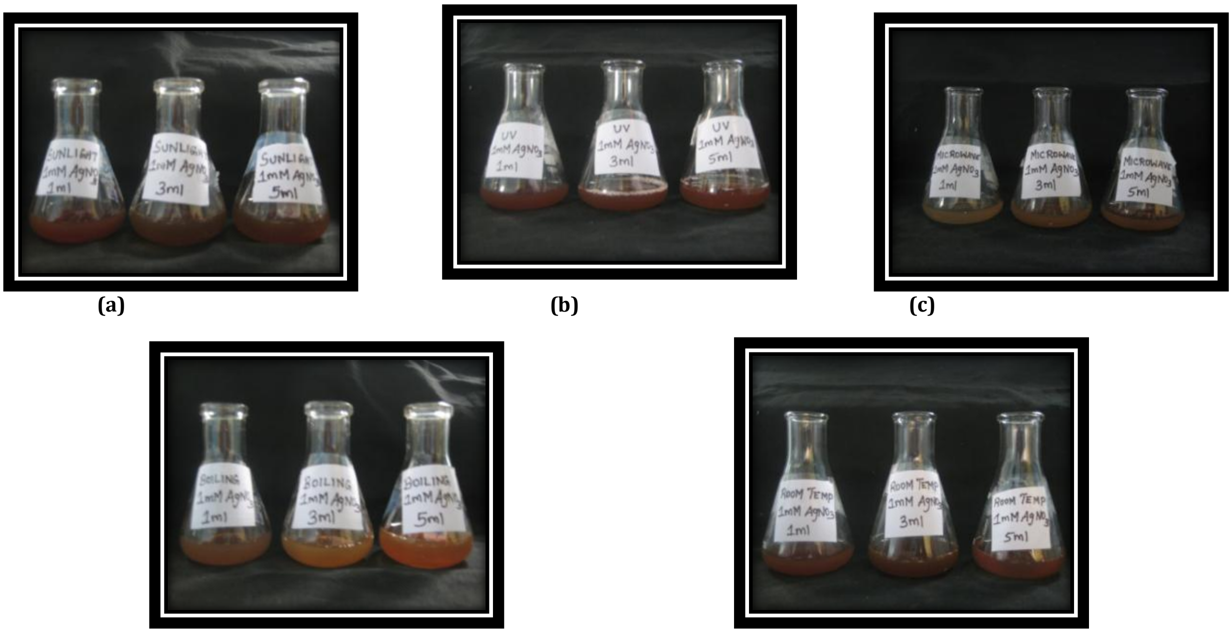
Characterization of silver nanoparticles by Fourier Transform Infrared spectroscopy (FTIR)

The dried silver nanoparticles were subjected to FTIR analysis by Potassium Bromide pellet (FTIR grade) method in 1: 100 ratios and spectrum was recorded in Nicolet Impact 400 FT-IR Spectrophotometer using diffuse reflectance mode.

RESULTS

Biosynthesis of silver nanoparticles

Plant materials were collected and plant leaf extracts were prepared both by conventional and homogenization methods. Biosynthesis of silver nanoparticles by the filtrate of *Morinda pubescens* was confirmed by change in the colour of the filtrate to brown after addition of silver nitrate and it was optimized under various conditions as Sun Light, UV, Microwave, Direct Boiling, and Room Temperature (Fig. 1a, 1b, 1c, 1d, 1e). The obtained nanoparticles were recovered and stored (Fig. 2). This resulted due to excitation of surface plasmon vibrations in the silver nanoparticles.



(a) (b) (c) (d) (e)

Fig. 1: Synthesis of silver nanoparticles under (a)Sunlight irradiation (b)UV irradiation (c)Microwave irradiation (d)Direct Boiling (e)Room temperature.

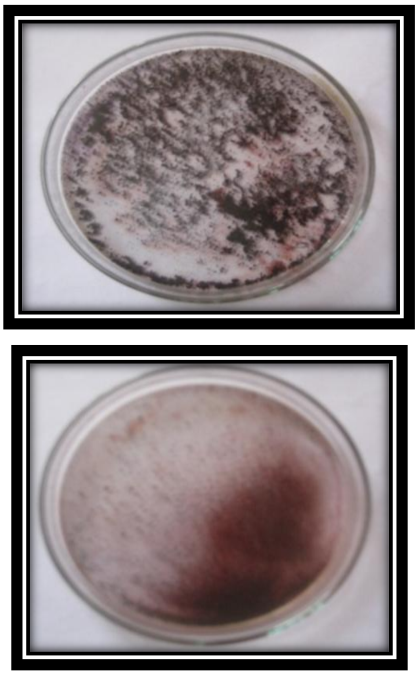


Fig. 2: Recovery of silver nanoparticles.

UV-VIS spectral analysis

The bioreduction of silver in the filtrate reaction solution was monitored by periodic sampling of the reaction mixture at regular intervals by using UV-Vis spectroscopy. Control flasks maintained with silver nitrate solution (without plant filtrates) did not show any change of colour and its absorbance maximum was found to be at 340nm, which was specific for silver nitrate solution. Sunlight irradiated reaction mixture with plant filtrate and silver nitrate showed a strong characteristic absorbance peak at around 430nm (Fig. 3). Analysis by spectrophotometer was made up to 8hrs.

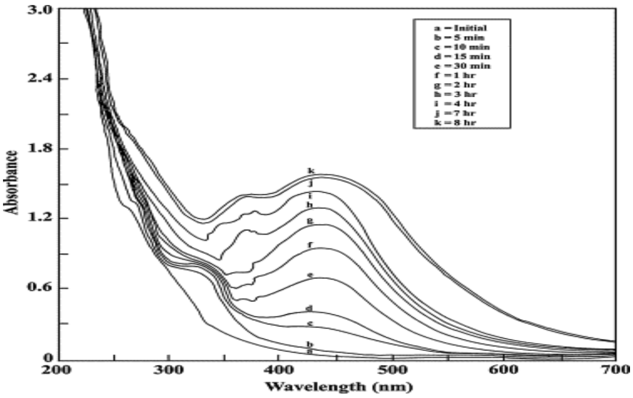


Fig. 3: UV-Vis spectroscopy of silver nanoparticles.

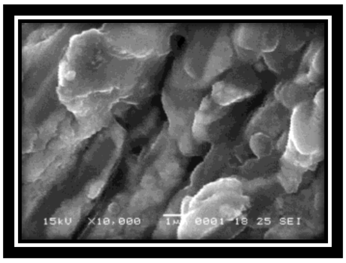
CHARACTERIZATION OF SILVER NANOPARTICLES

SEM Analysis

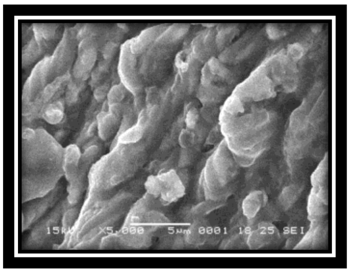
Scanning electron microscopic analysis of the silver nitrate solution (Control) and reduced form of silver nitrate solution were clearly distinguishable owing to their size difference. It was clear from the SEM pictures that control silver nitrate particles were more than 1000nm size, where as silver particles in the bioreduced colloidal suspensions measured 15-20nm in size. Fig. 4 is the SEM of bioreduced silver nitrate.

TEM Analysis

TEM analysis revealed that the synthesized nanoparticles were stable in solution at room temperature. The size of nanoparticle ranged from 20-40nm (Fig. 5). The decrease in anisotropy and particle size was evident from the images. The TEM images indicated equally spherical shaped orthorhombic crystals.



(a)



(b)

Fig. 4: SEM analysis of (a) silver nanoparticle in 1µm and (b) silver nanoparticle in 5µm

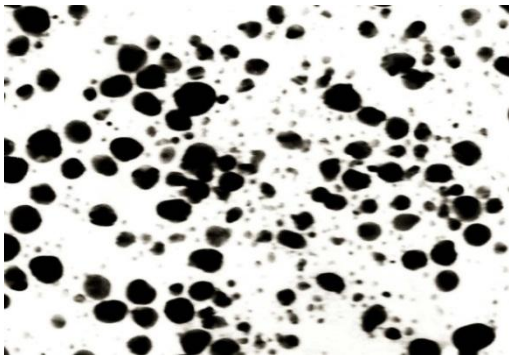


Fig. 5: TEM analysis of silver nanoparticle in 150nm.

XRD Analysis

XRD analysis showed distinct diffraction peaks which can be indexed the angle vales of (111), (200), (220), (240) crystalline planes of nano silver. This analysis revealed the orthorhombic crystals of silver nanoparticles. The high peaks in the analysis indicated the active silver composition with the indexing (Fig. 6).

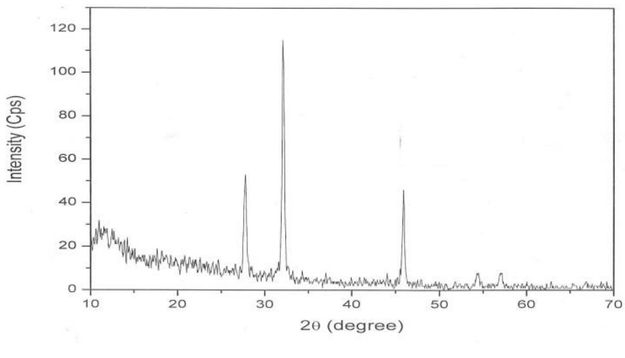


Fig. 6: XRD pattern of the silver nanoparticles.

FTIR Analysis

The presence of some functional groups as revealed by FTIR spectral analysis is shown in Fig. 7. The FTIR Spectral analysis of silver nanoparticles showed certain common absorption bands at 3398 cm-1, characteristic of υ (O-H) and υ (N-H) vibrational frequencies. Vibrational peaks between 2899 and 2977 cm-1 were characteristic of a υ (C-H) symmetrical vibration of saturated hydrocarbon. The vibrational frequency υ (C-O) was observed in the spectra of the extracts at 1047 and 1087 cm-1. Deviation from this region to a higher wave number was observed which was indicative of a secondary amide. These peaks were sharper than the υ (O-H) peaks due to reduction in hydrogen bonds which increased with electronegativity. Vibrational peaks at 1654 cm-1 in extract signified the possibility of an aromatic compound. Based on the physical state of the extracts and the characteristic features of the infrared vibrational peaks in the spectra, terpenoids, long chain fatty acids and secondary amide derivatives were the possible compounds in the obtained nanoparticles.

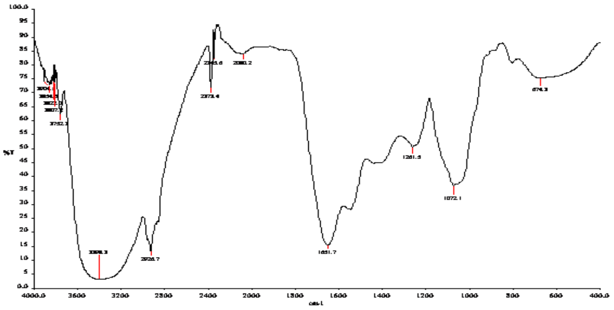


Fig. 7: FTIR spectrum of the silver nanoparticles

DISCUSSION

Silver nanoparticles exhibited yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles[5]. As the *Morinda pubescens* leaf extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from watery to yellowish brown due to reduction of silver ion; which denoted formation of silver nanoparticles. UV–Vis spectroscopy could be used to examine size- and shape-controlled nanoparticles in aqueous suspensions[11]. Absorption spectra of silver nanoparticles formed in the reaction media had absorbance peak at 430 nm and the broadening of peak indicated that the particles were polydispersed.

The biosynthesised silver nanostructure by employing *Morinda pubescens* plant leaf extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image and the structural view under the scanning electron microscope. The XRD pattern showed three intense peaks in the whole spectrum of 2θ value ranging from 10 to 70. Average size of the particles synthesized was 15nm with size range 10 to 50nm with cubic and orthorhombic crystals. The typical XRD pattern revealed that the sample contained a mixed phase (cubic and hexagonal) structures of silver nanoparticles. The average estimated particle size of this sample was 15 nm derived from the FWHM(full width at half maximum) of peak corresponding to 111 plane. The SEM and TEM images showing the high density silver nanoparticles synthesized by the *Morinda pubescens* plant leaf further confirmed the development of silver nanostructures.

FTIR analysis was used further for the characterization of the resulting nanoparticles. FTIR absorption spectra of silver nanoparticles showed absorbance bands (before bioreduction) in the region of 500–2000 cm-1 at 1697, 1618, 1514, 1332, 1226 cm-1. These absorbance bands were known to be associated with the stretching vibrations for –C C–C O, –C C– [(in-ring) aromatic], –C–C– [(in-ring) aromatic], C–O (esters, ethers) and C–O (polyols), respectively[12]. In particular, the 1226 cm-1 band arose most probably from the C–O group of polyols such as hydroxyflavones and catechins. The total disappearance of this band after the bioreduction might be due to the fact that the polyols were mainly responsible for the reduction of silver ions, whereby they themselves got oxidized to unsaturated carbonyl groups leading to a broad peak at 1650cm-1 for reduction of silver.

Reduction of silver ions present in the aqueous solution of silver complex during the reaction with the ingredients present in the *Morinda pubescens* plant leaf extract as observed by the UV-Vis spectroscopy revealed the presence of silver nanoparticles . The XRD, SEM, TEM analyses showed the particle size between 25-50nm as well the cubic structure of the nanoparticles. FTIR analysis confirmed that the bioreduction of silver ions to silver nanoparticles was due to the reduction by capping material of plant extract. The present study, thus showed a simple green route for rapid and economical synthesis of silver nanoparticles.

CONCLUSION

The bio-reduction of aqueous silver ions by the leaf extract of the *Morinda pubescens* plant had been demonstrated. The reduction of the metal ions through the leaf extract led to the formation of silver nanoparticles of fairly well-defined dimensions. In the present study it was found that leaves of *Morinda pubescens* could be a good source for the synthesis of silver nanoparticles through a green chemistry approach with several advantages.

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