

Biosynthesis and Characterization of Silver and Gold Nanoparticles Using Aqueous Leaf Extraction of *Phyllanthus amarus* Schum. & Thonn

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Abstract: Metal nanostructures have unusual physicochemical properties and biological activities compared to their bulk parent materials. Thus in recent years a number of physical, chemical and biological techniques were applied for the development of metal nanoparticles (NPs). In this study, we synthesized silver and gold nanoparticles from leaf extracts of *Phyllanthus amarus*. This plant belongs to Family Phyllanthaceae and the extracts of this herb have promising medicinal properties for a wide range of human diseases. The resulting silver and gold nanoparticles were characterized using UV-Vis spectroscopy, Scanning electron microscopy (SEM), X-ray diffraction (XRD), Energy dispersive X-ray (EDX), Atomic force microscopy (AFM), Particle size analyzer and Fourier transform infrared spectroscopy (FTIR) methods. Silver and gold nanoparticle suspensions gave maximum UV-Vis absorbance at 420 and 580 nm and XRD data illustrated characteristic diffraction patterns of the elemental phases. Particle size was determined using AFM (Atomic force microscopy) and Particle size analyzer, estimated from the peak profiles. FTIR spectra of the leaf extract before and after the synthesis of nanoparticles were determined to allow identification of possible functional groups responsible for the bioconversion of metal ions to metal nanoparticles.

Key words: Biosynthesis • Characterizations • Ag NPs • Au NPs • *Phyllanthus amarus*

INTRODUCTION

Nanoparticles are being viewed as fundamental building blocks of nanotechnology. The most important and distinct property of nanoparticles is that they exhibit larger surface area to volume ratio. The most effectively studied nanoparticles today are those made from noble metals, in particular Ag, Pt, Au and Pd. Metal nanoparticles have tremendous applications in the area of catalysis, optoelectronics, diagnostic biological probes and display devices. Among the above four, silver nanoparticles play a significant role in the field of biology and medicine. In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and ecofriendliness. Although biosynthesis of silver and gold nanoparticles by plants such as *Alfalfa*, [1] *Emblica officianalis*, [2] *Carica papaya*, [3] *Parthenium hysterophorus*, [4] *Diopyros kaki*, [5] *Azadirachta indica*, [6] *Hibiscus rosasinensis* [7] and *Capsicum annum* [8] have been reported, the potential

plants as biological materials for the synthesis of nanoparticles is yet to be fully explored. Using plants for nanoparticle synthesis can be advantageous over other biological processes because it eliminates the elaborate process of maintaining cell cultures and can also be suitably scaled up for large-scale nanoparticle synthesis. [9] Gardea-Torresdey *et al.*, [10, 11] demonstrated gold and silver nanoparticle synthesis within live alfalfa plants from solid media. Extracellular nanoparticle synthesis using plant leaf extracts rather than whole plants would be more economical owing to easier downstream processing. Pioneering works on nanoparticle synthesis using plant extracts have been carried out by Sastry and others [9, 12-17] who reported that nanoparticles can be synthesized using plant extracts at rates comparable to those of chemical methods. The shape of nanoparticles plays a crucial role in the modulation of their optical properties. Gold nanotriangles were formed when lemongrass (*Cymbopogon flexuosus*) leaf extract was reacted with aqueous AuCl₄ ions [14]. Gold and silver

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nanotriangles, in particular, are promising because they may find potential applications in the treatment of cancer hyperthermia and in infrared radiation absorbent optical coatings [17]. According to Kasthuri *et al.*, [27] the anisotropic gold and spherical-quasi-spherical silver nanoparticles (NPs) were synthesized by reducing aqueous chloroauric acid (HAuCl_4) and silver nitrate (AgNO_3) solution with the extract of phyllanthin at room temperature. The rate of reduction of HAuCl_4 is greater than the AgNO_3 at constant amount of phyllanthin extract. The size and shape of the NPs can be controlled by varying the concentration of phyllanthin extract and thereby to tune their optical properties in the near-infrared region of the electromagnetic spectrum. In this study, we synthesized Extracellular biological synthesis of silver and gold nanoparticles from aqueous leaf extract of *Phyllanthus amarus*.

MATERIALS AND METHODS

Experimental: Silver nitrate and Chloro auric acid were obtained from Sigma-Aldrich, Bangalore. All glasswares had been washed with distilled water and dried using a hot air oven before use. Fresh leaves of *P. amarus* had been collected from Karunya University Campus, Coimbatore, Tamil Nadu, India.

Preparation of the Extract: The fresh leaves of *P. amarus* were washed several times with ultra pure water to remove dust. Leaf extract used was prepared from 5 g of thoroughly washed leaves were heated in 50 ml ultrapure water for 5 min in an Erlenmayer flask using a water bath. The filtered leaf extract was stored at -15°C for further use, being usable for 1 week.

Synthesis of Nanoparticles: 1mM of AgNO_3 was reduced using 50 ml of 5% leaf extract at room temperature, resulting in a dark brown solution indicating the formation of Silver (Ag NPs) nanoparticles Figure 1b. Similarly, 1 mM of chloroauric acid was reduced using 50 ml of 5% leaf extract at room temperature, resulting in a dark ruby pink-red solution indicating the formation of gold (Au NPs) nanoparticles Figure 1c.

UV-Vis Spectra Analysis: Synthesis of Ag and Au NPs by reducing the respective metal ion solution with leaves extract may be easily observed by UV-vis spectroscopy. The absorption spectra of leaves extract quantities and metal concentrations were measured using a Perkin-Elmer Lambda-45 spectrophotometer in 300–1000 nm range.

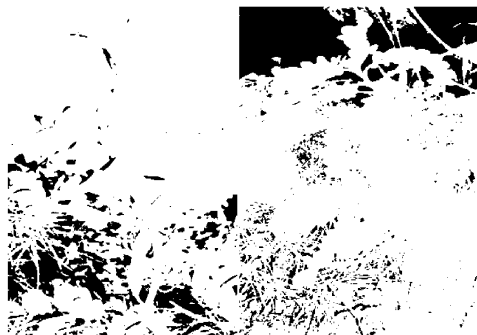


Fig. 1: Habit of *Phyllanthus amarus*

Naturally synthesized Ag Nps (420 nm) and Au NPs (580 nm) of diameters gave sharp peaks in the visible region of the electromagnetic spectrum.

Scanning Electron Microscopy (SEM): The morphological characterizations of the samples were done using SEM (JEOL model 6390). In the SEM an electron beam is focused into affine probe and subsequently raster scanned over a small rectangular area. As the beam interacts with the sample it creates various signals (secondary electrons, internal currents, photon emission, etc.), all of which can be appropriately detected.

X-Ray Diffraction (XRD): To determine the structural characterization of the nanoparticles by using X-ray diffractometer (XRD-6000 Shimadzu). The intensity of the diffracted X rays is measured as a function of the diffraction angle 2θ and the specimen's orientation. This diffraction pattern is used to identify the specimen's crystalline phases and to measure its structural properties (which is measured with great accuracy) and the size and orientation of crystallites (small crystalline regions). XRD can also determine concentration profiles, film thicknesses and atomic arrangements in amorphous materials and multilayers.

Energy Dispersive X-ray Spectroscopy (EDX Analysis): The presence of elemental gold was determined. The samples were dried at room temperature and then analyzed for samples composition of the synthesized Nps.

Atomic Force Microscopy (AFM): To know the exact particle size and nanosize effect the samples were characterized using Atomic force microscope (Nanonics imaging MN1000) which measures the atomic range of particles using tapping mode.

Particle Size Analysis: The particle size ranges of the nanoparticles were determined by using Particle size analyzer (Malvern Zetasizer nanosizer). Particle sizes were arrived based on measuring the time dependent fluctuation of scattering of laser light by the nanoparticles.

Fourier Transform Infrared Spectroscopy (FTIR): Samples were measured by Shimadzu 8400s and using spectral range of $4000\text{--}400\text{ cm}^{-1}$ with resolution of 4 cm^{-1} . Powder samples for the FTIR were prepared similarly as for powder diffraction measurements. The FTIR spectra of leaf extracts taken before and after synthesis of Ag and Au NPs were analyzed to study the possible functional groups for the formation of Ag and Au Nps.

RESULT AND DISCUSSION

UV-Vis and Scanning Electron Microscopy: Formation of silver and gold nanoparticles from 1 mM solution of Silver nitrate and Chloroauric acid was confirmed using UV-Vis spectral analysis. Ag and Au NPs having free electrons, give rise to a surface plasmon resonance (SPR) absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with the light wave [18-22]. Surface plasmon resonance spectra for Ag NPs are obtained at 420 nm Figure 2a with brown and for Au NPs are obtained at 580nm Figure 2b with dark pink ruby red. A rapid increase in the synthesis of nanoparticles was observed with an increase in reaction time. The concentration of the extract also plays a major role as it is responsible for the synthesis of symmetrical nanoparticles. As metal nanoparticles can be synthesized by reducing metal ions using some chemical molecules, in green synthesis, it is believed that the natural material extract acts as reducing agent for the generation of metal nanoparticles. SEM images of bio-synthesized Ag and Au NPs were observed Figure 3a and 3b. Size of nanoparticles obtained was found to be between 32nm-53nm for Ag NPs and 65nm-99nm for Au NPs as confirmed by the SEM image analysis.

X-Ray Diffraction: Nanoparticles in XRD patterns exhibited several different size dependant features leading to anomalous peak position height and width. XRD analysis is mainly taken to study the crystalline nature of the nanoparticles [23, 24]. XRD analysis in Figure 4a shows cubic structure (# 87-0718) matches the values (38.4000) (44.5000) (64.8) (77.6) in hkl plane (111) (200) (220) (311) as explained in the figure related to Ag and

Figure 4b shows cubic structure (# 04-0784) and matches the values (38.4000) (44.2000) (64.3966) (77.6000) (81.7187) in hkl plane (111) (200) (220) (311) (222) as explained in the figure related to Au. The remaining peaks may be due to (*Phyllanthus amarus*).

Energy Dispersive X-ray Spectroscopy: The presence of the elemental silver and gold can be seen in the graph presented by the EDX analysis, which indicated the reduction of silver and gold ions Figure 5a and 5b. The EDX profile showed a strong elemental signal along with weak oxygen, which may have originated from the biomolecules bound to the surface of the nanoparticles [25]. It has been reported that nanoparticles synthesized using plant extracts are surrounded by a thin layer of some capping organic material from the plant leaf broth that remains stable in the solution even after synthesis.

Atomic Force Microscopy: Figure 6a and 6b shows the AFM image of silver and gold nanoparticles synthesized with 5% *Phyllanthus amarus* leaf extract was taken at 55°C . From the image it can be concluded that a nanoplate structure was formed proving this method to be appropriate and successful for the synthesis of nanoparticles [26].

Particle Size Analyser: In Figure 7a particle size range of Ag NPs is 192.7 nm diameter and width about 25.21 nm and Figure 7b particle size range of Au NPs is 464.4 nm diameter (nm) and width about 50.94 nm. Shape of the plate and spherical structures could be controlled by changing the reaction temperature and leaf broth concentration.

Fourier Transform Infrared Spectroscopy: FT-IR spectra of the samples were measured using Perkin-Elmer Spectrum. One instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets. Powder samples for the FT-IR was prepared similarly as for powder diffraction measurements. The FT-IR spectra of plant extracts taken before and after synthesis of nanoparticles were analyzed which discussed for the possible functional groups for the formation of nanoparticles. FTIR measurements were carried out to identify the possible biomolecules in the leaf extract responsible for the reduction of ions and also the capping agents responsible for the stability of the biogenic nanoparticle solution. Figure 9 represents the FTIR spectrum of leaf extract plant extract which shows prominent absorption bands at, 1693 cm^{-1} , 1397 cm^{-1} , 432 cm^{-1} . The shoulder at 1693 cm^{-1} is



Fig. 2: (a) Aqueous leaf extract of *P. amarus*. (b) Changing color from pale yellow to brown on adding 1mM AgNO_3 . (c) Changing color from pale yellow to purple ruby red on adding 1mM H_2AuCl_4 .

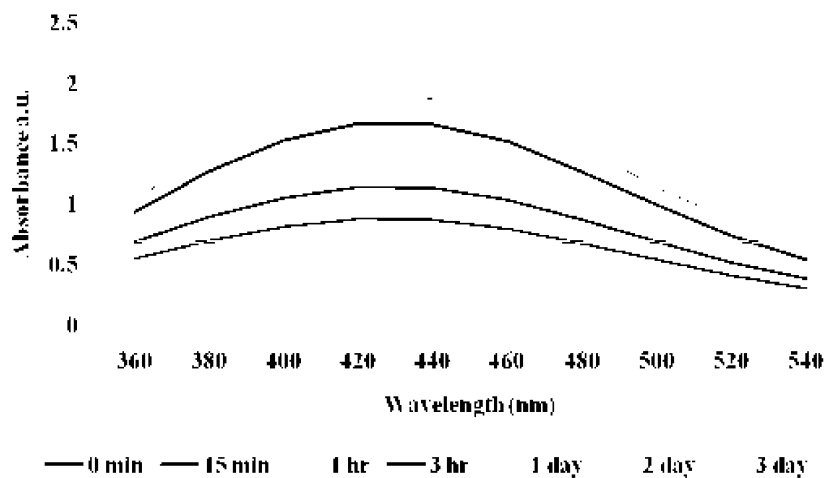


Fig. 3a: UV-Visible analysis for the synthesis of Silver nanoparticles

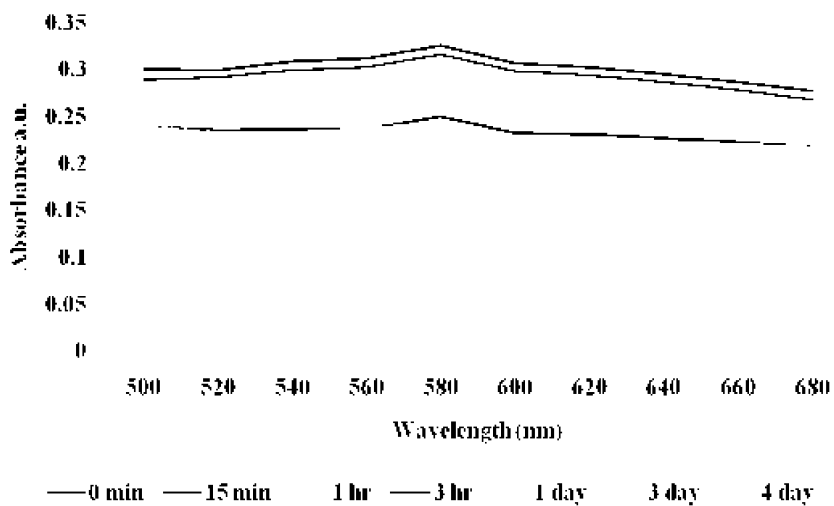


Fig. 3b: UV-Visible analysis for the synthesis of Gold nanoparticles

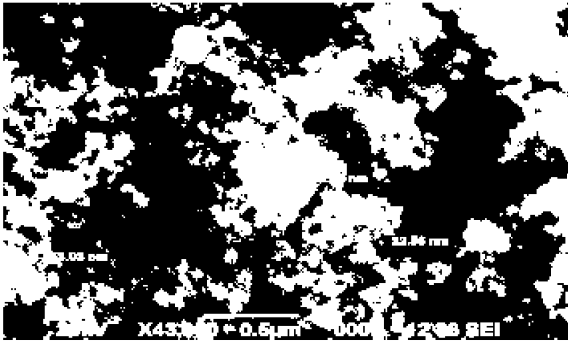


Fig. 4a: Scanning electron microscopy image of the silver nanoparticles of *P. amarus* at 55°C

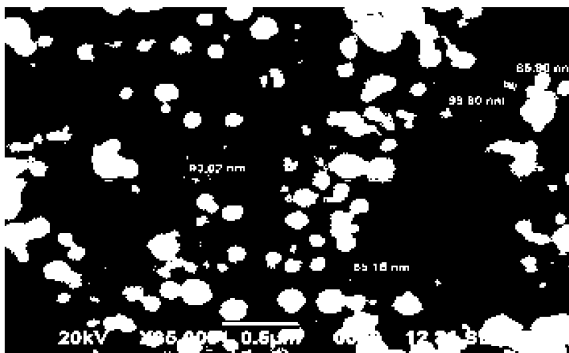


Fig. 4b: Scanning electron microscopy image of the gold nanoparticles of *P. amarus* at 55°C

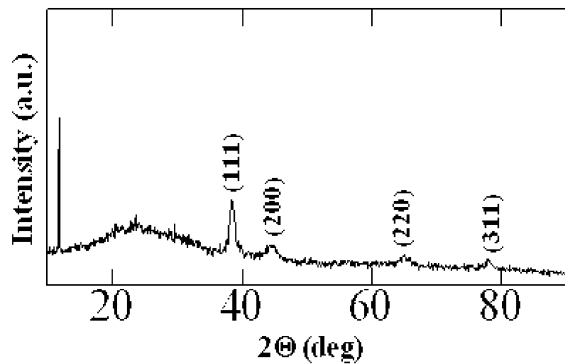


Fig. 5a: X-ray diffraction pattern of Silver nanoparticles

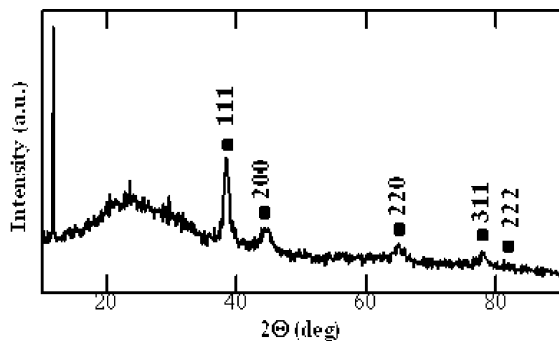


Fig. 5b: X-ray diffraction pattern of Gold nanoparticles

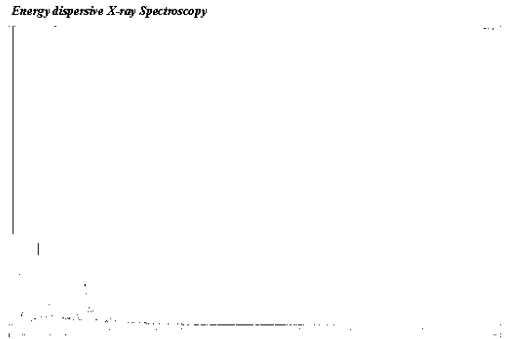


Fig. 6a: Energy dispersive X-ray Spectroscopy of Ag NPs (*P. amarus*)

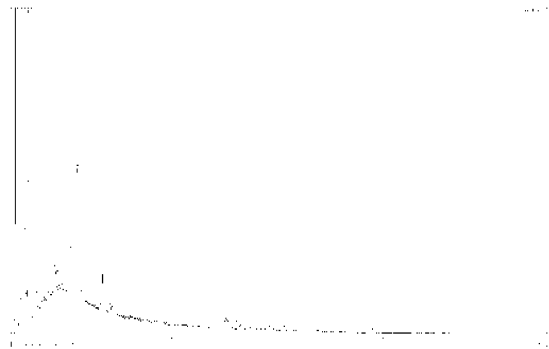


Fig. 6b: Energy dispersive X-ray Spectroscopy of Au NPs (*P. amarus*)

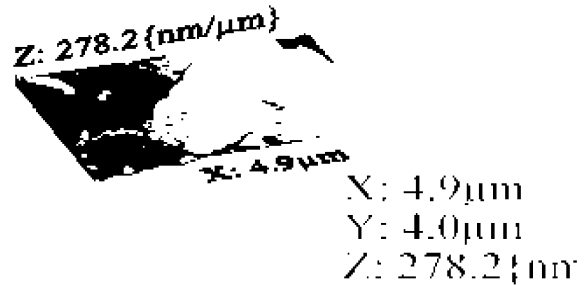


Fig. 7a: Atomic force microscopy image of Silver nanoparticles (*P. amarus*)

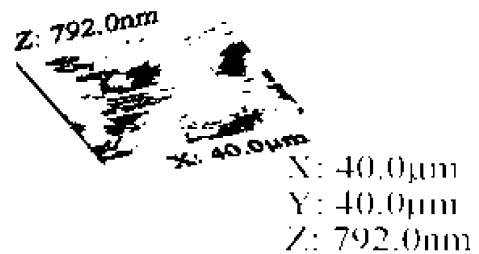


Fig. 7b: Atomic force microscopy image of gold nanoparticles (*P. amarus*)

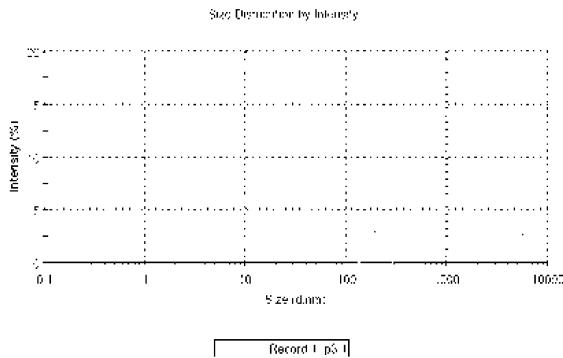


Fig. 8a: Particle size of Silver nanoparticles (*P. amarus*)

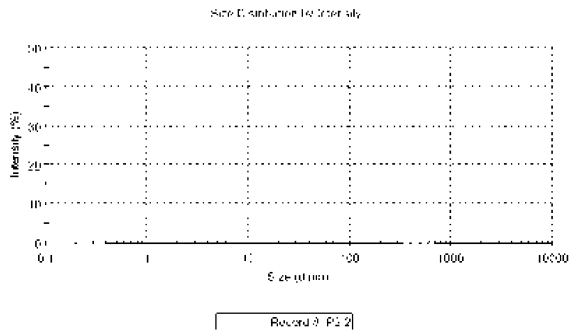


Fig. 8b: Particle size of gold nanoparticles (*P. amarus*)

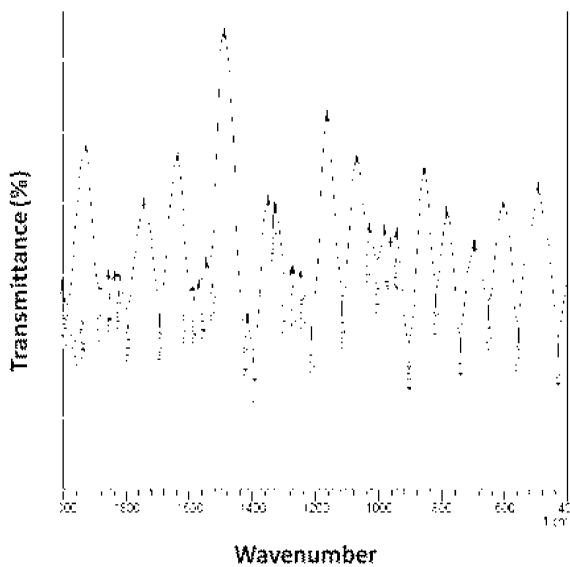


Fig. 9: Fourier Transform Infrared Spectroscopy absorption spectra of the *P. amarus* leaf extract before nanoparticle synthesis

characteristic of strong peak of carbonyl functional group. The peak value of 1397 cm^{-1} indicates the presence of alkyl halide groups. The broad stretch at 432 cm^{-1}

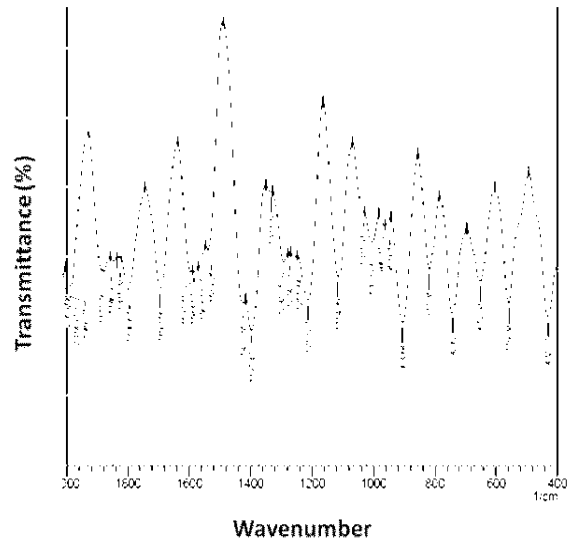


Fig. 10a: Fourier transform Infrared Spectroscopy absorption spectra of the *P. amarus* leaf biomass after bioreduction of Silver nanoparticles

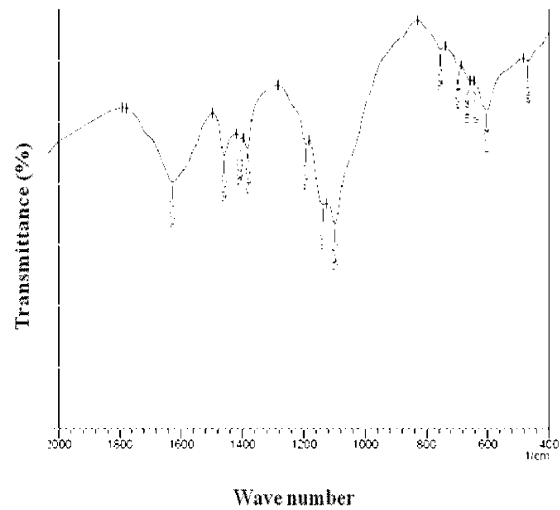


Fig. 10b: Fourier Transform Infrared Spectroscopy absorption spectra of the *P. amarus* leaf extract after bioreduction using gold nanoparticles

formation is indicated the presence of alkyl halides. Figure 10a represents the FTIR spectrum of the leaf extract reduced silver with the absorption bands at 1670 cm^{-1} , 3244 cm^{-1} , 1397 cm^{-1} , 431 cm^{-1} . The shift in higher to lower wave numbers when compared with 1 and 2 is indicated the facilitation resonance for the binding and reduction of silver nanoparticles surface. These lower wave numbers confirmed the represented functional group combined with the silver nanoparticles and reduction occurred in that particular surface. Figure 10b

represents the FTIR spectrum of the leaf extract reduced gold with the absorption bands at 650.13 cm^{-1} , 1098 cm^{-1} , 1627 cm^{-1} , when compared with plant peaks the values are 652.93 cm^{-1} , 1114 cm^{-1} , 1627 cm^{-1} . It is visually shown that the values are reduced when compared with the normal values. The value of 652.93 cm^{-1} indicates the presence of alkyl halides. The broad absorption peak at 1114 cm^{-1} indicates the presence of alcoholic groups. The absorption peak at 1627 cm^{-1} confirms the presence of stretch at alkenes. The lower wave numbers confirmed that the represented functional group combined with the gold nanoparticles and reduction occurred in that particular surface.

CONCLUSION

P. amarus leaf extract was found suitable for the green synthesis of Ag and Au NPs. The reduction of Silver and Gold ions by the leaf extract resulted in the formation of stable nanoparticles with spherical and cubic morphologies which ranged from 32-53 nm for Ag and 65-99 for Au in size. The concentration of leaf extract and metal ions play an important role in the green synthesis of Ag and Au NPs. The spectroscopic characterizations using UV-Vis, SEM, XRD, EDX, AFM and Particle size analyzer were useful in proving the formation of nanoparticles and also in confirming their size, shape and composition. FTIR evidenced the formation and stability of the bio-synthesized Ag and Au NPs which can be studied further to understand the chemical and molecular interactions which could be responsible for nanoparticle synthesis. Thus this simple and rapid method of green synthesis of Ag and Au NPs can further be applied in various biomedical and biotechnological fields and their properties and applications can further be explored.

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