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Original Research Article

Green Synthesis of Silver Nanoparticles from *Phyllanthus amarus* and their Antibacterial and Antioxidant Properties

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A B S T R A C T

An environmental friendly approach is employed to synthesize silver nanoparticles. The biomolecules found in plants induce the reduction of Ag+ ions from silver nitrate to silver nanoparticles (AgNPs). UV-visible spectrum of the aqueous medium containing silver ions demonstrated a peak at 404nm corresponding to the plasmon absorbance of silver nanoparticles. Fourier Transform Infra-Red spectroscopy was done to find the functional groups present. Antioxidant activities were done using DPPH antioxidant assay and Hydrogen Peroxide assay. Plants during glycolysis produce a large amount of H+ ions along with NAD which acts as a strong redoxing agent; this seems to be responsible for the formation of AgNPs. AgNPs produced show good antimicrobial activity against common pathogens. Green synthesis of silver nanoparticles exhibits an important eco-friendly and useful to the environment. UV-visible spectrophotometer used to predict that where the synthesis has been done or not. Whereas the organic functional groups were determined by FT-IR with different wave number and it has determined by the functional group data analysis. Keywords: DPPH, FT-IR, Silver nanoparticles, NAD.

Keywords

DPPH;
FT- IR:
Silver Nanoparticles;
NAD.

Introduction

Nanotechnology is a fast growing field and nanoparticles are viewed as fundamental building block of nanotechnology. The most important and distinct property of nanoparticles is that they exhibit larger surface area to volume ratio (Albrecht *et al.,* 2006). The most effectively studied nanoparticles today are those made form noble metals, in particular Ag, Pt, Au and Pd. An important aspect of nanotechnology concerns the development of experimental processes for the synthesis of nanoparticles of different sizes, shape, and controlled disparity (GarderToressgay *et al* ., 2003).

The metal nanoparticles have tremendous application in the area of catalysis, optical,

electric, magnetic properties, diagnostics biological probes and display devices. Silver nanoparticles play a significant role in the field of biology and medicine. Nanomaterials due to their sheer size show unique and considerably change physical, chemical, and biological properties compared to their macro scale counter parts (GarderToressgay *et al.,* 2003).

Biological methods are considered safe and ecologically sound for the nanomaterial fabrication as an alternative to conventional physical and chemical methods. Biological routes to the synthesis of these particles have been proposed by exploiting microorganisms and by vascular plants. (Jha *et al.,* 2009; Spring *et al.,* 1995). The function of these material depend on their composition and structure .Plants have been reported to be used for synthesis of metal nanoparticles of gold and silver and of a gold –silver –copper alloy.(Anderson *et al.,* - Cao *et al.,* 2002). Colloidal silver is of particular interest because of its distinctive properties such as good conductivity, chemical stability, and catalytic and antibacterial activity (Cao *et al.,* 2002-Rosi *et al.,* 2005).

India has great potential for bio prospecting because of its rich biodiversity. Advances in biotechnology have increased the value of plants genetic resources (Belloni *et al.,* 2003). In recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco friendliness. Using plants for nanoparticles 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 nanoparticles synthesis (GarderToressgay *et al.,* 2003).

Metal nanoparticles with at least one dimension approximately 1-100 nm had received considerable attention in both scientific and technological are technological areas due to their unique and unusual physio-chemical properties with that of bulk materials. Due to the specific size, shape and distribution nanoparticles are used in the production of novel systems. Literature reveals a promising medical application of silver nanoparticles synthesized using herbal extracts have been reported to have good anti-bacterial, anti-fungal and antioxidant properties (Banerjee *et al.,* 2011; Elumalai *et al.,* 2006). In this study we synthesised silver nanoparticles from aqueous leaf extract of *Phyllanthus amarus.*

Materials and Methods

Sample Collection

Silver nitrate was obtained from Sigma-Aldrich, Bangalore. All the glasswares had been washed with distilled water and dried using a hot air oven before use. Fresh leaves of *P.amarus* have been collected from KSRCT campus, Tiruchengode.

Figure.1 Fresh leaves of *P.amarus*

Preparation of Extract

Freshly collected *Phyllanthus amarus* leaves were shad dried and powdered. 0.5g of the powdered samples were boiled for 10 min in 100ml sterile distilled water and filtered through what man filter paper (pore size 25 µm). The filtrate was further filtered through 0.6 µm sized filter paper. The filtrate was used for the present study.

Synthesis of Nanoparticles

1mM of silver nanoparticles was reduced using 100ml of 5% leaf extract at room temperature for 3days. After 3 days, it was resulting in the dark brown solutions indicating the formation of silver nanoparticles.

UV-Vis Spectra Analysis

Synthesis of silver nanoparticles 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 concentration was measured using a Perkin- Elmer Lamda- 45 spectrophotometer in 300-1000 nm range.

Fourier Transform Infrared Spectroscopy (FTIR)

Samples were measured by Shimadzu 8400s and using spectral range of 4000-400 $cm^{-1}$with resolution of 4 cm^{-1} Powder samples for the FTIR were prepared similarly as for powder diffraction measurements.

The FTIR spectra of leaf extract taken before and after synthesis of silver nanoparticles were analysed to study the possible functional groups for the formation of silver nanoparticles.

Antibacterial Activity

Anti-bacterial activity of the silver nanoparticles was screened against *Bacillus subtilis* and *Escherichia coli.* Briefly, overnight cultures of the bacterial strains were prepared in Nutrient broth. 100µl of synthesized silver nanoparticles were filled in to the well and kept for incubation overnight at 30°c.

Antioxidant Activity

DPPH Assay

0.6g,1.2g,1.8g,2.4g &3.0g of leaf was taken.6ml of ethanol was added and kept in the water bath for 10 min at 30ºc. 1ml solution was taken from the plant extract and 3ml of DPPH was added.Then it was incubated in room temperature for 30min.And reading was taken at 517nmusing UV-Vis spectroscopy. Ascorbic acid serves as a blank.

Hydrogen Peroxide Assay

Phosphate buffer was prepared and 0.01ml of hydrogen peroxide was added in 10ml of phosphate buffer. Then 0.6g, 1.2g, 1.8g, 2.4g &3.0g of leaf was taken and 6ml of ethanol was added and kept in the water bath for 10 min at 30°c.1ml leaf extracts were taken and 0.6ml solution of H_2O_2 was added and it was incubated in room temperature for 30min and reading was taken at 510nm using UV-Vis spectroscopy. 0.01ml H_2O_2 in 10ml of phosphate buffer serves as blank.

Results and Discussion

UV-Vis Spectra Analysis:Formation of silver nanoparticles from 1mM solution of Silver nitrate was confirmed using UV-Vis

spectra analysis. AgNPs 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. Surface Plasmon resonance spectra for AgNPs are obtained at 404nm with brown colour. 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 biosynthesis, it is believed that the natural material extract acts as reducing agent for the generation of metal nanoparticles.

The synthesised silver nanoparticles synthesized using *Phyllanthus amarus* showed strong bands at 3383.46cm^{-1} and 1639.36cm^{-1} corresponds to stretch in OH bond (3200-3600).The level of C=O is in 1639.36cm^{-1} .The peak which was obtained 2960.24cm^{-1} and 2851.45cm^{-1} shows the presence of absorbance Alkane region (C-H).FTIR demonstrated that the chemical of nanoparticles synthesized by *Phyllanthus amarus* was non homogenous.

Synthesisedsilver nanoparticles showed better anti-bacterial activity against the growth of common pathogens. The pathogens, *Escherichia coli* and *Bacillus subtilis* were found to be sensitive against the compound. Zone of inhibition was found to be 2mm.

Antioxidant Activity

DPPH Assay

Free radical scavenging activity of the silver nanoparticles was assessed by DPPH solution exhibited a deep purple colour with a maximum absorbance at 517nm. The disappearance of purple colour on adding synthesized silver nanoparticles might due to presence of antioxidant in the medium. AgNPs on DPPH radical was found to increase with increase in concentration, showing a maximum of81.39%at 500µg/ml.

DPPH radical scavenging activity (%) = [(control absorbance)-(sample absorbance)]/
[control absorbance]*100

Hydrogen Peroxide Assay

Hydrogen peroxide assay was done and antioxidant activity was examined at 510nm with the reduction of hydrogen ions.

Phyllanthus amarus leaf extract was found suitable for the green synthesis of AgNPs. The reduction of the Silver ions by the leaf extract resulted in the formation of stable nanoparticles with spherical and cubic morphologies which ranged from for silver in size. The concentration of the leaf extract and metal ions play an important role in the biosynthesis of silver nanoparticles. The spectroscopic characterizations using UV-Vis, Particle size analyser, Antimicrobial Activity, Antioxidant Activity were useful in proving the formation of nanoparticles and composition. FTIR evidenced the formation and stability of the bio-synthesized silver nanoparticles which can be studied further to understand the responsible for nanoparticles synthesis. Thus, this simple and rapid method of biosynthesis of AgNPs can further be applied in various biomedical and biotechnological fields and their properties applications can further be explored.

Figure.1 UV Vis Spectra Analysis

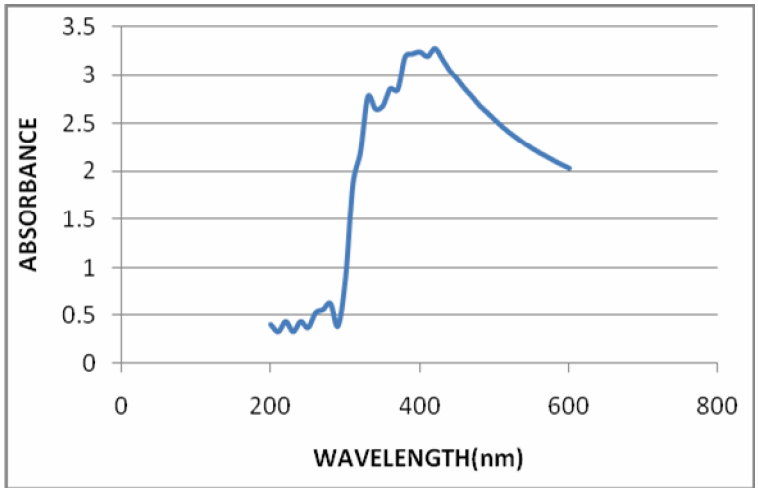


Figure.2 Fourier Transform Infrared Spectroscopy (FTIR**)**

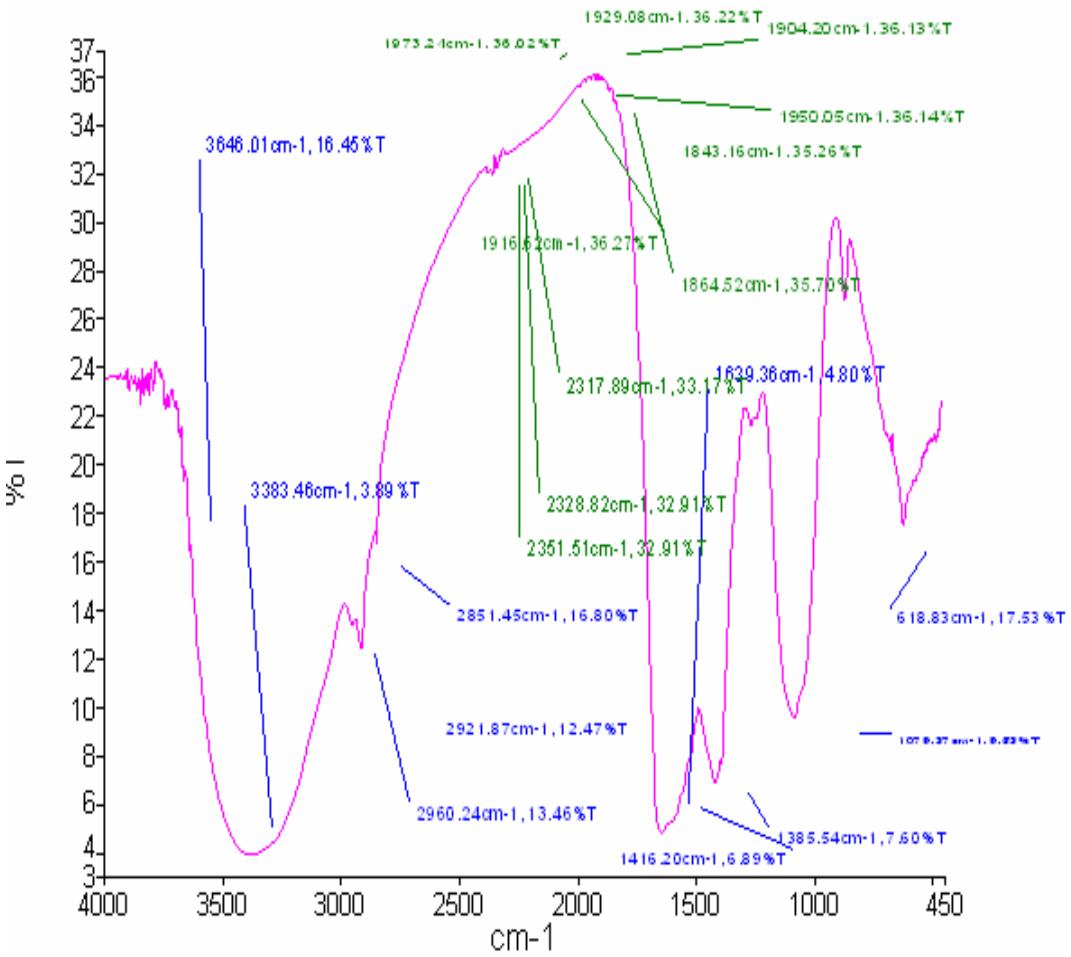


Figure.3 DPPH Assay

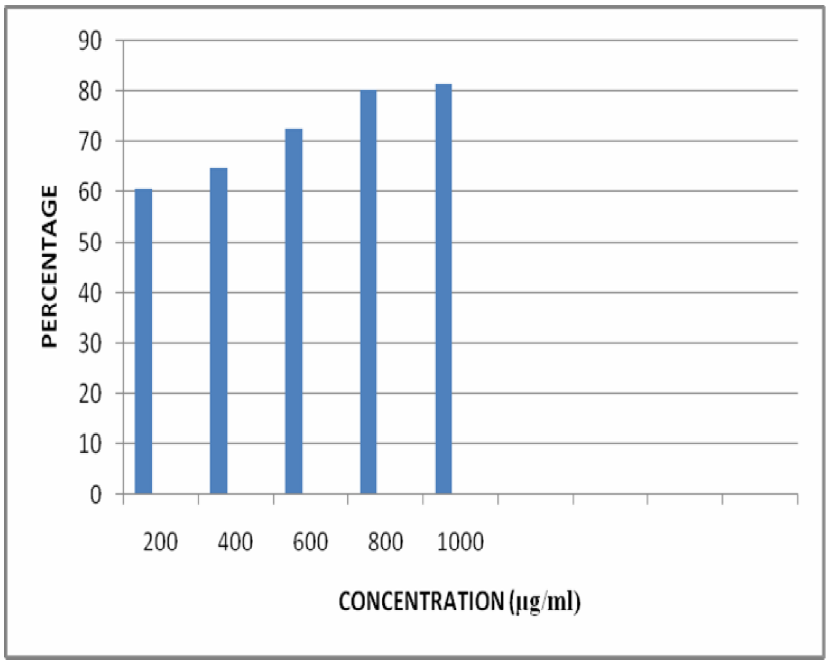
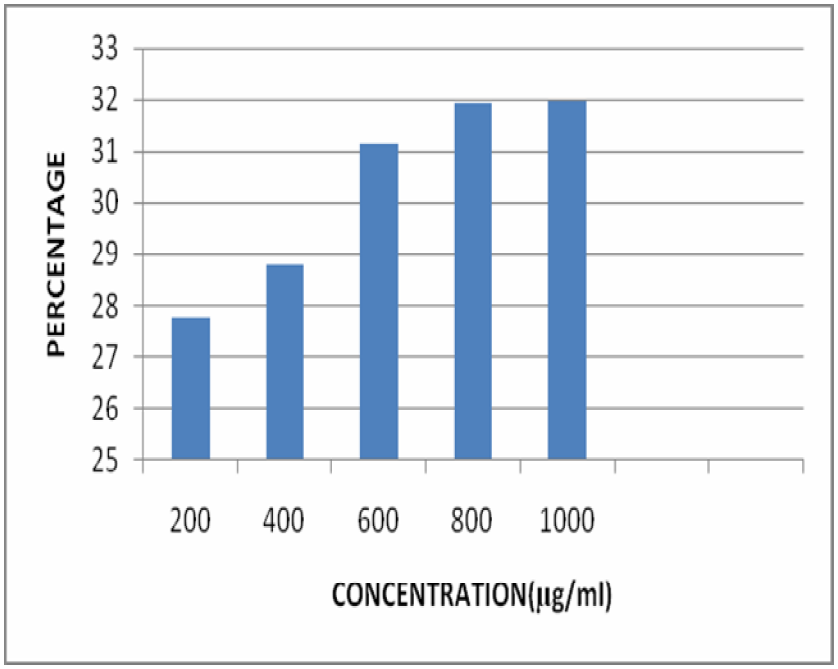


Figure.4 Hydrogen Peroxide Assay



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