ORIGINAL ARTICLE

Green approach for the synthesis of silver nanoparticles using *Bryophyllum pinnatum*

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Abstract

Introduction: Nanotechnology has opened up novel dimensions in the field of biotechnology and medicine. Green synthesis of silver nanoparticles (AgNPs) is a clean, cost effective, and non-toxic over synthetic methods. Silver is the metal of choice as they hold the promise to kill microbes effectively. AgNPs have been recently known to be a promising antimicrobial agent that acts on a broad range of target sites both extracellularly and intracellularly. Green synthesis of AgNPs has been estimated to be rich with phytochemicals such as alkaloids, triterpenes, flavonoids, glycosides, steroids lipids, and organic acids that are extracted from various medicinal plants. **Materials and Methods:** An aim to synthesize and optimize the AgNPs of *Bryophyllum pinnatum* leaf extract within 10 min at microwave (100 W) temperature conditions was carried out. The synthesized nanoparticles were characterized using ultraviolet–visible spectrophotometer, scanning electron microscopy, and Fourier transform infrared (FT-IR). **Results:** The carbonyl group that of amino acid residues has a power to bind with silver which suggested this of a layer covering AgNPs and acts as a capping agent and prevents agglomeration and assists in resist changes due to medium. The silver nanoparticles thus formed were well capped which were observed by FT-IR and showed antibacterial activity against *Escherichia coli* and *Bacillus subtilis.* **Conclusion:** The present investigation has evaluated that leaf extract of *B. pinnatum* has a potential source of reducing and capping agent for the synthesis of AgNPs. The synthesized AgNPs showed a strong antibacterial activity which is very important from the aspects of its biomedical application.

Key words: *Bryophyllum pinnatum*, Fourier transform infrared, green synthesis, nanotechnology, phytochemicals, scanning electron microscopy

INTRODUCTION

Nanotechnology provides the ability to engineer the properties of materials by controlling their size, and this has driven research toward a multitude of potential uses for nanomaterials.[1] Metal nanoparticles have a high specific surface area and a high fraction of surface atoms. Because of the unique physicochemical characteristics of nanoparticles, including catalytic activity, optical properties, electronic properties, antibacterial properties, and magnetic properties, they are gaining the interest of scientist for their novel methods of synthesis.[2,3] Some of the engineered nanoparticles are being developed for various applications to replace the use of more hazardous chemicals.[4] Among the synthetic methods used for the preparation of silver nanoparticles (AgNPs), certain chemicals such as $NaBH_4$, citrate, or ascorbate are said to be most common reducing agent.[3] The leaf extract of medicinal plants serve as reducing agent as well as capping agent in the synthesis of silver nanoparticles which are eco friendly.[5] Based on that such reducing agents may be associated with environmental toxicity or biological hazards, the development of a green synthesis approach for AgNPs is desired.[6,13] AgNPs suppose to have very large surface area which results in greater biochemical reactivity, catalytic activity, and atomic behavior compared to large particles of the similar chemical composition.[7] The structure of gold nanoparticles synthesized on the silicon surface is studied by pulsed laser ablation and magnetron sputtering. The surface morphology is examined by scanning electron microscopy (SEM). The structure of gold nanoparticles is analyzed by transmission electron microscopy and electron

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diffraction. Thermal annealing helps to control the size and structure of nanoparticles.[8]

In the synthesis of AgNPs, the phytochemicals, proteins and enzymes present in the plant extract play a major role as reducing agents along with electron-shuttling compounds. Since then, various herbs are employed for the synthesis of nanoparticles. Since few years due to the promising potential of antimicrobial property of medicinal plants have attracted the attention of pharmaceutical and scientific communities towards plant derived substances for the synthesis of AgNPs.[13] The antimicrobial properties of plant oils and extracts have laid the basis of many applications. The synthesis of nanoparticles using plant extracts having antimicrobial properties which are termed as green synthesis. In this study, we aim to synthesize and optimize the silver nanoparticles of *Bryophyllum pinnatum* leaf extract.[9,10] This plant is vegetative propagated herb found in tropical areas of India. This plant has medicinal properties and finds application in folk medicine. The preparation of AgNPs or antimicrobial activity of *B. pinnatum is* less investigated and can be explored to great extent.

MATERIALS AND METHODS

Preparation of Leaf Extract

Fifty grams of leaves were washed thrice with distilled water to remove the surface impurities. The leaves were chopped finely and macerated with the help of mortar and pestle. After macerating, 250 ml of distilled water is added and mixed well. The mixture is incubated at 60°C up to 1 h. The extract thus obtained was cooled and filtered with Whatman filter paper- l and used freshly.

Preparation of 1 mm silver nitrate ($AgNO_3$) Solution

To prepare 1 mM $AgNO_3$ solution, 0.l698 g of $AgNO_3$ is added to l000 ml of distilled water which is kept in dark to avoid photochemical reactions and for further use.

Synthesis of Silver Nanoparticles

Fresh leaf extract of 5 ml was added to 45 ml of 1 mM $AgNO_3$ solution for bioreduction action at room temperature and change in the color was observed.

Optimization of Synthesis Process

Volumetric ratio of leaf extract and $AgNO_3$ solution

Different concentrations of the leaf extract and AgNO solution (1:1 1:2, 1:3, 1:4, and 1:5) are used for the biosynthesis of the AgNPs. Time taken to attain an absorption peak at 425 nm was recorded.

Microwave-assisted biosynthesis of AgNPs

The biosynthesis was achieved under microwave condition kept at 100 W power. The color change and the absorbance of the reaction mixture were observed for every 30 s spectrophotometrically.

Water bath-assisted biosynthesis of AgNPs

The biosynthesis of AgNPs was carried out using a water bath which was supported with 80°C temperature till the reaction mixture changes to brownish-red color and the ultraviolet (UV)–visible spectrum was recorded.

Autoclave-assisted biosynthesis of silver nanoparticles

Preparation of AgNPs was carried out under autoclave condition supported with 125°C and 5–7 lbs pressure, until a reddish-brown color appears. The UV–visible spectrum was noted.

UV–Visible Spectrophotometry

The reduction of $AgNO_3$ to AgNPs using aqueous leaf extract was observed by measuring the UV–visible spectrum of the resultant mixture after diluting a small aliquot of the sample with deionized water twice. The measurements are recorded on CORBET UV–visible spectrophotometer operated at a resolution of 1 nm.

Fourier transform infrared (FT-IR) Analysis

FT-IR measurement was carried out for both the extract and AgNPs to observe and identify the possible bioactive molecules which is responsible for the reduction of the Ag+ ions and the capping of the bio reduced AgNPs by the leaf extract, in the diffuse reflectance mode at a resolution of 4 cm^{-1} using potassium bromide (KBr) pellets and the wavelength was recorded at interval of 4000 to 400 cm^{-1}.

SEM-EDX

A drop of colloidal solution of AgNPs was placed on carbon-coated copper grid and later it is dried in air, before the sample is transferred to the microscope which is operated at an accelerated voltage of 5 kV [Figure 1].

Antimicrobial Assay

Antimicrobial activity of biosynthesized AgNPs is carried out against these *Escherichia coli*, *Bacillus subtilis*, and *Aspergillus niger* with different concentrations of AgNPs by well-diffusion method and zone of inhibition is observed.

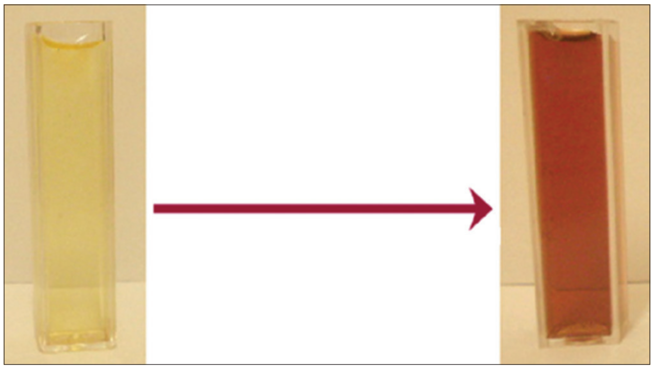

Figure 1: Bioreduction at room temperature

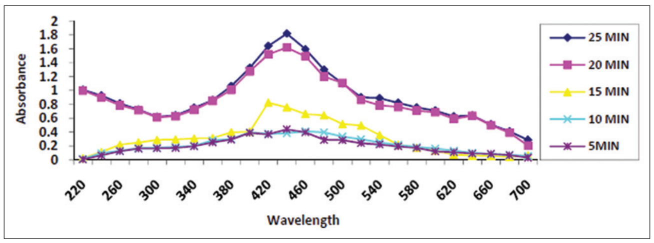

Figure 2: Absorption peak observed at 430 nm

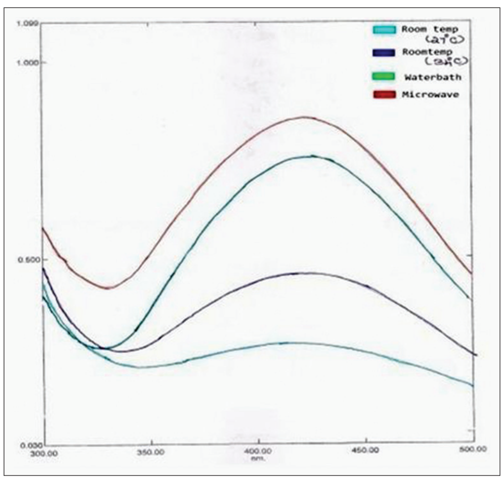

Figure 3: Absorption spectra recorded at different temperature conditions

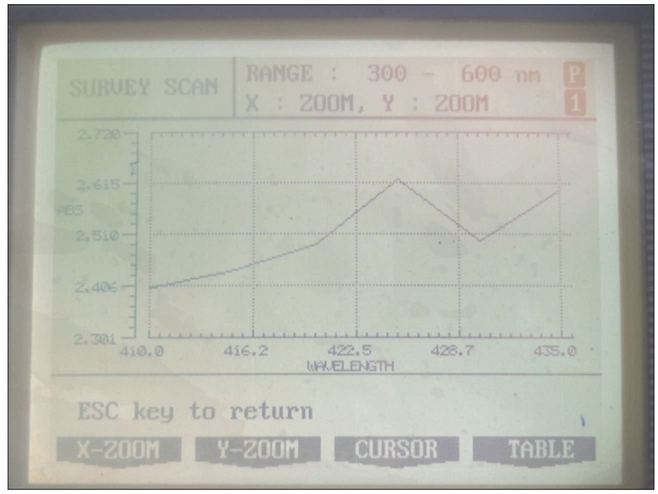

Figure 4: Absorption peak at 425 nm

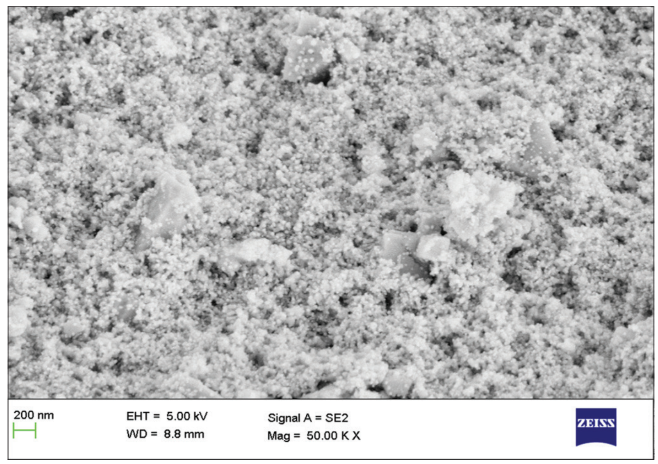

Figure 5: Ultramicrograph of silver nanoparticles at 50 Kx

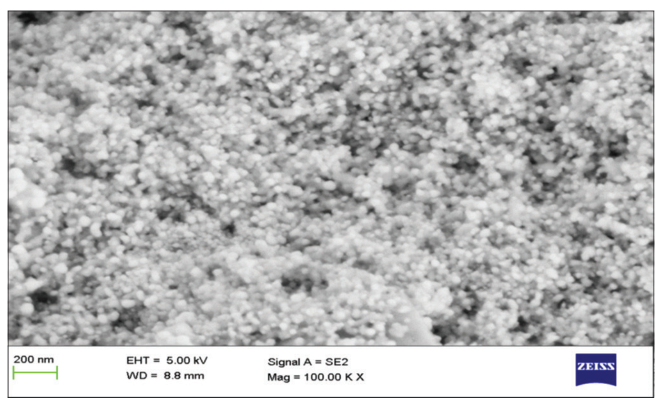

Figure 6: Ultramicrograph of silver nanoparticles at 100 Kx

RESULTS AND DISCUSSION

Synthesis of Silver Nanoparticles

From the reference, 10 ml of leaf extract is mixed with 40 ml of Ag NO_3 solution and keeping at room temperature change in the color is observed, and UV–visible spectra are recorded.

Biosynthesis at Different Heating Conditions

Absorption spectra recorded at different temperature conditions [Figures 2 and 3].

Confirmatory Test and Characterization of Synthesized AgNPs

Absorption peak at 425 nm due to surface plasmon resonance confirms the presence of AgNPs [Figure 4].

SEM

SEM gave an insight of the morphology and details of size of the AgNPs.[9,11] The results showed that the diameter of synthesized nanoparticles was about −20 nm and the shape was spherical, as shown in Figures 5-7.

The carbonyl group of amino acid residues has a strong binding ability with silver which was confirmed by the study done by FT-IR spectroscopic analysis [Figures 8 and 9] that suggested that the formation of a layer covering AgNPs and acting as a capping agent to prevent agglomeration and gave stability to the medium.[12]

Antimicrobial Assay

E. coli

Biosynthesized AgNPs were analyzed for their antimicrobial activity against *E. coli* by disk diffusion method [Figure 10]. When 1 mg/ml concentration of silver nanoparticles is used, it showed the zone of inhibition. It was observed that microbial growth of *E. coli* was dependent on AgNP concentration. The zone of inhibition increases with increased volume of AgNPs and compared with reference drug (RD, streptomycin). The zone of inhibition range was 5 mm for 50 µl, 18 mm for 100 µl, and 19 mm for 150 µl, these results indicated that the silver nanoparticles synthesized by *B. pinnatum* have strong antibacterial effect similar to reference drug.

B. subtilis

The synthesized nanoparticles (1 mg/ml) showed inhibition zone against *B. subtilis* [Figure 11]. The zone of inhibition range was 10 mm for 50 µl, 20 mm for 100 µl, and 18 mm for 150 µl. The result obtained shows that the AgNPs synthesized by *B. pinnatum* have strong antibacterial effect against *B. subtilis.*

DISCUSSION

The AgNPs synthesized from *B. pinnatum* showed zone of inhibition against *B. subtilis* as the flavonoids present in the leaves have a potential to reduce silver ion leading to the formation of AgNPs. This is in accordance with the study done by Huag *et al*. (2011) who showed that flavonoids present in *Cacumen platycladi* extract are responsible for the reduction of silver ions which are significant antibacterial activity.

Synthesized nanoparticles formed by biosynthetic methods from *B. pinnatum* showed the diameter of about 20 nm with spherical shape which was observed from the micrograph records of unpublished SEM. This result is similar to Iravani *et al*. (2014) where biosynthesis of AgNPs has demonstrated the bioreductive synthesis of AgNPs using *F. oxysporum* by reaction with $AgNO_3$ solution (1 mM) which was been analyzed using SEM.

The observations from FT-IR spectrum suggested that the layer covering AgNPs and capping agents present in the plant extract prevent agglomeration and give stability to the

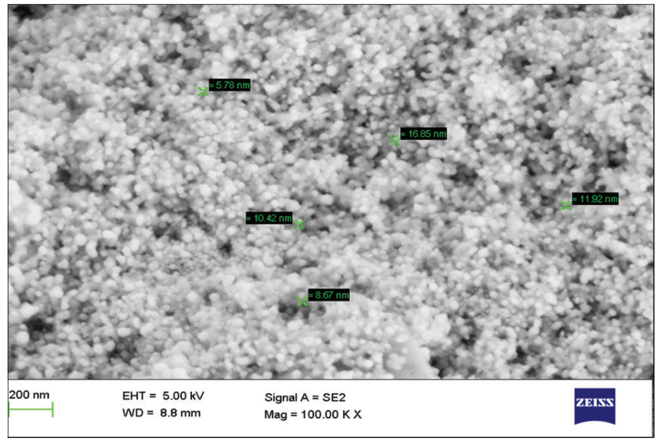

Figure 7: Ultramicrograph of silver nanoparticles at 100 Kx showing 5–20 nm diameter size

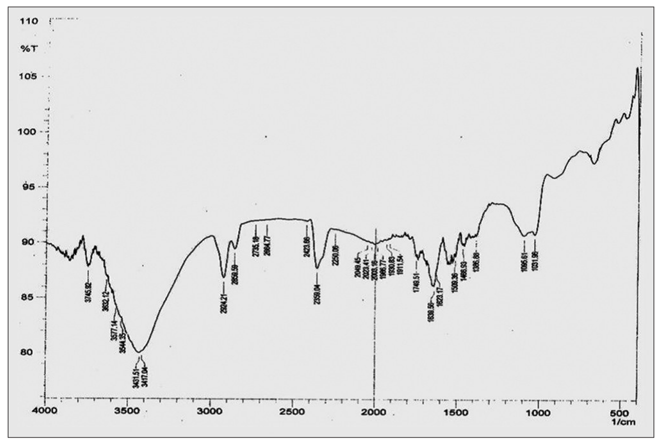

Figure 8: Fourier transform infrared spectrum of *Bryophyllum pinnatum* leaf extract

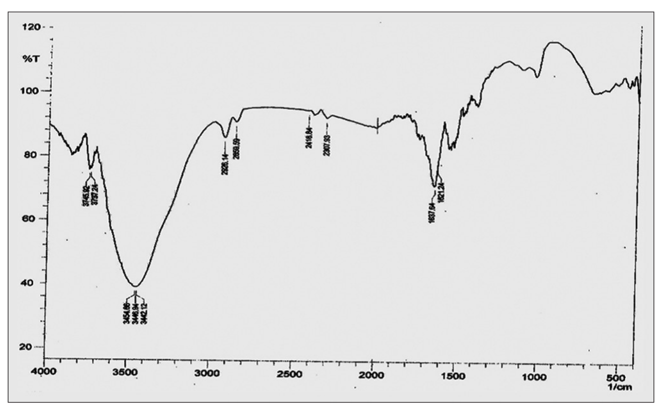

Figure 9: Fourier transform infrared spectrum of biosynthesized silver nanoparticles

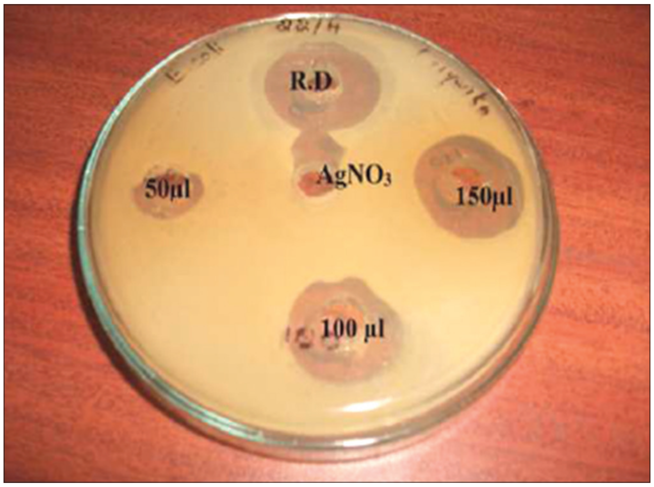

Figure 10: Zone of inhibition formed from *Escherichia coli*

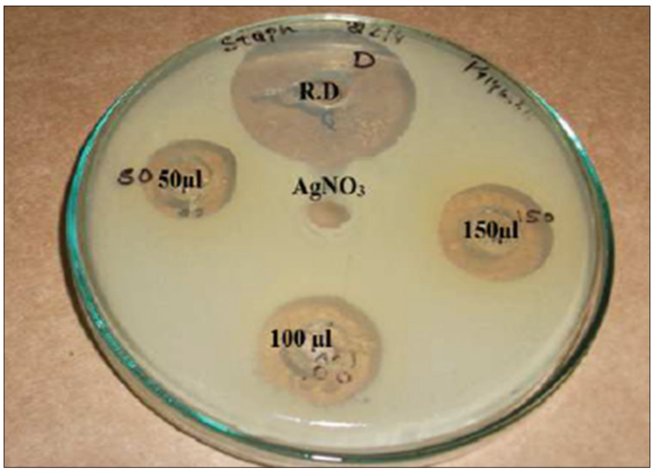

Figure 11: Zone of inhibition formed from *B. sub*

particles formed which is accordance with the study done by Ashokkumar *et al*., (2015), the stable and spherical silver nanoparticles from *Anser indicus* demonstrated the presence of carbohydrates and flavonoids in the extract acts as reducing and capping agents.

Synthetic methods used for the preparation of AgNPs, certain chemicals such as $NaBH_4$, citrate, or ascorbate are said to be most common reducing agent. Such reducing agents may be associated with environmental toxicity or biological hazards, the development of a green synthesis approach for AgNPs is desired and contains natural phytochemicals present in *B. pinnatum* leaves extract such as tannin, saponin, alkaloids, phenols, flavonoids, Vitamin C, etc., and the flavonoids present are responsible for the bioreduction of $AgNO_3$ to nanoparticles in accordance with the study done by Masum *et al*. (2019), the synthesis and characterization of AgNPs from *Phyllanthus emblica* fruit extract reported that it is advantageous over the chemically synthesized nanoparticles that contain toxic chemicals that are expensive ecologically harmful.

CONCLUSION

The study showed that it is possible to obtain biosynthetic AgNPs using an aqueous extract of *B. pinnatum*. The biosynthesized AgNPs show a significant antimicrobial effect against E. coli and *B. subtilis* at lower concentrations than chemically synthesized AgNPs.

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