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Biosynthesis of Silver Nanoparticles Using *Selaginella bryopteris* Plant Extracts and Studies of Their Antimicrobial and Photocatalytic Activities

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RESEARCH ARTICLE

The present study focuses on the green synthesis of silver nanoparticles (AgNPs) using aqueous extract of *S. bryopteris* which is a lithophyte with remarkable resurrection capabilities. These biosynthesized NPs were characterized with the help of UV-vis spectrophotometer, X-ray diffraction (XRD), FT-IR and Transmission electron microscopy (TEM). Formation of AgNPs was confirmed by UV-Visible spectrophotometer analysis which showed surface plasmon resonance (SPR) at around 420 nm. The TEM images showed the nanoparticles to be polydispersed, nearly spherical in shape and have sizes in range 4–30 nm. The synthesized AgNPs possessed high antibacterial activity as well as photocatalytic dye degradation properties under solar light irradiation in the absence of chemical reducing reagents. Stability of bio-reduced silver nanoparticles was analyzed using UV-vis absorption spectra, and their antimicrobial activity was screened against various gram-positive bacteria.

Keywords: Biosynthesis, Silver Nanoparticles, *S. bryopteris* Extract, Characterization, Antimicrobial Activity, Photodegradation.

1. INTRODUCTION

Nanoparticles usually referred as particles with a size from 1 to 100 nm.[1] As compared to the bulk, nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Wide varieties of synthetic strategies, including both physical and chemical methods, have been employed to synthesize nanoparticles. Even though chemical approaches are the most popular methods for the production of nanoparticles, use of toxic chemicals are often required in the synthesis protocol. Hence, there is a growing need to develop environmentally friendly processes of nanoparticles synthesis that do not use toxic chemicals. Biological methods of nanoparticles synthesis[2] using microorganism,[3,4] enzyme,[5] plant or plant extract,[6–9] fruit and fruit peels[10,11] have been suggested as possible ecofriendly alternatives to chemical and physical methods. Use of plant extract for the synthesis of nanoparticles could be advantageous over other environmentally benign biological processes by eliminating the elaborate process of maintaining cell cultures.[12] It can also be suitably scaled up for large-scale synthesis of nanoparticles.

In recent years, biosynthesis of silver nanoparticles by plants such as *Blighia sapida*,[13] *Tridax procumbens*,[14] *Tephrosia purpurea*,[15] *Ocimum sanctum* (Tulsi),[16] *Solanum xanthocarpum*,[17] *Pinus desiflora* (Pine), *Diopyros kaki, Ginko biloba, Magnolia kobus, Platanus orientalis*,[18] *Acalypha indica*,[19] *aloe vera*,[20] *Basella alba*,[21] *Coriandrum Sativum*,[22] *Sesuvium portulacastrum* (salt-marsh plant),[23] *Memecylon edule* (Melastomataceae),[24] *Ceratonia siliqua* (carob),[25] *Arbutus unedo*,[26] *Stevia rebaudiana*,[27] *Rosa rugosa*,[28] *Artemisia nilagirica*,[29] *Ficus benghalensis*,[30] *Azadirachta indica*,[31] *Atrocarpus altilis*[32] *Coccinia grandis*[33] and several others[34] have been reported and a great deal of work has been done to investigate both the photo degradation, targeting carcinogens,[35] antimicrobial properties.[36–38] In continuation to our on-going interest in the development of newer synthetic methodology,[39–42] herein we wish report a simple, mild and ecofriendly synthesis of AgNPs and investigation of their antimicrobial and catalytic photodegration properties.

To the best of our knowledge no work has been done for the synthesis of AgNPs using aqueous extract of

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S. bryopteris, a traditional lithophytic herb that for centuries has occupied a prime place among the most sought-after herbs in Indian mythology as '*Sanjeevani*' (one that infuses life) by virtue of its resurrection properties.[43] Many studies have described numerous and varied biological and medicinal properties of *S. bryopteris*.[44–47] Extracts of *S. bryopteris* were shown to have antimicrobial, antioxidant, anticarcinogenic, antiplasmodial and leishmanicidal activity. In this study, we describe the use of *S. bryopteris* as a reducing and stabilizing agent for the bio-inspired synthesis of AgNPs at room temperature. The obtained AgNPs were characterized fully by UV-visible spectroscopy, FT-IR, TEM, XRD, etc. Besides, the as-synthesized AgNPs were studied to evaluate their antimicrobial and photo catalytic activity in degradation of MB dye.

2. MATERIALS AND METHODS

2.1. Materials

S. bryopteris was procured from a local market of Silchar, Assam. Silver nitrate was bought from Fischer Scientific (India), Methylene Blue (MB) and Eosin dye were obtained from SRL (India) and used for experimentations without further purification. Adsorption spectra were recorded on a Carry Varian-450 UV-visible spectrophotometer. XRD measurements were carried out on a PAN-Alytical X'Pert Pro. TEM images were obtained using JEOL, JEM2100 equipment. FT-IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer.

2.2. Preparation of *S. bryopteris* Extract

S. bryopteris plant was washed properly with deionized water to remove all the impurities and finally dried under sunlight to remove all the moisture. The dried materials were ground to fine powder using mixer grinder. 20 gm of plant was weighed and transferred to a 500 ml round bottom flask. To it was added 100 ml of distilled water and boiled under refluxed condition for 2 hours. The extract obtained was filtered through Whatman No. 1 filter paper and the filtrate was collected in 250 mL Erlenmeyer flask and at stored in refrigerator at 4 °C for further use and analysis.

2.3. Biosynthesis of AgNPs

In a typical procedure for the synthesis of AgNPs, 5 ml of *S. bryopteris* plant extract was mixed with 50 ml of aqueous solution of $AgNO_3$ (1 mM solution) and stirred for 12 hours at room temperature. The progress of the reaction was monitored by observing the color change as well as by UV-visible spectrum of the reaction solution. Gradually initial pale yellow solution changed to a reddish brown color, indicating formation of AgNPs. The AgNPs obtained by were centrifuged at 15,000 rpm for 5 min and subsequently dispersed in sterile distilled water.

2.4. Catalytic Performance Test

5 mL of biosynthesized colloidal solution of AgNPs was added to 50 mL (2 mg/L) aqueous solution of MB or Eosin Y dye were added and the reactants were quickly mixed by stirring. Afterwards the dispersion was put under the sunlight and monitored. At a specific time intervals, aliquots of 3 mL suspension were collected and the absorbance was measured using UV-visible spectrophotometer. A control reaction was also maintained without AgNPs.

2.5. Screening of Antibacterial Property of Synthesized AgNPs

Antibacterial activity of the synthesized AgNPs was studied by disc diffusion method[48,49] against ATCC bacterial samples; *L. acidophilus (ATCC 314)*, *B. pumilus (ATCC 14884) and A. viscosus (ATCC 15987)*. The ATCC samples were cultured in nutrient broth for 3 day at 30 °C. The broth culture was used for growth of these pathogenic bacteria for antibacterial assay. Approximately 1 ml of bacterial cultures of the strains was spread over Petri plate containing Nutrient agar medium to create a confluent lawn of bacterial growth. Sterile filter paper (Whatman filter paper No. 1) was cut into small disc shape measuring 5 mm in diameter. The discs were then dipped into to the plant extracts and AgNPs for 15 min (until the disc were completely soaked). The discs were then placed on to the medium (3 discs/plate) and then incubated at 30 °C for 3 days.

3. RESULTS AND DISCUSSION

3.1. UV-Visible Spectral Analysis

The primary detection was done by simple visual observation. The change in color of the reaction solution from pale yellow to a dark brown with the increase in time provided evidence of the formation of AgNPs. The observed color changes were due to the excitation of surface plasmon resonance (SPR) with the AgNPs. The SPR of the nanoparticles produced a peak centered at around 420 nm, indicating the reduction of silver nitrate into AgNPs. UV-vis absorbance of the reaction mixture was taken at an interval of 2 h (Fig. 1). The steady rise in intensity of SPR suggested gradual increase in the yield of AgNPs with respect to time.

3.2. FT-IR and XRD Spectrum

The FT-IR spectroscopic studies were performed to investigate interaction between the surface of AgNPs and the possible organic functional groups of constituent compounds present in the *S. bryopteris* plant extract. The FT-IR spectrum of aqueous extract of *S. bryopteris* was shown in Figure 2(a). The absorption bands at 3435 cm^{-1} and 1096 cm^{-1} are due to N–H and C–O stretching vibration respectively. The peak for carbonyl group was observed at 1737 cm^{-1}. The absorption peak at 1619 cm^{-1} and 1096 cm^{-1} may be assigned to the amide I of the

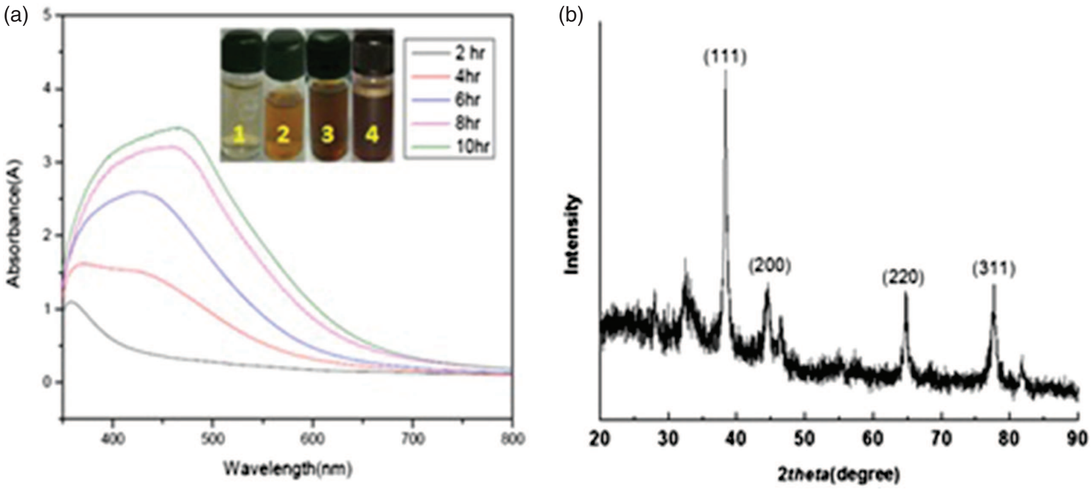

Fig. 1. (a) UV-visible of AgNPs (inset (1) Plant extract, (2) AgNPs after 4 h, (3) AgNPs after 8 h, (3) AgNPs after 10 h), (b) XRD pattern of AgNPs.

polypeptides and symmetric stretching arising from the carboxylate groups in the amino acid residues of the protein molecules. Results of the FT-IR study of biosynthesized AgNPs showed slight deviation from that of plant extract and gave sharp prominent absorption peaks located 3436, 2923, 2849, 1630, 1520, 1461, 1384, 1324, 1088 cm^{-1} (Fig. 2(b)). Interestingly, here the carbonyl stretching band, at around 1737 cm^{-1} in case of plant extract only, was not observed anymore. The FT-IR spectroscopic study confirmed that the carbonyl group of amino acid residues has a strong binding ability with silver, suggesting the formation of a layer covering silver nanoparticles and acting as a capping agent to prevent agglomeration of nanoparticles and provide stability to the medium.[50]

The powder XRD patterns were recorded for the identification of phase exhibited by the AgNPs. It showed diffraction peaks at 2θ values of 38.32°, 44.54°, 64.70° and 77.67° corresponding to (111), (200), (220) and (311) Bragg reflections, respectively, which may be indexed based on the face-centered cubic structure of silver (Fig. 1(b)).

3.3. TEM and SAED Analysis

The TEM image (Figs. 3(a and b)) showed that the synthesized AgNPs were polydispersed and were predominantly spherical and oval in shape with particle size range from 5 to 40 nm. The HR-TEM image showed the lattice fringes between the two adjacent planes to be 0.23 nm apart which corresponds to the interplanar separation of the (111) plane of the face-centered cubic AgNPs (Fig. 3(c)). The crystalline structure of the synthesized nanoparticles was also

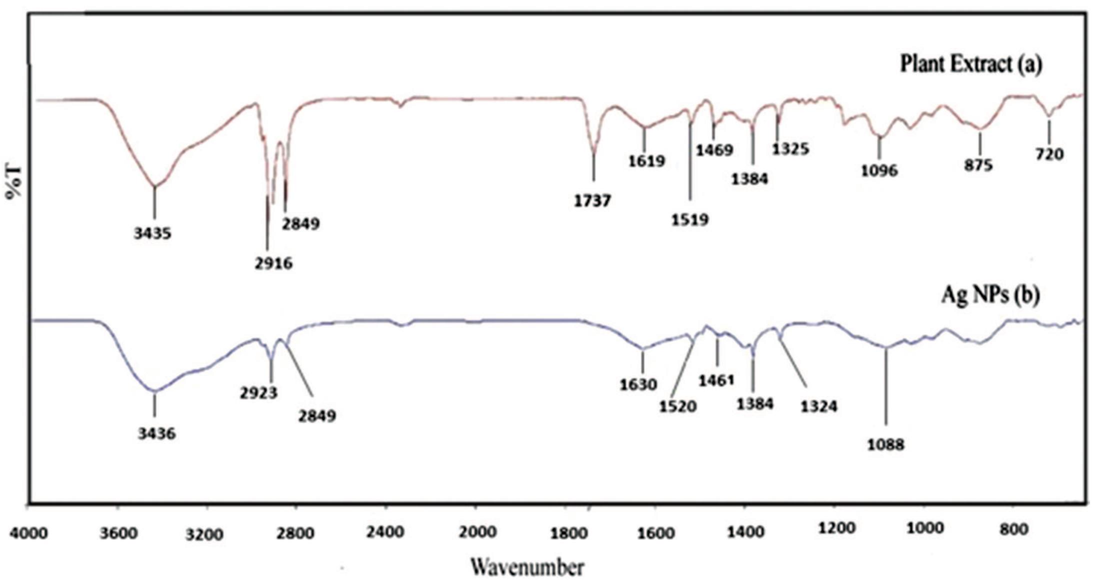

Fig. 2. FT-IR spectrums of (a) plant extract (b) AgNPs.

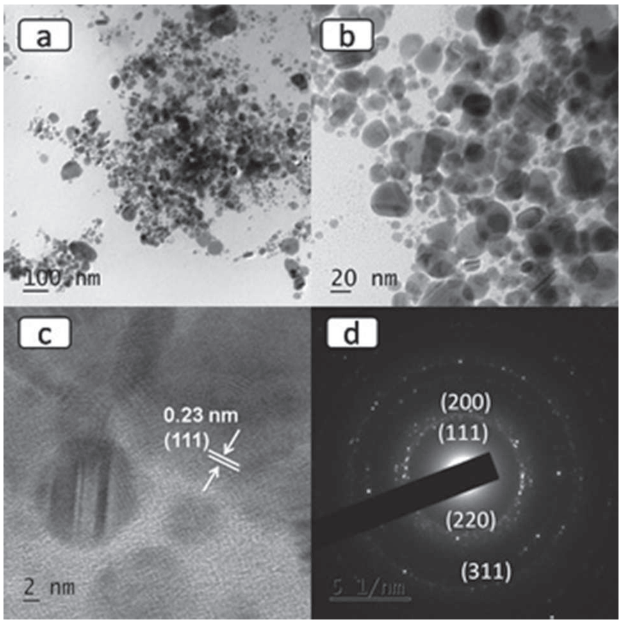

Fig. 3. (a, b) TEM images, (c) HRTEM image and (d) SAED pattern of AgNPs.

confirmed by the selected area electron diffraction (SAED) pattern as shown in Figure 3(d).

3.4. Screening of Antibacterial Property of Synthesized Silver Nano Particle

Antibacterial activity of the synthesized AgNPs was studied against bacterial samples; *L. acidophilus, B. pumilus* and *A. viscosus* (Fig. 4). The formation of halo zone around the disc was scored as positive antibacterial activity while those without halo zone were scored as negative. Control was maintained using Rifampicin disc

Table I. Antibacterial activity of control (rifampicin), plant extract and AgNPs samples against test pathogen (inhibition zone).

Organism	Control (rifampicin) inhibition zone (mm)	Plant extract inhibition zone (mm)	AgNPs inhibition zone (mm)
B. pumilus	Positive 20	Positive 7	Positive 17
P. acidophilus	Negative	Negative	Negative
A. viscosus	Positive 16	Negative	Positive 13

(30 mcg/disc) as antibacterial agent. The inhibition zone was measured using a metre ruler and the mean value for each organism was recorded and expressed in millimeters.

As can be seen from Table I, AgNPs showed antibacterial activity against *B. pumilus* and *A. viscosus* while plant extract showed antibacterial activity against *B. pumilus* only. The AgNPs also showed higher antibacterial activity than the plant extract (with respect to their zone of inhibition). Thus, it may serve as a more potent antibacterial agent. The positive control (Rifampicin) showed higher antibacterial activity against *B. pumilus* and *A. viscosus* as compared to AgNPs and plant extract. However, all the samples viz. AgNPs, plant extract and positive control (antibiotic) do not show any inhibitory action against *L. acidophilus*.

3.5. The Catalytic Efficiency Towards Degradation of Methylene Blue (MB) and Eosin Yellow (EY)

MB and EY were chosen for evaluating the photocatalytic activities of the synthesized AgNPs in aqueous medium under solar irradiation (Fig. 5). The characteristic absorption peak of MB and EY solution were found to be 664 nm and 516 nm respectively. Degradation of MB was visualized by decrease in peak intensity within 1 h of incubation

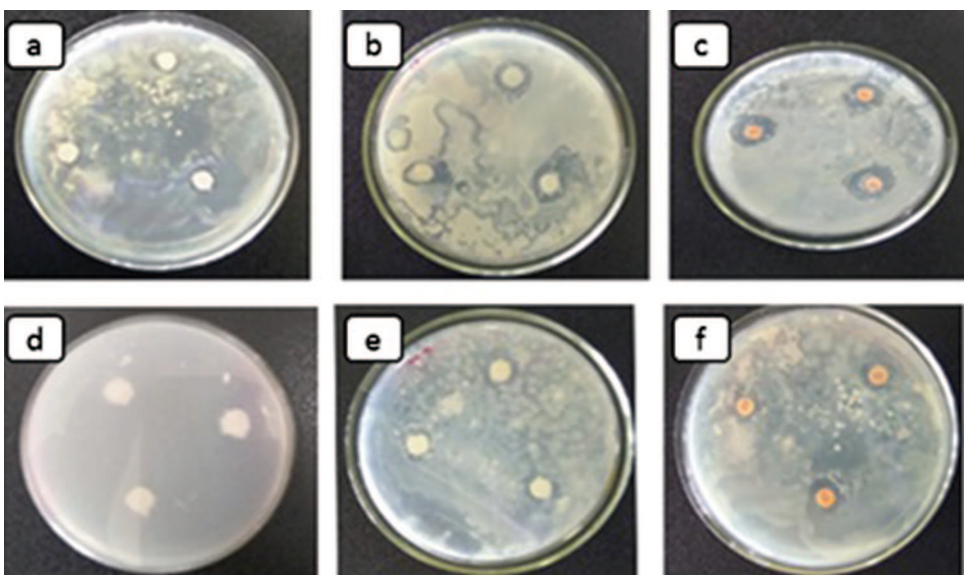

Fig. 4. Antibacterial activity test showing positive results; (a) *B. pumilus* against plant extract; (b) *B. pumilus* against AgNPs; (c) *B. pumilus* against control; (d) *A. viscosus* against plant extract; (e) *A. viscosus* against AgNPs; (f) *A. viscosus* against control.

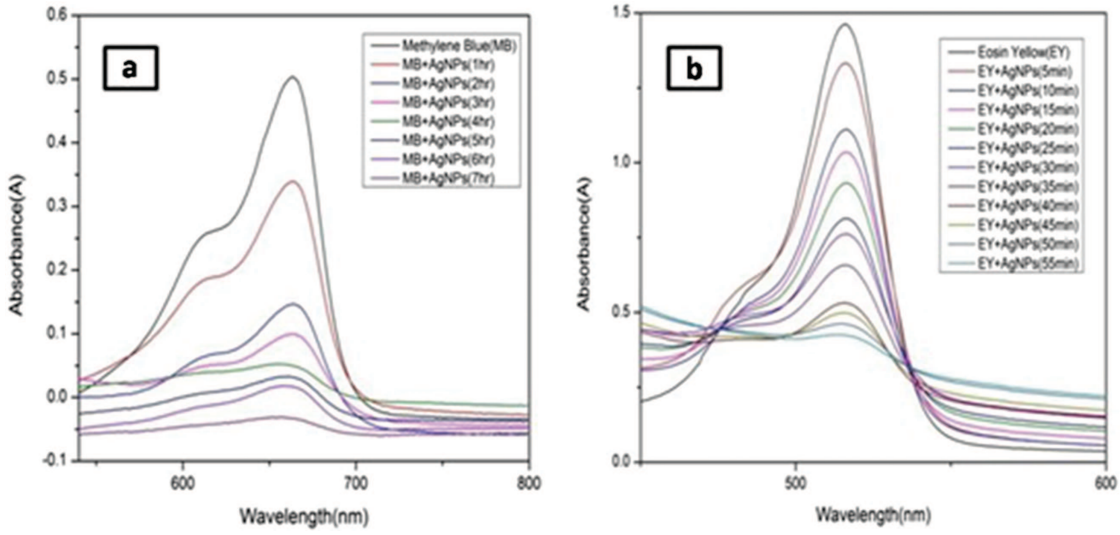

Fig. 5. UV-visible absorption spectra of degradation of (a) MB (Methylene Blue) and (b) Eosin Yellow in the presence of AgNPs.

time and completely degraded in 7 h; whereas degradation of EY completed within 55 mins, as shown in Figures 5(a) and (b) respectively. There is no considerable shift in peak position for both MB and EY solution without exposure to AgNPs. The dye degradation does not take place immediately and was initially low and further increased with constant increase in time. The photocatalytic properties of Ag nanoparticles in visible light may be due to excitation of SPR.[45] It was found that when the dye solutions were kept under sunlight in the absence of AgNPs, the dyes showed no degradation. Similarly, dyes showed almost negligible degradation when placed in dark without sunlight in presence of NPs.

4. CONCLUSIONS

In summary, we have reported the bio-inspired synthesis of silver nano particles by reducing silver nitrate salts using aqueous extract of *S. bryopteris*. The biomolecules present in *S. bryopteris* extract acted as both reducing and stabilizing agents, thereby eluding the requirement of external reducing agents. The antibacterial activity of the as-synthesized AgNPs was further studied. The nanoparticles obtained demonstrated high antibacterial activity against varieties of gram-positive bacteria and also showed photocatalytic dye degradation activity for MB and EY under solar light illumination without any requirements of chemical reducing reagents. We anticipated that the utilization of bio-inspired procedure opens new possibilities for the design of ideal catalyst which exhibits both high activity and stability.

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