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Photoinduced green synthesis of silver nanoparticles using aqueous extract of *Physalis angulata* and its antibacterial and antioxidant activity

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ABSTRACT

The present work discusses the establishment of green route for the rapid synthesis of silver nanoparticles (AgNPs) using aqueous extract of *Physalis angulata* (AEP) leaves which act as a reducing as well as a capping agent. The change in color from watery to dark brown confirmed the synthesis of AgNPs. A characteristic surface plasmon resonance (SPR) band at 436 nm advocated the presence of AgNPs. The synthesis process was optimized using one factor at a time approach where 2.0 mmol L^{-1} $AgNO_3$ concentration, 4.5% (v/v) of AEP inoculum dose and 10 min of sunlight exposure were found to be the optimum conditions. The synthesized AgNPs was characterized by several characterizing techniques such as Transmission Electron Microscopy (TEM), Selected Area Electron Diffraction (SAED), Scanning Electron Microscopy (SEM), Energy Dispersive X-Ray (EDX), X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and Atomic Force Microscopy (AFM) analysis. The synthesized AgNPs showed strong antibacterial activity against both Gram-negative and Gram-positive bacteria as well as antioxidant activity. Herein, an effort was also made to propose the mechanism of biosynthesis, antibacterial activity as well as antioxidant activity.

1. Introduction

At present, the development of green route for the synthesis of nobel metal nanoparticles (NMNPs) has gained much attention due to their unique properties. Among these NMNPs, silver nanoparticles (AgNPs) possess potent antibacterial, antiviral and anticancer properties [1–3]. In addition to this, AgNPs are also utilized for biosensing, catalysis, wound dressings, medicine, coating surgical instruments and water treatment [4,5].

The green route of AgNPs synthesis avoids the use of toxic chemicals and extreme temperature and pressure condition against conventional chemical and physical route [6,7]. Recently, the use of aqueous solution of plant extracts for the photoinduced biosynthesis of AgNPs have become advantageous over other biological systems because they are simple, eco-friendly and economical in nature which requires less time and eliminates the need of culture, aseptic condition and maintenance [8]. The plant extracts are reported to contain various phytochemicals such as terpenoids, flavonoids, phenol derivatives, plant enzymes and their derivatives which act as bioreductant in the presence of metal salts [9]. Currently, the plant extract has been extensively used as a bioreducing agent for the synthesis of AgNPs [10,11]. Recently, we have synthesized AgNPs using an aqueous extract of *Euphorbia hirta, Xanthium strumarium* and *Polyalthia longifolia* using photo-induced method [12–14]. Sahu et al. have also reported the synthesis of AgNPs using aqueous extract of *Cynodon dactylon* under bright sunlight radiation [15].

Physalis angulata is an annual herb which belongs to family solanaceae and indigenous to many parts of the tropical areas of Africa, Asia and America [16]. It is commonly known as camapu which is a common weed of cultivated fields, waste lands and forest fringes. This herb plays a major role in traditional medicine to treat the diseases like malaria, asthma, hepatitis, dermatitis and rheumatism [17,18]. The phytochemical analysis of various parts of this plant showed the presence of several phytochemicals such as alkaloids, flavonoids, phenol, Saponin, tannins, phytosterols glycosides and sugars which act as both reducing as well as stabilizing agent [19,20].

The prime objective of the present study is photo-induced, one pot, green synthesis of stable AgNPs using aqueous leaf extract of

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P. angulata (AEP) and to investigate its antibacterial and antioxidant activity. This study describes the simple and eco-friendly method for synthesis of AgNPs.

2. Materials and method

The chemical Silver Nitrate ($AgNO_3$ analytical grade) was purchased from Sigma-Aldrich. Nutrient Agar (NA) was used as a maintenance media for all bacterial cultures and Mueller Hinton Agar (MHA) for assessment of the antimicrobial activity of AgNPs was procured from Hi-Media (Mumbai, India). Glutaraldehyde and Ethanol (Sigma-Aldrich) were used for bacterial fixation. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Methanol, Hydrogen Peroxide and Ascorbic Acid were procured from Merck, India. The Luria-Bertani (L.B.) media and Bacteriological agar were purchased from Himedia Lab. Ltd., Mumbai. The fresh leaves of *P. angulata* were collected from Indian Institute of Technology (Banaras Hindu University), Varanasi, India. The bacterial strain *Escherichia coli* MTCC 739 (ATCC 10536) and *Staphylococcus aureus* NCIM 5021 (ATCC 25923) were purchased from the Institute of Microbial Technology (Chandigarh, India) and National Chemical Laboratory (Pune, India) respectively.

2.1. Preparation of leaf extract

The collected fresh leaves of the *P. angulata* were washed several times with deionized water to remove all the adhered impurities. After that, the leaves were completely air dried to remove moisture and cut into fine pieces. Thereafter, 25 g fine cut leaves were boiled for 10 min in 100 mL of deionized water at 80 °C. Then the aqueous extract of *P. angulata* (AEP) was collected by filtering through Whatman filter paper No. 1 (Scheme S1).

2.2. Synthesis of silver nanoparticles

For the synthesis of AgNPs, variable inoculums dose (v/v) 0.5%, 1.5%, 2.5%, 3.5%, 4.5%, 5.5% and 6.5% of AEP were added into each reaction volume containing 100 mL aqueous solution of 1 mmol L^{-1} $AgNO_3$. The experiments for the synthesis of AgNPs were carried out in both bright sunlight and in dark condition. The temperature of the ambient environment in bright sunlight and solar intensity were 38 °C and 71000 lux respectively whereas the temperature of the dark condition and light intensity were 33 °C and 0 lux respectively. When the reaction mixture containing $AgNO_3$ and inoculums dose exposed to bright sunlight, the color of the solution changed from colorless to reddish-brown instantaneously whereas the reaction mixture kept in dark condition exhibited no change in color even up to 6 h which advocated the importance of photocatalytic action of sunlight on synthesis of AgNPs. Thus, all the AgNPs synthesis experiments were conducted in bright sunlight. In order to optimize the different process variables which affect the AgNPs synthesis, one factor at a time approach was adopted. These process variables were sunlight exposure time, inoculums dose and $AgNO_3$ concentration which were optimized from 0 to 14 min, 0.5% to 6.5% (v/v) and 1 mmol L^{-1} to 5 mmol L^{-1} respectively. The AgNPs thus synthesized were purified by centrifuging at 15000 g for 15 min and subsequently redispersing in deionized water to remove the uncoordinated biological molecules. This process was repeated four times and after drying the final mass of AgNPs was collected.

2.3. Characterization of AgNPs

The optical properties of AgNPs were studied using UV–visible spectrophotometry (Evolution 201, Thermo Scientific) and Fourier transform infrared spectrophotometer (FTIR, Perkin Elmer Spectrum 100) in the range of 300 to 700 nm and 4000–400 cm^{-1} respectively. The FTIR study was carried out using powdered AgNPs. The X-ray Diffractometer (XRD, Rigaku Miniflex II) having Cu Kα radiation source and Ni filter was used to determine the crystalline nature of AgNPs in range of 20° to 80° at a scanning rate of 10° min^{-1} at 39 kv and 14 mA with step width 0.02 °. The crystalline nature of AgNPs was also confirmed by Selected Area Electron Diffraction (SAED) pattern. The morphology and size of AgNPs were initially determined by Scanning Electron Microscopy equipped with energy-dispersive X-ray analysis (SEM-EDX, Hitachi H-7100). The elemental composition and purity of the powdered samples were confirmed by EDX analysis. The morphology and size of the biosynthesized AgNPs was further analyzed by Transmission Electron Microscopy (TEM) carried out on TECNAI 20 G2-electron microscope operated at accelerating voltage 200 kV. The surface texture of AEP synthesized AgNPs was analyzed using Atomic Force Microscopy (AFM, NTMDT) working in contact mode. The intensity of sunlight was measure using Light Meter (LX-101A, Lutron). For the TEM and AFM analysis, one drop of the optimized AgNPs was casted over cupper grid and small glass cover respectively and imaged.

2.4. Assessment of antibacterial activity of AgNPs

2.4.1. Determination of minimum inhibitory concentrations of AgNPs

To determine the minimum inhibitory concentrations (MICs), a Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli.*) were exposed against AEP synthesized AgNPs. For this, the turbidity of standard bacterial populations was measured with a spectrophotometer. Further, the susceptibility tests of AgNPs in standard broth were performed by the twofold microdilution method. In brief, the mid-log phase (1×10^6 cells/mL) bacterial strains were selected and diluted in fresh Mueller Hinton Broth (MHB). After that, from the diluted cell suspension, 0.1 mL was dispensed into each well of a 96-well microtiter plate. Then the cells of *E. coli* and *S. aureus* were exposed to different AgNPs concentration (0.1%, 0.08%, 0.05%, 0.03%, 0.01%, 0.006% and 0.003%) (w/v). The bacterial growth was monitored by monitoring the absorbance at 600 nm by a microplate reader and the lowest concentration of the AgNPs that inhibited the visible growth of the bacteria was determined as MIC [21,22]. The AgNPs concentration that reduced the number of susceptible cells by less than 20% after 24 h of incubation was considered as MIC. The viability assays were carried out with different concentrations of AgNPs which were made by dissolving known concentration of it in to distilled water.

2.4.2. Cell viability test

The cell viability of *E. coli* and *S. aureus* was determined by growing them in Mueller Hinton Broth (MHB) at 37 °C with continuous shaking at 150 g for overnight. Thereafter, the cells were regrown in fresh medium for 4 h and suspended in saline solution. A cell suspension consisting of 10^6 cells/mL was incubated with a concentration of 0.69 μg/mL AgNPs for *E. coli* and 0.84 μg/mL AgNPs for *S. aureus* at 37 °C without shaking. The loss of cell viability of *E. coli* and *S. aureus* was evaluated by colony counting method in which 100 μ L solution (10 fold diluted) of both cells were spread onto LB plates separately and left to grow overnight at 37 °C. The colonies were counted and compared with control plates to determine degree of inhibition in cell growth. The isotonic saline solution without AgNPs based materials was used as a control. All the tests were carried out in triplicates and results were reported in regarding the value of three independent experiments.