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Temperature-dependent green biosynthesis and characterization of silver nanoparticles using balloon flower plants and their antibacterial potential

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ABSTRACT

Environmentally benign silver nanoparticles (Ag NPs) were synthesized at different temperatures using leaf extract of *Platycodon grandiflorum*. The apparent UV–Vis spectrum maxima clearly displayed plasmon resonance bands at 442 and 457 nm for nanoparticles synthesized at 37 °C and 50 °C, respectively. The morphology of Ag NPs were investigated by FESEM, FETEM and AFM analysis and the results showed the formation of uniform spherical nanoparticles with average sizes of 19 and 21 nm for Ag NPs produced at 37 °C and 50 °C, respectively, with slightly larger nanoparticles formed at 50 °C owing to agglomeration. Further characterization by XRD, XPS, and FTIR confirmed their crystal nature, while stability was confirmed by zeta potential analysis. The strong XRD diffraction pattern confirmed the face-central cubic crystalline nature of the nanoparticles. Moreover, XPS revealed Ag 3d doublet ($3d_{5/2}$ and $3d_{3/2}$) with C1s peaks of the catalysts that were turned into C–H (283.68) C=C, C–C (284.16 eV), C–O (285.34 eV), and C=O (288.55 eV). The O1s spectra were also exhibited with binding energy at 530.94, 531.96 and 532.53 for AgO, C–O and C=O, respectively, and FTIR confirmed the peaks of plant extract appeared on Ag NPs. Overall, Ag NPs synthesized at 50 °C had good shape and structure with a high stability (−5.23). The nanoparticles also exhibited antibacterial activity against *Escherichia coli* and *Bacillus subtilis*. The biosynthesized Ag NPs have the potential for use in various applications, particularly in the field of biomedicine.

1. Introduction

Nanotechnology is a rapidly developing field that is being applied to a variety of materials and devices, with applications in the medical, biological mechanical, electronic and environmental industries [1–5]. There are several approaches to synthesis of nanoparticles, including physical, chemical and biological [6–9], with the biological method being non-toxic and economically viable [6,10–14]. Recently, many nanoparticles, including silver nanoparticles (Ag NPs), have been synthesized from various biological sources such as bacteria, fungi, algae and plants [6,15–17]. Silver nanoparticles are extensively used in several applications for their antibacterial, antiviral, anticancer, and antileishmanial activity, as well as for biochemical detection and catalysis, wastewater treatment and medicine [11,18,19]. Biosynthetic method can also be a simple one step process for production of Ag NPs [20] and mainly used in the medical field to remove pathogenic microorganisms from the environment. Silver has potential to inhibit microorganisms, and this effect has been shown to further increase after transformation into Ag NPs [21].

Platycodon grandiflorum, which is commonly known as the balloon flower, belongs to the family Campanulaceae and is widespread in East Asia, including South Korea. Traditionally, this plant is used in Korea to treat many diseases, including asthma, tuberculosis, diabetes and inflammatory diseases [22,23]. In addition, this plant is used as an ingredient in many edible dishes. The antioxidant properties of *P. grandiflorum* extracts are of a great

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interest because of their potential use as natural replacements for synthetic additives [24]. Different parts of *P. grandiflorum* (e.g., stems, leaves, seeds and roots) contain many natural compounds that have anti-inflammatory, antioxidant and antimicrobial activities [25,26]. Many researchers have reported the synthesis of Ag NPs from different plant materials, including *Pongamia pinnata, Carica papaya, Hibiscus rosasinensis,* and *Chenopodium album* [21,27–30]. However, there have been no investigations of the use of *P. grandiflorum* to synthesize Ag NPs. Therefore, the present study was conducted to investigate the synthesis of Ag NPs from *P. grandiflorum* and to characterize their antibacterial activity against pathogenic bacterial strains.

2. Materials and methods

The plant *P. grandiflorum* was collected from a park in Incheon, South Korea. Silver nitrate and filter membranes were purchased from Sigma Aldrich (USA) and Sartorius Stedim Biotech (Germany), respectively. Luria Bertani (LB) agar and LB broth media were purchased from Biopure Reagent, Korea. Other reagents used were of analytical grade and all reagents were stored as recommended by the manufacturer.

2.1. Preparation of plant extract

The fresh leaves of plants (Fig. 1; inset) were cut into small pieces and washed thoroughly with tap water followed by sterile distilled water to remove impurities. The washed leaves were then dried, after which 5 g of leaves were weighed and placed in 50 ml of distilled water in a 100 ml beaker and boiled for 20 min. After boiling, the extract was cooled and filtered through the Whatman No. 1 filter paper. The filtered solution was filtered again through a filter membrane (0.45 μm size) to give the final extract, which was stored at 4 °C until further use as a reducing and capping agent.

2.2. Biosynthesis of Ag NPs

The Ag NPs were synthesized as previously described, with slight modification [31]. Briefly, about 30 ml of 1 mM $AgNO_3$ solution was mixed with 8 ml of plant extract in a 100 ml Erlenmeyer flask and then incubated at 37 °C and 50 °C for 20 min. After incubation, the color changed from pale yellow to brownish yellow, indicating the formation of Ag NPs. The reaction mixture was then centrifuged at 10,000 × g for 10 min. The precipitate was then suspended in distilled water, after which the tube was centrifuged again under the same conditions. The final obtained precipitate was subsequently dried and stored at 4 °C. A control experiment was conducted without the addition of leaf extract.

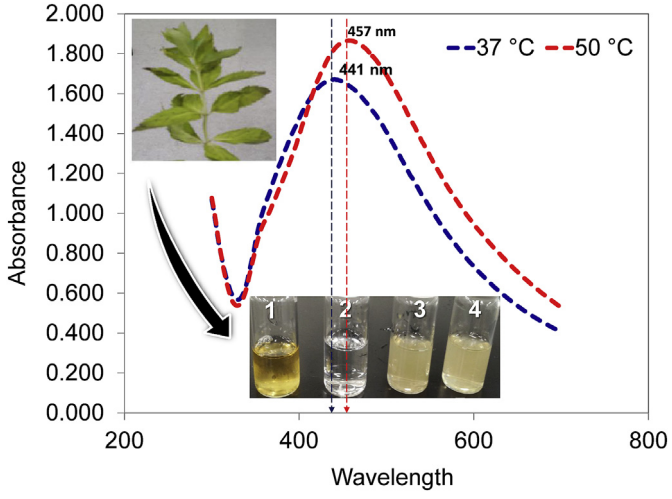

Fig. 1. UV–Vis spectrum showing the formation of Ag NPs at 37 °C and 50 °C. Insets show 1) Leaf extract; 2) $AgNO_3$ solution; 3) Ag NPs formed at 37 °C; and 4) Ag NPs formed at 50 °C. Photograph of *P. grandiflorum* is also inserted.

2.3. Characterization of Ag NPs

The formation of Ag NPs was confirmed by UV–Vis spectrophotometry analysis (Jasco V-770, UV-VIS spectrophotometer) at 300–700 nm. The morphology and size were determined by field emission-scanning electron microscopy (FESEM; Hitachi, S-4300SE, Japan). Energy dispersive X-ray (EDX; EDAX, USA) analysis was conducted to identify the elemental compositions, including silver from the sample. Field emission-transmission electron microscopy (FETEM) was performed using a JEM 2100F microscope (Jeol, Japan). Briefly, samples were prepared by placing a drop of Ag NP solution on a carbon-coated copper grid and then drying the samples using a vacuum desiccator. The images of the nanoparticles were recorded at various magnifications. The size and shape of the Ag NPs were also determined using an Atomic Force Microscope (Nano Scope, Ica, Vecco, USA). Ag NP samples were prepared on a glass slide and scanned with an AFM to analyze the morphology of the Ag NPs.

The synthesized Ag NPs were analyzed by X-ray-powder diffraction using a DMAX-2500 (Rigaku, Japan). XRD system equipped with a Nickel filter and a Cu Kα (1.54059 Å) radiation source. The diffraction angle was varied in the range of 10–90° and the scanning rate was 0.5/s. X-ray photoelectron spectroscopy (XPS; Thermo Scientific, K-Alpha, UK) was used to analyze the elemental and chemical bonding on the surface of the Ag NPs. The size distribution and stability of Ag NPs were determined by dynamic light scattering (DLS) using the zeta potential and an ELS-Z particle size analyzer (Photal Otsuka Electronics, Japan). Fourier transform infrared (FTIR) spectroscopy was employed to examine the molecular configuration of Ag NPs. This analysis was conducted using a Vertex 80 V FTIR system (Bruker, Germany) to record the infrared spectrum of Ag NPs by either absorption or emission of a sample. It was necessary to make a KBr pellet with Ag NPs to study the FTIR spectrum from 4000 to 400 cm^{-1}. Analyses of the FTIR spectra of leaf extract alone and Ag NPs were conducted.

2.4. Antimicrobial activity

The antimicrobial activity of the synthesized Ag NPs was checked against pathogenic test organisms such as *Escherichia coli* and *Bacillus subtilis* [32]. Both strains were grown in LB broth for 24 h at 37 °C and then spread on LB agar plates using a sterile glass spreader. Next, sterile paper discs (6 mm diameter) were placed on inoculated plates, after which the Ag NP samples were loaded onto each disc. Similarly, a control was prepared using sterile water. All plates were subsequently incubated for 24 h at 37 °C, after which the antibacterial activity was determined by measuring the zone of inhibition formed around the discs.

3. Results and discussion

Plants have been extensively used to synthesize different metal nanoparticles, including silver nanoparticles (Ag NPs), because plant extracts contain various active ingredients such as terpenoids, flavonoids, poly-phenols and plant enzymes and their derivatives, which act as reductants in the presence of metal ions [18,23,33,34]. Therefore, the present study was conducted to synthesis of Ag NPs using the extract of *P. grandiflorum* and confirmed by UV–Vis