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Green synthesis of silver nanoparticles using tissue extract of weaver ant larvae

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ABSTRACT

Green synthesis of silver nanoparticles (AgNPs) has gained widespread interest as the alternative method to use natural, non-toxic substances to facilitate the formation of AgNPs. This work reported on the alternative source of reducing and stabilizing agents from tissue extract of weaver ant (*Oecophylla smaragdina*) larvae. The formation of AgNPs in the reaction containing silver nitrate, the tissue extract, and glucose at 60 °C was indicated by the surface plasmon resonance at 428 nm. Transmission electron microscopy images revealed spherical AgNPs with the average diameter of 67.4 nm. The synthesized AgNPs exhibited potent antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli* with the minimum bactericidal concentrations equally at 16 μg/mL.

1. Introduction

The antibacterial activity of AgNPs has been attracted many interests especially for commercial applications in textile industry [1], food packaging [2], medical devices [3], and antimicrobial products [4]. Several methods to produce AgNPs were reported such as chemical reduction [5], microwave irradiation [6], photoreduction [7], thermal decomposition [8], and green synthesis [9]. Although the chemical reduction is the simplest and widely used, it relies on toxic chemicals unsafe to the environment. In order to minimize the environmental effects, a green synthesis is proposed as the alternative, eco-friendly, and cost-effective method. Plant extracts derived from various species and parts of plants were most studied for green syntheses of AgNPs, which several phytochemicals, such as alkaloids, tannins, phenolics, saponins, terpenoids, and vitamins, are functioned as reducing agents [10].

A tissue extract of insect larvae is one of the interesting sources of biomolecules that could function as reducing and stabilizing agents for a green synthesis of AgNPs. The side chains of tyrosine, phenylalanine, and tryptophan can act as reducing agents, while a complex structure of proteins can stabilize nanoparticles [11]. Weaver ants (*O. smaragdina*) are naturally abundant in South Asia and Australia. Their larvae contain many proteins essential for larval development and nest construction, which are rich essential amino acids, retinol, tocopherol, thiamine, niacin, riboflavin, and ascorbic acid at several times higher than those of domestic fowl eggs [12]. Thus, this work is interested in the tissue extract of weaver ant larvae as the alternative for a green synthesis of AgNPs.

2. Materials and methods

2.1. Synthesis and characterization of AgNPs

Ground tissues of weaver ant larvae were extracted in water (5 g/10 mL). After centrifuging at 12,000×*g* for 5 min, the supernatant was collected and determined the protein concentration using the Bradford assay (Bio-Rad, USA). The extracted proteins were separated on a 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gel.

To synthesize AgNPs, the reactions containing 19 mL the tissue extract (0.18–0.72 mg/mL) and 0.5 mL $AgNO_3$ (1 M) with or without 1 mL glucose (2 M) were incubated at 60 °C for 24 h in dark. The formation of AgNPs was monitored for 48 h by measuring the absorbance at 300–900 nm. The morphology of AgNPs was analyzed by a transmission electron microscope (TEM; FEI, USA) operating at 200 kV. The crystalline structure was studied by TEM-selected area electron diffraction (SAED) operating at 200 kV. The elemental composition was analyzed by energy-dispersive X-ray (EDX) operating at 200 kV accelerating voltage. Their sizes were determined using a particle analyzer (Beckman-Coulter, USA).

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2.2. Antibacterial activity

The antibacterial activities of AgNPs against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were analyzed by disc diffusion method [13]. A bacterial colony was cultured in Mueller-Hinton (MH) broth at 37 °C for 6 h. After adjusting to 0.5 McFarland, the culture (0.1 mL) was mixed with MH-agar (20 mL) for preparing culture plates. The sterile discs (6 mm, Whatman No.1) impregnated with AgNPs (50 μg), water, or ampicillin (25 μg) were incubated on culture plates at 37 °C for 24 h to determine the bacterial inhibition zones.

The minimal inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of AgNPs against both bacteria were analyzed by incubating AgNPs (2–32 μg/mL in MH-broth) and bacteria (5×10^5 CFU/mL) at 37 °C for 24 h to examine the bacterial growth at the optical density (600 nm). The MIC was the lowest concentration of AgNPs visually inhibiting 99% bacterial growth. The cultures (100 μL) at MIC and two higher concentrations were cultured on MH-agar plates at 37 °C for 24 h to determine the MBC, the lowest concentration of AgNPs killing 100% of the initial bacteria.

3. Results and discussion

3.1. A green synthesis of AgNPs

The proteins of the tissue extract of weaver ant larvae were visualized on a 12.5% SDS–PAGE gel (Fig. 1a). Among the detected proteins in a range of 18–235 kDa, the 165-kDa protein was the dominant band, followed by the 76-kDa, 98-kDa, 44-kDa, and 27-kDa proteins, respectively. The extraction yielded the average of 12 mg protein per 1 g weaver ant larvae (wet weight).

To investigate reducing and stabilizing functions of the tissue extract, the formation of AgNPs were compared in the reactions containing the tissue extract and $AgNO_3$ in the presence or absence of glucose. In addition to the observed color (dark brown), the absorption spectra in a range of 300–900 nm were examined for a formation of AgNPs. Fig. 1b shows the UV–Vis spectra of the synthesized AgNPs at 24 h using different concentrations of the tissue extract. The formation of AgNPs was observed in the reactions containing glucose and the tissue extract at 0.36 and 0.72 mg/mL, but not at 0.18 mg/mL, as determined by the surface plasmon resonance (SPR) peak at 428 nm. Without the tissue extract, no SPR peak was detected, suggesting no formation of stabilized AgNPs. These results suggested that the tissue extract of weaver ant larvae efficiently functioned as a stabilizing agent but not as a reducing agent since glucose was still required in the reaction. Although the weaver ant larvae contain tryptophan (3.03 mg/100 g) and ascorbic acid (12.8 mg/100 g) that potentially function as reducing agents [12], their reducing activity might be low and possibly reduced in the reaction at 60 °C.

The formation of AgNPs in a time course of 48 h is shown in Fig. 1c. The absorption values at 428 nm were increased according to the reaction times (12–36 h), suggesting increasing formation of AgNPs. At 48 h, the dropped intensity and shifted SPR peak were likely caused by an aggregation of AgNPs [14]. In this work, the optimized condition was to use 0.36 mg/mL tissue extract and 36 h incubation, which yielded 0.58 ± 0.01 g AgNPs.

3.2. Characterization of AgNPs

Spherical AgNPs were observed in TEM images (Fig. 2a). The hydrodynamic size of the synthesized AgNPs was 67.4 ± 38.7 nm (Fig. 2b). The identity of AgNPs was confirmed by TEM-SAED and EDX analyses. In Fig. 2c, the *d*-spacing of 0.237, 0.204, 0.144 and 0.124 determined by TEM-SAED analysis was consistent with the

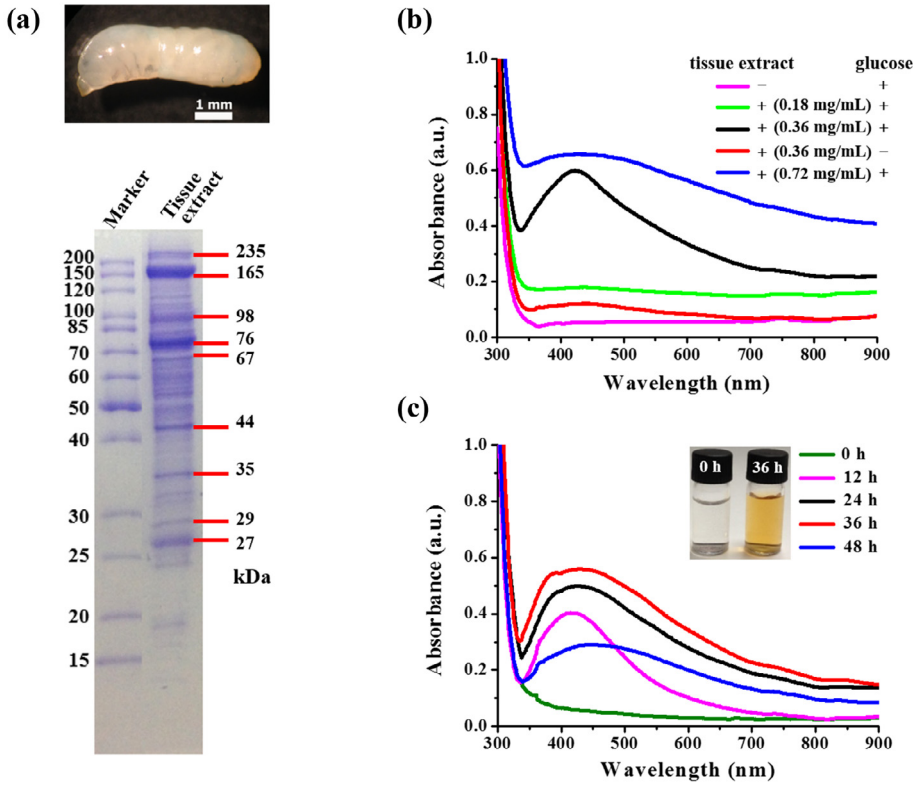

Fig. 1. (a) Proteins of the tissue extract visualized on a 12.5% SDS-PAGE gel, (b) the formation of AgNPs using different concentrations of the tissue extract, and (c) the formation of AgNPs monitored for 48 h.