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Fluorescent light mediated a green synthesis of silver nanoparticles using the protein extract of weaver ant larvae

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ABSTRACT

Alternative to crude plant extracts, a crude protein extract derived from animal cells is one of the potential sources of biomolecules for mediating a reduction of silver ions and a formation of silver nanoparticles (AgNPs) under a mild condition, which very few works have been reported. This work demonstrated a use of the protein extract of weaver ant larvae as a bio-facilitator for a simple, green synthesis of AgNPs under fluorescent light at room temperature. The protein extract of weaver ant larvae exhibited the reducing and antioxidant activities, which assisted a formation of AgNPs in the reaction containing only silver nitrate under light exposure. Transmission electron microscopy images revealed the dispersed, spherical AgNPs with an average size of 7.87 ± 2.54 nm. The maximum surface plasmon resonance (SPR) band of the synthesized AgNPs was at 435 nm. The energy-dispersive X-ray analysis revealed that silver was a major element of the particles. The identity of AgNPs was confirmed by X-ray diffraction pattern, selected area electron diffraction and high resolution transmission electron microscopy analyses, which demonstrated the planes of face centered cubic silver. The synthesized AgNPs showed antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus* with the minimum bactericidal concentration (MBC) values equally at 250 μg/ml, suggesting their potential application as an effective antibacterial agent.

1. Introduction

Due to unique catalytic, optical, thermal and electrical properties of nanoparticles (NPs), their syntheses and applications have attracted the interests of many researchers [1]. NPs have been used for numerous applications, for instance drug delivery [2], medical devices [3], biosensing [4], catalysis [5] and water treatment [6]. To supply a high demand of NPs in the past few decades, several methods have been developed, including physical methods (microwave radiation, ultrasonic irradiation, radiolysis and photochemical synthesis), chemical methods (chemical reduction and electrochemical synthesis), and biological methods (microorganisms, plant extracts and biomolecules) [7,8]. Among these methods, chemical synthesis is the most common approach because of its simple process and efficient production of highly dispersed, small and uniform nanoparticles. However, this method cannot avoid the use of hazardous chemicals, making the NPs often not suitable for biological purposes [9]. Therefore, green synthesis of NPs has been proposed as an alternative approach, which is required no sophisticated instrumentation, technical expertise and excessive use of hazardous chemicals. It is also proved to be more economical than other methods [10]. A green production of NPs have been reported by using living organisms including bacteria [11], fungi [12], algae [13] and plants [14,15]. Alternatively, biomolecules and extractions of microorganisms and plants have been used as reducing and stabilizing agents to mediate a formation of NPs [16,17].

Silver nanoparticles (AgNPs) are well-known for their antibacterial activity to a broad spectrum of bacteria [18]. They have been comprised in many commercial products, such as plastics [19], food packaging [20], antiseptic sprays [21], catheters [22], bandages [23] and textiles [24]. Syntheses of AgNPs are often hobbled by the easy oxidization weakness, causing the loss of their antibacterial activity. To overcome this problem, different organic and inorganic templates have been employed to stabilize AgNPs through the formation of nanocomposites. However, those methods traditionally need complex and tedious procedures, and face problems of high cost and poor biocompatibility. Consequently, many plant extracts derived from vegetables and herbal plants were reported as the alternative chemicals for a green synthesis of AgNPs [25]. In addition, few proteins and certain amino acid were reported to aid as stabilizing and reducing agents for a production of AgNPs; casein [26], silk sericin [27], silk fibroin [28], egg white [29] and tryptophan [30]. Through a use of edible proteins, a green production of AgNPs should produce less or no toxic residue and be cost-effective, therefore the

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abundant and edible proteins of weaver ant larvae was purposed in this study.

Weaver ants (*Oecophylla smaragdina*) are abundant in Southern India, Australia and Southeast Asia. The larvae of weaver ants mainly contain proteins and essential amino acids for larvae development and nest construction, especially high contents of tryptophan, leucine, threonine, methionine and lysine. Moreover, they also contain retinol, tocopherol, thiamine, niacin, riboflavin and ascorbic acid, which are several times higher than those in domestic fowl eggs [31]. Another important protein produced by weaver ant larvae is fibroin, the small fibrous protein used for a nest construction, which comprises of high amount of acidic amino acid and few glycine residue [32]. The plenty produced proteins in the larvae may be potentially served as reducing and/or stabilizing agents for green synthesis of AgNPs.

In this article, we demonstrated a simple green synthesis approach of AgNPs by using a protein extract of weaver ant larvae as a biotemplate at room temperature under a fluorescent light exposure to activate the protein activity. The antioxidant and reducing properties of the protein extract were also investigated. The characterization and antibacterial activity of the synthesized AgNPs were examined.

2. Materials and Methods

2.1. Materials

Fresh weaver ant larvae (*O. smaragdina*) were collected from trees in the area of Suranaree University of Technology, Nakhon Ratchasima, Thailand. All chemicals used were of analytical grade. D-glucose and Muller Hinton (MH) medium were purchased from VWR (Belgium) and Merck (Germany), respectively. Silver nitrate ($AgNO_3$) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (USA).

2.2. Preparation of the Protein Extract of Weaver Ant Larvae

Fresh weaver ant larvae (5 g) were frozen in liquid nitrogen and finely ground to obtain a homogeneous powder. Deionized water (10 ml) was added and incubated at 4 °C for 5 min. The supernatant containing water-soluble proteins was harvested by centrifugation at 12,000 $\times g$ for 5 min at 4 °C. The protein concentration was determined by Bio-Rad protein assay (Bio-Rad, USA). The obtained proteins were separated on a 12.5% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and stained with Coomassie brilliant blue R-250 dye.

2.3. Determination of Reducing Power

The reducing power of the protein extract was determined by a modified method of Ferreira and colleagues [33]. Different concentrations of the protein extract (2.5 ml) were mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min prior to an addition of 2.5 ml of 10% trichloroacetic acid (*w*/*v*). The mixture was centrifuged at 3000 $\times g$ for 10 min. The upper layer of the solution (1.25 ml) was mixed with distilled water (1.25 ml) and a freshly prepared 0.1% of ferric chloride (0.25 ml). Color changes were monitored at the absorbance of 700 nm. All determinations were performed in four replications.

2.4. Determination of DPPH Radical Scavenging Activity

The antioxidant activity of the protein extract of weaver ant larvae was determined using DPPH as described by Brand-Williams and colleagues [34] with slight modifications. Briefly, 150 μl of 1×10^{-4} M DPPH solution was added to 10 μl of the protein extract. After the mixture was well mixed for 2 min, the absorbance at 515 nm was measured, which an ascorbic acid was used as the standard. The DPPH radical scavenging activity of the sample was calculated by the following equation, where Ac and As are the absorbance values of the control and sample, respectively.

$$\text{DPPH radical scavenging activity } (\%) = 100 \times (Ac - As) / Ac.$$

The antioxidant activity of the protein extract was expressed as IC_{50}, the concentration of the sample required to scavenge half of DPPH free radical. The IC_{50} is calculated from the graph plotting scavenging activity against sample concentrations, which all determinations were performed in four replications.

2.5. Synthesis of AgNPs by Using the Protein Extract of Weaver Ant Larvae

To synthesize AgNPs, the reaction contained the protein extract of weaver ant larvae (0.36 mg/ml), 1 M $AgNO_3$ and distilled water using a volume ratio of 19:1:0.5. The reaction contained an addition of a reducing sugar, 1 ml of 2 M aqueous solution of glucose, was also performed. The reaction mixtures were exposed to light using a fluorescent lamp (400 Lux, Philips, Thailand) at room temperature for 6, 12, 24, 48, 60 and 72 h. A formation of AgNPs was monitored by the absorbance scanning from 300 to 900 nm using a spectrophotometer (Analytikjena Specord® 250 Plus, Germany). In addition, the reactions containing 1 M $AgNO_3$ and different concentrations of the protein extract of weaver ant larvae (0.09, 0.18, 0.36, 0.72 and 1.44 mg/ml) were also performed for 72 h. A formation of AgNPs was also evaluated in a condition without light, which the reaction contained 1 M $AgNO_3$ and the protein extract of weaver ant larvae (0.36 mg/ml) and incubated for 72 h.

2.6. Characterization of the Synthesized AgNPs

The morphology and size of the synthesized AgNPs were determined by a transmission electron microscope (TEM) using a Tecnai G2 20 S-Twin (FEI, USA) with operating at accelerating voltage 200 kV. The sample was prepared by placing a drop of colloidal solution on a carbon-coated copper grid and dried at room temperature before transferring it to the microscope.

The crystalline nature of the synthesized AgNPs was analyzed by selected area electron diffraction (SAED) pattern and high resolution transmission electron microscope (HR-TEM) using a Tecnai G2 S-Twin TEM operating at 200 kV with LaB_6 filament. All images were recorded with a Gatan Orius 200 CCD Camera (Gatan, USA).

Elemental composition of the synthesized AgNPs was characterized by energy-dispersive X-ray (EDX) spectroscopy carried out on a Tecnai G2 20 S-Twin (FEI, USA). EDX analysis was equipped with an EDAX r-TEM SUTW detector (FEI, USA) operated at accelerating voltage 10 kV.

Crystalline nature of the synthesized AgNPs was characterized by using the X-ray diffraction (XRD) pattern (D8 Advance, Bruker, UK) of Cu kα radiation ($\lambda = 1.5418$ Å) with a step size 0.02° within the 2θ range of the 30–80 rad. Operating X-ray tube of voltage and current were 40 kV and 40 mA, respectively. The sample was coated on a glass coverslip and dried at room temperature before analyzing.

2.7. Evaluation of Antibacterial Activity

The antibacterial activity of AgNPs was analyzed by a disc diffusion method [35] against Gram-negative (*Escherichia coli*, ATCC 25922) and Gram-positive (*Staphylococcus aureus*, ATCC 25923) bacteria. A single colony of each bacteria was initially cultured in MH broth at 37 °C and shaken at 200 rpm for 6 h. The turbidity of the bacterial culture was adjusted to equal 0.5 McFarland standard as 1×10^8 colony-forming units/ml (CFU/ml). The bacteria culture (0.1 ml) mixed with MH agar (20 ml) at 45 °C was poured into a petri dish plate. The filter papers (Whatman No. 1) were punched into circular discs (6 mm in diameter) and sterilized by autoclaving at 121 °C. Five μl of suspended AgNPs (5 μg/μl) were added onto each circular disc and dried in a laminar flow cabinet.