Research Article

Synthesis of Biogenic Silver Nanoparticles with Eco-Friendly Processes Using Ganoderma lucidum Extract and Evaluation of Their Theranostic Applications

Vinh Phu Nguyen,1,2 Hieu Le Trung,1 Thu Huong Nguyen,1 DongQuy Hoang1,3,4 and Thai Hoa Tran1

1Department of Chemistry, University of Sciences, Hue University, 77 Nguyen Hue Street, Hue City 530000, Vietnam
2Faculty of Basic Sciences, University of Medicine and Pharmacy, Hue University, 06 Ngo Quyen Street, Hue City 530000, Vietnam
3Faculty of Materials Science and Technology, University of Science, Vietnam National University Ho Chi Minh City, 700000, Vietnam
4Vietnam National University Ho Chi Minh City, 700000, Vietnam

Correspondence should be addressed to DongQuy Hoang; htdquy@hcmus.edu.vn and Thai Hoa Tran; thhaihoa@hueuni.edu.vn

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The green synthesis of silver nanoparticles (AgNPs) using plant extract, the cost-effective solution, and the abundance and environmental issue have been gaining much attention to scientists. Ganoderma lucidum (GL) commonly known as Lingzhi in Chinese and Reishi in Japanese, with a proven anticancer benefit, is discovered in the buffer zone of Bach Ma National Park, Nam Dong district, Thua Thien Hue province. In this work, the AgNPs were synthesized in a simple and effective biochemical reduction process using GL which is one of the biological organisms, as a reducing and stable agent. The optimum conditions of various experimental parameters such as pH, reaction time, concentration, and temperature were investigated. Obtained AgNPs were characterized by UV-Vis, FTIR, SEM, energy-dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD), and transmission electron microscopy (TEM). The effects of AgNP/GL materials and GL aqueous extraction on the antiproliferative activities of HepG2 and MCF-7 cells were studied. The novel AgNP/GL-based multicomponent suspension is a key compound that could find a good application in the medical and pharmaceutical sciences.

1. Introduction

Cancer is a disease caused when the normal cell proliferation control is lost. Human hepatocellular carcinoma (HepG2) is one of the types of cancer which causes a number of deaths with cirrhosis in each year [1]. Besides, breast cancer (MCF-7) is the cancer that forms in the cells of the breast and immense among women in the world, recording the second public inception of death in women with 16% of all female cancers [2]. In recent years, although there are a number of methods for treating cancer disease, they spend a lot of money cost and have more weak points such as the limitation on distinguishing between normal and diseased cells and elimination from the human body [3]. To solve this phenomenon, developing a new material with the ability to hold and release drugs in environments with suitable pH and temperature is essential [4]. Metal nanomaterials have received a great deal of scientist’s concern [5].

Noble metal nanomaterials such as Au, Ag, and Pt with size particles between 1 and 100 nanometers have distinguished properties such as the chemical and optical properties due to a surface plasmon resonance (SPR) [6–9]. Silver metal has attracted substantial attention because of a potential application in different areas such as antibacterial agents, catalysis, biological and chemical sensors, water treatment, and biomedicine [10–14]. Besides, the nanosized silver particles (AgNPs) have the ability to inhibit a variety of cancer cells especially HepG2 and MCF-7 [15, 16] and the
The anticancer activity of AgNPs was dependent on the size, shape, and surface charge of these particles [17].

In general, the AgNPs are synthesized by two main methods: the physical and chemical methods [18]. Although the particles synthesized by these two methods have defined shape and size, the procedures require a lot of expensive equipment and often use toxic and hazardous chemicals, which are considered highly harmful to human health [19, 20]. Hence, a novel green strategy for producing AgNPs has been developed by using extracted solutions from natural resources such as bacteria [21], fungi [22, 23], marine algae [24], lichens [25], and plants [26]. These extracted solutions not only are biocompatible and nontoxic to the human body but also play an important factor in reducing and protecting agents in the process of synthesis of AgNPs [27, 28]. Moreover, this method also provides an advance over other methods because it could be cataloged, eco-friendly, and low cost [29].

Ganoderma lucidum (GL) has been known as a medical mushroom and applied to traditional medicine for past centuries [30] and is one of the biological resources for the synthesis of AgNPs. Although GL contains different natural compounds, polysaccharide and triterpenoids are the most important components [31] and these compounds cause biological activities such as antiallergic [32], anti-inflammatory [33], antiviral [34], anticancer [35], and antidiabetes [36], which contributes to health benefits.

In this research, the AgNPs were synthesized by the “green method” and the process was carried out by a reduction reaction of silver nitrate using the aqueous extraction of G. lucidum as a reducing and protecting agent. This research focused on the impact of the reaction parameters such as temperature, time, concentration of silver nitrate, and pH value. The AgNPs were characterized in the aspects of the morphological properties, structure, and size distribution. The preliminary in vitro cytotoxicity was investigated on both human hepatocellular carcinoma (HepG2) and human breast cancer cell (MCF-7) via the MTT method.

2. Experimental Methods

2.1. Materials. The fruit bodies of GL collected from the buffer zone of Bach Ma National Park from Nam Dong district, Thua Thien Hue province, were dried and ground into powder. Silver nitrate (AgNO₃·5H₂O, 98%), ammonium hydroxide (NH₄OH, 25%), nitric acid (HNO₃, 90%), and ethanol (C₂H₅OH, 98%) were purchased from Xilong Chemical Co., Ltd. Human hepatocellular carcinoma (HepG2) and human breast carcinoma (MCF-7), Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Chemical Company, USA. All chemicals were used without further purification.

2.2. Preparation of Aqueous Extraction of Ganoderma lucidum. Aqueous extraction of GL was prepared by using the reflux method [37]. A 2.0 g of mushroom powder was added to 200 mL of distilled water. The mixture was heated to 85°C for 4 h under stirring condition. A yellowish solution was then centrifuged with 4300 rpm for 20 min. The solution was concentrated to 50 mL. In addition, four times the volume of 96% ethanol was added to the concentrated solution. The mixture was placed in a refrigerator for 24 h.
subsequently centrifuging (4300 rpm, 20 min). The precipitate was washed with acetone and dried at 75°C for 6 h. The dried aqueous extraction was stored for further experiments.

2.3. Biosynthesis of Silver Nanoparticles. In a typical process, the dry aqueous extract of *G. lucidum* was redissolved in distilled water to obtain an aqueous extraction solution of GL (A-GL) with a concentration of 1 g·mL⁻¹. 90 mL of this solution was mixed with 10 mL aqueous solution of silver nitrate with different concentrations. The reaction mixture was conducted under the stirring condition at 120 rpm. The pH of the mixture was adjusted by adding dropwise ammonia solution (25%, v/v) or nitric acid solution 0.01 M. The optimum condition for the synthesis process of AgNPs was studied by varying some reaction factors such as pH (3, 5, 7, 9, and 11), time (0.5 to 7 h), concentration of silver nitrate (0.5, 1, and 1.5 mM), and reaction temperature (65, 75, 85, and 95°C). To obtain AgNPs, the suspension was precipitated overnight by ethanol 95% (v/v), followed by centrifugation (4000 rpm, 20 min). The precipitate was dried at 75°C under vacuum for 24 h. The obtained pellets were symbolized as AgNPs/GL and stored for further studies.

2.4. Characterization of Silver Nanoparticles. The optical properties of AgNPs/GL were investigated by measuring optical absorption spectra in the UV-Vis region with a V-630 spectrometer. The crystal phase of products was characterized by X-ray diffraction (XRD, D8-BRUKER, Germany) equipped with Cu Kα radiation (λ = 1.5406 Å). The morphology and particle size distribution of the synthesis products were observed by transmission electron microscopy (TEM, JEOL-1010, Japan); also, the surface characteristics

![Graph](image-url)
were assessed using a scanning electron microscope (SEM) (JEOL JSM-6510, Japan). The presence of silver elements was confirmed by energy-dispersive X-ray spectroscopy (EDX). To investigate the functional groups of both A-GL and AgNPs/GL, a Fourier-transform infrared (FTIR) study was carried out with the R-Prestige-21 Shimadzu FTIR spectrophotometer, using KBr pellet method.

2.5. Cell Culture and Cytotoxicity Studies

2.5.1. Culture of Cells. HepG2 and MCF-7 cells were cultivated in DMEM medium, complemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 μg/mL) in the standard condition (37°C, 98% humidity, 5% CO₂, and absolutely sterile). After 24 h incubation, the cells were trypsinized, centrifuged, and washed with sterile PBS buffer solution. The viable cell count and viability were estimated using Trypan blue exclusion method. These cells having the viabilities above 95% were used to study the cytotoxicity assessment [38].

2.5.2. Cytotoxicity Assessment by MTT Assay. Firstly, the cytotoxic activities of G. lucidum aqueous extract and AgNPs/GL on HepG2 and MCF-7 were determined using MTT assay [39, 40]; the tumor cells were seeded with density 3 x 10⁴ cells/mL in 96-well plates and each well was filled with 200 μL of cell suspension (HepG2 and MCF-7). This was followed by incubation of plates at 37°C with 5% CO₂ overnight to allow cell attachment. After that, the G. lucidum aqueous extract and AgNP/GL suspension with the concentrations of 2, 8, 32, and 128 μg·mL⁻¹ were added into appropriate wells. These wells were incubated at 37°C, 5% CO₂ in 72 h. The control sample and blank sample were the well with culture medium and the well containing cells without samples, respectively. The experiments were repeated three times for validity. After the incubation period, 200 μL of MTT (5 mg·L⁻¹) was added to each well and it was continuously incubated for 4 h. The culture medium was removed, and 100 μL of DMSO was added to each well. The TECAN microplate reader was used to measure the absorbance of each well at the wavelength 540 nm. The proliferation of each cell line was expressed by the percentage of cell viability.

Each concentration of treated cells, control sample, and blank sample was repeated three times for validity. After 72 h, 200 μL of MTT (5 mg·L⁻¹) was added to each well and it was incubated for 4 h. The medium was removed, and 100 μL of DMSO was added to each well. The absorbance of the 96-well plates was measured at 540 nm using a TECAN microplate reader. The effect of the sample on the proliferation of HepG2 and MCF-7 was expressed as the percentage of growth inhibition cell, using the following formula:

\[
\%\text{Inhibition} = \frac{A_{\text{treated cell}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \cdot 100\%.
\]
where $A_{\text{treated\ cell}}$, $A_{\text{control}}$, and $A_{\text{blank}}$ were absorbance peaks of treated cell sample, control sample, and blank sample, respectively. IC$_{50}$ values were defined as sample concentration inhibiting 50% of cell growth, and they were obtained from the linear regression of calibration curve by using Microsoft Excel software.

3. Results and Discussion

3.1. Visual Observation. The process of biosynthesis of AgNPs/GL is shown in Figure 1. The color of the GL aqueous extract was yellow before its treatment with silver nitrate solution. After reaction, the color of the mixture became dark brown. This observation is contributed to confirm the formation of AgNPs due to the surface plasmon resonance. Moreover, the UV-Vis spectra of samples had the surface plasmon resonance peak of mixture reaction at a wavelength around 407 nm, which indicates the formation of AgNPs.

3.2. Reaction Condition Optimization. The pH was investigated in order to find the suitable pH for the formation of AgNPs. The pH of the solution was adjusted from its original value of pH 7 to the desired pH (3, 5, 9, and 11) by a dropwise addition of nitric acid (HNO$_3$) or ammonium hydroxide (NH$_4$OH) dilute solutions. The other chemical parameters were constant: $C_{\text{AgNO}_3} = 1 \text{mM}$, reaction time: 4 h, and reaction temperature: 75°C. The UV-Vis spectra in different pH are shown in Figure 2(a). At the pH values of 3 and 5, the SPR peaks were not detected, indicating that the acidic condition was not suitable for generating the AgNPs. This phenomenon could be explained that the reducing power of these functional groups such as hydroxyl and carbonyl in the acidic environment is declined and so that it cannot be able to reduce silver ions [41]. Besides, in the pH range from 7 to 11, the color of AgNPs was turned to deep brown and the UV-Vis spectra presented SPR peaks from 405 to 415 nm, demonstrating that the AgNPs were formed. In neutral and alkaline conditions, both reducing and protecting abilities become more efficient. Therefore, more silver nanoparticles were generated and the agglomeration between these particles was prevented [41, 42]. By comparing the shape and peak intensities between UV-Vis spectra, the sample synthesized at pH 9 was narrow and had the highest intensity. Thus, the synthesis process of AgNPs achieved a high performance and these particles were

Table 1: The maximum absorbance of sample at different temperatures and storage times.

| Temperature (°C) | Initially | 2 weeks | 4 weeks | 6 weeks |
|-----------------|----------|---------|---------|---------|
| 65              | 0.1764   | 0.1464  | 0.1032  | Agglomeration |
| 75              | 0.4924   | 0.4124  | Agglomeration | Agglomeration |
| 85              | 1.4374   | 1.4314  | 1.4234  | 1.4144  |
| 95              | 1.4597   | 1.4317  | 1.4226  | 1.3882  |

Figure 5: UV-Vis spectra of AgNPs/GL obtained by varying the temperature reaction from 65 to 95°C.

Figure 6: UV-Vis spectra of AgNP/GL colloidal solution at different storage times.

Figure 7: XRD pattern of AgNPs/GL.
obtained with uniform size distribution. Hence, the alkaline condition was suitable for synthesizing AgNPs and pH 9 was selected as the optimum pH value. The morphology and particle size of AgNPs/GL synthesized with pH 9 were confirmed by TEM image and shown in Figure 2(b). It can be seen that the AgNPs/GL obtained was spherical and uniform. The histogram size distribution curve of AgNPs/GL (pH 9) is shown in Figure 2(c) with the particle size around 10.72 nm.

In the process synthesis of AgNPs/GL, reaction time was also an important parameter. Therefore, reaction time was studied with the other factors keeping intact: $C_{AgNO_3} = 1$ mM, reaction temperature: 75°C, and pH 9 (optimum pH). Figure 3 shows the absorption spectra of AgNPs at different reaction times from 30 min to 7 h. It is clear that in the first 30 min, the UV-Vis spectrum shows a broader peak with a lower intensity. By increasing the reaction time, the solution changed from pale-yellow to brown and dark brown and the intensity of peaks was also increased, which indicates that the AgNPs were generated continuously. From 5 to 7 h, the intensity peak at $\lambda_{max}$ value (407 nm) slightly increased and remained unchanged at 6 h so that this leads to the completion of AgNP formation in this solution. Therefore, the optimum reaction time was 6 h and chosen for further studies.

The concentration of silver nitrate is one of the important parameters affecting the size and productivity of AgNPs. In terms of studying the effect of ion silver, the concentration of ion silver is varied from 0.5 to 1.5 mM and the other experimental conditions were kept steady (pH 9, 75°C, and 6 h). The UV-Vis spectra in Figure 4(a) show the samples of AgNPs in different concentrations. By changing the concentration of silver ion from 0.5 to 1 mM, the intensity of SPR peak would be increased significantly, which leads to more silver atoms being generated with high levels of productivity. However, at high concentrations (1.5 mM), the maximum wavelength shifted to the red shift and the peak became broader. This observation could be explained by the assumption that at this concentration, although the rate of reduction was increased, the size of silver particles was increased due to the agglomeration of these clusters. Figure 4(b) shows the TEM image of AgNPs with the concentration of silver nitrate.
1.5 mM, and it was compared with the sample synthesized at 1 mM (Figure 2(b)). It can be seen that the AgNPs obtained at 1.5 mM were larger and nonuniform, which reasserts that the quantity of ion silver in the reaction solution influenced the agglomeration and the size of AgNPs. Therefore, the optimum concentration of silver nitrate was 1 mM.

Reaction temperature is also an important parameter impacting on the synthesis of AgNPs. Hence, four different temperatures from 65 to 95°C were investigated and the other parameters were chosen as the optimal values in previous studies. The UV-Vis spectra of silver colloidal solutions at four different temperatures are shown in Figure 5. The result
silver concentration: 1 mM, reaction temperature: 85°C. The AgNPs/GL colloidal solution was synthesized with optimal condition as follows: pH 9, temperature, AgNPs were the most durable at reaction temperature, agglomerated. Therefore, in the range of investigated temperatures, the polysaccharides in fungal aqueous extract. In the spectra of GL aqueous extraction, the composition of AgNPs/GL. The EDX analysis illustrates the purity and the complete chemical composition of silver nanoparticles synthesized. The EDX spectrum reveals the presence of some organic compounds in A-GL containing functional groups such as hydroxyl and carbonyl decorating onto the surface of AgNPs; hence, this prevents agglomeration between these particles.

3.4. Characteristics of the Obtained Materials. In optimized synthetic condition, the AgNPs/GL were obtained and characterized with some techniques such as XRD, TEM, EDX, and FTIR.

The X-ray diffraction (XRD) pattern was used to analyze the crystalline nature and identify the phases presented in the prepared sample. The XRD pattern of the AgNPs/GL in Figure 7 presented the typical diffraction peaks at 37.96°, 44.12°, 64.26°, and 77.25° which corresponded to (111), (200), (220), and (311) planes of faced-centered cubic silver (JCPDS card no. 04-0783).

The morphology and particle size of AgNPs/GL were investigated by SEM and TEM techniques. The SEM image showed in Figure 8 that there were AgNPs attaching to the surface of the macromolecule of GL aqueous extract and distributing uniformly. Besides, Figure 8 shows the TEM image of AgNPs/GL revealing the shape and the size distribution of as-prepared nanoparticles and these particles were spherical and quite uniform. These results demonstrated a good agreement with the results of UV-Vis spectra. Moreover, in order to analyze the size distribution of AgNPs, the diameter of approximately 170 particles was determined, and then, the data was demonstrated by a histogram. The diameter of the particle of the AgNPs was around 6.96 ± 2.29 nm.

The elemental composition was determined by using EDX as presented in Figure 9. The EDX spectrum reveals the purity and the complete chemical composition of silver nanoparticles synthesized. The EDX analysis illustrates the percentage of relative composition of elements found in AgNPs/GL (Figure 9(b)) such as C, O, Mg, P, S, K, Ca, and Ag. In fact, in the composition of AgNPs/GL, there were not only silver metal but also other chemical elements serving as capping organic agent bond to the surface of silver nanoparticles. This phenomenon was verified by the analysis of the composition of aqueous extraction of GL, and the result is shown in Figure 9(a). The composition of GL aqueous extract is similar to the other chemical elements found in the composition of AgNPs/GL.

Figure 10 shows the FTIR spectrum of AgNPs/GL and GL aqueous extract. In the spectra of GL aqueous extraction, the absorption peak observed at 3408 cm⁻¹ was assigned to the stretching vibration of the hydroxyl group. The peak at 2914 cm⁻¹ was attributed to the methylene group, and the peak at 1614 cm⁻¹ could be ascribed to the carbonyl group (C=O) stretching vibration considering the presence of hydroxyl hemiacetal groups. In addition, the peak that appeared near 2918 cm⁻¹ confirmed the C-N stretching vibrations of aliphatic amines of protein and the peak at 1415 cm⁻¹ related to the C-H bending vibration peak [45, 46]. For the spectra of AgNPs/GL, it was exhibited similarity.
with the FTIR result of GL aqueous extraction and there was a new peak around 1743 cm\(^{-1}\) relating to the carboxylic ester groups. Moreover, the intensity peak of the carbonyl group (≈1614 cm\(^{-1}\)) was decreased strongly and become wider in comparison with the spectrum of GL aqueous extraction. This observation could be justified by the assumption that some carbonyl groups were oxidized to carboxylic ester groups, which involved reducing ion Ag(I) to Ag(0). The FTIR spectroscopic studies illustrated that the presence of functional groups has the ability to form the layer covering the silver nanoparticles to prevent the agglomeration and stabilizing the silver nanoparticles.

3.5. Determination of Cell Viability. The antiproliferative effect of AgNPs/GL and GL aqueous extract was studied by MTT assay in both cell lines (HepG2 and MCF-7), and the results are shown in Figure 11.

Overall, it is clear that AgNPs/GL inhibited effectively the proliferation of HepG2 cells and MCF-7 cells, compared to the inhibitory ability of GL aqueous extract. In terms of HepG2 cells, GL aqueous extract could not inhibit HepG2 cells with concentrations below 32 μg·mL\(^{-1}\) and exhibited negligible cytotoxic effect with only 19% inhibition with 128 μg·mL\(^{-1}\). However, in terms of AgNPs/GL, by increasing the concentration from 2 to 128 μg·mL\(^{-1}\), the percentage of the inhibition growth rose significantly and reached a peak at 128 μg·mL\(^{-1}\) concentration with 97% inhibition. The same pattern could be seen for the results of MCF-7 cells with a lower percentage of inhibition. Moreover, the IC\(_{50}\) value of AgNPs/GL for HepG2 cells was lower than once for MCF-7 cells with 21.85 ± 0.2 μg·mL\(^{-1}\) and 67.77 ± 1.44 μg·mL\(^{-1}\), respectively. In summary, by utilizing MTT assay for studying the in vitro cytotoxicity, AgNPs/GL seems to reduce the proliferation of HepG2 cells and MCF-7 cells, thereby having potential application on cancer treatment.

4. Conclusions

In this study, we demonstrated a novel and easy process for the synthesis of AgNPs using aqueous extraction of *G. lucidum*. The reaction parameters affecting the particle size and productive reaction such as pH, reaction time, concentration, and temperature were investigated. The results revealed that pH 9, silver concentration of 1 mM, reaction temperature at 85°C, and reaction time of 6h were the optimal conditions for the synthesis of AgNPs. The obtained particles were stable and characterized by UV-Vis, SEM, TEM, XRD, EDX, and FTIR. Furthermore, the antiproliferative effect of AgNPs/GL on two cell lines HepG2 and MCF-7 was studied and the results showed that cancer cells were more susceptible to AgNPs/GL with IC\(_{50}\) value of 21.85 μg·mL\(^{-1}\) for HepG2 and 67.77 μg·mL\(^{-1}\) for MCF-7. The results proposed a potential of utilizing AgNPs/GL in the medical fields, especially cancer treatment in two cell lines HepG2 and MCF-7.

Data Availability

Data is available upon reasonable request.

Conflicts of Interest

There are no conflicts of interest to declare.

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