Biosynthesis, Characteristics and Antibacterial Activity of Silver Nanoparticles Using Lemon Citrus Latifolia Extract

Duong H. T. Linh¹, Nguyen P. Anh¹, Truong T. A. Mi¹, Nguyen T. Tinh¹, Hoang T. Cuong¹, Tran L. Quynh¹, Nguyen T. T. Van¹, Nguyen V. Minh² and Nguyen Tri¹,*

¹Institute of Chemical Technology, 01 Mac Dinh Chi Str., HCM City, Vietnam, 70100
²Open University Ho Chi Minh City, 97 Vo Van Tan Str., HCM City, Vietnam, 70100

Silver nanoparticles (AgNPs) were synthesized from aqueous AgNO₃ precursor via an effective and eco-friendly method using Lemon Citrus Latifolia (LCL) extract as the reducing and stabilizing agent under sunlight condition. The AgNPs formation was confirmed by ultraviolet-visible absorption spectroscopy at the wavelength of 400–450 nm. The appropriate conditions and positive effect of direct sunlight on the AgNPs preparation were revealed clearly. The synthesized AgNPs were characterized using multitechniques X-ray diffraction revealed that the AgNPs had the crystalline nature of face-centered cubic structure. Scanning electron microscopy and transmission electron microscopy showed the obtained AgNPs was spherical and the size distribution was uniform with the nanosize of 4–24 nm. The obtained AgNPs solution showed an effective antibacterial activity against E. coli, B. subtilis and B. cereus with the average diameter of inhibition zones over 15 mm.

Keywords: biosynthesis, silver nanoparticles, antibacterial activity, Lemon Citrus Latifolia extract

1. Introduction

Recently, various techniques have been developed to synthesize metal nanoparticles (NPs), such as chemical reduction,1–3 electrochemical decomposition,4 thermal decomposition,5 radiation,6 ultrasound7 and microwave.8 Although these methods have performed effectively, they are associated with several limitations including using toxic chemicals, high operational cost and energy. Therefore, the development of cost-effective and eco-friendly NPs synthesis has been a challenge. Biosynthesis of NPs using microorganisms,9 plant extracts9–11 and natural polymers9 as reducing agents has emerged as a promising alternative for AgNPs preparation. The exploit of green chemistry for AgNPs synthesis has obtained substantial achievements in the latest years. Most of reviews for biosynthesis of AgNPs focused on utilizing several plant and microbial sources, in which seeds,10 leaf,11 bark,12 stem13 and fruit extracts14 have been effectively used.

Lemon Citrus Latifolia (LCL) is a member of the citrus genera. Its extract contains various vitamins, citric acid, flavonoids, phenolic compounds and minerals.15–17 There are also numerous studies on AgNPs synthesis using lemon extract, but these studies were not comprehensive and systematic, especially in the microbial activities.

In the present study, AgNPs were synthesized by the reducing reaction of AgNO₃ using LCL extract. Effects of synthesis duration, volume ratio of AgNO₃/LCL extract, stirring rate, AgNO₃ solution concentration and temperature were assessed. Physico-chemical properties and antibacterial behaviors of the obtained AgNPs were investigated and evaluated.

2. Experimental

2.1 Preparation of extract

Fresh Lemon Citrus Latifolia fruits were collected from Ben Tre Province, Vietnam. After washed and drained three times with deionized water, they were squeezed to obtain solution. The solution was centrifuged at 5,000 rpm for 30 minutes to remove the residues, followed by the vacuum filtration through 0.22 µm filters to obtain LCL extract which was preserved at 4°C for further experiments. AgNO₃ (Merck, >99.8%) was used as a precursor for AgNPs synthesis.

2.2 Synthesis of AgNPs

The synthesis volume was conducted with 50 mL/batch. To survey the effect of direct sunlight on the AgNPs formation, two experiments were performed with different conditions with and without sunlight exposure. The other factors, including the synthesis duration of 2 hours, volume ratio of AgNO₃ solution/LCL extract of 3.5/1.5, stirring rate of 300 rpm, AgNO₃ solution concentration of 1.75 mM and the temperature of 40°C, were fixed.

Further investigation on AgNPs synthesis under sunlight illumination was also carried out by adjusting the AgNO₃ concentration, volume ratio of AgNO₃ solution/LCL extract, stirring rate, duration and temperature of synthesis.

2.3 Characterization

The presence of silver nanoparticles in the solution was determined by UV-Vis spectrophotometer (UV–1800 instrument) at the wavelength of 200 to 800 nm. Before analyzing, the samples were diluted 5 times. The morphology of AgNPs was characterized by scanning electron microscopy (SEM) on Hitachi S–4800 instrument and transmission electron microscopy (TEM) using JEM–1400 instrument. Based on TEM image, by using the ImageJ software, size distribution of AgNPs sample was determined. Crystalline phases of the prepared AgNPs powder dried at 60°C were investigated by X-ray diffraction (XRD) using Bruker D2 Phaser powder diffractometer. The relation between AgNPs and LCL Extract was confirmed using Fourier transform infrared spectroscopy (FTIR) carried out on a Tensor 27-Bruker spectrophotometer operated in the range of 400–4,000 cm⁻¹.

*Corresponding author, E-mail: ntri@ict.vast.vn

©2018 The Japan Institute of Metals and Materials
2.4 Antibacterial activity

The as-prepared AgNPs have been tested for antibacterial activity with E. coli (ATCC 25922), B. subtilis (ATCC 19659) and B. cereus (ATCC 11778) using agar well diffusion method. Pre-cultivation of the bacteria was conducted for 24 hours at room temperature. After 24 hours, Mueller Hinton Agar plates were prepared and four wells of 6 mm diameter were made using gel puncture on each agar plate. 70 µL of AgNPs solution was poured into three out of the four wells in each plate. The remaining was used as a control. After 15 minutes for AgNPs to diffuse into the agar, the samples incubated at 37°C for 24 hours. The results were obtained by measuring the diameter of the inhibition zone around the agar.

3. Results and Discussions

3.1 Biosynthesis of AgNPs

Effects of different factors on AgNPs biosynthesis were shown in Fig. 1. UV-Vis spectra analysis (Fig. 1(a)) showed no remarkable difference in the absorbance patterns of initial solution and the one synthesized in the dark during 120 minutes at room temperature. It indicated that no formation of AgNPs had taken place without sunlight. Meanwhile, it was found that the rate of AgNPs formation increased with the presence of sunlight in 120 minutes, the band indicating AgNPs of the range of 400–450 nm was observed. According to authors, the formation of silver nanoparticles under sunlight took place in four main steps: The first step was the photo-activation including the photosensitization of polyphenolic compounds (flavonoids) present in the extract. After the reactant mixture was exposed to sunlight, the flavonoid molecules absorbed the photons and got excited. The second step involved the electron donation of debonding of O–H group of the excited molecules for the reduction of Ag⁺ to Ag°. The third step involved the nucleation of Ag° atoms to form nanoclusters, and the fourth step involved the simultaneous growth of the nanoparticles in the nucleated nanoclusters to form the nanoparticles.

![Fig. 1 Effect of direct sunlight on AgNPs synthesis.](image)

Effects of different factors on AgNPs biosynthesis under direct sunlight were shown in Fig. 2. Figure 2(a) shows that with the volume ratio of AgNO₃ solution/LCL extract (V₁/Vₑₓ) of 3.5/1.5, the formation of AgNPs got the highest performance. The components of the extract effectively reduced Ag⁺ to Ag° with high extract concentrations, and provided enough capping agents for the stabilization of AgNPs through steric hindrance, thus, prevented their aggregation.

The stirring rate was increased from 200 up to 300 rpm leading to an increase in the formation rate of AgNPs (Fig. 2(b)). However, the formation decreased sharply when the stirring rate continuously risen up to 400 rpm. The strong stirring power resulted in an effective synthesis of nanoparticles because high rates of heat and mass transfer were essential in the process, especially on larger scale. Because of the increase in nanostructure concentration, the vigorous mixing was essential to provide uniform heat and mass transfer. However, when the stirring rate was too high (400 rpm), it could break the AgNPs structure and the parabola phenomena was occurred, leading to the reduction in the efficiency of the process. So, the stirring rate of 300 rpm was appropriate for the synthesis process.

AgNPs increased as raising the initial Ag⁺ concentrations from 1.5 to 1.75 mM (Fig. 2(c)), but decreased at 2.0 mM. Because AgNPs agglomerated when silver concentrations were too high, reducing the efficiency of the process. AgNPs synthesis was performed at different temperatures in the range of 35 to 50°C (Fig. 2(d)). It was observed that the absorbance increased significantly with the increasing temperature from 35 up to 45°C. It can be explained that the higher temperature led to an increase in the reduction rate of Ag⁺ ions. No changes in the width and the maximum wavelength of absorbance peaks observed at various temperatures indicated that the temperature only affected the amount, not the size, of the formed AgNPs. The absorbance decreased significantly when the temperature increased to 50°C. This may be due to the fact that compounds acting as reducing agents in LCL extract have significantly altered the properties at the temperature of 50°C. So, the suitable temperature for AgNPs synthesis was 45°C.

The absorbance peak was broaden indicating high conversion of silver ions to metallic silver nanoparticles when the synthesis duration increased (Fig. 2(e)). Prolonging the reaction time up to 90 minutes led to outstanding enhancement in the plasmon intensity, indicating that large amounts of silver ions were reduced and consumed for AgNPs formation. With the duration up to 120 minutes, the formed AgNPs were nearly unchanged that could be attributed to aggregation of the formed silver nanoparticles. Therefore, 90 minutes were chosen as the optimal reduction time.

To sum up, the suitable conditions for AgNPs synthesis using LCL extract were determined: sunlight exposure, AgNO₃ concentration of 1.75 mM, volume ratio of AgNO₃ solution/LCL extract of 3.5/1.5, stirring rate of 300 rpm, synthesis duration of 90 minutes and temperature of 45°C.

3.2 Characteristics of the synthesized AgNPs

FT-IR spectra of lemon extract, AgNPs solution synthesized and AgNPs powder dried at 60°C were depicted in Fig. 3. The spectrum for LCL extract showed typical broad
band of O-H stretching (3600–3300 cm⁻¹) and C=O stretching (1736–1710 cm⁻¹), suggesting the presence of carboxylic acids (citric-main component and ascorbic acids) in the extract²¹,²²) that acted as both the reducing and stabilizing agents. The difference of the peaks was not significant between the samples of pure LCL extract and AgNPs solution. This could be that the compounds of lemon extract covered the peaks of the silver nanoparticles by the functional groups contained in the extract, so the formation of the silver nanoparticles peaks was unclear. However, when compared to pure LCL extract, FT-IR spectrum of AgNPs solution showed weak signals, proving that the components of the extract had reacted to form silver nanoparticles.²³ Therefore, it can be argued that the bio-organics present in LCL extract along with citric acid as the main reductant

![Fig. 2 UV-Vis spectral analysis of AgNPs suspension samples synthesized with direct sunlight at different conditions. (a) Effect of volume ratio between AgNO₃ solution and extract (VAg/VExt). (b) Effect of stirring rate. (c) Effect of AgNO₃ concentration. (d) Effect of the temperature. (e) Effect of synthesis duration.](image)

![Fig. 3 FT-IR spectra of LCL extract and AgNPs samples.](image)
synergistically aid formation of AgNPs. On FT-IR spectrum of AgNPs powder, a bands centered at 2950 cm⁻¹, 1810 cm⁻¹, and 1420 cm⁻¹ were assigned to the axial stretching of C–H bonds, the axial stretching of C=O bonds of the acetamide groups, and the symmetric angular deformation of CH₃, respectively. Meanwhile, the absorption at 1340–1380 cm⁻¹ has been attributed to CH bending and CH₂ wagging, the 1200–1300 cm⁻¹ bands are associated with N–H bend amines. There was also a broad band of 1153–897 cm⁻¹ indicating the polysaccharide skeleton, including the vibrations of the glycoside bonds, C–O and C–O–C stretching.²⁴)

XRD pattern of AgNPs powder (Fig. 4) shows diffraction peaks at 2θ = 38.23°, 43.78°, 64.54°, and 77.13° which correspond to the (111), (200), (220), and (311) reflection planes of face-centered cubic phase of crystalline silver. Besides, a few intense and unassigned peaks at 2θ = 28.06°, 32.35°, 46.36°, and 54.87° were also noticed in the vicinity of silver peaks. These sharp Bragg peaks are due to the organic compounds which are present in the lemon extract and responsible for silver ions reduction and stabilization of nanoparticles. The intensity of the Bragg reflections suggests strong X-ray scattering centers in the crystalline phase and could possibly arise from the compounds in the nanoparticles during the synthesis. This XRD pattern is similar to those of the works.²⁵,²⁶) Based on XRD result, the average crystal size, of the sample at 2θ = 38.23°, determined according to Scherrer’s equation²⁷) was 9.8 nm.

SEM and TEM images (Fig. 5 and 6) and the size distribution histogram (Fig. 7) of AgNPs sample synthesized using LCL extract shows that the nanoparticles were spherical and uniform in size distribution. Based on the size distribution histograms, the average diameter of AgNPs was determined at 11.9 nm. The results were consistent with the XRD diffraction of the samples. Compared with previous studies,²⁸) the size of silver nanoparticles synthesized in this work using LCL extract was smaller and more uniform than AgNPs synthesized from Acalypha Indica (20–30 nm), Artemisia Nilagirica (70–90 nm), Boswelliaovali Foliolata (30–40 nm), Camelia Sinensis (30–40 nm), Cassia Fistula (50–60 nm), Chenopodiummurale (30–50 nm), Cinnamomum Camphora (55–80 nm), Dioscoreaalbulifera (35–60 nm) and Eucalyptus Hybrid (50–150 nm).

3.3 Antibacterial activity

Figure 8 shows the inhibition zone of synthesized AgNPs sample against three bacterial strains. The average inhibition zone diameters against E. coli, B. subtilis and B. cereus of synthesized AgNPs using of LCL Extract were determined at 20.0, 15.0 and 16.7 mm, respectively. The average inhibition
zone diameter against \textit{E. coli} of this work was higher than that of other studies such as \textit{Pomegranate} seeds (2.2 mm),\textsuperscript{39} \textit{Nicotiana tobacco} leaf (4.0 mm),\textsuperscript{30} \textit{lemon} extract (3.0 mm)\textsuperscript{31} and \textit{Neem} leaves (6.0 mm).\textsuperscript{32} The antibacterial activity of AgNPs synthesized from of LCL extract was also higher than one from the exopolysacharides\textsuperscript{33} against \textit{E. coli} (10 mm) and \textit{B. cereus} (10 mm), and from \textit{Capsicum Frutescence} extract\textsuperscript{34} against \textit{E. coli} (11.5 mm) and \textit{B. subtilis} (10.5 mm). It can be clearly found that synthesized AgNPs using LCL extract could be useful as a reducing agent exhibited highly bactericidal activity.

4. Conclusion

This work demonstrated that \textit{Lemon Citrus Latifolia} extract was available for producing silver nanoparticles through a very efficient and time saving process of 90 minutes. The appropriate conditions for AgNPs synthesis using this extract have been proposed. Obtained AgNPs were spherical and uniform in size distribution with particle sizes range of 4–24 nm. The average particle size were approxiamte 12 nm. Biosynthesized AgNPs exhibited great antimicrobial activities against \textit{E. coli}, \textit{B. subtilis} and \textit{B. cereus}. The results of this study proved that synthesized AgNPs using \textit{Lemon Citrus Latifolia} extract could be useful for the development of newer and more effective antibacterial agents.

Acknowledgments

This research is funded by The Incubator for Young Science and Technology Program organized by HCM City Department of Science and Technology and HCM City Youth Union in 2018.

REFERENCES