Scientific paper

Green Synthesis of Silver Nanoparticles Using Nelumbo *nucifera* Seed Extract and its Antibacterial Activity

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Abstract

Silver nanoparticles (AgNPs) were synthesized using a *Nelumbo nucifera* dry seed extract, which is a simple, non-toxic, eco-friendly "green material". The synthesized nanoparticles were confirmed by the color changes and characterized by UV-visible spectroscopy. The AgNPs were stable at room temperature for 2 months. Scanning electron microscopy (SEM) revealed the formation of well-dispersed and spherical shapes. Transmission electron microscopy (TEM) of the synthesized AgNPs showed the formation of spherical nanoparticles, 5.03–16.62 nm in size. Fourier transform infrared spectroscopy (FTIR) indicated the involvement of amine, aromatic and alkynes groups in the synthetic process. X-ray diffraction (XRD) confirmed the crystalline nature of AgNPs. These AgNPs were highly toxic to found to Gram negative bacteria.

Keywords: *Nelumbo nucifera* seeds, TEM, FTIR, XRD, antibacterial activity

1. Introduction

The application of nanoscale materials and structures, which by definition should fall in the range between 1 to 100 nanometers (nm) is an emerging area of nanoscience and nanotechnology. Nanomaterials often show unique and considerably different physical, chemical and biological properties compared to their bulk counterparts.[1] Recently, microorganisms such as bacteria,[2–4] fungi,[5–7] yeast,[8] actinomycetes[9] and plant extracts,[10–12] have been used as nanoparticles. Although the above biological methods aer rapid, simple, inexpensive, single step, sustainable and eco-friendly process, the culturing of microbes is a time- consuming and complicated process. The biosynthesis of AgNPs using lotus seeds can potentially eliminate these problems.

Silver in colloidal state exhibits distinctive properties, such as good conductivity, chemical stability, catalytic and antimicrobial activity. The synthesis of AgNPs using a plant system is a useful technology, which have many practical applications in the drug delivery, electronics, optics, diagnosis, tissue engineering, catalysis, antimicrobial activities, environment and biotechnology.[13–15]

Nelumbo nucifera Gaertn. (Nymphaeaceae) also known as sacred lotus is a large aquatic herb with stout, creeping rhizome. The seeds are of great importance to East Asian cuisine and have been used extensively in Chinese medicine and in Chinese desserts. *Nelumbo nucifera* was reported to posses' antidiarrhoeal,[16] psychopharmacological,[17] diuretic,[18] antipyretic,[19] antimicrobial,[20–22] hypoglycemic.[23] *Nelumbo nucifera* seeds are commonly used as a traditional remedy for the following: treatment of tissue inflammation, cancer, as antiemetic and children as a diuretic, as a refrigerant from a cooling medicine for skin disease and leprosy; and as an antidote to poisons.[24,25] The seeds are reported to have hepatoprotective and free radical scavenging activity,[26] antifertility activity[27]

and suppress cell cycle progression, cytokine genes expression, and cell proliferation in human peripheral blood mononuclear cells.[28]

In the present study, AgNPs were synthesized using *Nelumbo nucifera* seeds. The AgNPs were characterized using a range of characterization techniques. The antimicrobial activity of AgNPs was assessed against a range of pathogenic gram-positive and gram-negative bacteria.

2. Experimental

2. 1. Preparation of Seed Extract

Nelumbo nucifera dry seeds were collected from a supermarket in Ho Chi Minh City, Viet Nam. 10 g of dry seeds were added to 100 ml of distilled water and boiled for 25 min. The above cooled content was filtered through Whatmann filter paper 55 mm. The filtrate was used as a reducing agent for the preparation of nanoparticles.

2. 2. Synthesis of Silver Nanoparticles

5 ml of *Nelumbo nucifera* dry seed extract was added with the 95 ml of aqueous solution of 1 mM silver nitrate for reduction of Ag^+ ions and incubated at room temperature for 1 hrs. The reduction of pure Ag^+ ions was monitored by measuring the absorption of the reaction medium at wavelengths of 400–450 nm using a UV-vis spectrophotometer.

2. 3. Characterization of AgNPs

UV-Vis spectral analysis was performed on a Cary-4000 spectrophotometer. The morphology of the prepared AgNPs were observed by SEM (JSM 7401F (JEOL) and TEM (JEM-1400, JEOL). The AgNPs structure and composition were analyzed by XRD (AXS D8 Advance). Further characterization was performed using FTIR (Bruker Germany).

2. 4. Well Diffusion Method[28]

Pure cultures of the microbes were sub cultured on agar medium (Mueller Hinton for bacteria). Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Wells, 5 mm in diameter, were made on nutrient agar plates using sterilized glass column. Using a micropipette, 40 μL of nanoparticle solution was poured onto each well on all plates. After incubation at 37 °C for 24 hrs, the different zones of inhibition of microbes were measured.

3. Results and Discussion

3. 1. UV-Vis Spectroscopy

The study focused on the development of an eco-friendly method for the production of nanostructured AgNPs using green chemistry. After mixing the seed extract with a 1 mM $AgNO_3$ solution, the color began to change from brown to orange and finally to a dark orange color at 70° C for 30 min, indicating the formation of AgNPs which was determined by UV-vis spectroscopy (Fig 1).

Figure 1. Color change brown to orange obsreved indicating the formation of AgNPs

UV-vis spectroscopy is an important technique for establishing the formation and stability of metal nanoparticles in aqueous solutions. UV-vis spectroscopy revealed a strong Plasmon resonance, which was centered approximately at 445 nm (Fig 2). In the present study, the reaction mixtures showed a single SPR band, revealing spherical shape of the AgNPs, which was further confirmed by SEM and TEM.

The stability of the synthesized AgNPs was monitored regularly for approximately 2 months. The AgNPs solution was extremely stable at room temperature with no evidence of flocculation, as determined by UV-vis spectrophotometry.

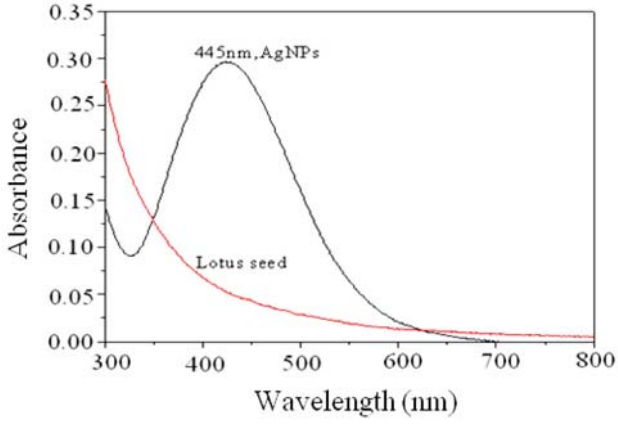

Figure 2. UV-Vis absorption spectra of green synthesized AgNPs

3. 2. XRD, FTIR, SEM and TEM Studies

The AgNPs structure green synthesized using the *Nelumbo nucifera* dry seed extract was confirmed by the characteristic peaks observed in the XRD pattern (Fig 3). The XRD pattern revealed three intense peaks in the entire spectrum with the peaks at 38.16°, 44.54° and 64.58° 2θ assigned the 111, 200 and 220 planes for the AgNPs, respectively, indicating a crystalline structure.

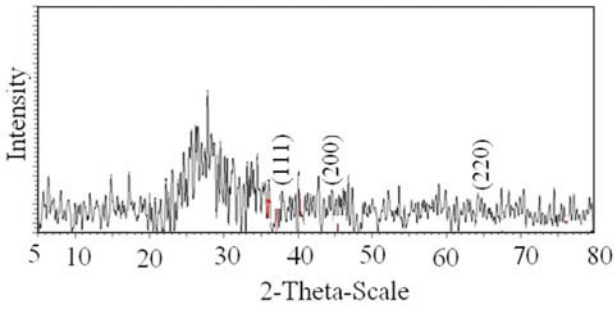

Figure 3. XRD pattern of AgNPs synthesized by *Nelumbo nucifera* seed extract

FTIR spectroscopy was carried out to identify the possible bio-molecules responsible for capping and reducing agent for the AgNPs synthesized by *Nelumbo nucifera* dry seed extract. The FTIR spectrum of the AgNPs revealed strong IR bands at 3436, 2077, 1637 and 684 cm^{-1} (Fig 4). The strong broad band at 3436 cm^{-1} was assigned to the N-H stretching mode in the linkage of proteins.[29] The peak at 1637 cm^{-1} corresponding to the C = O stretching mode in amine I group is commonly found in proteins[30] indicating the presence of proteins as the capping agents for AgNPs. The peaks at 684 cm^{-1} and 2077 cm^{-1} were assigned to the C-H bending mode of an aromatic and the C ≡ C stretching band of alkynes, respectively.

The formation of AgNPs from silver nitrate in the presence of the dry seed extract was confirmed by SEM and TEM. SEM (Fig 5) revealed spherical AgNPs and

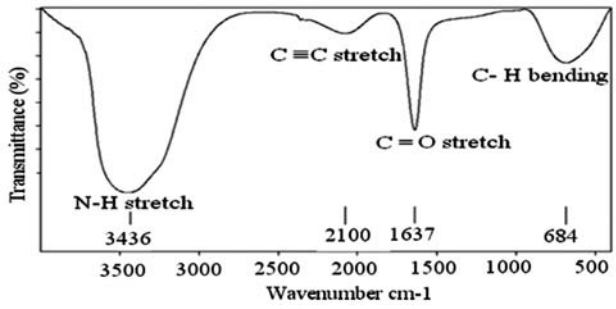

Figure 4. FTIR spectra of AgNPs using Nelumbo nucifera seed extract

with a uniform size distribution. TEM (Fig 6) showed that the AgNPs were predominantly spherical and irregular in shape with a size ranging from 2.76 nm to 16.62 nm (mean 7.07 nm).

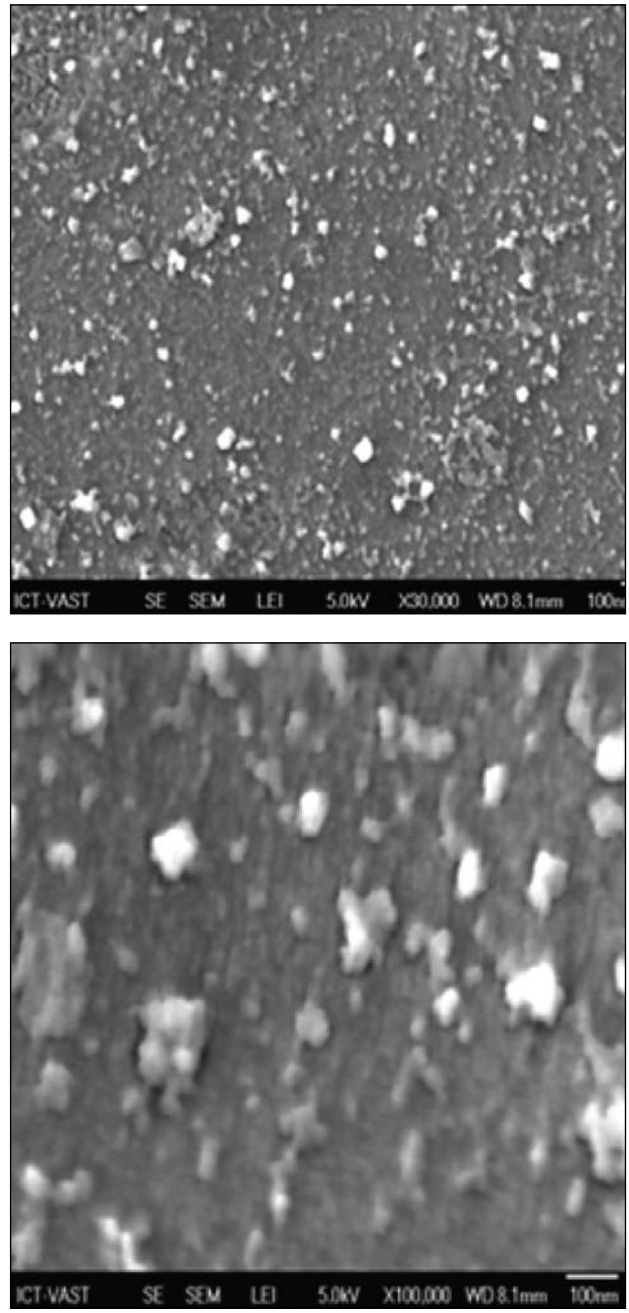

Figure 5. SEM omages of AgNPs synthesized from *Nelumbo nucifera* seed extract

3. 3. Antibacterial Studies

Silver has antibacterial properties and has been used in many medical applications. Green synthesized AgNPs were found to be highly toxic to gram negative bacteria

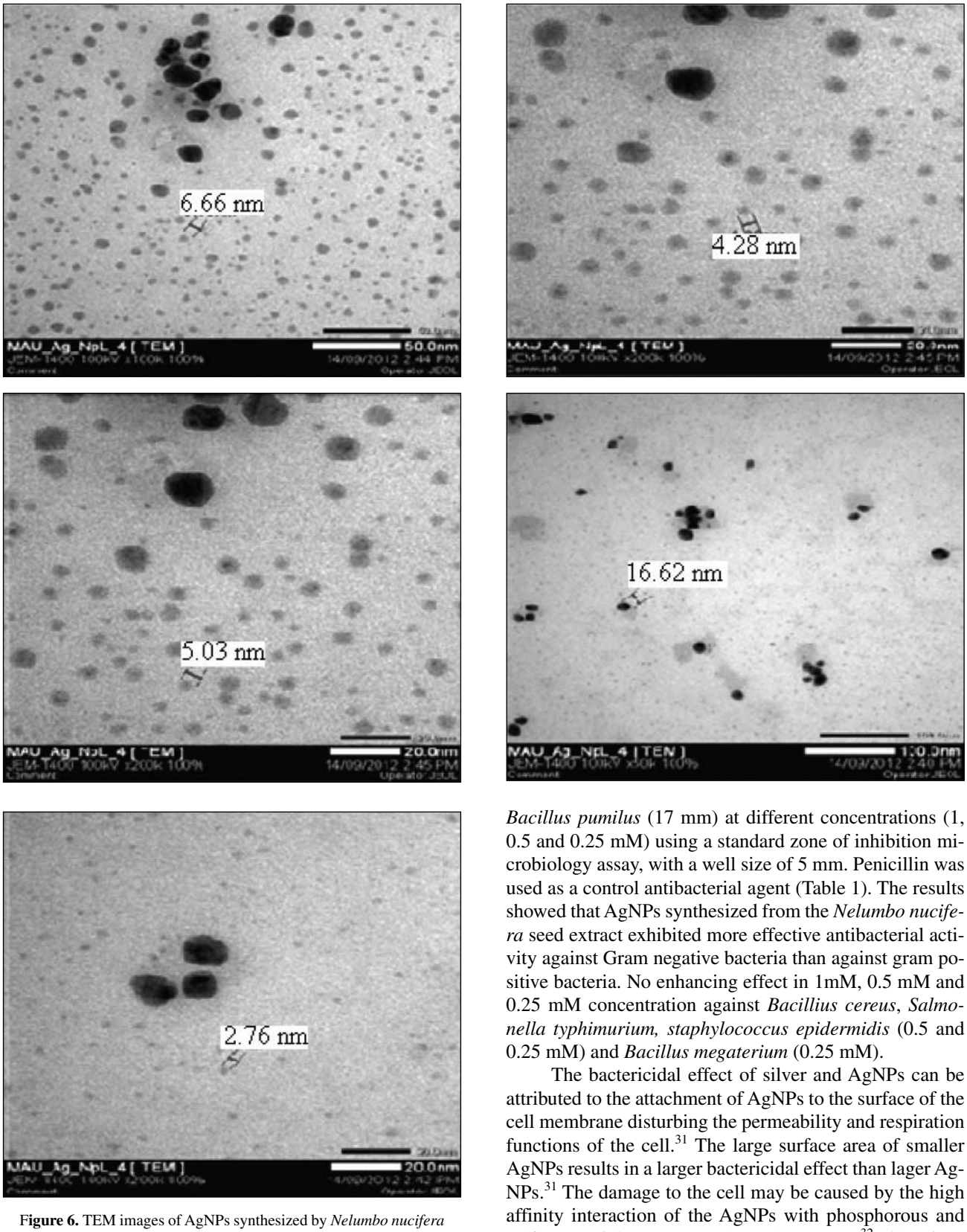

Figure 6. TEM images of AgNPs synthesized by *Nelumbo nucifera* seed extract

such as *Escherichia coli* (22 mm), gram positive bacteria *Bacillus subtillis* (20 mm), *Proteus cereus* (20 mm) and *Bacillus pumilus* (17 mm) at different concentrations (1, 0.5 and 0.25 mM) using a standard zone of inhibition microbiology assay, with a well size of 5 mm. Penicillin was used as a control antibacterial agent (Table 1). The results showed that AgNPs synthesized from the *Nelumbo nucifera* seed extract exhibited more effective antibacterial activity against Gram negative bacteria than against gram positive bacteria. No enhancing effect in 1mM, 0.5 mM and 0.25 mM concentration against *Bacillius cereus*, *Salmonella typhimurium, staphylococcus epidermidis* (0.5 and 0.25 mM) and *Bacillus megaterium* (0.25 mM).

The bactericidal effect of silver and AgNPs can be attributed to the attachment of AgNPs to the surface of the cell membrane disturbing the permeability and respiration functions of the cell.[31] The large surface area of smaller AgNPs results in a larger bactericidal effect than lager AgNPs.[31] The damage to the cell may be caused by the high affinity interaction of the AgNPs with phosphorous and sulfur containing compounds, such as DNA.[32] Silver ions strongly interact with the available thiol groups of the biomolecule to inactivate the bacteria.[33] Gram-negative bacteria have a lipopolysaccharide layer at the exterior, fol-

Table 1. Mean zone of inhibition (mm) of AgNPs synthesized using seed extract of lotus and penicillin against 9different bacterial species (well diameter 5 mm).

Name of the bacteria	Penicillin	1mM	0.5 mM	0.25 mM
Bacillus subtillis	15 ± 0.33	20 ± 0.57	15 ± 0.17	11 ± 0.31
Staphylococcus aureus	13 ± 0.11	11 ± 0.31	11 ± 0.31	9 ± 0.28
Escherichia coli	25 ± 0.05	22 ± 0.28	20 ± 0.11	16 ± 0.34
Bacillus pumilus	30 ± 0.63	17 ± 0.40	15 ± 0.23	13 ± 0.11
Bacillus megaterium	21 ± 0.17	9 ± 0.28	9 ± 0.57	–
Bacillus cereus	10 ± 0.28	–	–	–
Proteus cereus	28 ± 0.05	20 ± 0.51	15 ± 0.34	12 ± 0.17
Staphylococcus epidermidis	15 ± 0.57	10 ± 0.82	–	–
Salmonella typhimurium	11 ± 0.11	–	–	–

lowed underneath by a thin (7–8 nm) layer of peptidoglycan consisting of linear polysaccharide chains cross-linked by short peptides to form a three dimensional rigid structure.[34] Although lipopolysaccharides are composed of covalently linked lipids and polysaccharides, they lack strength and rigidity. The negative charges on the lipopolysaccharides are attracted towards the weak positive charges available on the AgNPs,[35] thereby contributing to the sequestration of free Ag^+ ions. Therefore, gram-positive bacteria may allow less Ag^+ to reach the cytoplasmic membrane than the gram-negative bacteria.

4. Conclusion

In this study, spherically shaped AgNPs were synthesized using a *Nelumbo nucifera* dry seed extract at room temperature. The AgNPs were stable without using any toxic chemicals. The spherical shaped AgNPs ranged in size from 2.76 to 16.62 nm. The AgNPs showed effective antibacterial activity against gram negative bacteria. The AgNPs prepared using a seed extract has the desired quality with a low price and convenient methodology.

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Povzetek

Srebrove nanodelce smo sintetizirali z uporabo ekstrakta suhih semen rastline *Nelumbo nucifera.* Metoda je preprosta in okolju prijazna. Pri sintezi nanodelcev smo opazili značilno spremembo barve in jih nadalje karakterizirali z UV-VIS spektroskopijo. Srebrovi nanodelci so stabilni pri sobni temperaturi dva meseca. Z vrstično elektronsko spektroskopijo (SEM) smo prikazali nastanek dobro razpršenih delcev sferične oblike. S transmisijsko elektronsko spektroskopijo (TEM) pa smo ocenili velikost delcev, ki se je gibala med 5,03–16,62 nm. Infrardeči spektri (FTIR) kažejo na vključenost amino, aromatskih in alkilnih skupin v sintezni proces. Z rentgensko praškovno analizo smo potrdili kristaliničnost nanodelcev. Antibakterijska aktivnost srebrovih nanodelcev je velika v primeru Gram negativnih bakterij.