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Biosynthesis and Characterization of Silver Nanoparticles using Agrocybe Cylindracea, and their Antibacterial Activity

Amira A. El-Fallal[1*], Mahmoud M. Nour El- Dein[1] and Maha H. Ebeed[*1]

[1]Department of Botany and Microbioligy, Faculty of Science, Damietta University, New Damietta, Egypt

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[*] Corresponding author's E-mail: mahaebeed18@gmail.com

Abstract

Mushrooms are of great interest in nanotechnology. Antibiotics resistance is a serious condition that threatens the treatment of many diseases caused by bacteria. So, it becomes persistent to find new antibacterial agents. Silver nanoparticles (AgNPs) showed a good antibacterial activity and the green synthesis of silver nanoparticles is considered a safe method. In this study AgNPs were biologically synthesized from mycelial free filtrate of an Egyptian mushroom *Agrocybe cylindracea.* The shift in reaction color from pale yellow to dark brown served as a primary indication for the creation of nanoparticles. The production of AgNPs was further confirmed using UV- visible spectrophotometer and the synthesis process was optimized at different parameters. The best concentration of silver nitrate, pH and temperature values were 4 mM, 7 and 40 °C, respectively. Transmission electron microscopy (TEM), Fourier transform infrared (FTIR), and Zetasizer analyses were used to investigate the characteristics of the produced nanoparticles. TEM studies showed that the size of synthesized AgNPs was 3.47 – 13.99 nm. FTIR studies showed the presence of some functional groups (O-H, C-H and C=C) which might be involved in the reduction of silver nitrate to silver ion and stabilization of nanoparticles. Zeta potential of the nanoparticles was -3.57. Antibacterial activity of synthesized AgNPs was demonstrated against different harmful microorganisms; *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and the best antibacterial activity was recorded against *P. aeruginosa*.

Keywords: Green synthesis, Silver nanoparticles, Antibacterial activity, Mushroom.

Introduction

Materials containing nanoparticles have at least one dimension less than 100 nm. When compared to their bulk equivalents, they exhibit distinct properties including melting point, wetability, electrical and thermal conductivity, catalytic activity, light absorption and scattering that distinguish them from the rest **(Jeevanandam *et al.*, 2018)**.

In comparison to the traditional ways of physical and chemical tactics, biological synthesis of nanoparticles is currently of significant interest throughout the world since it provides a safer environmental-friendly technology and biocompatible applications.

Mushrooms are of great interest. In biosynthesis of nanoparticles. Mushrooms have a significant nutritional and therapeutic value, as well as phytochemicals, polysaccharides, protein, enzymes, glucan, and other bioactive substances, making them suitable precursors for nanoparticle production. Mushrooms namely *Pleurotus sajor-caju, Pleurotus florida, Ganoderma lucidum* and *Microporus xanthopus* have been used for the production of AgNPs **(Nithya and Ragunathan, 2009)**, **(Bhat *et al.,* 2011)**, **(Karwa *et al.,* 2011)** , **(Balashanmugam *et al.*, 2013)**.

Several mushroom species, including *Agaricus, Calocybe, Pleurotus, Ganoderma, Lentinula,* and *Volvariella*, have been utilised to synthesise metallic nanoparticles, and their antibacterial, antioxidant, anticancer, anti-inflammatory, and other therapeutic value has been investigated **(Adebayo *et al.*, 2021)**.

A substantial amount of interest has been generated in the therapeutic applications of silver nanoparticles as a result of the emergence and spread of bacterial resistance to antibiotics, which has limited the efficacy of antibiotic therapies for infectious diseases **(Panáček *et al.*, 2018)**. When administered at non-cytotoxic levels to human cells, silver nanoparticles show outstanding bactericidal activity. They also considerably increase the antibacterial activity of standard medicines, even against multiresistant bacteria, by acting in synergistic fashion **(Panáček *et al.*, 2016)**.

It had been demonstrated that nanoparticles could be produced by reduction of Ag+ utilising a variety of reducing agents, including chemical agents, fungus extracts as biological agents, and irradiation as a physical agent **(Ayuk *et al.*, 2017)**. Nowadays, biological synthesis is favoured because it is safe, clean, inexpensive, and readily scaled up for the production of high-quality nanoparticles on a large scale **(Salem and Fouda, 2021)**.

The purpose of this study is to determine the efficacy of using a mycelium-free filtrate of an Egyptian mushroom as kind of reducing and capping substance in the mycosynthesis of AgNPs and characterize and optimize the resultant AgNPs. The study extended to its antibacterial activity against some pathogenic bacteria.

Materials and methods

The source of the tested fungus

The mushroom was common in Mansoura countryside often in clumps on deciduous trees and on trunk of *Salix safsaf* trees. It is isolated and identified as *Agrocybe cylindracea* (*Agrocybe aegerita*) based on morphological, characteristics by El-Fallal **(2003) .** The culture was routinely maintained on potato dextrose agar, being subcultured at 3-months intervals.

Biosynthesis of AgNPs

After letting the fungal culture to grow in liquid medium containing (5g/L malt extract and 10g/L glucose) for 5 days at 25°C on a 150 rpm shaking incubator, the filtrate was passed through Whatman filter paper No. 3 to achieve the desired level of AgNP biosynthesis **(Shivashankar *et al.*, 2013)**.

Optimization of extracellular biosynthesis of silver nanoparticles

Numerous factors influence the biogenesis, shape, and size of metal nanoparticles. The influence of pH and temperature on the biogenesis of Ag nanoparticles, as well as the concentration of the substrate ($AgNO_3$) and varied incubation durations, was investigated.

Nanoparticles biosynthesis in all tests was detected and confirmed by using the UV-visible spectrophotometer (Jasco V-630) in the range of 350–700 nm **(Chou *et al.*, 2005)**.

Characterization of silver nanoparticles

Transmission electron microscopy (TEM) was used to characterize the nanoparticles using a JEOL JEM-2100 apparatus set to 200 KV and was carried out according to **(Jain *et al.*, 2011)** at Alexandria University and Fourier transform infrared spectroscopy (FTIR) (Jasco, Japan) **(Siddique *et al.*, 2013)** at Faculty of Science, Damietta university. The recorded correlation functions and measured particles motilities were converted into zeta potentials using the Malvern Dispersion software **(Hanaor *et al.*, 2012)**. Zeta potential range (mV): mV and was measured at Mansoura university.

Antibacterial activity

Ag nanoparticles' inhibitory effect on three pathogenic organisms, including Gram positive bacteria (*Staphylococcus aureus* ATCC25923

and Gram-negative bacteria (*Klebsiella pneumoniae* ATCC33495 and *Pseudomonas aeruginosa* ATCC27853) was evaluated. All of the bacterial strains were generously donated by the culture collection of the Microbiology Laboratory at Damietta University, Egypt's Faculty of Science. The assay was carried out using agar well diffusion method on nutrient agar plates **(Deans and Ritchie, 1987)**. Five milliliters of nutrient broth (NB) was dispersed in test tubes and the cultures were cultured at 37 degrees Celsius for an overnight period. In the next step, 100 mL of each actively growing culture with a cell count of about 10^8 cells/mL was placed over nutrient agar plates with three wells (7 mm) punched for the addition of AgNPs solution was spread over the plates. Incubation at 37°C for 24 hours with the Ag nanoparticle solutions (50, 100, and 150 g/ml) was used to quantify antibacterial activity based on the zones of inhibition.

Results and Discussion

Synthesis of AgNPs

The green synthesis of silver nanoparticles has many advantages over other synthesis methods as decreasing cost of production and environmental pollution, reduced toxicity, and enhanced biological compatibility **(Duan *et al.*, 2015)**. Biosynthesis of nanoparticles using mushroom has emerged as an interesting and ecofriendly field of research. Synthesized nanoparticles using mushrooms exhibited increased stability, extended shelf life, and increased biological activity **(Kalia and Kaur, 2018)**. It was also demonstrates by Madhanraj et al. **(2017)** a low-cost and environmentally safe method of biogenesis of AgNPs employing basidiomycetes mushroom fungal strains *Pleurotus citrinopileatus, Pleurotus eous, Pleurotus cystidiosis, Pleurotus ostreatus, Pleurotus eryngii, Pleurotus flabellatus, Pleurotus florida, Pleurotus pulmonarius,* and *Schizophyllum commune* are some of the *Pleurotus* species. *Agrocybe aegerita,* an edible mushroom, has been shown to have anticancer properties through the synthesis of silver nanoparticles **(Nagalakshmi, 2015)**.

AgNPs was synthesized by mixing *Agrocybe cylindracea* mycelial free filtrate with $AgNO_3$ and incubating in dark conditions. The colour shift from light yellow to dark brown verified the creation of silver nanoparticles, while no change was observed to mycelial free filtrate or silver nitrate solution in similar conditions as shown in **(Fig. 1).**

The color change to yellowish brown indicated the formation of silver nanoparticles **(Bhat *et al.*, 2011)**. This change happened due to the excitation of plasmon vibration as described by Mulvaney **(1996)**.

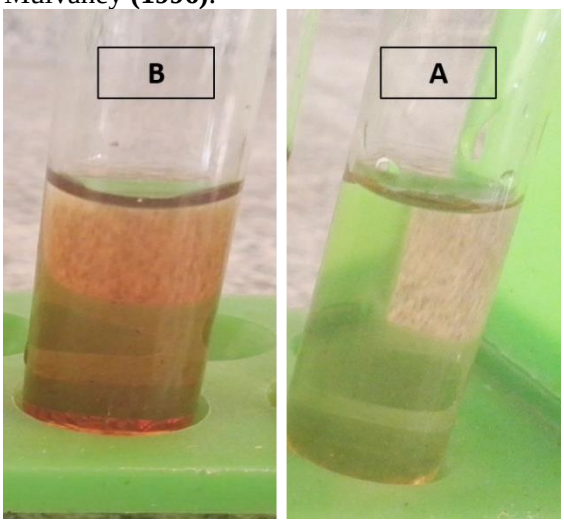

Fig. 1. Synthesis of silver nanoparticles by *Agrocybe cylindracea* and detecting of synthesis by colour change from pale yellow to brown color. (A): *Agrocybe cylindracea* mycelial free filtrate. (B): Reaction mixture (mycelial free filtrate + $AgNO_3$).

Characterization by Uv-visible spectrophotometer and optimization of different parameters:

The UV-vis spectroscopy is an important technique for indication and characterization of silver nanoparticles. Synthesis of AgNPs was detected by using UV- visible spectrophotometer in the range of 370 – 700 nm. A single peak appeared in the range of 420 – 450 nm which is a confirmation of spherical AgNPs synthesis. Mirunalini et al.**(2012)** who has synthesized AgNPs using edible mushroom illustrated that silver nanoparticles absorbed at visible zone of the electromagnetic spectrum at 380-450 nm. Jyoti et al. **(2016)** suggested that surface plasmon resonance at 410 – 450 nm may attribute to spherical nanoparticles.

Many factors were tuned to get the optimum AgNPs production. It was found that the optimum $AgNO_3$ concentration for nanoparticles synthesis was 4 mM (**Fig. 2A)**. Shivashankar et al. **(Shivashankar *et al.*, 2013)** *found that 5mM and 3mM concentrations of $AgNO_3$* were optimum for biosynthesis of

AgNPs from *Pleurotus djamor and Hypsizygus ulmarius respectively.* For pH value, there were no change in colour in the acidic conditions (pH 3 – 5). The change of colour appears at pH 6 and increased gradually. At pH 8 the nanoparticles started to aggregate at the bottom of the test tube, so pH 7 was taken as the optimum value for AgNPs synthesis from *Agrocybe cylindracea* (**Fig. 2B)**. This result is consistent with the findings of Balakumaran et al., **(2015)** who found that at acidic pH (1–4), no colour change occurred, and brown colour development started at pH 5 and 6, rising in intensity as pH increased; nonetheless, monodispersed and stable silver nanoparticles were formed at pH 7.

It was found that the optimum incubation period for AgNPs synthesis was 5 days, after which the nanoparticles started to aggregate (**Fig. 2C)**. Osorio-Echavarría et al., **(2021)** found that the optimal reaction time for AgNP production employing the white rot fungus Anamorphous *Bjerkandera* sp. R1 was 144 hours. The optimum temperature for the synthesis process was 40° C (**Fig. 2D).** which agree with *Khan and Jameel **(2016)** who found that 40 °C is the optimum temperature for synthesis of AgNPs.*

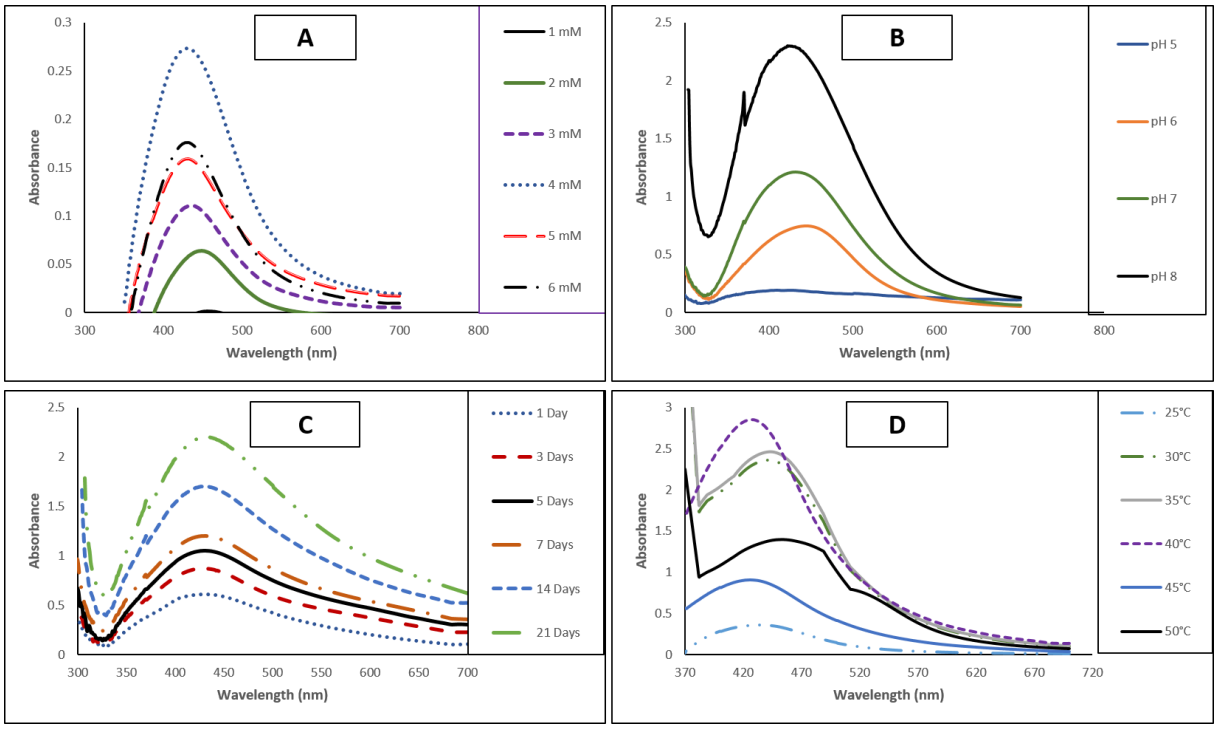

Fig. 2. Optimization of biosynthesis of silver nanoparticles by *Agrocybe cylindracea*. (A) Effect of $AgNO_3$ concentration. (B) Effect of pH values. (C) Effect of incubation period. (D) Effect of temperature

TEM analysis

The TEM data gave information on the biologically generated Ag nanoparticles' shape, size, and dispersion. The TEM analysis showed that synthesized AgNPs by mycelial-free filtrate *Agrocybe cylindracea* were spherical in shape with size ranged from 3.47 – 13.99 nm and the nanoparticles were well dispersed and no particle adhesion or agglomeration was observed as appeared in (**Fig. 3)**. The present recorded size of AgNPs is smaller than that recorded by Nagalakshmi **(2015)** who found silver nanoparticles produced by *Agrocybe aegerita* were spherical and have size 29.9 nm. It could be attributed to the use of fruit bodies and the Egyptian strain is different. Similarly, Philip **(2009)** recorded that silver nanoparticles produced by *Volvariella volvacea* are spherical with size approximately 15 nm. The TEM image also showed well dispersed particles. The spaces between particles may be due to the stabilization with capping agents which agreed with the study by Devika et al. **(2012)** on *Pleurotus ostreatus.*

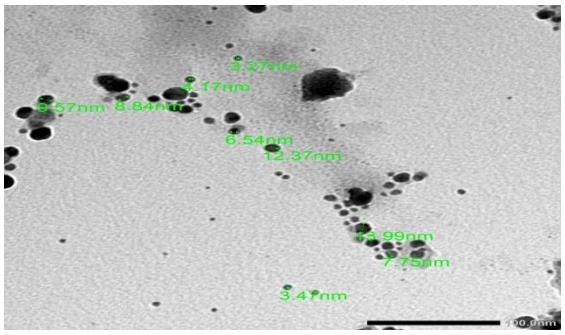

Fig. 3. Transmission electron microscope image of synthesized AgNPs by *Agrocybe cylindracea*.

FTIR analysis

The FTIR spectroscopy is used for identifying the biomolecules surrounding the nanoparticles and acts as capping and stabilizing agents. The FTIR spectrum shows bands at 3388.3 corresponding to O-H stretching, 2934.1 corresponding to C-H stretching peak, 1635.3 stretching vibrations of the carbonyl group (C=O), 1247.7 corresponding to C-O structure and 1030.7 which may be ascribed to –C-O-C or –C-O- bonds as presented in (**Fig. 4) (Arun *et al.*, 2014)**, **(Manzoor-Ul-Haq *et al.*, 2014)**. The results confirmed the prescence of capping agents which may be involved in the stability of AgNPs, which is consistent with the findings of Elumalai et al. **(2012)**, Bhat et al. **(2011)** on *Pleurotus florida* and *Agrocybe aegerita* by Nagalakshmi **(2015)**.

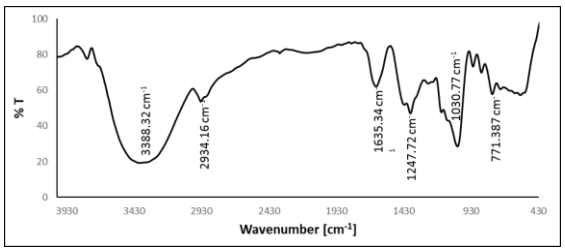

Fig. 4. FTIR spectrum of biosynthesized AgNPs by *Agrocybe cylindracea*.

Zeta potential

The biosynthesized AgNPs had a negative charge with a zeta potential value − 3.75 mV as represented in **Fig.5.** The repulsion between the produced silver nanoparticles is shown by the negative zeta potential **(Elamawi *et al.*, 2018)**.

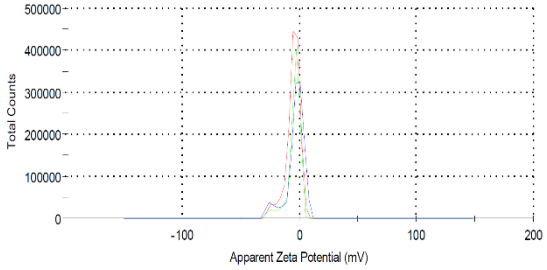

Fig. 5. Zeta potential of synthesized AgNPs by *Agrocybe cylindracea*.

Antibacterial activity

Antimicrobial activity of produced AgNPs was determined using the agar well diffusion technique (**Fig. 6).** The antibacterial activity of the produced AgNPs was strongest against *Pseudomonas aeruginosa* with clear zone diameter 15.33 mm using 150 µg/ml AgNPs. The antimicrobial potential of the synthesized AgNPs increased by increasing the concentration as presented in table (1).

AgNPs sythesized using *Pleurotus ostreatus* showed inhibition zone against bacteria *S. aureus* (12.7 mm), and *P. aeruginosa* (13 mm) **(Al-Bahrani *et al.*, 2017)**. The inhibition zone measured by Mohanta et al. **(2018)** was 11 mm when test the AgNPs synthesized by the wild mushroom *Ganoderma sessiliforme* against *E. coli.*

In the study done by Shivashankar et al. **(2013)** *Pleurotus pulmonarius AgNPs showed inhibition zone of 0.15 cm against Staphylococcus aureus at 0.5mg/ml and 0.17 cm against Pseudomonas aeruginosa at 0.4 mg/ml.* Silver nanoparticles' mechanism of action against bacteria is not fully understood at this time but it could be explained that antibacterial activity result from gaps induced by nanoparticles in the cell membrane or interaction of Ag ions with sulfhydryl groups of enzymes that disrupt metabolism and lead to cell death **(Dizaj et al., 2014)**.

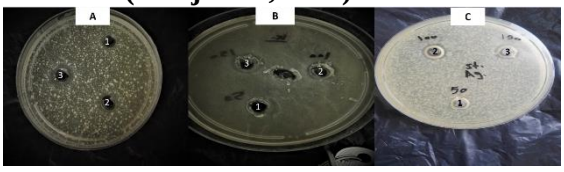

Fig. 6. Antibacterial potential of synthesized AgNPs by *Agrocybe cylindracea* against pathogenic bacteria: (A) *P. aeruginosa*, (B) *K. pneumoniae*, (C) *S. aureus.* (1): 50 µg/ml, (2): 100 µg/ml, (3): 150 µg/ml.

Table 1: Antimicrobial potential of synthesized AgNPs and Penicillin as positive control and mycelial free filtrate as negative control. Zone of inhibition expressed in mean diameter ± standard deviation.

Treatment	Zone of inhibition (mm)		
	S. aureus	*K. pneumoniae*	*P.aeruginosa*
Negative control (mycelial free filtrate)	0.0	0.0	0.0
Positive control Penicillin (500 µg/mL)	16.7 ± 0.06	0.0	33.8 ± 0.3
AgNPs (50 µg/mL)	10.1 ± 0.43	9.97 ± 0.25	11.7 ± 0.2
AgNPs (100 µg/mL)	11.9 ± 0.26	11.53 ± 0.28	14.33 ± 0.58
AgNPs (150 µg/mL)	12.5 ± 0.26	12.36 ± 0.16	15.33 ± 0.58

Conclusion

The synthesis of silver nanoparticles from *Agrocybe cylindracea* mycelial free filtrate provides simple and efficient methods for the synthesis of nanomaterials, according to this study. The characterization of Ag+ ions confirms the reduction of silver ions into stable spherical particles with modest sizes ranging from 3.47 to 13.99 nm. There was no particle adhesion or agglomeration, and they were properly disseminated. The silver nanoparticles that were created were repellent to one another. The optimal conditions for *Agrocybe cylindracea* silver nanoparticle production were pH 7 at 40° C after 5 days. The recorded antimicrobial effectiveness of biosynthesized AgNPs against pathogenic microorganisms because of their great antibacterial efficacy, small size, and durability, silver nanoparticles generated by *Agrocybe cylindracea*, AgNPs are regarded promising in medical applications and biotechnology.

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الملخص العربي

عنوان البحث: التخليق الحيوي لجزيئات الفضة النانوية وتوصيفها باستخدام أجروسيبي سيلندركا، ونشاطها المضاد للبكتريا

أميرة علي الفلال[١]، محمود متولي نور الدين[١]، مها هاني عبيد[١]
[١] قسم النبات والميكروبيولوجي، كلية العلوم ، جامعة دمياط ، مدينة دمياط الجديدة ، مصر

الغرض من البحث هو تخليق جزيئات الفضة النانوية باستخدام ناتج فطر أجروسايبي سيلندركا كعامل مختزل لنترات الفضة. أضيف مستخلص الفطر إلى محلول نترات الفضة ، وحُضّن في الظلام عند درجة حرارة ٣٠ درجة مئوية، وقد كان تغير اللون من الأصفر الباهت إلى البني دليلاً على تكون جزيئات الفضة النانوية.
تأكد تكوين جزيئات النانو باستخدام أطياف الأشعة فوق البنفسجية- المرئية في الطيف ٣٥٠ – ٧٠٠ نانومتر ، وتم تحسين عملية الإنتاج عن طريق تغيير ظروف التفاعل (تركيز محلول نترات الفضة – درجة الحرارة – الرقم الهيدروجيني – فترة التحضين). وُجد أن أفضل تركيز من نترات الفضة لإنتاج جزيئات النانو هو ٤ ملي مول، والرقم الهيدروجيني المناسب للإنتاج هو ٧، ودرجة الحرارة المُثلى هي ٤٠ درجة مئوية.
استُخدم جهاز الميكروسكوب الإلكتروني النافذ لتحديد شكل وحجم جزيئات النانو المتكونة، وأظهر التحليل أن حجم جزيئات النانو يتراوح بين ٣,٤٧ – ١٣,٩٩ نانومتر. كما أُجريت قياسات FTIR وأشارت النتائج إلى وجود بعض المجموعات الوظيفية التي قد تكون مسؤولة عن عملية اختزال وثبات جزيئات النانو المتكونة.
تم قياس النشاط المضاد للبكتريا لجزيئات النانو المتكونة على بعض البكتريا المُمرضة مثل استافيلوكوكس أوريس و زيدومونس ارجنوزا وكلبسيلا نيمونيا، وأظهرت جزيئات الفضة نشاطاً جيداً ضد البكتريا المذكورة.