

Environmentally benign synthesis of silver nanoparticles using tea leaf extract and their antibacterial potential

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Abstract

The escalating global burden of antibiotic-resistant bacterial infections has intensified the search for sustainable antimicrobial nanomaterials that can be produced without hazardous chemical reagents. In this study, we report a simple, rapid and environmentally benign route to silver nanoparticles (AgNPs) that combines tea leaf extract (*Camellia sinensis*) as a natural reducing/capping agent with sodium oleate as an auxiliary stabilizer, a dual-agent strategy that remains comparatively underexplored relative to single-extract green-synthesis protocols and that couples the biomolecules-driven reduction of tea polyphenols with the colloidal stability conferred by an anionic surfactant. Nanoparticle formation was first signaled by a visible color change of the reaction mixture from colorless to brown-black, consistent with localized surface plasmon resonance and was confirmed by a characteristic UV-Vis absorption maximum at approximately 415nm. A comprehensive, multi-technique protocols, X-ray diffraction, thermogravimetric analysis, scanning electron microscopy, transmission electron microscopy, energy-dispersive X-ray spectroscopy, and photoluminescence spectroscopy, established the formation of highly crystalline, face-centered-cubic, spherical and well-dispersed AgNPs in the 5–20 nm size range, capped by a thermally robust organic phytochemical layer stable upto 600°C. The biosynthesized AgNPs exhibited broad-spectrum antibacterial activity against clinically relevant gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Klebsiella* and *Pseudomonas aeruginosa*) strains in well-diffusion assays, notably including inhibition of a β -lactamase-positive *Klebsiella* isolate. Therefore, these results position tea-extract/oleate-mediated AgNPs as a low-cost, scalable and biocompatible nanomaterials platform with clear translational relevance for antimicrobial applications at a time when resistance to conventional antibiotics is rising.

1. Introduction

Recent advances in nanotechnology have driven extensive research on metal nanoparticles owing to their unique physicochemical properties at the nanoscale. Their high surface area-to-volume, tunable optical characteristics, and enhanced biological reactivity have enabled applications in spanning medicine, catalysis, sensing, and environmental remediation [1–3]. Among metal nanoparticles, silver nanoparticles (AgNPs) occupy a particularly prominent position because of their potent antimicrobial, catalytic, optical, and anticancer properties [4–7]. Their distinctive optical signature arises from localized surface plasmon resonance (LSPR), generated by the collective oscillation of conduction electrons at the nanoparticle surface upon interaction with incident light [8]. Conventional routes to AgNPs including chemical reduction, electrochemical synthesis, photochemical methods, and thermal decomposition [9–11]. These limitations have motivated the development of green synthesis strategies that employ biological materials as safer, more sustainable alternatives to conventional chemical reduction [12–14]. Plant extracts are especially well suited to this purpose, as they are rich in naturally occurring phytochemicals such as polyphenols, flavonoids, alkaloids, proteins, and carbohydrates, that can simultaneously act as reducing and stabilizing agents [15–17]. Tea leaf extract is particularly attractive for this purpose because of its high content of catechins and other polyphenolic compounds, which are capable of reducing Ag^+ ions to metallic silver concurrently stabilizing the synthesized particles [18–20].

Beyond their eco-friendly mode of synthesis, tea-mediated AgNPs have attracted considerable interest for their enhanced biological performance and broad-spectrum antimicrobial activity [13, 18, 21]. Their nanoscale dimensions afford a large surface area that facilitates intimate interaction with microbial cell membranes, promoting membrane disruption, generation of reactive oxygen species, and interference with essential cellular processes [22]. Phytochemical constituents derived from tea extract not only mediate nanoparticle formation, but may also improve the stability and biocompatibility of the resulting nanoparticles [23]. Given these advantages,

green-synthesized AgNPs have attracted growing attention as candidate materials for antimicrobial coatings, wound-healing formulations, waste water treatment, drug delivery, and a range of biomedical applications [24–27].

However, most report of tea-mediated AgNP synthesis rely on the extract alone as both reductant and capping agent, leaving the potential benefits of pairing a phytochemical reductant with an auxiliary colloidal stabilizer comparatively unexplored. In the present work, we address this gap by combining tea leaf extract with sodium oleate, an anionic surfactant during AgNP formation, a dual-agent approach intended to couple efficient biomolecules driven reduction with improved colloidal stabilization. We further evaluate the resulting nanoparticles using an integrated characterizations suite (UV-Vis, XRD, TGA, SEM, TEM, EDX and PL) that is broader than typically reported for tea-mediated AgNPs and we assess their antibacterial efficacy against a clinically relevant bacterial panel that includes a β -lactamase-positive strain, thereby situating the synthesized nanoparticles within the context of the growing antimicrobial resistance challenges. Therefore, these results establish an environmentally benign and structurally well-characterized synthesis route to biologically active AgNPs with significant antimicrobial and therapeutic potential.

2. Experimental

2.1. Materials

Fresh tea leaves (*Camellia sinensis*) were collected from tea garden of Golaghat district, Assam, India. Silver nitrate (AgNO_3), and Sodium oleate ($\text{C}_{18}\text{H}_{33}\text{NaO}_2$) was of analytical grade, purchased from Sigma-Aldrich, India. Distilled water was used for the preparation of all solutions. All the glassware thoroughly washed with water prior to use for the experiment. Standard microbiological media and analytical grade chemicals were employed for antibacterial experiments.

2.2. Preparation of tea extracts

Tea leaf extract was prepared by adding 10 g of fresh tea leaves to 100 mL of distilled water and heating the mixture at 80°C for 15 min. The extract was cooled to room temperature and filtered through Whatman filter paper to obtain a clear solution, which was stored at room temperature and subsequently used as a natural reducing and stabilizing agent for AgNPs synthesis.

2.3. Greener synthesis of AgNPs

AgNPs were synthesized using tea leaf extract as a natural reducing and stabilizing agent via a green synthesis approach. An aqueous solution containing AgNO_3 (40 mL, 4.3 mM) and sodium oleate (0.2721 g, 0.05 M) was prepared and stirred continuously. Freshly prepared tea leaf extract (15mL) was then added dropwise under vigorous stirring at room temperature. The solution progressively changes colour from colorless to brownish-black, indicating reduction of Ag^+ ions and the formation of AgNPs. Stirring was continued for 30 min to ensure complete reduction and stabilization. The resulting fine, shining silver particles were separated by filtration, washed repeatedly with distilled water to remove unreacted impurities and dried in air.

2.3. Characterization

UV-visible spectra were recorded using Perkin Elmer, lambda 25 spectrophotometer. The SEM-EDX characterization was performed on a JEOL JSM-6510 scanning electron microscope fitted with an energy dispersive X-ray spectrometer operating at 20 kV; samples were on conductive carbon adhesive tape and gold-

coated prior to imaging. TEM images were acquired on JEOL JEM-2100 transmission electron microscope, with samples dispersed in water by ultrasonication and deposited on copper-coated grids. XRD measurements were recorded over a 2θ range of $5\text{--}80^\circ$ with a scan rate of 4° min^{-1} using a Rigaku Miniflex-II diffractometer with Ni-filtered Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$). TGA was performed on a Shimadzu DTG-60H at a heating rate of $20^\circ \text{ C min}^{-1}$ from 50 to 800° C under N_2 atmosphere. PL spectra were measured on a HITACHI F-2500 fluorescence spectrometer using quartz cuvettes of 1 mm path length, with an excitation at 280 nm in special-grade CHCl_3 .

2.4. Antibacterial assay

Bactericidal activity against standard Gram-negative (*E. coli* ATCC 25922, *P. aeruginosa* PA01, *Klebsiella* sp. β -Lactamase-positive) and Gram-positive (*S. aureus* ATCC 25923) bacterial strains were assessed using the well-diffusion methods.²¹ The clear zone of bacterial inhibition, indicative of antibacterial potential was measured in mm using a zone-diameter scale. Briefly, 100 μL of bacterial suspension ($5 \times 10^6 \text{ cells mL}^{-1}$) was spread onto overnight-dried Muller-Hinton agar plates, and wells were bored into the agar. AgNPs solutions (1 mg mL^{-1} in DMSO) were loaded into the wells, and plates were incubated at 37° C for 24 h. Standard antibiotic disc served as the positive control, while 100 μL of DMSO loaded into a well served as the negative control; nitrofurantoin was used as the reference antibiotic.

3. Results and Discussions

A simple, rapid, and eco-friendly method was developed for the synthesis of AgNPs using green tea leaf extract as a natural reducing and stabilizing agent, supplemented with sodium oleate to enhance colloidal stability. The phytochemical constituents of the tea extract played a central role in reducing Ag^+ to metallic silver and in stabilizing the resulting nanoparticles [28]. The use of an aqueous green tea leaf extract and water as the reaction medium offers clear advantages over conventional chemical methods that rely on toxic reducing agents and organic solvents [29] and the combined use of a phytochemical reductant with an auxiliary surfactant provides an additional, comparatively underexplored handle for controlling particle stability without compromising the green character of the synthesis.

3.1. UV-visible spectral analysis

The UV-visible absorption spectrum of AgNPs in aqueous medium is shown in Fig. 1A. A strong UV-visible absorption band centered at 415 nm was observed, corresponding to the surface plasmon resonance (SPR) of AgNPs [30]. The relatively narrow, single and symmetric nature is consistent with the formation of small, predominantly spherical nanoparticles with a comparatively narrow size distribution, and provides an initial, non-destructive optical signature that corroborates the morphological data obtained subsequently from electron microscopy.

3.2. X-ray diffraction Studies

The crystalline nature of the silver nanoparticles was confirmed by XRD pattern shown in Fig. 1B. The pattern exhibits a number of Bragg reflections indexable to the face-centered cubic (fcc) structure of metallic silver, in good agreement with the standard pattern for silver (JCPDS file no. 04-0783). Four sharp, well-defined diffraction peaks were observed at 2θ values of 38.10° , 44.34° , 64.50° , 77.40° corresponding to respectively to the (111), (200), (220) and (311) reflections of fcc silver. Using the Debye-Scherrer equation, the average crystallite size was estimated at approximately 20 nm, consistent with the particle size range obtained independently from TEM analysis and leading mutual support to the two measurements [31].

3.3. Thermogravimetric Analysis

The TGA thermogram (Fig. 1C) shows a major weight loss over the range of 50–440°C, attributable to the removal of physically absorbed moisture together with the thermal decomposition of the organic phytochemical constituents that cap and stabilize the AgNP surface. Beyond this range, no significant further weight loss was observed, indicating good thermal stability of the synthesized AgNPs upto 600°C. This thermal profile is consistent with the presence of a robust organic capping layer derived from the tea extract and sodium oleate, which is expected to contribute both to colloidal stability during synthesis and to the long-term stability of the isolated nanoparticles [32].

3.4. SEM-EDX analysis of AgNPs

Surface morphology examined by SEM at different magnifications reveals a predominantly spherical particle shape. Figure 2 shows that the AgNPs possess a smooth surface and are well dispersed, with no evidence of significant agglomeration, consistent with effective capping by the tea phytochemicals and sodium oleate. Energy dispersive X-ray spectroscopy (Fig. 2) confirms the elemental composition of the nanoparticles. A strong signal at approximately 3.0KeV, characteristic of metallic silver, dominates the spectrum. In addition, carbon and oxygen signal were detected, indicating the presence of biomolecules from the plant extract capping the nanoparticle surface, a finding that both prevents agglomeration and enhances colloidal activity [33].

3.5. TEM analysis of AgNPs

TEM imaging (Fig. 2) provided further insight into the microstructure and size of the AgNPs. The micrographs confirm that the nanoparticles are predominantly spherical and polydisperse with an estimated average size range of 5–20 nm, which is in good agreement with the crystallite size derived from XRD analysis (Section 3.2) and reinforcing the internal consistency of the structural characterization [34].

3.6. PL analysis

Figure 3 presents the room-temperature PL emission spectrum of the synthesized AgNPs. Noble-metal nanoparticles exhibit photoluminescence arising from the radiative recombination of conduction band electrons near the Fermi level with holes in the sp or d-bands.

The observed visible luminescence of the AgNPs is primarily attributed to excitation of electrons from the filled d-band to higher energy states above the Fermi level, followed by radiative relaxation [35].

The comparison of the present study with representative reports on the green synthesis of silver nanoparticles is summarized in Table 1, highlighting the advantages of the tea leaf extract-sodium oleate dual-agent system in achieving improved nanoparticle stability, comprehensive physicochemical characterization, and promising broad-spectrum antibacterial performance over previously reported plant-mediated approaches.

Table 1

Comparison of representative plant-mediated AgNPs with the silver nanoparticles synthesized in the present study.

Plant extract / reducing agent	Additional stabilizer	Particle size (nm)	Characterization	Antibacterial evaluation	Distinct feature	Ref.
Tea polyphenols / tea extract (<i>Camellia sinensis</i>)	None	5–25	UV-Vis, TEM, XRD	Not primarily evaluated; biocompatibility studied	Early demonstration of controllable AgNP formation using tea polyphenols	[36]
Green tea extract (<i>Camellia sinensis</i>)	PEG in selected formulations	20–40	UV-Vis, XRD, TEM, SEM, FTIR	<i>E. coli</i> , <i>S. aureus</i> and other bacteria	Green tea-mediated synthesis with antibacterial activity and improved biocompatibility	[37]
Tea leaf extract (<i>Camellia sinensis</i>)	None	10–50	UV-Vis, XRD, TEM	<i>E. coli</i> , <i>S. aureus</i>	Conventional tea-mediated green synthesis	[38]
Tea extract (<i>Camellia sinensis</i>)	None	Nanometre range	UV-Vis, XRD, SEM/TEM and other physicochemical analyses	ESBL-producing Gram-negative pathogens	Activity against ESBL-producing antibiotic-resistant bacteria	[39]
Leaf extracts (<i>Musa balbisiana</i> , <i>Azadirachta indica</i> , <i>Ocimum tenuiflorum</i>)	None	80–200	UV-Vis, DLS, SEM, TEM, EDS, FTIR	<i>E. coli</i> , <i>Bacillus</i> sp.	Comparative synthesis using three readily available Indian plants	[40]
Aloe vera extract	None	70.7–192.0	UV-Vis, XRD, SEM, TEM	<i>P. aeruginosa</i> , <i>S. epidermidis</i>	Hydrothermal plant-mediated synthesis with antibacterial activity	[41]
Medicinal plant extracts (multiple species)	None	Variable	UV-Vis, TEM, XRD, SEM/EDX	Gram-positive and Gram-negative bacteria	Comparative evaluation of AgNP morphology and antibacterial efficacy from different plant extracts	[42]
Present work: tea leaf extract (<i>Camellia sinensis</i>)	Sodium oleate	5–20	UV-Vis, XRD, SEM, TEM, EDX, TGA, PL	<i>E. coli</i>, <i>S. aureus</i>, β-lactamase-positive <i>Klebsiella</i> sp., <i>P. aeruginosa</i>	Dual-agent green synthesis combining tea phytochemicals with sodium oleate for	This work

Plant extract / reducing agent	Additional stabilizer	Particle size (nm)	Characterization	Antibacterial evaluation	Distinct feature	Ref.
					stabilization, with comprehensive characterization and activity against a β -lactamase-positive strain	

3.7. Antibacterial activity

Antibacterial performance is a key characteristic underpinning the biological applicability of any nanomaterials. Nano-silver is among the most extensively studied universal antibacterial agent [43–45], as silver-based compounds are strongly toxic to microorganisms and exhibit potent germicidal effects. The antibacterial activity of synthesized AgNPs is illustrated in Table 2.

Table 2
Antibacterial activity of the synthesized AgNPs against selected bacterial strains determined by the agar well diffusion method

Bacterial Strains	Gram reaction	Zone of Inhibition (in mm)
<i>E. coli</i>	Gram-negative	12
<i>S. aureus</i>	Gram-positive	14
<i>Klebsiella</i> sp.	Gram-negative	11
<i>Pseudomonas aeruginosa</i>	Gram-negative	10

The AgNPs exhibited broad-spectrum antibacterial activity, inhibiting both gram-positive and gram-negative bacteria pathogens with the largest zone of inhibition observed against *S. aureus* (14 mm) followed by *E. coli* (12 mm), *Klebsiella* sp. (11 mm),and *P. aeruginosa* (10 mm). Notably, inhibitory activity was retained against the β -lactamase-positive *Klebsiella* isolate, a strain characterized by resistance to β -lactam antibiotics through enzymatic drug inactivation.

This results (Fig. 4) is of particular translational significance: it indicates that the antibacterial mechanisms of the biosynthesized AgNPs, generally attributed to disruption of bacterial membrane integrity, generation f reactive oxygen species, and interference with intracellular processes is not compromised by the β -lacctam-resistance determinant, supporting the potential of tea-extract/oleate-mediated AgNPs as an antimicrobial strategy complementary to conventional antibiotics in the context of rising antimicrobial resistance.

4. Conclusions

A simple, rapid, eco-friendly and cost-effective route was developed for the green synthesis of silver nanoparticles using tea leaf extract (*Camellia sinensis*) as a natural reducig agent together with sodium oleate as an auxiliary stabilizer, a dual agent strategy that broadens the toolbox available for tea-mediated AgNPs synthesis beyond single-extract protocols. AgNPs formation was confirmed by the characteristics SPR absorption band at 415 nm in the UV-Vis spectrum and an integrated characterization approach spanning XRD, SEM, TEM, EDX, TGA and PL

analysis established the formation of stable, highly crystalline, spherical and well-dispersed nanoparticles with sizes ranging from 5 to 20 nm, capped by a thermally robust organic layer. The biosynthesized AgNPs exhibited significant, broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacterial strains, *E. coli* ATCC 25922, *P. aeruginosa* PAO1, β -lactamase-positive *Klebsiella* sp. and *S. aureus* ATCC 25923 with retained efficacy against a β -lactamase-positive isolate underscoring their relevance to the challenge of antimicrobial resistance. Therefore, this study demonstrates that tea leaf extract/oleate-mediated AgNPs constitute an environmentally benign, structurally well-defined and scalable nanomaterials with promising broad-spectrum antimicrobial applications, motivating future work on their mechanistic mode of action, in vivo efficacy and formulation into antimicrobial coatings or wound-care products.

Declarations

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Data availability

Data will be made available on request from the corresponding author.

Ethics approval

The plant material used in this study was identified as *Camellia sinensis* by Dr Mohd. Asif, Drug Standardization Research Institute, CCRUM, Ministry of Ayush, Ghaziabad, U.P, India. The collection and use of the plant material complied with applicable local and national guidelines. No human participants or live vertebrate animals were involved in this study; therefore, ethical approval was not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Consent to publish

Not applicable.

Competing interests

The authors declare no competing interests.

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Figures

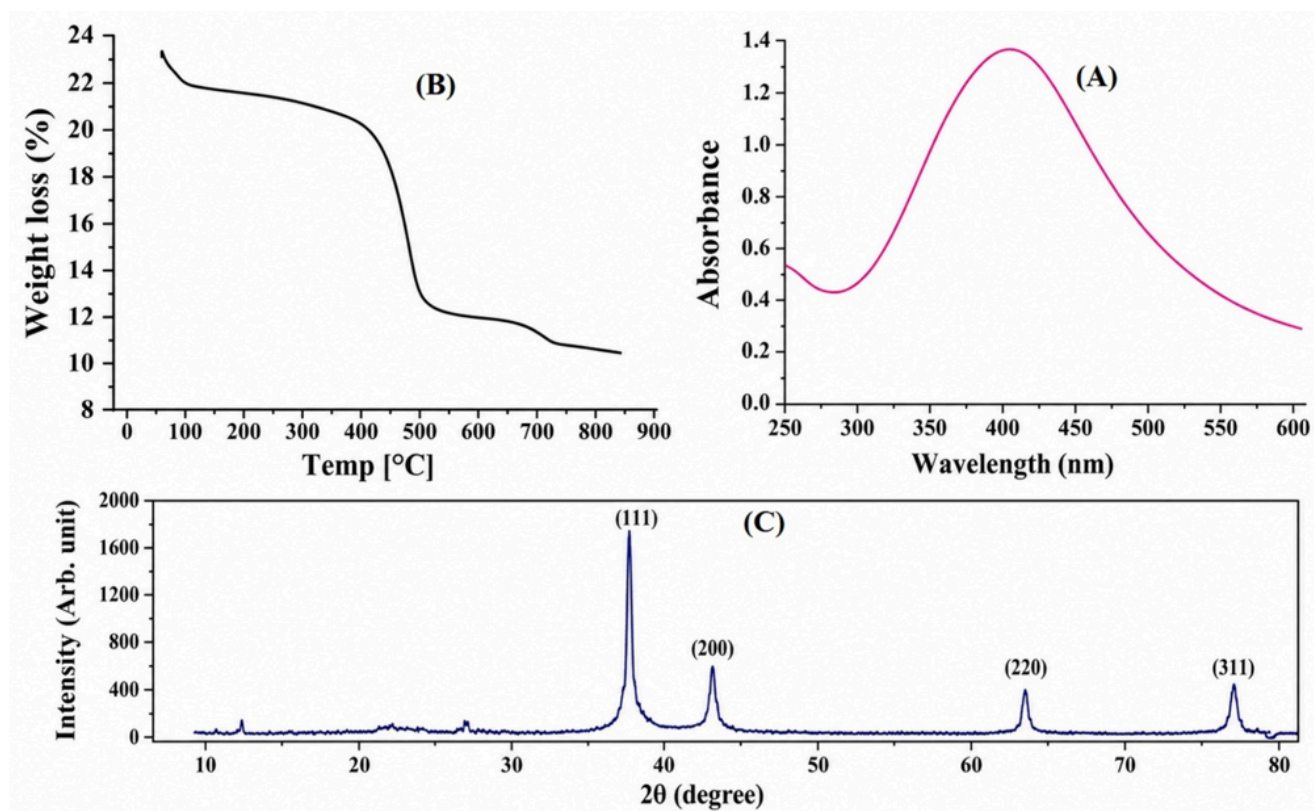


Figure 1

Characterization of the synthesized nanoparticles: (A) UV-Vis absorption spectrum; (B) TGA curve showing thermal stability and (C) XRD pattern confirming the crystalline structure with indexed diffraction planes (111), (200) and (311).

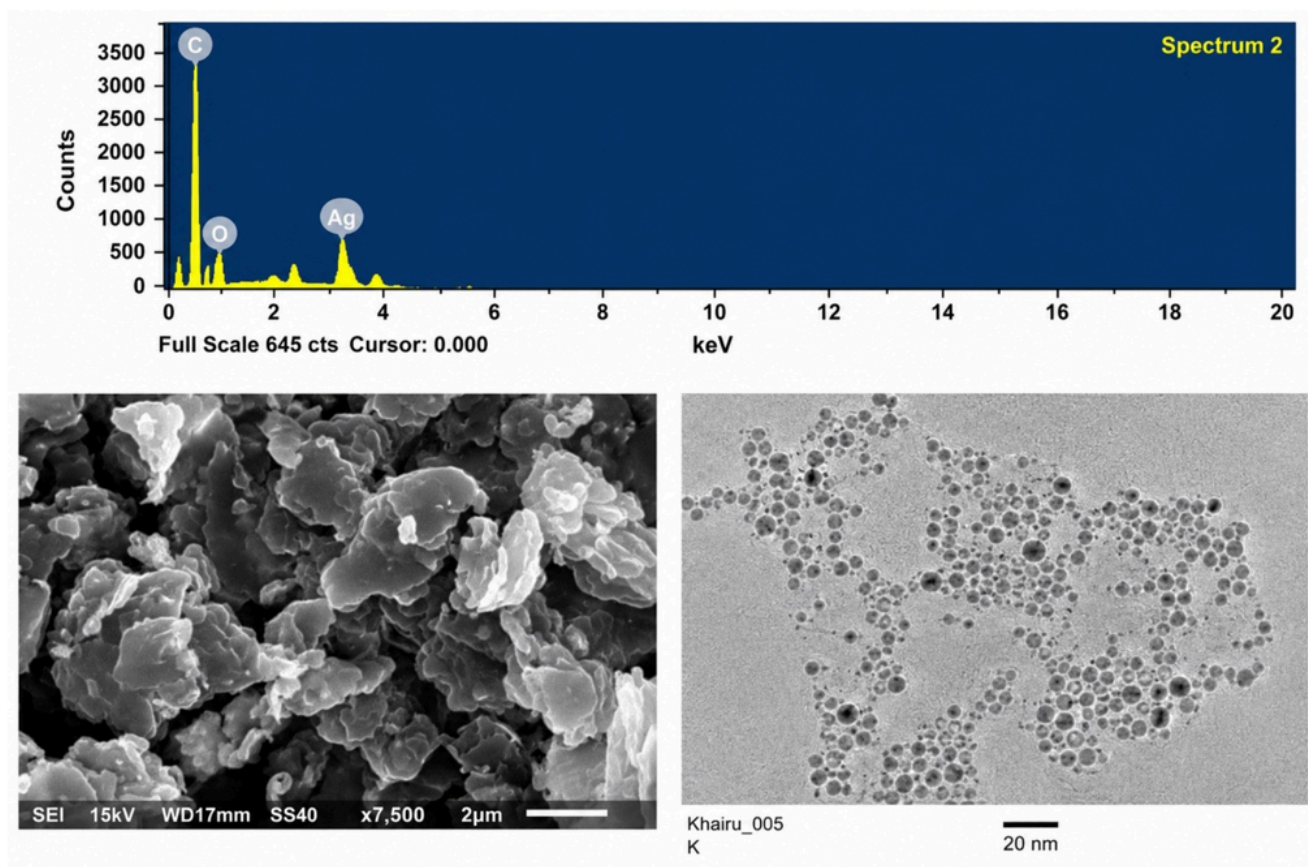


Figure 2

Characterization of synthesized AgNPs showing the EDX spectrum (top), SEM micrograph (bottom left) and TEM micrograph (bottom right).

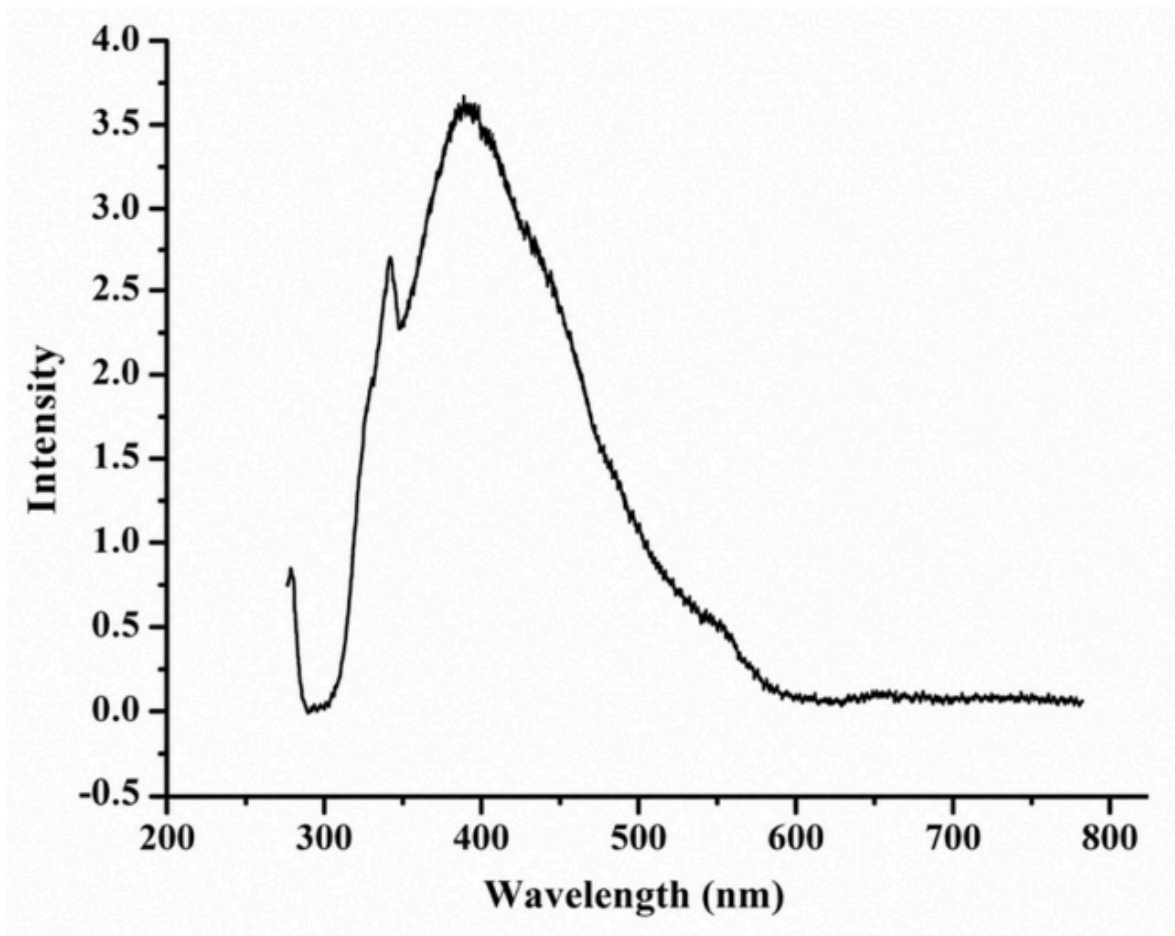


Figure 3

Photoluminescence (PL) emission spectrum of the synthesized AgNPs

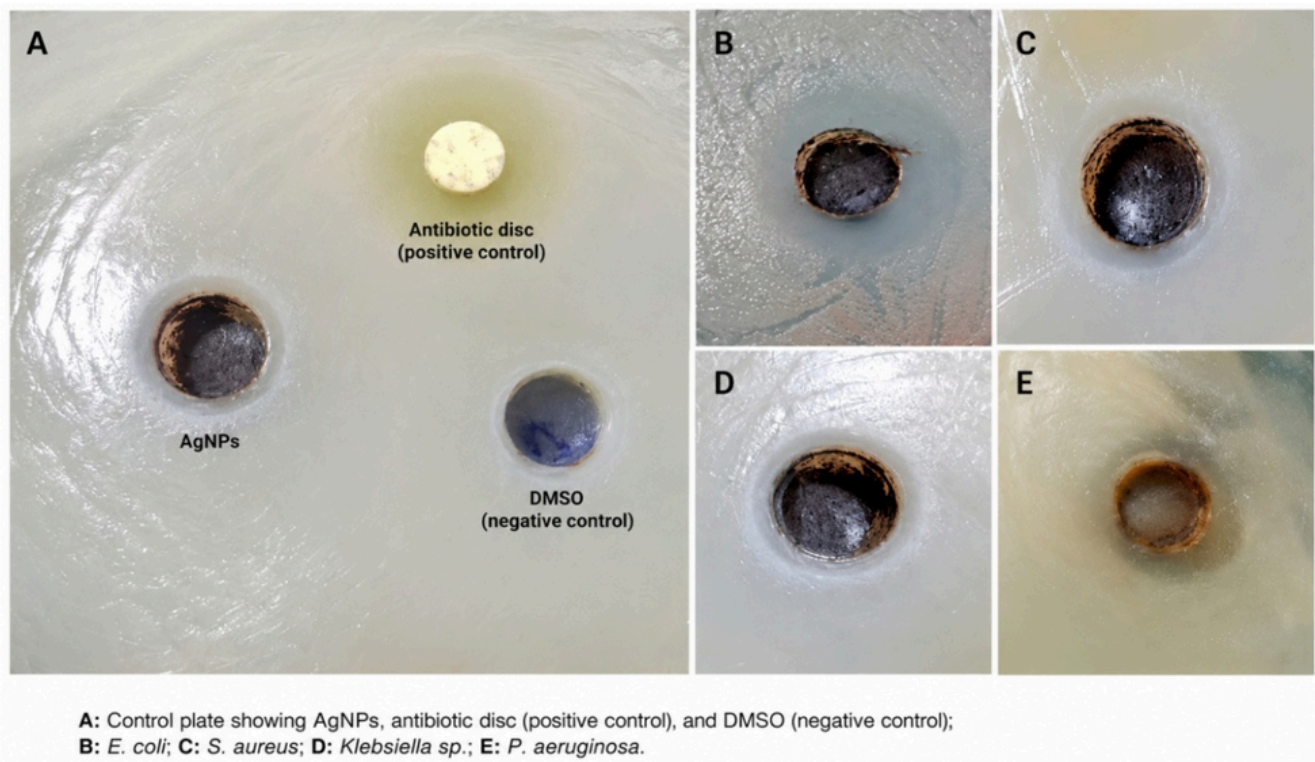


Figure 4

Antibacterial activity of the synthesized AgNPs evaluated by the agar well diffusion method. (A) Representative assay showing AgNPs, antibiotic disc (positive control), and DMSO (negative control). Zones of inhibition against; (B) *Escherichia coli*; (C) *Staphylococcus aureus*; (D) *Klebsiella* sp.; and (E) *Pseudomonas aeruginosa*.