

Biofabrication And Antitumor Activity Of Silver Nanoparticles Utilizing Novel *Nostoc sp.* Bahar M

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Mashaël Mohammed Bin-Meferij¹

Reham Samir Hamida²

¹Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia; ²Molecular Biology Unit, Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt

Background: Over recent years, green chemistry procedures have been developed to synthesize nanoparticles in eco-friendlier and less expensive ways. These procedures use natural sources such as bacteria, fungi, yeast, plants, actinomycetes, algae, or cyanobacteria, or use biomolecules such as proteins, vitamins, or pigments instead of chemical materials to fabricate salt precursors into nanoparticles.

Methodology: In the current investigation, we developed an effective, inexpensive, non-toxic method to synthesize silver nanoparticles (SNPs) using the cellular extract of a novel strain of cyanobacterium, *Nostoc sp.* Bahar M. SNPs were characterized using ultraviolet-visible spectroscopy, Fourier-transform infrared spectroscopy, X-ray diffraction, scanning electron microscopy, and transmission electron microscopy. The antitumor properties of the biogenic SNPs were tested against Caco-2 cells using a cell proliferation assay and inverted light microscopy.

Results: The new strain *Nostoc sp.* Bahar M was able to fabricate small SNPs from silver nitrate through an eco-friendly and inexpensive biosynthesis process. SNPs synthesis was accompanied by a color transformation from pale yellow to dark brown. Ultraviolet spectroscopy showed an absorption peak at 403 nm, confirming SNPs formation. X-ray diffraction analysis indicated that the SNPs had a face-centered cubic crystalline structure. Fourier-transform infrared spectroscopy was used to identify a protein that may play an important role in SNPs biosynthesis. Scanning and transmission electron micrographs showed that the SNPs were uniformly distributed and spherical in shape, with an average diameter of 14.9 nm. Cytotoxicity assays showed that SNPs exhibited a significant dose-dependent cytotoxic activity against human colon cancer cells with an IC₅₀ of 150 µg/mL.

Conclusion: *Nostoc sp.* Bahar M provided an eco-friendly route for fabricating SNPs, which have cytotoxic activity toward Caco-2 cells.

Keywords: cyanobacteria, nanotechnology, eco-friendly, Caco-2 cells, cytotoxic activity

Introduction

Nanotechnology and its products have improved daily life and enhanced industrial and commercial development in various sectors.¹ For example, nanoparticles (NPs) have advanced the fields of bio-imaging² and drug delivery.³ Different methods have been employed to successfully synthesize NPs, including chemical, physical, and green chemistry methods.⁴ The green synthesis approach of nanoparticles has been centered comparatively with other traditional routes due to its unique properties that have enhanced its industrial applications.⁵ Green synthesis of NPs involves using microorganisms,^{5,6} plants,^{7,8} enzymes,⁹ or vitamins¹⁰ to fabricate precursor materials into NPs under suitable production conditions.¹¹ To be considered a nanomaterial or

Correspondence: Reham Samir Hamida
Department of Molecular Biology, Faculty of Science, Alexandria University, Baghdad St, Moharam Bek, Alexandria, Egypt
Tel +201156298937
Email reham.hussein@alexu.edu.eg

nanoparticle, the material or particle must have at least one dimension in the size range of 1 to 100 nm.¹²

Compared to chemical and physical methods, biological synthesis of NPs is reliable, cost-effective,¹³ cytotoxic to microorganisms,¹⁴ and cytotoxic to tumor cells.¹⁵ Furthermore, chemical synthesis of metal NPs creates highly toxic by-products, requires a high energy input, and is expensive to perform.^{16,17}

Several organisms are able to produce metallic NPs,^{18,19} including yeast, bacteria, actinomycetes, fungi, plants, algae, and cyanobacteria.²⁰ Many researchers have begun to use cyanobacteria to manufacture NPs.^{21,22}

Cyanobacteria are a large, ancient group of photoautotrophic prokaryotes.²³ They are a valuable source of many chemicals, pharmaceuticals, biofuels, pigments, and proteins.²⁴ *Nostoc* is a genus of cyanobacteria that lives in various ecological habitats such as soil and hot springs.²⁵ *Nostoc* is able to control its habitats by producing bio-active compounds,²⁶ making this microorganism a valuable reservoir of diverse products.²⁷ These compounds possess a variety of biological activities and chemical structures, and include cyclic and linear lipopeptides, fatty acids, alkaloids, and other organic chemicals.²⁸ Many reports have mentioned the antibacterial,²⁹ antifungal,³⁰ and cytotoxic³¹ properties of these compounds. Extract from blue green algae contains several types of biomolecules that can perform reduction and capping reactions in the reduction and the stabilization processes of synthesizing NPs.^{24,32} Many cyanobacteria, including *Anabaena sp.* and *Spirulina sp.*, have been reported to fabricate intracellular silver and gold NPs.^{21,33} Silver NPs (SNPs) were synthesized by whole cells of non-nitrogen-fixing cyanobacteria such as *Plectonema boryanum* and the marine *Oscillatoria willei*.^{34,35} Recently, SNPs have been synthesized using cell extracts of *Nostoc sp.* HKAR-2 and the extracted phycobilin pigment of *Nostoc sp.*^{36,37}

Biogenic SNPs have been shown to have antimicrobial,³⁸ anticancer,³⁹ anti-inflammatory,⁴⁰ and anti-diabetic⁴¹ properties. In addition, these particles have been used to develop medicine and in various industries.^{42,43} They are used in water purification,⁴⁴ textile engineering,⁴⁵ detergents, and personal care products such as soaps,⁴⁶ and can act as biosensors, surgical instruments, bone cement, and surgical masks.⁴⁷

Cancer is a disease with devastating effects on human health. It is estimated to be the second most common cause of death worldwide.⁴⁸ New compounds or methods to reduce the global effects of this disease are constantly pursued. Green NPs have been reported to prevent and inhibit the progression of malignant cells.^{49,50} For

example, SNPs synthesized by *Nostoc sp.* HKAR-2 showed significant cytotoxicity towards the human breast cancer cell line MCF-7.³⁶

In the present work, a novel cyanobacteria strain *Nostoc sp.* Bahar M was used to synthesize SNPs in an eco-friendly manner. The biofabricated SNPs were characterized and their anticancer activity toward the human colon cancer cell line Caco-2 was evaluated.

Materials And Methods

Material

SNPs were synthesized from *Nostoc sp.* extract. Silver nitrate (AgNO₃) and cell culture materials were purchased from Sigma-Aldrich (St. Louis, MO, USA). Colon carcinoma cells (Caco-2) were purchased from the Medical Research Institute (Alexandria, Egypt).

Methods

Sample Collection And Purification

Cyanobacteria isolates were collected from soil in Alexandria, Egypt and kept sterile until reaching the laboratory. Samples were purified following the method developed by Bolch et al.⁵¹ Briefly, samples were diluted 15-fold using sterile BG11 media (pH = 7). Drops of the diluted samples were spread on BG11 agar plates and incubated at typical temperature and under light intensity of 2000 ± 200 lux provided by a cool white fluorescent lamp. An inverted light microscope was used to identify plates containing purified colonies suitable for large scale growth. Colonies were inoculated into 500 mL sterile flasks containing aqueous modified BG11 media without any nitrogen source and incubated with agitation for 15 days at room temperature.

Identification Of *Nostoc sp*

Morphological Examination

Aliquots of the cyanobacteria were examined using an inverted light microscope (Optika, Ponteranica BG, Italy). Taxonomic identification of the isolates was based on their morphology.⁵²

Molecular Identification

DNA extraction. DNA was extracted from the microorganisms following the method of Singh et al.⁵³ Purified DNA was analyzed by gel electrophoresis (ReadyAgarose™ Precast Gel System Bio-Rad Laboratories, Inc., California, USA) to check for DNA contamination or fragmentation.

Polymerase chain reaction amplification and Sanger's dideoxynucleotide sequencing of 16S rRNA. DNA concentration was measured with a spectrophotometer (Jenway, Staffordshire, OSA, UK). DNA was amplified by polymerase chain reaction (PCR) (Multigene Optimax, Labnet International, Inc., Edison, NJ, USA) using suitable primers; the forward primer was 5'-AGAGTTTGATCMTGGCTCAG-3' (position 8 in the 16S rRNA gene according to *E. coli* numbering) and the reverse primer was 3'TACGGYAC CTTGTTACGACTT-5' (position 1514 in the 16S rRNA gene according to *E. coli* numbering). After gel electrophoresis to confirm the success of the previous steps, the amplified DNA was stored in nuclease-free water and sequenced using an ABI 3730 DNA sequencer (Thermo Fisher Scientific, Waltham, MA, USA).

Phylogenetic analysis. Genetic data were analyzed using a BLAST search (<http://www.ncbi.nlm.nih.gov/>) to compare this sequence with other cyanobacterial 16S rRNA sequences in GenBank. This phylogenetic analysis was performed to identify the relation between *Nostoc sp.* and the closely related cyanobacteria. Multi-sequence alignment was performed using Clustal W (<https://www.genome.jp/tools-bin/clustalw>) and the phylogenetic tree was created using MEGA software version 4.0.2.

Preparation Of Cyanobacteria Extract

After two weeks of growth, the purified *Nostoc sp.* culture was harvested and washed by centrifugation at 4000 rpm. The biomass was freeze-dried and ground using a mortar and pestle, and 20 mg of the resulting fine powder was suspended in 20 mL of sterile water. This mixture was incubated at 30°C for 24 h, then filtered with Whatman filter paper no.1 (Camlab, Cambridge, United Kingdom).

SNPs Fabrication By Cyanobacteria

For SNPs synthesis, 10 mL of the filtrate was mixed with 90 mL of 1 mM AgNO₃ solution at room temperature for 24 h in the dark. Another sterile flask of AgNO₃ solution without cyanobacteria filtrate was prepared as a positive control. After 24 h, the mixtures were centrifuged at 4°C for 30 min at 10,000 rpm. The resultant pellet was washed at least three times to eliminate uncapping nanomaterial and spread on sterile plates to dry at 40°C for 24 h. Dried powder was collected for further study.

Physicochemical Analysis Of The Biogenic SNPs

UV-Visible Spectroscopy

After the biofabrication process, aliquots (1 mL) of the SNPs mixture were taken for UV monitoring using a UV

spectrophotometer (UV1800 PC spectrophotometer, Shimadzu, Japan) from 300 to 700 nm.

X-Ray Diffraction

X-ray diffraction (XRD) (X-ray 7000, Shimadzu, Japan) was performed to confirm the crystalline nature of the biogenic SNPs. Biogenic SNPs were drop-coated onto a silica plate with intermittent drying to obtain a thick coat of SNPs for XRD. Diffraction patterns were obtained at 30 kV and 30 mA in Cu, K-alpha radiation, with a Bragg angle (2θ) range of 0° to 80° and scan speed 4 deg/min.

Fourier-Transform Infrared Spectroscopy

Fourier-transform infrared (FTIR) spectroscopy was used to detect the biomolecules in the extracts responsible for NPs synthesis. Samples were prepared by diluting biogenic SNPs powders 1:100 in potassium bromide. Diffuse reflectance FTIR (Shimadzu, Japan) was measured from 400 to 4000 cm⁻¹ at a resolution of 4 cm⁻¹. FTIR results were compared to a reference chart to identify functional groups in the target sample.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) (Jeol, Tokyo, Japan) was used to characterize SNPs morphology. Briefly, a small amount of an aqueous solution of SNPs was placed onto a carbon coated copper grid, and extra solution was removed from the grid with blotting paper. The thin film on the grid was dried under a mercury lamp for 5 min before examination.

Transmission Electron Microscopy

Transmission electron microscopy (TEM) images were obtained to examine the shape and size of the biosynthesized SNPs. TEM (Jeol, Tokyo, Japan) was performed at an accelerating voltage of 200 kV. After sonication for 5 min, a drop of SNPs solution was placed on a carbon coated copper grid and dried under an infrared lamp before TEM evaluation.

Cell Culture

In brief, the Caco-2 cell line was maintained in Dulbecco's modified Eagle's Minimum Essential Medium with 10% fetal bovine serum and 50 IU/mL penicillin and streptomycin. Cells were allowed to grow in a humidified atmosphere containing 5% CO₂ and 95% air at 37°C. Confluent cells were passaged with trypsin-EDTA.

Cell Proliferation Assay

The cytotoxicity of biogenic SNPs synthesized by *Nostoc* sp. was evaluated after 24 h of incubation against Caco-2 cells with the MTT tetrazolium reduction assay. Cells were seeded in 96-well plates with 7×10^3 cells/well. SNPs were filtered through 0.22 μm sterile filters. After achieving cell culture confluence, cells were subjected to different SNPs concentrations from 31.25 $\mu\text{g/mL}$ to 1000 $\mu\text{g/mL}$. After 24 h the medium was discarded and replaced with 100 μL of fresh culture medium, and 10 μL of 12 mM MTT stock solution was added to each well. As a negative control, 10 μL of 12 mM MTT stock solution was added to 100 μL of the medium alone. Cells were incubated for 4 h at 37°C and 100 μL DMSO was added to each well with thorough mixing to dissolve the formazan crystal. The absorbance intensity was measured at 570 nm by enzyme-linked immunosorbent assay (ELISA) (Bio-Rad Laboratories, Hercules, CA, USA). In ELISA, absorbance is directly related to the number of viable cells, allowing the half-maximal growth inhibitory concentration (IC_{50}) to be estimated.

Morphological Observation Of Cells

Cell morphology was examined before and after SNPs treatment using an inverted light microscope (Optika, Ponteranica BG, Italy) at 400X magnification. Cells were seeded onto 12-well plates to be sub-confluent, treated with biogenic SNPs at IC_{50} and IC_{25} (150 $\mu\text{g/mL}$ and 75 $\mu\text{g/mL}$, respectively), and incubated for 24 h at 37°C. Untreated control cells were also incubated for 24 h.

Statistical Analysis

All results are presented as mean values of three independent replicates (mean \pm SD) and statistical analyses were done by one-way ANOVA analysis of variance using Prism 6 software (GraphPad Software Inc., La Jolla, CA, USA). Values were considered statistically significant at $P < 0.01$ and $P < 0.001$. Origin8 (OriginLab Corporation, Northampton, MA, USA) and ImageJ (National Institutes of Health, Bethesda, MD, USA) were also used to analyze NPs.

Results And Discussion

Inverted light micrographs of *Nostoc* sp. showed barrel-shaped trichomes (Figure 1A). In addition, several spherical cells enveloped by a sheath were observed, and these cells may be coccoid cells (Figure 1B). These data are in agreement with data reported by

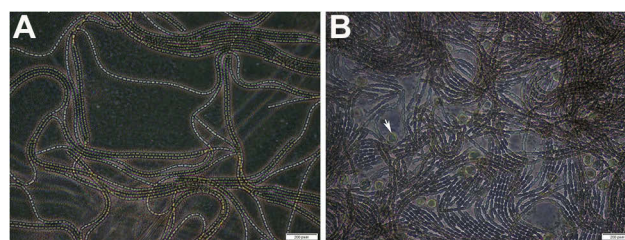


Figure 1 Inverted light micrographs of *Nostoc* Bahar M strain.

Notes: (A) Barrel-shaped *Nostoc* trichomes. (B) Coccoid cells (arrow) surrounded by a sheath.

Nowruzi et al²⁵ who identified and studied the toxicity of *Nostoc* sp.

In the current study, whole genomic DNA was extracted, purified, and used as a PCR template. Agarose gel electrophoresis of the resultant amplicon (1.5 kb) of the selected isolate is shown in (Figure 2). The phylogeny of the selected strain was analyzed using a multi-sequence alignment program, and the results were used to create a phylogenetic tree (Figure 3). The AM1 strain was 97% similar to *Nostoc* sp. Bahar M.²⁵ Sequence data for the selected isolate was deposited in GenBank as MN423202.

The primary indicator of SNPs synthesis was the color change from greenish-brown to pale yellow, gradually

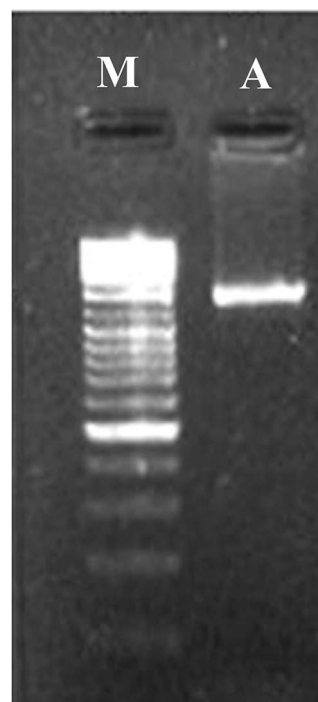


Figure 2 Electrophoresis of the extracted genomic DNA in a 1% agarose gel.

Notes: (A) 16S rDNA amplified by PCR from genomic DNA of *Nostoc*. (M) Molecular marker 1500 bp.

Abbreviation: PCR, polymerase chain reaction.

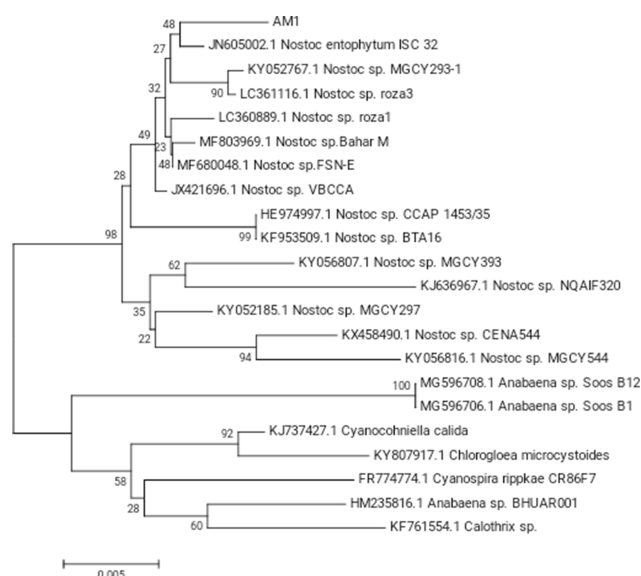


Figure 3 The phylogenetic tree of *Nostoc* sp. based on partial sequencing of 16S rRNA.

becoming dark brown with increased incubation time. This color change is due to the excitation of surface plasmon resonance (SPR). The biogenic SNPs had a peak at 403 nm (Figure 4), indicating SNPs fabrication from bulk AgNO_3 . This finding agrees with Ahmed et al, who reported that biogenic SNPs synthesized by *Nostoc* sp. showed SPR peaks centered at 400–402 nm.⁵⁴ On the other hand, other publications that used *Nostoc linckia*, and *Nostoc commune* to synthesize SNPs reported SPR peaks at 415 and 435 nm, respectively.^{55,56} Furthermore, SNPs produced from the reaction of phycoerythrin and an aqueous AgNO_3 solution had a distinct peak at 430 nm.³⁷

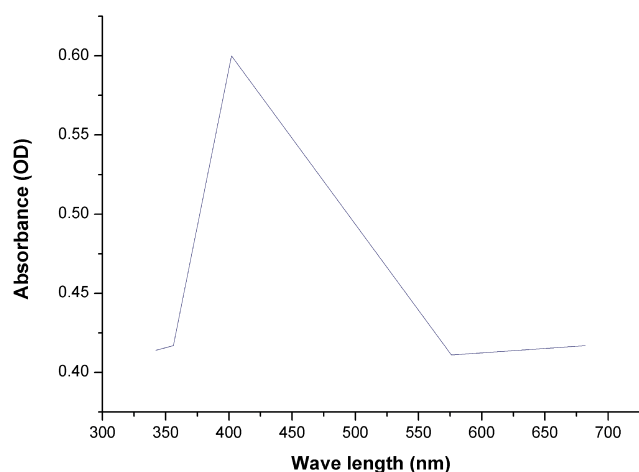


Figure 4 UV-vis absorption spectra of biogenic SNPs synthesized by *Nostoc* sp.

Note: The plasmon resonance of SNPs at 403 nm.

Abbreviations: UV-vis, ultraviolet-visible; SNPs, silver nanoparticles.

The different SPR values may be related to the different strains of *Nostoc* sp. and different biomolecules involved in producing SNPs.

The XRD pattern of SNPs synthesized by *Nostoc* sp. Bahar M showed four intense peaks (Figure 5) with 2θ values of 38.2, 45.3, 67.44, and 75.25°, related to the silver (111), (200), (220), and (300) planes, respectively. Comparisons of each spectrum with standards from the Joint Committee on Powder Diffraction Standards library helped determine that the SNPs were crystalline. The current results are similar to the 2θ values of SNPs fabricated by *Streptomyces* sp. and *Coleus aromaticus*, which

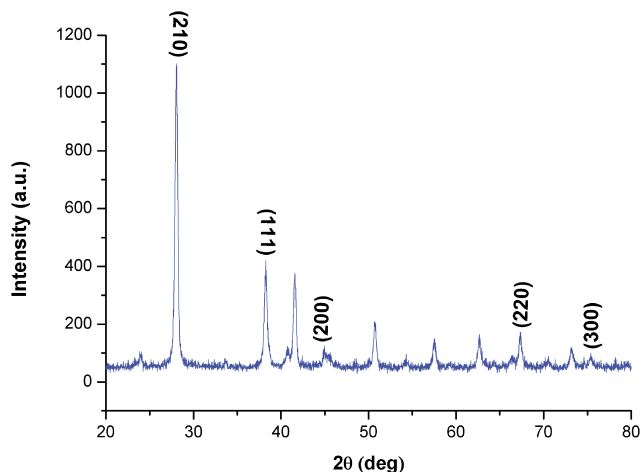


Figure 5 XRD pattern of biogenic SNPs synthesized by *Nostoc* sp.

Note: Four peaks at 3.5 KeV ranging from 0° to 80° at 2θ values for SNPs.

Abbreviations: XRD, x-ray diffraction; SNPs, silver nanoparticles.

were reported to be 45.3, 27.51, 75.2, and 67.44°. ^{57,58} A sharp peak at 2θ of 28° was recorded by XRD. This peak might be related to the crystallization of biogenic groups on the NP surface. ⁵⁹ XRD provides information about the shape, size, and orientation of the SNPs. ³⁷ SNP crystal size, calculated from the highest intensive signal (111) using the Scherrer equation, ⁶⁰ $D = 0.9\lambda/\beta \cos \theta$, was found to be 3.8 nm. The interplanar distance of crystal particles (d), calculated using Bragg's law, $d = n \lambda/2 \sin \theta$, was found to be 0.26 nm which is corresponding to the (111) plane of metallic silver.

FTIR analysis distinctly showed the spectral wave-numbers of the bioactive groups surrounding the biogenic SNPs that were responsible for SNPs fabrication and stabilization. FTIR spectroscopy of reduced SNPs (Figure 6) showed 14 absorption peaks, including peaks at 1119.01 cm^{-1} (C-O secondary alcohol), 1397.07 cm^{-1} (O-H carboxylic acid), 1632.35 cm^{-1} (N-H stretching amine), 1777.14 cm^{-1} (C=O stretching anhydride or vinyl/phenyl ester), 2114.44 cm^{-1} (N=C=S stretching isothiocyanate), 2946.98 cm^{-1} (stretching O-H carboxylic acid or N-H amine salt), 3460.32 cm^{-1} (O-H stretching alcohol), and 842.59 cm^{-1} (stretching alkene C=C). Based on the FTIR results, the aromatic compound may represent the reducing ligand of the SNPs during the synthesis process. Moreover, the existence of amine groups on the NP surface was supported by FTIR analysis, indicating that proteins may act as capping ligands and prevent agglomeration of reduced SNPs. ^{55,61} Gole et al ⁶² explained that proteins can bind NPs through negatively charged carboxylate groups, cysteine residues, or free amine groups in the proteins.

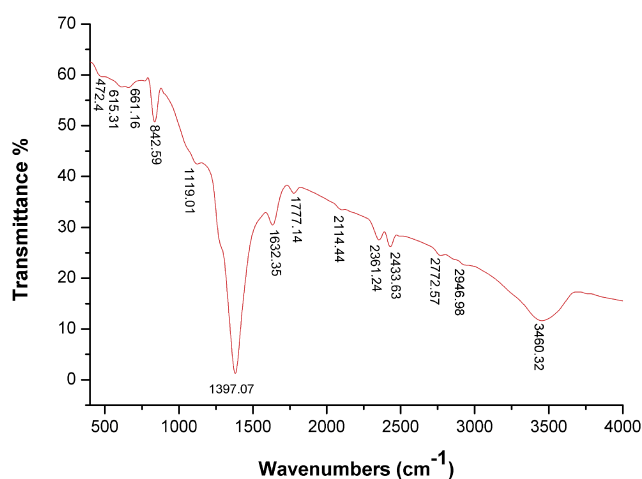


Figure 6 FTIR spectra of biogenic SNPs.

Abbreviations: FTIR, Fourier-transform infrared; SNPs, silver nanoparticles.

Sastry et al ⁶³ emphasized that functional groups such as $-\text{C}-\text{O}-\text{C}-$, $-\text{C}=\text{C}-$ and $-\text{C}-\text{O}-$ that exist in heterocyclic compounds like proteins play important roles in stabilizing and reducing NPs. Although the exact mechanism by which biomolecules in the cell extract are involved in NP synthesis is poorly understood, El-Batal et al ⁶⁴ revealed that an NADH-dependent reductase was critical in SNPs biosynthesis. El-Naggar et al ⁶¹ also stated that biomolecules (eg proteins, enzymes, amino acids, carbohydrates, photosynthetic pigments, carotenoids, and vitamins) present in cell extracts have been implicated in the reduction of Ag^+ ions.

SEM micrographs revealed that SNPs fabricated by *Nostoc sp.* had a spherical shape and ranged from 30 to 50 nm, with some SNPs forming larger agglomerates (Figure 7). NPs agglomeration may be due to the aqueous extract of *Nostoc sp.* around the SNPs. ⁶⁵ Sonker et al ³⁶ used SEM imaging to determine that the size of silver synthesized by *Nostoc sp.* HKAR-2 ranged from 51 to 100 nm, while Anderson et al ⁶⁶ reported that the hydrodynamic diameter of SNPs synthesized by seaweed extract was between 20 and 95 nm. Moreover, Al Rashid et al mentioned that SEM micrograph of biosynthesized SNPs by *Momordica charantia* fruit extract showed that SNPs were spherical and have diameter range from 78.5 to 100 nm. ⁶⁷ It is noteworthy that the Bahar M strain can reduce AgNO_3 into smaller size SNPs with less size range, a property that highlights the possible applications of SNPs synthesized by *Nostoc sp.* Bahar M in diverse fields.

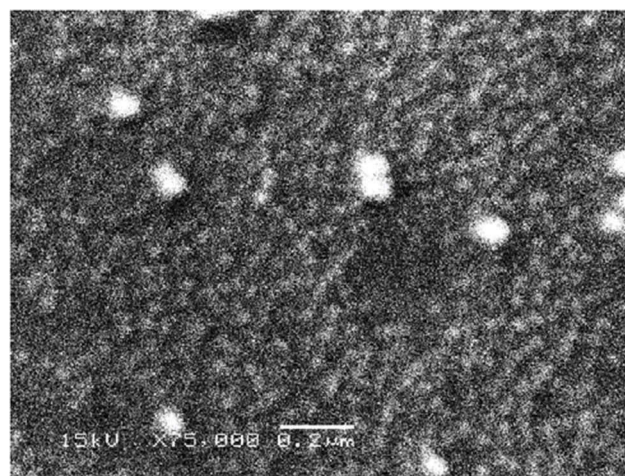


Figure 7 SEM examination of SNPs.

Notes: SEM image showing the spherical shape of SNPs, with some SNPs aggregations. Scale bar, 0.2 μm .

Abbreviations: SEM, scanning electron microscope; SNPs, silver nanoparticles.

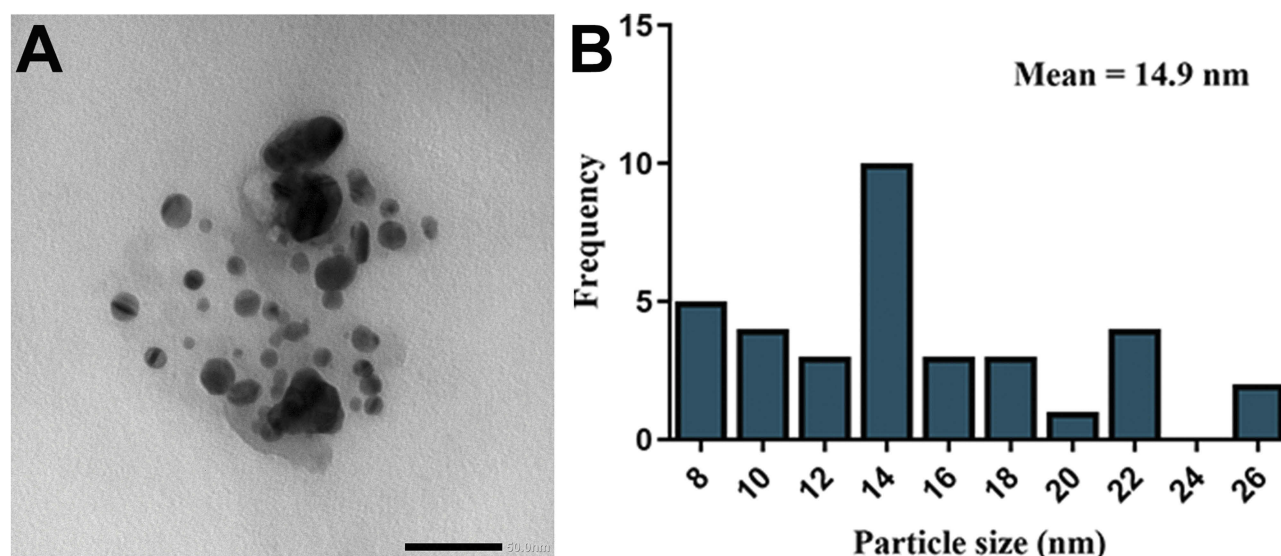


Figure 8 TEM examination and size distribution of SNPs.

Notes: (A) TEM micrograph exhibiting the spherical shape of SNPs with size range of 8.5 to 26.44 nm. Scale bar, 50 nm. (B) SNPs size distribution from TEM analysis, with average size of 14.9 nm.

Abbreviations: TEM, transmission electron microscope; SNPs, silver nanoparticles.

TEM was employed to characterize the size and shape of synthesized SNPs. TEM images revealed that biogenic SNPs had a spherical shape (Figure 8A), consistent with the shape of an SPR band in the UV-visible (UV-vis) spectrum. Moreover, the NP size ranged from 8.5 to 26.44 nm, with an average particle size of 14.9 nm (Figure 8B).³⁷ Also, TEM micrograph showed that the inter particle distance was equal to 31.4 nm. Morsy et al⁵⁵ reported that the SNPs fabricated using cellular extract of *Nostoc commune* were spherical and had sizes from 15 to 51 nm. The current results compared with data from Morsy et al⁵⁵ demonstrate the potential of the *Nostoc* Bahar M strain to fabricate smaller SNPs. Creating particles have smaller nano-size scale will encourage researchers to use various cyanobacteria for biosynthesis of different NPs.

To the best of our knowledge, this is the first report regarding the anticancer activity of SNPs biosynthesized using the cell extract of *Nostoc sp.* Bahar M on Caco-2 cells. SNPs suppressed cell proliferation in a dose-dependent manner compared with untreated cells (Figure 9A). The IC₅₀ of SNPs was 150 µg/mL (Figure 9B). Martins et al⁶⁸ tested the influence of a polyelectrolyte complex containing SNPs against Caco-2 cells and healthy African green monkey cells (VERO cells) and found that the SNPs exhibited cytotoxic effects on Caco-2 cells, but not VERO cells, at concentrations above 100 µg/mL. Song et al⁶⁹ reported that SNPs showed slight cytotoxic activity against

Caco-2 cells at 10, 25, 50, 100, and 200 µg/mL. Our results indicate that SNPs synthesized using *Nostoc sp.* Bahar M are more potent antitumor agents against Caco-2 cells than the chemically synthesized SNPs studied by Martins et al⁶⁸ and Song et al.⁶⁹

Control cells showed a normal distribution, with a confluent monolayer of cells attached to the plate and a small distance between cells (Figure 10A). Caco-2 cells treated with SNPs at IC₂₅ exhibited moderate morphological changes, including cell detachment, shrinkage, and clustering, with restricted cell spreading (Figure 10B). However, Caco-2 cells treated with SNPs at IC₅₀ exhibited more drastic changes, including cell rounding and features characteristic of apoptotic cell death, in a dose-dependent manner (Figure 10C). SNP-treated colon cells exhibited morphological changes such as shrinkage, irregular shape and plasma membrane reupture.^{69,70} Likewise, Böhmert et al used inverted light microscopy to examine Caco-2 cells after SNP treatment and reported altered cell shape and cell adhesion capacity compared to control cells.⁷¹

The MTT data and cytomorphological observations indicate that the cytotoxic effect of SNPs toward Caco-2 cells may result from an interaction of SNPs with different organelles such as the mitochondria, nucleus, or endoplasmic reticulum, or through direct interaction with biomolecules such as DNA, enzymes, or proteins. This interaction may affect cell membrane integrity, lactate dehydrogenase

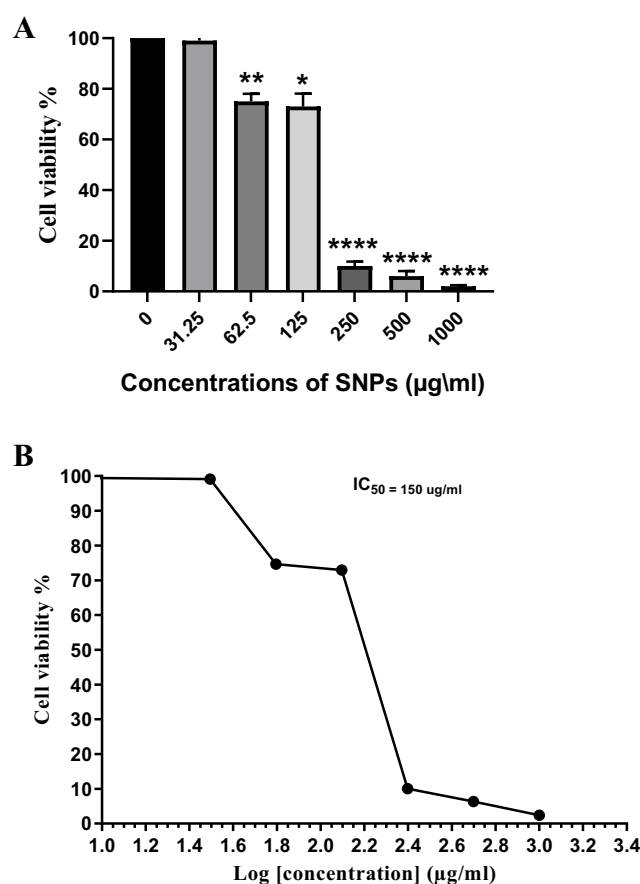


Figure 9 Dose-dependent growth inhibition of Caco-2 cells by SNPs.

Notes: (A) Antiproliferative effect of SNPs synthesized by *Nostoc* sp. against Caco-2 cell line expressed as percentage cell viability. (B) Caco-2 cell viability percentage and the log concentration at which cell viability is reduced to 50%. Data from at least three independent experiments performed in at least triplicate are presented as mean \pm SD; *P* value was calculated versus control cells: **P* < 0.05; ***P* < 0.01 and *****P* < 0.001.

Abbreviations: SNPs, silver nanoparticles; SD, standard deviation.

levels, and mitochondrial permeability, causing oxidative stress and cell death.³⁷

Conclusion

Nanomaterials represent an alternative strategy for cancer treatment to overcome multidrug resistance and several drawbacks of traditional therapies. Problems with nanomaterial production, including toxic by-products of their synthesis, limit their applications. A green chemistry approach to NPs synthesis offers a solution to safely produce NPs. The current study demonstrated the eco-friendly synthesis of SNPs using cellular extract from a new strain of cyanobacteria, *Nostoc* sp. Bahar M. These SNPs are spherical with diameters of 8.5 to 26.44 nm. The biogenic SNPs had potent antitumor activity against Caco-2 cells, and cytotoxicity data showed that it would be possible to utilize SNPs as

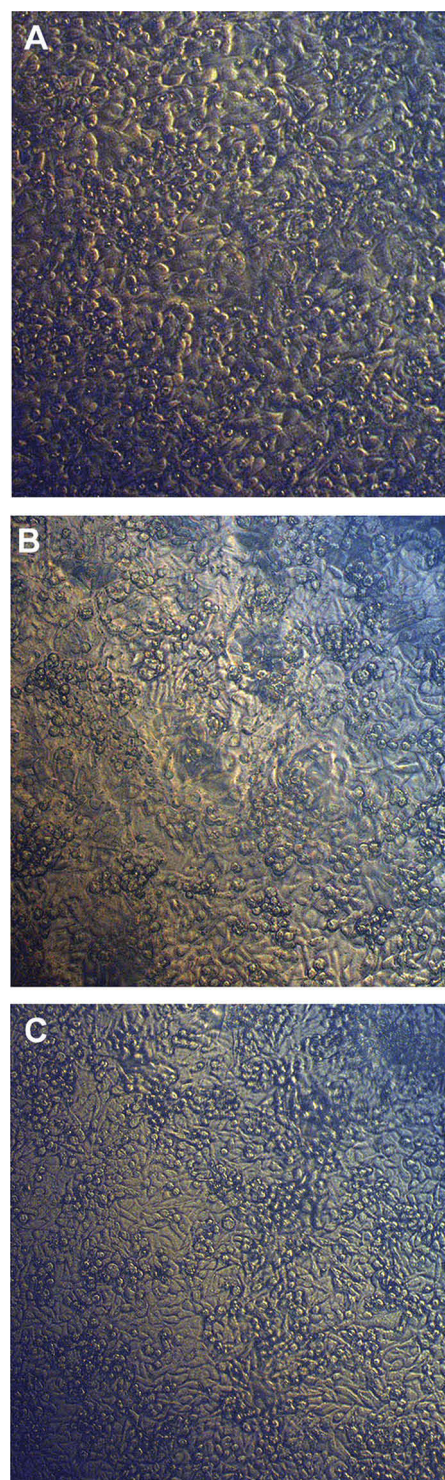


Figure 10 Inverted light micrographs of Caco-2 cells.

Notes: Morphological changes in Caco-2 cells before and after being treated with SNPs. (A) Control, (B) Caco-2 cells treated with IC₂₅ SNPs (75 µg/mL), (C) Caco-2 cells treated with IC₅₀ SNPs (150 µg/mL).

Abbreviation: SNPs, silver nanoparticles.

an antitumor agent against cancerous cells. Further research is needed to understand the mode of action of these NPs as anticancer agents.

Ethics Approval And Informed Consent

The current study followed the Research Ethical Committee guidelines published by the National Health and Medical Research Council and the Ministry of Health and Population in Egypt. The Department of Zoology (Science Faculty, Alexandria University) and the Department of Cancer Management and Research (Medical Research Institute, Alexandria University) granted permission for this work.

Abbreviations

NPs, Nanoparticles; SNPs, Silver nanoparticles; AgNO₃, Silver nitrate; PCR, Polymerase chain reaction; nm, Nanometer; min, Minute; μ L, Microliter; mg, Milligram; mL, Milliliter; μ g, Microgram; mM, Millimolar; h, Hour; cm⁻¹, Inverse centimeter; rpm, Revolutions per minute; SEM, Scanning electron microscope; TEM, Transmission electron microscope; DLS, Dynamic light scattering; FTIR, Fourier-transform infrared spectroscopy; XRD, X-ray diffraction; kV, Kilovolt; kb, Kilobase; SPR, Surface plasmon resonance; ELISA, Enzyme-linked immunosorbent assay; UV-vis, Ultraviolet-visible spectroscopy.

Data Availability

The data supporting this article are available in [Figures 1–10](#). The data sets analyzed in the present study are available from the corresponding author on reasonable request.

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Author Contributions

All authors contributed to data analysis and drafting or revising the article. All authors gave final approval of the published article and are accountable for all aspects of the work.

Disclosure

This research was included in a request for patent 119410021 from King Abdulaziz City for Science and Technology, submitted 5/9/2019, titled "Biosynthesis of silver nanoparticles as anticancer agents" Patents.kacst.edu.sa. The authors report no other conflicts of interest in this work.

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