ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Biosynthesized Silver Nanoparticles Using *Alnus nitida* Leaf Extract as a Potential Antioxidant and Anticancer Agent

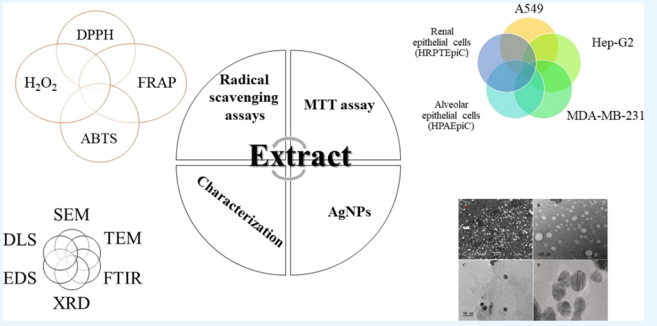
Fazli Khuda,* Meshal Gul, Atif Ali Khan Khalil, Sajid Ali, Naveed Ullah, Muhammad Shafiq Khan, Shabnam Nazir, Sumaira Irum Khan, Sultan Mehtap Büyüker, Saud Almawash,* Muhammad Shafique, and Sayed Afzal Shah

Cite This: *ACS Omega* 2023, 8, 30221−30230

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Biogenic synthesis of silver nanoparticles (AgNPs) using plant extracts is gaining attention as a substitute to the conventional physical and chemical synthesis methods. This study reports a facile, cost-effective, and ecofriendly synthesis of AgNPs using leaf extract of *Alnus nitida* (*A. nitida*) and their antioxidant and antiproliferative activities. The biosynthesized AgNPs were characterized using various analytical techniques including UV−visible spectroscopy, energy-dispersive spectrometry, scanning electron microscopy (SEM), Fourier transform infrared (FTIR), X-ray diffraction (XRD), and dynamic light scattering. The antioxidant and cytotoxic potential of the extract and AgNPs was evaluated using different in vitro models. The UV−vis analysis revealed a surface plasmon resonance peak of 400 nm corresponding to the synthesis of AgNPs. SEM analysis confirmed the formation of heterogeneously dispersed particles of nano size, while the XRD and FTIR spectra confirmed the crystallinity and existence of different functional groups that helped in capping and stability of AgNPs. The antioxidant activity of AgNPs and extract, studied by 1,1-diphenyl 2-picryl hydrazyl (DPPH), fluorescence recovery after photobleaching (FRAP), 2, 2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and H_2O_2 scavenging assays, showed a dose-dependent effect. The AgNPs at 1000 μg/mL significantly scavenged DPPH, FRAP, ABTS, and H_2O_2 by 66.45, 74.65, 78.81, and 72.56% with an average IC_{50} value of 33.31, 18.50, 16.46, and 15.65 μg/mL, respectively. The cytotoxic potential investigated by MTT assay revealed promising antiproliferative effects against different cancer cell lines. The IC_{50} values of AgNPs on MDA-MB-231, A549, and Hep-G2 cells were 14.88, 3.6, and 5.38 μg/mL, respectively. The results showed that AgNPs were more effective against lung and hepatocellular carcinoma. The selectivity index showed that AgNPs remained highly selective in retarding the growth of A549 and Hep-G2 cells as compared to normal cell lines HPAEpiC and HRPTEpiC. Overall, this study showed that biosynthesized AgNPs were associated with considerable antioxidant and cytotoxic effects. Our work suggests that *A. nitida*-mediated AgNPs should be evaluated further in order to develop safe and effective formulations for the treatment of different degenerative diseases.



1. INTRODUCTION

Cancer is one of the most devastating diseases, characterized by the uncontrolled division of mutated cells. Its pathogenesis is very complex and commonly caused by the genetic deregulation or mutation that results from exposure to environmental pollutants or xenobiotics.[1] The generation of free radicals in the human body is considered to be one of the major reasons for the development of cancer. The reactive oxygen species (ROS) produced during aerobic metabolism can injure various cellular biomolecules, causing damage to organs and tissues, causing a variety of degenerating disorders including cancer.[2] Antioxidants are capable of stabilizing ROS by donating electrons and thus inhibit its detrimental effects. Antioxidants include both endogenous and exogenous molecules. Endogenous antioxidants are synthesized by the body and include superoxide dismutase, while exogenous antioxidants are derived from both plants and animal sources. Polyphenols (flavonoids, lignans, and anthocyanins), vitamins (C and E), and carotenoids (xanthophylls) are some of the important natural antioxidants obtained from plant sources. Among these, vitamins E and C and β-carotenes have shown beneficial effects against breast, ovarian, and colorectal cancer.[3] Besides these, conventional therapies for the treatment of cancer includes chemotherapy, radiation, and surgery. Despite advances in cancer therapy, patient mortality is often high and

Received: April 28, 2023
Accepted: July 19, 2023
Published: August 14, 2023

there is a great risk of severe side effects. In addition, most chemotherapeutic agents have poor water solubility. Due to lower oral bioavailability, these drugs are administered in higher doses to achieve effective plasma concentrations, which may lead to adverse effects or systemic toxicity. Due to its small size and high surface area, nanoparticles (NPs) possess excellent solubility and dissolution profiles that not only improve the bioavailability of conventional chemotherapeutic agents but also reduce the dose and associated side effects.[4]

To date, different NPs have been reported including silver, gold, copper, carbon, zinc oxide, and titanium oxide.[5−7] Among these, silver NPs (AgNPs) are used in different fields such as catalysis, food industry, electronics, clothing, printing, photography, and dentistry. In addition, it has diverse biomedical applications such as antimicrobial, anticancer, antioxidant, anti-inflammatory, and antidiabetic.[8] Besides this, AgNPs can be exploited and used in water treatment, wound dressing ointments, food containers, and many more.[9] Various methods including physical, chemical, biological, and green approaches are adopted for the synthesis of metallic NPs.[10] Among biological methods, plant-mediated synthesis is a simple, rapid, and environment-friendly technique that utilizes various plant parts such as stem, bark, flowers, fruit, leaves, and seed extracts.[11,12] Nowadays, industrial wastes like sugarcane bagasse, rice, and coffee husk are also gaining popularity for the synthesis of NPs.[13−15] Moreover, various animal by-products such as sheep blood serum have been used for the synthesis of NPs.[16] Due to its availability and cost-effectiveness, plant extracts have gained popularity for research purposes. A large number of plants such as *Eugenia roxburghii*,[8] *Salacia oblonga*,[17] *Rubus ellipticus*,[18] *Protium serratum*,[10] *Teucrium apollinis*,[19] *Pueraria tuberosa*,[20] *Reynoutria japonica*,[21] and *Ganoderma applanatum*[22] have been used for the synthesis of metal NPs with improved biological activities. However, the production of AgNPs from indigenous plant species such as *A. nitida* has not been investigated to a large extent.

Alnus nitida (Spach) Endl. (family Betulaceae) (Figure 1), commonly known as Sharol in Urdu and Geiray in Pashto, is a

Figure 1. *Alnus nitida* (Spach) Endl. (photograph courtesy of Dr. Fazli Khuda; the photo is from a free domain).

deciduous tree that can grow 20 meters or more. The genus *Alnus*, native to Pakistan, Nepal, and the Western Himalayas, prefer to live along the banks of rivers. In Pakistan, it is mainly found in Murree, Dir, and Swat valley. Diarylheptanoids are the major phytochemicals isolated from *alnus* species. Besides this, it also contains flavonoids, glycosides, phenols, alkaloids, coumarins, saponins, anthraquinones, and tannins.[23] Traditionally, *alnus* species has been widely used for the treatment of several diseases including diabetes, fever, hemorrhages, diarrhea, inflammation, influenza, and different malignancies such as lungs, liver, and uterine cancer. These activities are attributed to the presence of diarylheptanoids.[24−26] The antioxidant and hepatoprotective effects of this plant is attributed to the high concentration of its total phenols and flavonoids contents.[23] Similarly, the antimicrobial, insecticidal, and leishmanicidal activities may be due to the presence of various glycosides and resinous components.[27]

It has been documented that AgNPs have intrinsic anticancer properties through several mechanisms including formation of free radicals. The radicals are formed when AgNPs are taken up by the living cells, which initiates deregulation of several cellular pathways, leading to cell damage or death.[28] This study is designed to combine the anticancer intrinsic potential of AgNPs with the antiproliferative potential of phytochemicals present in *A. nitida* leaves for the effective treatment of a variety of cancers. In present research, a simple, cost-effective, and eco-friendly green method was used for the synthesis of AgNPs in which various phytochemicals of the studied plant reduced Ag^+ ions into elemental AgNPs. Different analytical techniques including UV−visible and energy-dispersive spectrometry (EDS), scanning electron microscopy (SEM), Fourier transform infrared (FTIR), X-ray diffraction (XRD), and dynamic light scattering (DLS) were used for characterization of NPs. The synthesized NPs were investigated for antioxidant and anticancer potential using in vitro models.

2. MATERIALS AND METHODS

2.1. Materials. Analytical grade chemicals and reagents including hydrogen peroxide and 2, 2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1,1-diphenyl 2-picryl hydrazyl (DPPH), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), dimethyl sulfoxide (DMSO), silver nitrate, and phosphate buffer saline (PBS) were purchased from Sigma-Aldrich Chemicals Co., St. Louis, USA.

2.2. Collection of Plant Material and Preparation of Extract. Healthy leaves of *A. nitida* were collected from Sangota, Swat district (34.842727°N and 72.431089°E), Khyber Pakhtunkhwa (KPK), Pakistan, during the months of March to April 2022. The plant was collected and identified by Sayed Afzal Shah, Department of Biological Sciences, National University of Medical Sciences, Rawalpindi, Pakistan (Voucher no. NUMS00009). The leaves were washed with distilled water, dried in shade, powdered, and sieved through a 20 mm mesh. In order to make an aqueous extract, approximately, 50 g of the powdered material was soaked in distilled water (200 mL) and sonicated for 30 min at room temperature. The resulting extract was then centrifuged for 10 min in order to remove undesirable matters. The supernatant was finally filtered through Whatman No. 1 filter paper, and the clear extract was stored at 4 °C for further use.

2.3. Synthesis and Purification of AgNPs. For the biosynthesis of AgNPs, 1 mL of aqueous leaf extract was added to 9 mL of 2 mM silver nitrate ($AgNO_3$) aqueous solution in a 25 mL Erlenmeyer flask. On the other hand, 1 mL of aqueous leaf extract was mixed with 9 mL of distilled water, which serves as control. Both the solutions were thoroughly stirred in

https://doi.org/10.1021/acsomega.3c02928
ACS Omega 2023, 8, 30221−30230

dark conditions at 25 °C for about 2 h. The appearance of a light yellowish-green tint confirmed the reduction of Ag^+ ions to Ag^0, which was further confirmed by UV−vis spectroscopy. The colloidal dispersion was purified by centrifuge at 10,000 rpm for 10 min. at 10 °C. The clear supernatant was discarded, and the NPs were successively washed with sterile Milli-Q water to remove any traces of free ions and unutilized phytochemicals. The remaining pellets were freeze-dried and stored at 4 °C for further characterization and biological assays.[21]

2.4. Characterization of AgNPs. *2.4.1. UV−Vis Spectrophotometry.* The reduction of Ag^+ by aqueous leaf extract of *A. nitida* was monitored periodically using a UV−vis spectrophotometer (Lambda 35, Perkin-Elmer, USA) in the range of 350−800 nm. The UV−vis spectra of the reaction mixture were scanned as a function of reaction time with a resolution of 1 nm.

2.4.2. SEM and EDS Analysis. The nano-scale size, shape, and elemental composition of AgNPs were investigated using SEM (S-2500, Japan) integrated with EDS (NOVA-450 instrument).

2.4.3. FTIR Analysis. The FTIR spectra were generated to confirm the involvement of various functional groups such as −OH, C−H, and C−N in the synthesis and stabilization of NPs using an FTIR spectrophotometer (Perkin-Elmer, USA) by adopting the KBr pellet method. The analysis was performed in the 400−4000 cm^{-1} spectral range.

2.4.4. XRD Analysis. The crystallinity of the synthesized NPs was investigated using XRD (JDX-3532 JEOL, Japan) with Cu Kα radiation in θ-2θ configurations at a specific voltage and current (40 kV and 30 mA). Debye−Scherrer's equation was used to calculate the size of the synthesized crystals.

$$D(\text{nm}) = \left(\frac{0.9\lambda}{\beta\cos\theta}\right)$$

where D is the crystal size; β is the full-width at half maximum (FWHM); λ is the X-ray wavelength; θ is the diffraction angle.

2.4.5. Dynamic Light Scattering. The average size, zeta potential, and polydispersity index (PDI) of AgNPs were measured using Zetasizer (Malvern, UK). The sample was diluted with PBS (0.2 M, pH 7.2), sampled in DLS cuvettes, and investigated for the average size and size distribution with a scattering angle of 90. Moreover, zeta potential was measured by PALS technology.

2.5. Antioxidant Assays. *2.5.1. DPPH· Scavenging Assay.* DPPH free radical scavenging effects of *A. nitida* extract and biosynthesized AgNPs were assessed using a conventional DPPH assay.[29] Briefly, 0.3 mM DPPH solution in ethanol was prepared, and various concentrations of AgNPs and crude extract (62.5, 125, 250, 500, and 1000 μg/mL) were mixed with DPPH (100 μL) solution. The mixture was vortex-mixed and allowed to incubate for 30 min at 23 °C in a dark environment. DPPH solution without samples was considered as blank. Subsequently, the reaction mixture was centrifuged at 3000 rpm for 5 min, and the absorbance of the supernatant layer was measured at a specific wavelength of 517 nm using a microplate reader against a blank. Ascorbic acid was used as reference standard. Using the formula, the percent radical scavenging activity (RSA) was calculated.

$$\%\text{RSA} = \frac{\text{Control}_{\text{Abs}} - \text{Sample}_{\text{Abs}}}{\text{Control}_{\text{Abs}}} \times 100$$

2.5.2. Fluorescence Recovery after Photobleaching Scavenging Assay. Fluorescence recovery after photobleaching (FRAP) assay was performed using a reported method.[21] This assay was used to determine the reducing power of crude extract and AgNPs using ascorbic acid as reference standard. Compounds with antioxidant potential are considered to reduce Fe^{3+} to Fe^{2+} ions; the latter form a blue complex (Fe^{2+}/TPTZ), which enhances the total absorbance at 593 nm. The reaction mixture primarily consists of TPTZ solution (0.25 mL; 10 mM) in HCl (40 mM), FeCl3 (0.25 mL; 20 mM), acetate buffer (2.5 mL; 300 mM, pH 3.6), crude extract, and/or AgNPs at different concentrations (62.5, 125, 250, 500, and 100 μg/mL). The FRAP reagent (170 μL), AgNPs, and/or extract (20 μL) were mixed in a 96-well plate and incubated for 30 min in a dark place. Following incubation for 30 min, the maximum absorbance was measured at 593 nm, and % RSA of the extract and AgNPs was determined. The assay was performed in triplicate.

2.5.3. ABTS Scavenging Assay. This is one of the most common antioxidant assays used for the assessment of free radicles. Briefly, a working solution of $ABTS^{\cdot+}$ radicals was prepared by the reaction between ABTS (7 mM) and potassium persulfate (2.5 mM) at 1:1 (v/v) ratio. The reaction mixture was kept in dark at room temperature for about 15 h. This solution was diluted with ethyl alcohol until an absorbance of 0.70 was recorded at 734 nm. Each well of the microplate reader was then added with the plant extract or AgNPs (20 μL) and ABTS solution (200 μL). The mixture was incubated in dark for 30 min, and the absorbance was calculated at 734 nm. Ascorbic acid was used as a standard.[21]

2.5.4. Hydrogen Peroxide (H_2O_2) Scavenging Assay. In this assay, different concentrations of *A. nitida* extracts and its NPs (0.1 mL) were added to phosphate buffer (0.4 mL; 50 mM; pH 7.4) in separate tubes. To start the chemical reaction, 0.6 mL of H_2O_2 (2 mM) solution was added to it. The reaction mixture was vortex-mixed and allowed to incubate in the dark for 30 min at 37 °C. The maximum absorbance was measured at 560 nm. Sodium pyruvate was used as a reference drug. The assays was performed in triplicate.[30]

2.6. Cytotoxicity Assay. The cytotoxic activity of *A. nitida* and its corresponding NPs were assessed using MTT assay.[31] Selected cancer cell lines including breast adenocarcinoma (MDA-MB-231), lung adenocarcinoma (A549), and hepatocellular carcinoma (Hep-G2) cells were cultured with DMEM medium supplemented with FBS (10%) and penicillin−streptomycin (1% v/v) solutions. The cells were incubated at 37 °C with 5% CO_2. Cells showing 90% confluency were selected and subsequently suspended in the RPMI-1640 media. After an overnight incubation, the selected cells were treated with different concentrations of crude extract and/or AgNPs. Following further incubation (37 °C; 5% CO_2) for 24 h, the MTT solution (25 μL) in PB was added to each well and allowed to incubate for 30 min. The resulting MTT formazan was solubilized by the addition of 1 mL of DMSO. Using a microplate reader, the absorbance of the formazan product was recorded at 490 nm. Finally, cell viability was determined using the formula:

https://doi.org/10.1021/acsomega.3c02928
ACS Omega 2023, 8, 30221−30230

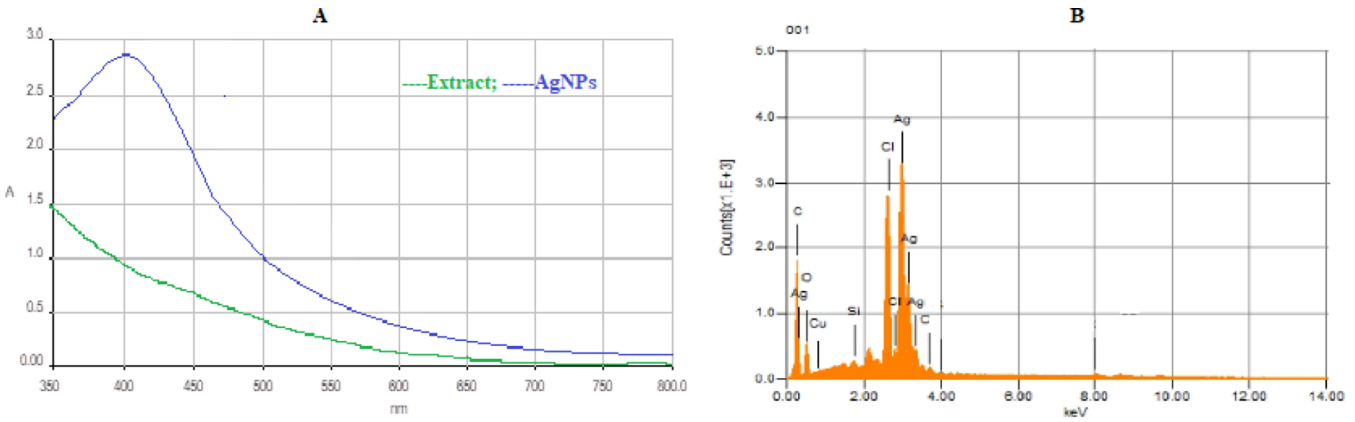


Figure 2. (A) UV−vis and (B) EDS spectra of AgNPs.

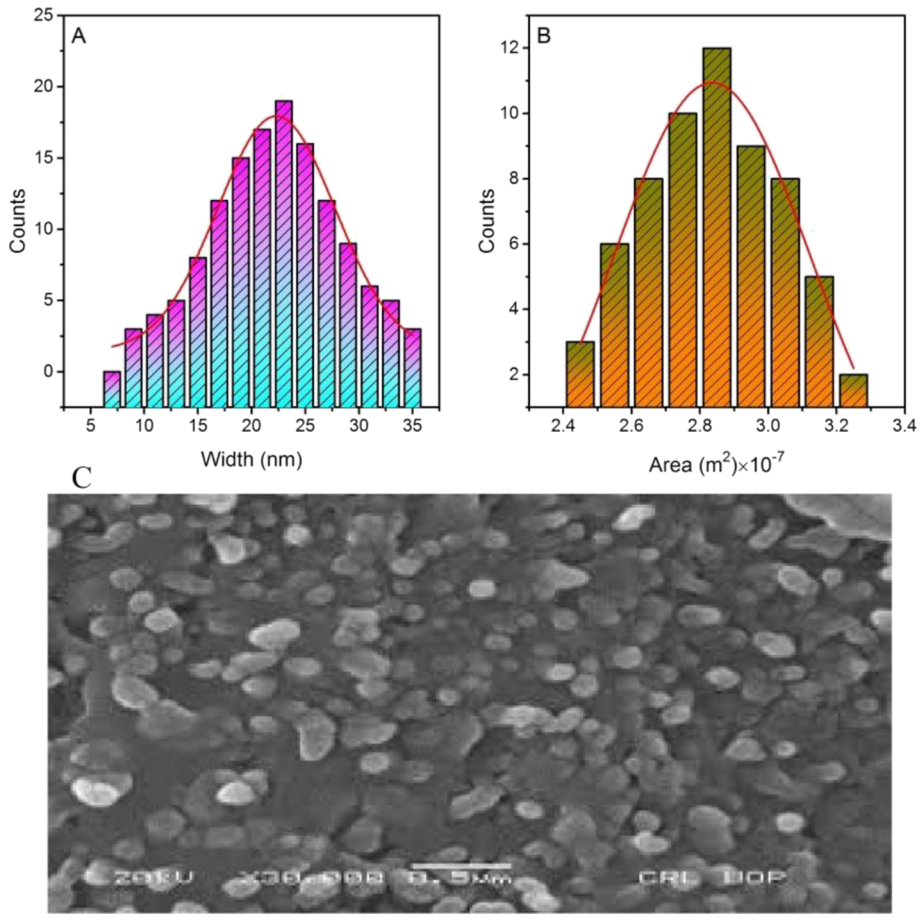


Figure 3. (A) Average width, (B) average area, and (C) SEM images of AgNPs.

$$\%\text{Viable cells} = \left(\frac{\text{abs}_{\text{sample}} - \text{abs}_{\text{blank}}}{\text{abs}_{\text{control}} - \text{abs}_{\text{blank}}}\right) \times 100$$

The assay was performed in triplicate, and the concentrations of the test samples that induce 50% of cytotoxicity were calculated as IC_{50}. Moreover, the selectivity index (SI) of the samples was calculated in order to determine its specificity toward normal human cell lines.

2.7. Statistical Analysis. The data were analyzed using one-way analysis of variance (GraphPad Software). Results were presented as mean ± SEM.

3. RESULTS AND DISCUSSION

3.1. Synthesis of AgNPs. In the present study, AgNPs were successfully synthesized using *A. nitida* leaf extract. The synthesis of AgNPs was initially confirmed by visual observation of color change from light-green to yellowish brown. Synthesis of AgNPs through biological methods does not utilize chemical reagents that are hazardous to some extent. Biological methods are based on the use of plants, bacteria, virus, fungi, and algae that contain natural reducing agents. These agents reduce Ag to AgNPs. In addition to reducing and stabilizing agents, plants also contain a variety of therapeutic agents that coat the AgNPs and exert many effects such as

https://doi.org/10.1021/acsomega.3c02928
ACS Omega 2023, 8, 30221−30230

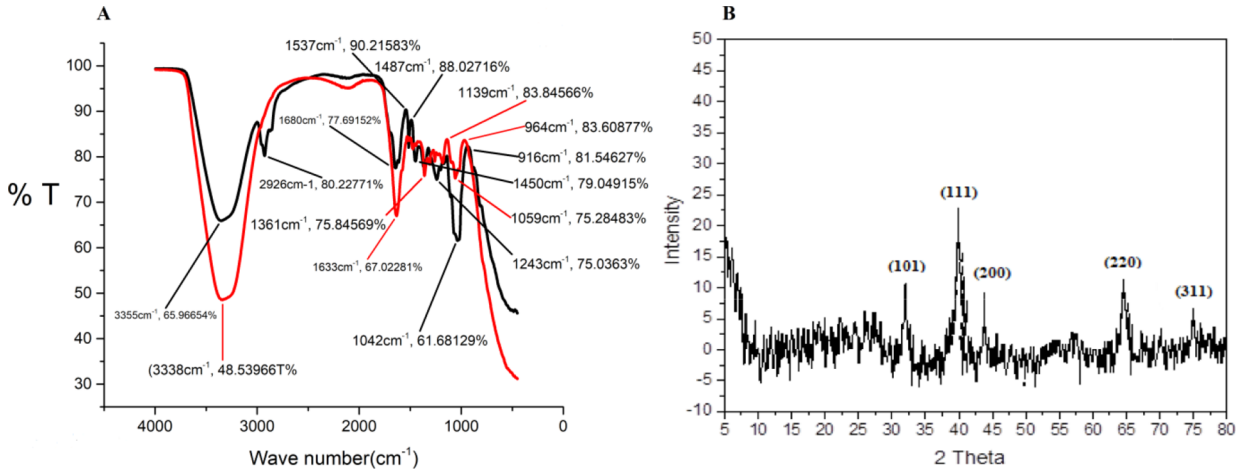


Figure 4. (A) FTIR spectra of extract (black) and AgNPs (red) and (B) XRD spectra of AgNPs.

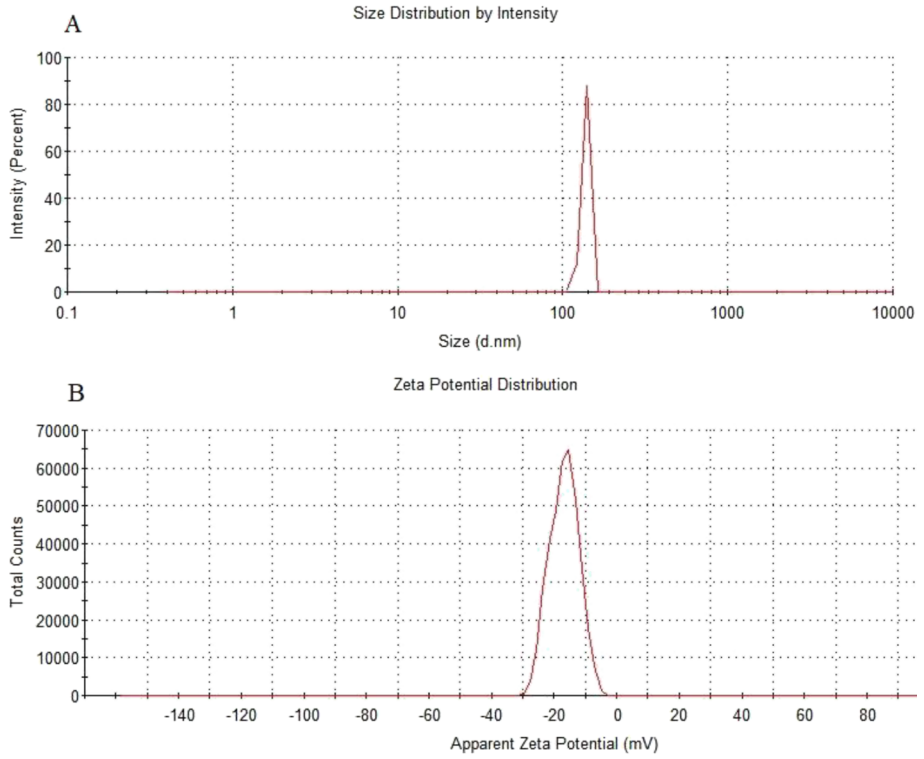


Figure 5. (A) Hydrodynamic size distribution and (B) zeta potential of AgNPs.

anticancer, antioxidant, anti-inflammatory, and antidiabetic. The intrinsic biological properties of silver along with plant phytochemicals often exert a synergistic action. The biofabrication of AgNPs using plants is gaining popularity because this method is comparatively simple, safe, and economical.[32]

3.2. Characterization of AgNPs. *3.2.1. UV−Vis Spectrophotometry.* The reduction of Ag to AgNPs was further confirmed from surface plasmon resonance (SPR) with a characteristic peak at 400 nm. No other peak was observed within this range, which confirms the formation of pure AgNPs (Figure 2A). The observed value was within the range reported previously.[8,17,18] UV−vis spectrophotometry is a valuable technique for the characterization and monitoring the synthesis of AgNPs. In addition to light scattering, AgNPs have unique optical properties that enable them to interact with specific wavelength of light. In AgNPs, both the valence and conduction bands are quite close, with freely moving electrons, which originate an SPR band at a specific wavelength. The absorption properties of AgNPs mainly depends on the particle shape and size. The SPR band for AgNPs is in the range 380−450, and it reflects the average size of NPs.[33]

3.2.2. EDS Analysis. EDS analysis was performed to investigate the elemental composition of AgNPs. A strong Ag peak was observed at 3 keV, which confirms the presence of Ag in the test sample. The spectrum also showed the presence of a trace amount of other elements such as O, Cu, Cl, Si, and C, which may be due to the adsorption of other biomolecules at the surface of AgNPs (Figure 2B).

3.2.3. SEM Analysis. Figure 3A,B depicts that the average width and area of the particles were 24 nm and 2.97 m^2, respectively. SEM analysis confirmed the synthesis of heterogeneously dispersed particles of variable sizes (Figure

https://doi.org/10.1021/acsomega.3c02928
ACS Omega 2023, 8, 30221−30230

3C). According to Doshi et al., the interaction of macrophages with AgNPs mainly depends on its size.[34] The ideal size for NPs is in the range 100−200 nm. Particles having a diameter of less than 5 nm are rapidly cleared from the general circulation by renal clearance. Similarly, particles with a diameter of more than 15 nm usually accumulate in different organs such as spleen and liver. Moreover, NPs possess greater solubility and dissolution rate, which enhance the oral bioavailability of drugs. In the present study, AgNPs with the desired size were produced using aqueous extract of *A. nitida*; therefore, we suggest that these NPs could be used in the development of more effective and safe formulations.

3.2.4. FTIR Analysis. The FTIR analysis was performed to find out the possible role of biomolecules involved in the synthesis of AgNPs. The FTIR spectra of the synthesized NPs are depicted in Figure 4A. The major peak at 3338 cm^{-1} corresponds to O−H stretching of phenolic and alcoholic functional groups. The presence of this band suggests the involvement of phenolics and flavonoids in the capping and stabilization of AgNPs. The band at 2926 cm^{-1} is assigned to C−H stretching of methoxy and methyl groups. Furthermore, the presence of C═O stretch at 1633 cm^{-1} confirmed the presence of esters and ketones in the synthesized NPs. The peak at 1713 cm^{-1} represents the C═O stretching of esters and ketones. The peaks at 1614−1515 cm^{-1} are assigned to aromatic Ar−C═C bond stretching, which are due to the presence of phenolic compounds at the surface of AgNPs. Another medium peak at 1361 cm^{-1} is due to C−N stretching, while the peak at 1042 cm^{-1} corresponds to (C−O) bond stretching of carbohydrates and esters. Bands at 888 cm^{-1} may be due to the stretching of glycosidic linkages. The presence of various functional groups such as phenols, alcohols, aldehydes, and other nitro compounds in the aqueous extract of *A. nitida* validates the stabilization and capping of synthesized AgNPs.

3.2.5. XRD Analysis. XRD was performed to investigate the crystallinity of the synthesized NPs. The XRD pattern showed characteristic peaks at 2θ values of 32.45, 38.15, 44.20, 64.45, and 77.20°, which confirmed the face center cubic structure of AgNPs (Figure 4B). Moreover, the mean crystallite size of the synthesized NPs was determined as 30.50 nm, using Debye−Scherrer's equation.

3.2.6. Dynamic Light Scattering. The mean size of AgNPs investigated by the DLS technique was 150.25 ± 5.30 (Figure 5A) with a PDI of 0.215 ± 0.024. The adsorption of different phytochemicals around the particles may form a capping layer, which prevents agglomeration and thus results in high dispersity of AgNPs.[20] The mean particle size determined by the DLS method is larger as compared to other techniques such as SEM analysis because it measures the hydrodynamic size in aqueous colloidal dispersion, which includes both the metallic core and the phytochemicals adsorbed on the NP surface. In contrast, SEM determines the size of NPs in the dried form. Moreover, zeta potential of AgNPs was −19.85 ± 2.35 mV (Figure 5B). The negative charge on the surface of AgNPs may be due to the adsorption of −OH groups. This high zeta potential of NPs produces electrostatic repulsion between NPs, which results in greater stability by preventing particles agglomeration.[35]

3.3. Antioxidant Assays. The antioxidant potential of aqueous extract of *A. nitida*, AgNPs, and standard was investigated using DPPH, FRAP, ABTS, and H_2O_2 models. The DPPH scavenging activity of the test samples using ascorbic acid as standard is shown in Table 1. The AgNPs at various concentrations (62.2, 125, 250, 500, and 1000 μg/mL) significantly scavenged DPPH by 11, 33.1, 45, 56, and 66.45%, respectively, with an average IC_{50} value of 33.31 μg/mL. However, these activities are comparatively less than that of ascorbic acid (IC_{50} 22.90 μg/mL). The crude extract exhibited lower inhibition effects at the same concentrations with an IC_{50} value of 37.35 μg/mL.

Table 1. DPPH Free Radical Scavenging Activity of Plant Extract, AgNPs, and Reference at Different Concentrations

concentration (μg/mL)	DPPH scavenging activity (%)		
	extract	AgNPs	ascorbic acid
62.5	4.12 ± 0.25	11.0 ± 1.8	26.0 ± 5.2
125	17.45 ± 2.10	33.1 ± 2.5	45.4 ± 6.2
250	28.55 ± 2.80	45.0 ± 2.0	60.1 ± 2.0
500	38.35 ± 3.45	56.0 ± 2.5	69.4 ± 3.8
1000	48.80 ± 3.55	66.45 ± 3.1	80 ± 5.2
IC_{50}	37.35	33.31	22.90

A dose-dependent inhibition was observed in the FRAP scavenging assay, which increased with the increase in dose of the AgNPs (Table 2). The AgNPs revealed 74% inhibition at a higher concentration of 1000 μg/mL with an IC_{50} value of 18.50 μg/mL. On the other hand, the crude extract and standard depicted 48 and 89% inhibition at the same concentration with IC_{50} values of 25.20 and 11 μg/mL, respectively.

Table 2. FRAP Scavenging Activity of Plant Extract, AgNPs, and Reference at Different Concentrations

concentration (μg/mL)	FRAP scavenging activity (%)		
	extract	AgNPs	ascorbic acid
62.5	17.25 ± 1.45	23.0 ± 1.22	34.10 ± 4.45
125	21.55 ± 1.50	32.21 ± 1.65	55.15 ± 2.52
250	29.60 ± 2.0	43.88 ± 3.85	68.25 ± 4.80
500	42.0 ± 2.55	60.75 ± 4.50	78.40 ± 4.35
1000	48.45 ± 4.30	74.65 ± 5.54	89.60 ± 6.45
IC_{50}	25.20	18.50	11.0

The ABTS scavenging potential of test samples is shown in Table 3. The aqueous extract, AgNPs, and ascorbic acid depicted 48.90, 78.81, and 85.70% inhibition at 1000 μg/mL, respectively. The corresponding IC_{50} values were 24.25, 16.46, and 10.10 μg/mL, respectively. The studied samples showed a dose-dependent inhibition activity. Moreover, the scavenging potential of AgNPs was comparable to that of the standard.

Table 3. ABTS Radical Scavenging Activity of Plant Extract, AgNPs, and Reference at Different Concentrations

concentration (μg/mL)	ABTS radical scavenging activity (%)		
	extract	AgNPs	ascorbic acid
62.5	5.18 ± 0.25	19.66 ± 0.88	23.45 ± 1.80
125	21.45 ± 1.90	34.20 ± 2.55	39.0 ± 4.22
250	34.70 ± 2.34	47.31 ± 5.11	56.38 ± 6.42
500	44.61 ± 4.75	60.0 ± 7.80	69.18 ± 8.11
1000	48.90 ± 6.12	78.81 ± 8.67	85.70 ± 10.73
IC_{50}	24.25	16.46	10.10

Table 4 represents the H_2O_2 scavenging activity of the studied samples. The AgNPs exhibited comparable scavenging effects to sodium pyruvate, the standard reference used. The

https://doi.org/10.1021/acsomega.3c02928
ACS Omega 2023, 8, 30221−30230

Table 4. Hydrogen Peroxide Scavenging Activity of Plant Extract, AgNPs, and Reference at Different Concentrations

concentration (μg/mL)	H_2O_2 scavenging activity (%)		
	extract	AgNPs	sodium pyruvate
62.5	9.23 ± 0.40	22.0 ± 0.80	30.41 ± 1.34
125	18.58 ± 1.12	32.56 ± 1.55	44.50 ± 1.89
250	22.40 ± 1.78	45.67 ± 3.0	53.51 ± 4.40
500	42.68 ± 2.34	61.90 ± 5.45	72.82 ± 6.0
1000	55.80 ± 4.40	72.56 ± 8.19	88.80 ± 9.45
IC_{50}	21.39	15.65	10.42

crude extract, AgNPs, and standard revealed maximum scavenging effects of 58, 72, and 88% at 1000 μg/mL, respectively. Their corresponding IC_{50} values were 21.39, 15.65, and 10.42 μg/mL, respectively.

In the biological system, ROS including −OH and O^{-2} radicals can cause oxidation of proteins and lipids, which may lead to DNA damage.[2,36] Normally, antioxidants present in the human body can scavenge free radicals and thus maintain a balance between anti-oxidation and oxidation. However, chronic exposure to alcohol, environmental toxins, radiation, and smoking induces the production of ROS, which disrupts this balance, resulting in some degenerative diseases.[37,38] The intake of natural antioxidants usually ameliorates the damage caused by oxidative stress via several mechanisms including inhibition of the initiation or propagation steps in oxidative chain reaction, scavenging of free radicals, and acting as reducing agents.[39] The natural antioxidants are mainly obtained from medical plants and food such as vegetables, mushrooms, cereals, spices, and several beverages.[40] Antioxidants from plant sources are mainly composed of vitamins (E & C), carotenoids (xanthophylls), and polyphenols such as anthocyanins, phenolics, stilbenes, and flavonoids.[36] These antioxidants exhibit a variety of biological effects such as anti-aging, anti-inflammatory, and anticancer.[41,42] Satpathy et al. have reported that the antioxidant activity of *Pueraria tuberosa*-mediated AgNPs is due to the presence of flavonoids and phenolics, adsorbed on the surface of NPs.[20] Another study demonstrated significant antioxidant effects of AgNPs synthesized from root extract of *Salacia oblonga*. The activity was attributed to the adsorption of various phytochemical and functional groups on the surfaces of AgNPs.[17] Our results further validate these studies. Synthetic antioxidants including butylated hydroxyl anisole, hydroxyl toluene, ascorbic acid, and gallic acid esters are cheap and effective; however, they are associated with several side effects such as carcinogenesis, and they are nonbiodegradable and pose environmental hazards. On the other hand, natural antioxidants are considered safe and effective; therefore, in recent years, the search for natural agents with antioxidant potential has been increased.[43]

In the present study, AgNPs demonstrated considerable antioxidant effects against DPPH, FRAP, ABTS, and H_2O_2 scavenging models in a dose-dependent manner. The results strongly recommend the use of *A. nitida*-mediated AgNPs as useful natural antioxidants for the treatment of different degenerative diseases caused by oxidative stress and other associated conditions.

3.4. Cytotoxicity Assay. The % cell viability of A549, Hep-G2, and MDA-MB-321 cell lines post treatment with fabricated AgNPs was investigated using MTT assay. The viability (%) of cancer cell lines treated with different concentrations of plant extract, AgNPs, and standards (cyclophosphamide and doxorubicin) is shown in Figures S1−S3. Similarly, the viability (%) of normal cell lines including alveolar (HPAEpiC) and renal primary epithelial (HRPTEpiC) cells is depicted in Figures S4 and S5, respectively. Moreover, micrographs of the treated and untreated cell lines A549, HepG2, MDA-MB-231, and HRPTEpiC are shown in Figures S6−S9, respectively. Post 24 h treatment of the studied cancer cell lines with *A. nitida* mediated AgNPs revealed that the viability of cells was dose-dependent. It was observed that the cytotoxic potential increased proportionally with increasing concentration of test samples, with maximum cell death occurred at 10 mg/mL dose. AgNPs revealed considerable cytotoxic effects against A549 and HepG2 cell lines with IC_{50} values 3.6 and 5.38 μg/mL, respectively, as compared to cyclophosphamide and doxorubicin, the reference standards (Figure 6).

In contrast, the aqueous extract demonstrated relatively poor cytotoxicity against the studied cells with IC_{50} values 10.23 (A549), 7.81 (Hep-G2), and 15 μg/mL (MDA-MB-231). The results suggest that AgNPs exhibit strong anti-proliferative effects against A549 and Hep-G2 cells. On the other hand, the IC_{50} values of AgNPs against normal cells HPAEpiC and HRPTEpiC were 17.88 and 16.82 μg/mL, respectively, as compared to standards. These results preliminarily confirmed the safety of AgNPs against normal human cells. To further explore the safety of AgNPs toward normal cell lines, and their SI values were determined. A value of 2 or more indicated high specificity. The SI values of the standard, aqueous extract, and its NPs against normal cells HPAEpiC are shown in Figure 7.

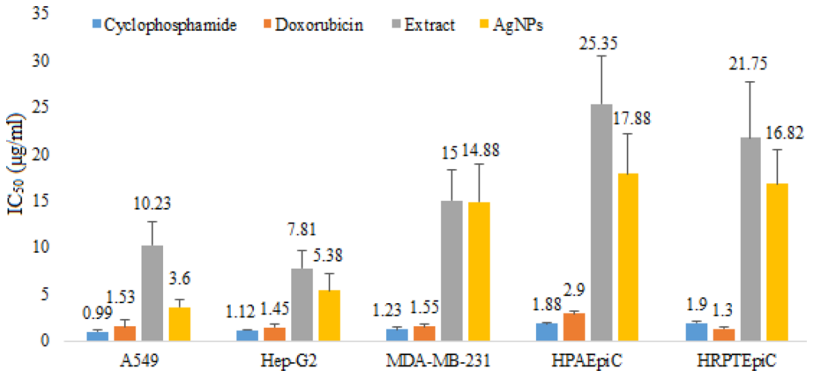


Figure 6. IC_{50} values of the extract, AgNPs, and standard against selected cell lines.

https://doi.org/10.1021/acsomega.3c02928
ACS Omega 2023, 8, 30221−30230

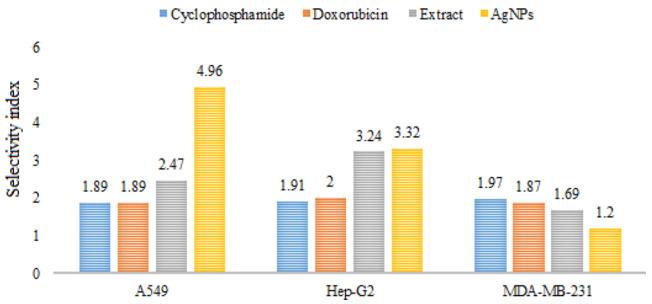


Figure 7. Standard, extract, and silver nanoparticle selectivity indices against HPAEpiC.

AgNPs revealed high specificity for lung adenocarcinoma cells (SI 4.96), followed by hepatocellular carcinoma cells (SI 3.32); however, it showed poor selectivity toward breast adenocarcinoma cells (SI 1.2). Comparable results were achieved when the samples were studied for its SI values using HRPTEpiC as normal cells (Figure 8). The results suggest that AgNPs possess highly specific cytotoxic potential toward A549 and Hep-G2 cells.

Cancer treatment is one of the most serious concerns globally, as it is the major cause of mortality. The synthesis of plant-mediated AgNPs is considered an innovative approach for the treatment of cancer due to two major reasons. Ag ions possess intrinsic cytotoxic potential and can be used as a carrier for anticancer drugs that may exert a synergistic effect.[28] It has been documented that the cytotoxic potential of AgNPs is mediated through Ag^+ ions. AgNPs facilitate the generation of ROS that leads to oxidative damage of cellular contents such as DNA, lipids, and proteins, which may lead to cell death.[44] *A. nitida* has been widely used by local communities in the treatment of several diseases including cancer. As previously mentioned, crude methanolic extract of this plant has shown cytotoxic potential against lung and uterine cancer. Moreover, different fractions of the stem bark have shown significant antioxidant effects in in vitro models. In animal models, crude extract remarkably ameliorated the carbon tetrachloride-induced liver damages.[23] In the present study, the cytotoxic potential of AgNPs may be due to the presence of diarylheptanoids, a group of natural products containing 1, 7-diphenylheptane skeleton, which appears to retard the growth of cancer cells.[45] Moreover, the phytochemicals and natural antioxidants play a significant role against oxidative stress-related diseases including cancer. There are several proposed mechanisms regarding the cytotoxic effects of AgNPs. They induce apoptosis by up- or downregulating the expression of important genes, such as p53. They may also exhibit cell cycle arrest or alter important signaling pathways including hypoxia-inducible factor (HIF). Several cancer cells experience sub-G1 arrest and apoptosis upon exposure to AgNPs. They may also inhibit angiogenesis and diminish distant metastasis.[46−49] The cytotoxic potential demonstrated by AgNPs is in close agreement with previous studies on A549, Hep-G2, MDA-MB-231, Hela, and MCF7 cell lines.[21,50,51] To produce a safe and efficient anticancer drug, other mechanisms for the anticancer actions of AgNPs must be investigated.

4. CONCLUSIONS

To the best of our knowledge, this is the first study to report the biosynthesis of AgNPs using *A. nitida* leaf extract and to investigate its antioxidant and antiproliferative effects in in vitro models. *A. nitida* contain different phytochemicals such as flavonoids and phenolics that reduced Ag^+ to Ag^0 and produced AgNPs with desirable properties, which were confirmed by several analytical methods such as UV−vis spectrometry, SEM, EDS, FTIR, XRD, and DLS. The synthesized AgNPs were predominantly spherical and crystalline with low PDI values. The AgNPs demonstrated strong antioxidant and cytotoxic effects as compared to standard. On the other hand, crude extract showed relatively weak effects. The enhanced biological activities of AgNPs can be attributed to its small size, large surface to volume ratio, specificity, excellent dissolution, and absorption properties. In conclusion, plant-mediated synthesis of AgNPs may be very useful in the effective treatment of degenerative disorders as compared to conventional therapies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c02928.

Cell viability (%) of test samples and standards and micrographs (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Fazli Khuda − *Department of Pharmacy, University of Peshawar, Peshawar 25120, Pakistan;* orcid.org/0000-

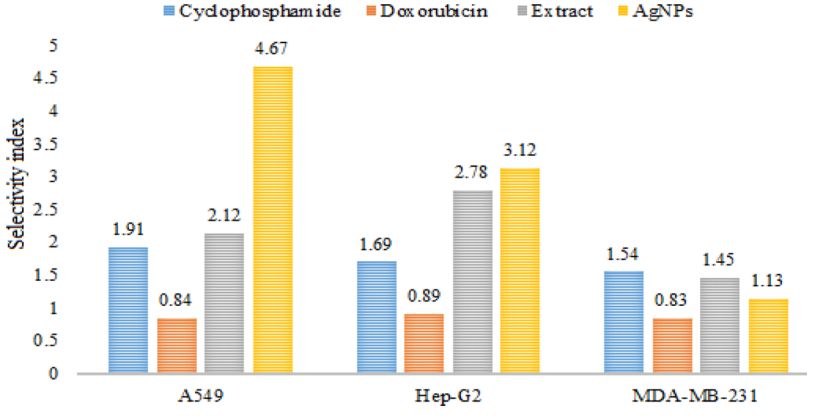


Figure 8. Standard, extract, and silver nanoparticle selectivity indices against HRPTEpiC.

https://doi.org/10.1021/acsomega.3c02928
ACS Omega 2023, 8, 30221−30230

0002-6847-1744; Phone: +92 91 9216750; Email: fazlikhuda@uop.edu.pk; Fax: +92 91 9218131

Saud Almawash − *Department of Pharmaceutical Sciences, College of Pharmacy, Shaqra University, Shaqra 11961, Saudi Arabia;* orcid.org/0000-0001-8999-3464; Phone: +966 56 555 2648; Email: salmawash@su.edu.sa

Authors

Meshal Gul − *Department of Pharmacy, University of Peshawar, Peshawar 25120, Pakistan*

Atif Ali Khan Khalil − *Department of Pharmacognosy, Institute of Pharmacy, Lahore College for Women University, Lahore 54000, Pakistan*

Sajid Ali − *Department of Biotechnology, Abdul Wali Khan University, Mardan 23200, Pakistan*

Naveed Ullah − *Department of Pharmacy, University of Swabi, Swabi 23430, Pakistan*

Muhammad Shafiq Khan − *Department of Pharmacy, Abbottabad University of Science and Technology, Havelian 22500, Pakistan*

Shabnam Nazir − *Department of Pharmacy, Kohat University of Science and Technology, Kohat 26000, Pakistan*

Sumaira Irum Khan − *Department of Pharmacy, Mirpur University of Science and Technology, Mirpur 10250, Pakistan*

Sultan Mehtap Büyüker − *Department of Pharmacy Services, Üsküdar University, İstanbul 34662, Turkey*

Muhammad Shafique − *Department of Pharmaceutical Sciences, College of Pharmacy, Shaqra University, Shaqra 11961, Saudi Arabia*

Sayed Afzal Shah − *Department of Biological Sciences, National University of Medical Sciences, Rawalpindi 46000, Pakistan*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c02928

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The author would like to thank the Deanship of Scientific Research at Shaqra University for supporting this work.

■ REFERENCES

(1) Gali-Muhtasib, H.; Chouaib, R. Nanoparticles in cancer treatment: Types and preparation methods. In *Nanoparticle Drug Delivery Systems for Cancer Treatment*, 1st ed.; Jenny Stanford Publishing: Boca Raton, FL, USA, 2020.

(2) Fang, Y. Z.; Yang, S.; Wu, G. Free radicals, antioxidants, and nutrition. *Nutrition* **2002**, *18*, 872−879.

(3) Xu, D. P.; Li, Y.; Meng, X.; Zhou, T.; Zhou, Y.; Zheng, J.; Zhang, J. J.; Li, H. B. Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. *Int. J. Mol. Sci.* **2017**, *18*, 96.

(4) Bhalani, D. V.; Nutan, B.; Kumar, A.; Singh Chandel, A. K. Bioavailability Enhancement Techniques for Poorly Aqueous Soluble Drugs and Therapeutics. *Biomedicines* **2022**, *10*, 2055.

(5) Hasan, K. M. F.; Horvath, P. G.; Alpar, T. Potential natural fibre polymeric nanobiocomposites: a review. *Polymer* **2022**, *12*, 1072.

(6) Hasan, K. M. F.; Horvath, P. G.; Alpar, T. Silk protein and its nanocomposites. In *Biopolymeric Nanomaterials: Fundamentals and Applications*; Shamsher, K., et al., Eds.; Elsevier: Amsterdam, Netherlands, 2021; pp 309−323.

(7) Hasan, K. M. F.; Horvath, P. G.; Alpar, T. Nanotechnology for waste wood recycling. In *Nanotechnology in Paper and Wood Engineering*; Woodhead Publishing: Duxford, United Kingdom, 2022; pp 61−80.

(8) Giri, A. K.; Jena, B.; Biswal, B.; Pradhan, A. K.; Arakha, M.; Acharya, S.; Acharya, L. Green synthesis and characterization of silver nanoparticles using *Eugenia roxburghii* DC. extract and activity against biofilm-producing bacteria. *Sci. Rep.* **2022**, *12*, 8383.

(9) Mohanta, Y. K.; Panda, S. K.; Jayabalan, R.; Sharma, N.; Bastia, A. K.; Mohanta, T. K. Antimicrobial, Antioxidant and Cytotoxic Activity of Silver Nanoparticles Synthesized by Leaf Extract of Erythrina suberosa (Roxb.). *Front. Mol. Biosci.* **2017**, *4*, 14.

(10) Mohanta, Y. K.; Panda, S. K.; Bastia, A. K.; Mohanta, T. K. Biosynthesis of Silver Nanoparticles from Protium serratum and Investigation of their Potential Impacts on Food Safety and Control. *Front. Microbiol.* **2017**, *8*, 626.

(11) Hasan, K. M. F.; Xiaoyi, L.; Shaoqin, Z.; Horvath, P. G.; Bak, M.; Bejó, L.; Sipos, G.; Alpar, T. Functional silver nanoparticles synthesis from sustainable point of view: 2000 to 2023 − A review on game changing materials. *Heliyon* **2022**, *8*, No. e12322.

(12) Biswas, K.; Mohanta, Y. K.; Kumar, V. B.; Hashem, A.; Abd Allah, E. F.; Mohanta, D.; Mohanta, T. K. Nutritional assessment study and role of green silver nanoparticles in shelf-life of coconut endosperm to develop as functional food. *Saudi J. Biol. Sci.* **2020**, *27*, 1280−1288.

(13) Adelere, I. A.; Lateef, A. A novel approach to the green synthesis of metallic nanoparticles: the use of agro-wastes, enzymes, and pigments. *Nanotechnol. Rev.* **2016**, *5*, 567−587.

(14) Aguda, O.; Lateef, A. Novel biosynthesis of silver nanoparticles through valorization of Parkia biglobosa fermented-seed wastewater: antimicrobial properties and nanotextile application. *Environ. Technol. Innov.* **2021**, *24*, No. 102077.

(15) Espindola-Gonzalez, A.; Martinez-Hernandez, A.; Angeles-Chavez, C. Novel crystalline SiO2 nanoparticles via annelids bioprocessing of agro-industrial wastes. *Nanoscale Res. Lett.* **2010**, *5*, 1408−1417.

(16) Kakakhel, M. A.; Wu, F.; Feng, H.; Hassan, Z.; Ali, I.; Saif, I.; Zaheer Ud Din, S.; Wang, W. Biological synthesis of silver nanoparticles using animal blood, their preventive efficiency of bacterial species, and ecotoxicity in common carp fish. *Microsc. Res. Tech.* **2021**, *84*, 1765−1774.

(17) Dugganaboyana, G. K.; Kumar, M. C.; Jain, A.; Kantharaju, R. M.; Nithya, R. R.; Ninganna, D.; Ahalliya, R. M.; Shati, A. A.; Alfaifi, M. Y.; Elbehairi, S. E. I.; Silina, E.; Stupin, V.; Velliyur, K. G.; Achar, R. R.; Shivamallu, C.; Kollur, S. P. Environmentally benign silver bionanomaterials as potent antioxidant, antibacterial, and antidiabetic agents: Green synthesis using Salacia oblonga root extract. *Front. Chem.* **2023**, *11*, No. 1114109.

(18) Khanal, L. N.; Sharma, K. R.; Paudyal, H.; Parajuli, K.; Dahal, B.; Ganga, G. C.; Pokharel, Y. R.; Kalauni, S. K. Green Synthesis of Silver Nanoparticles from Root Extracts of Rubus ellipticus Sm. and Comparison of Antioxidant and Antibacterial Activity. *J. Nanomater.* **2022**, *2022*, No. 1832587.

(19) Abdussalam-Mohammed, W.; Mohamed, L.; Abraheem, M. S.; Mansour, M. M. A.; Sherif, A. M. Biofabrication of Silver Nanoparticles Using Teucrium Apollinis Extract: Characterization, Stability, and Their Antibacterial Activities. *Chemistry* **2023**, *5*, 54−64.

(20) Satpathy, S.; Patra, A.; Ahirwar, B.; Hussain, M. D. Antioxidant and anticancer activities of green synthesized silver nanoparticles using aqueous extract of tubers of *Pueraria tuberosa*. *Artif. Cells Nanomed. Biotechnol.* **2018**, *46*, 71−85.

(21) Khuda, F.; Jamil, M.; Khalil, A. A. K.; Ullah, R.; Ullah, N.; Naureen, F.; Abbas, M.; Khan, M. S.; Ali, S.; Farooqi, H. M. U.; Ahn, M.-J. Assessment of antioxidant and cytotoxic potential of silver nanoparticles synthesized from root extract of Reynoutria japonica Houtt. *Arab. J. Chem.* **2022**, *15*, No. 104327.

(22) Mohanta, Y. K.; Singdevsachan, S. K.; Parida, U. K.; Panda, S. K.; Mohanta, T. K.; Bae, H. Green synthesis and antimicrobial activity of silver nanoparticles using wild medicinal mushroom Ganoderma applanatum (Pers.) Pat. from Similipal Biosphere Reserve, Odisha, India. *IET Nanobiotechnol.* **2016**, *10*, 184−189.

(23) Sajid, M.; Khan, M. R.; Shah, N. A.; Shah, S. A.; Ismail, H.; Younis, T.; Zahra, Z. Phytochemical, antioxidant and hepatoprotective effects of *Alnus nitida bark* in carbon tetrachloride challenged Sprague Dawley rats. *BMC Complement Altern. Med.* **2016**, *16*, 268.
(24) Shah, S. A.; Shah, N. A.; Ullah, S.; Alam, M. M.; Badshah, H.; Ullah, S.; Mumtaz, A. S. Documenting the indigenous knowledge on medicinal flora from communities residing near Swat River (Suvastu) and in high mountainous areas in Swat-Pakistan. *J. Ethnopharmacol.* **2016**, *182*, 67−79.
(25) Hammond, G. B.; Fernández, I. D.; Villegas, L. F.; Vaisberg, A. J. A survey of traditional medicinal plants from the Callejón de Huaylas, Department of Ancash, Perú. Potent anti-cancer activity of Alnus nitida against lung cancer cells; in vitro and in vivo studies. *J. Ethnopharmacol.* **1998**, *61*, 17−30.
(26) Tung, N. H.; Kwon, H.-J.; Kim, J.-H.; Ra, J. C.; Ding, Y.; Kim, J. A.; Kim, Y. H. Anti-influenza diarylheptanoids from the bark of *Alnus japonica*. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1000−1003.
(27) Batool, F.; Sabir, S. M.; Rocha, J.; Shah, A. H.; Saify, Z. S.; Ahmed, S. D. Evaluation of antioxidant and free radical scavenging activities of fruit extract from *Zanthoxylum alatum*: A commonly used spice from Pakistan. *Pak. J. Bot.* **2010**, *42*, 4299−4311.
(28) Gomes, H. I. O.; Martins, C. S. M.; Prior, J. A. V. Silver Nanoparticles as Carriers of Anticancer Drugs for Efficient Target Treatment of Cancer Cells. *Nanomaterials* **2021**, *11*, 964.
(29) Kedare, S. B.; Singh, R. P. Genesis and development of DPPH method of antioxidant assay. *J. Food Sci. Technol.* **2011**, *48*, 412−422.
(30) Chandan, T.; Moushumi, H.; Jayanta, B.; Gajurel, P. R. Antioxidant Activities and Phenolic Content of Piper wallichii (Miq.) Hand.-Mazz. *Int. J. Food Prop.* **2014**, *17*, 309−320.
(31) Khuda, F.; Alam, N.; Khalil, A. A. K.; Jan, A.; Naureen, F.; Ullah, Z.; Alotaibi, A.; Ullah, R.; Ullah, S.; Shah, Y.; Shah, S. I.; Büyüker, S. M. Screening of Rhamnus Purpurea (Edgew.) Leaves for Antimicrobial, Antioxidant, and Cytotoxic Potential. *ACS Omega* **2022**, *7*, 22977−22985.
(32) Das, R. K.; Pachapur, V. L.; Lonappan, L.; Naghdi, M.; Pulicharla, R.; Maiti, S.; Cledon, M.; Dalila, L. M. A.; Sarma, S. J.; Brar, S. K. Biological synthesis of metallic nanoparticles: Plants, animals and microbial aspects. *Nanotechnol. Environ. Eng.* **2017**, *2*, 18.
(33) Rahman, A.; Kumar, S.; Bafana, A.; Lin, J.; Dahoumane, S. A.; Jeffryes, C. A. Mechanistic view of the light-induced synthesis of silver nanoparticles using extracellular polymeric substances of chlamydomonas reinhardtii. *Molecules* **2019**, *24*, 3506.
(34) Doshi, N.; Mitragotri, S. Macrophages Recognize Size and Shape of Their Targets. *PLoS One* **2010**, *5*, No. e10051.
(35) Honary, S.; Zahir, F. Effect of zeta potential on the properties of nano-drug delivery systems − a review (Part 2). *Trop J Pharm Res.* **2013**, *12*, 265−273.
(36) Peng, C.; Wang, X.; Chen, J.; Jiao, R.; Wang, L.; Li, Y. M.; Zuo, Y.; Liu, Y.; Lei, L.; Ma, K. Y. Biology of ageing and role of dietary antioxidants. *BioMed Res. Int.* **2014**, *2014*, No. 831841.
(37) Li, S.; Tan, H. Y.; Wang, N.; Zhang, Z. J.; Lao, L.; Wong, C. W.; Feng, Y. The role of oxidative stress and antioxidants in liver diseases. *Int. J. Mol. Sci.* **2015**, *16*, 26087−26124.
(38) Wang, F.; Li, Y.; Zhang, Y. J.; Zhou, Y.; Li, S.; Li, H. B. Natural products for the prevention and treatment of hangover and alcohol use disorder. *Molecules* **2016**, *21*, 64.
(39) Baiano, A.; Nobile, M. A. Antioxidant compounds from vegetable matrices: Biosynthesis, occurrence, and extraction systems. *Crit. Rev. Food Sci. Nutr.* **2015**, *56*, 2053−2068.
(40) Cai, Y. Z.; Luo, Q.; Sun, M.; Corke, H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci.* **2004**, *74*, 2157−2184.
(41) Li, A. N.; Li, S.; Zhang, Y. J.; Xu, X. R.; Chen, Y. M.; Li, H. B. Resources and biological activities of natural polyphenols. *Nutrients* **2014**, *6*, 6020−6047.
(42) Zhou, Y.; Li, Y.; Zhou, T.; Zheng, J.; Li, S.; Li, H. B. Dietary natural products for prevention and treatment of liver cancer. *Nutrients* **2016**, *8*, 156.
(43) Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M. T. D.; Mazur, M.; Telser, J. Free radicals and antioxidants in normal physiological functions and human diseases. *Int. J. Biochem. Cell Biol.* **2007**, *39*, 44−84.
(44) Arunachalam, K. D.; Arun, L. B.; Annamalai, S. K.; Arunachalam, A. M. Potential anticancer properties of bioactive compounds of *Gymnemasylvestre* and its biofunctionalized silver nanoparticle. *Int. J. Nanomed.* **2014**, *10*, 31−41.
(45) Siddiqui, I. N.; Ahmad, V. U.; Zahoor, A.; Ahmed, A.; Khan, S. S.; Khan, A.; Hassan, Z. Two new diaryl heptanoids from Alnus nitida. *Nat. Prod. Commun.* **2010**, *5*, 1787−1788.
(46) Yin, M.; Xu, X.; Han, H.; Dai, J.; Sun, R.; Yang, L.; Xie, J.; Wang, Y. Preparation of Triangular Silver Nanoparticles and Their Biological Effects in the Treatment of Ovarian Cancer. *J. Ovarian Res.* **2022**, *15*, 121.
(47) Singh, D. K.; Pandey, D. K.; Yadav, R. R.; Singh, D. A Study of ZnO Nanoparticles and ZnO-EG Nanofluid. *J. Exp. Nanosci.* **2013**, *8*, 731−741.
(48) Kitimu, S. R.; Kirira, P.; Abdille, A. A.; Sokei, J.; Ochwang'i, D.; Mwitari, P.; Makanya, A.; Maina, N. Anti-Angiogenic and Anti-Metastatic Effects of Biogenic Silver Nanoparticles Synthesized Using *Azadirachta Indica*. *Adv. Biosci. Biotechnol.* **2022**, *13*, 188−206.
(49) Noorbazargan, H.; Amintehrani, S.; Dolatabadi, A.; Mashayekhi, A.; Khayam, N.; Moulavi, P.; Naghizadeh, M.; Mirzaie, A.; Mirzaei Rad, F.; Kavousi, M. Anti-Cancer & Anti-Metastasis Properties of Bioorganic-Capped Silver Nanoparticles Fabricated from Juniperus Chinensis Extract against Lung Cancer Cells. *AMB Express* **2021**, *11*, 61.
(50) Vivek, R.; Thangam, R.; Muthuchelian, K.; Gunasekaran, P.; Kaveri, K.; Kannan, S. Green biosynthesis of silver nanoparticles from *Annonasquamosa* leaf extract and its in vitro cytotoxic effect on MCF-7 cells. *Process Biochem.* **2012**, *47*, 2405.
(51) Suman, T. Y.; Rajasree, S. R.; Kanchana, A.; Elizabeth, S. B. Biosynthesis, characterization and cytotoxic effect of plant mediated silver nanoparticles using *Morindacitrifolia* root extract. *Colloids Surf., B* **2013**, *106*, 74−78.

https://doi.org/10.1021/acsomega.3c02928
ACS Omega 2023, 8, 30221−30230