

Green Synthesis of Silver Nanoparticles using Alkanna Tinctoria Aqueous Leaf Extract and its Antibacterial and Antioxidant Activity Against Cancer Cell Lines

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Abstract

Nanotechnology plays a pivotal role in advancing materials science, particularly through the development of nanoparticles, which are integral in a variety of industrial applications. This study focuses on silver nanoparticles (AgNPs), which are highly valued in multiple sectors due to their unique physical, chemical, and biological properties. We explore the green synthesis of AgNPs using the aqueous leaf extract of "*Alkanna tinctoria*". This method not only ensures a sustainable and eco-friendly approach but also facilitates the production of nanoparticles with controlled sizes and shapes, which are crucial for their efficacy and application versatility. The synthesized AgNPs were characterized using advanced techniques including X-ray Diffraction (XRD), UV-visible Spectroscopy (UV-Vis), Fourier Transform Infrared Spectroscopy (FT-IR), and Transmission Electron Microscopy (TEM). These characterizations confirm the successful synthesis and desired physicochemical properties of the AgNPs. The research highlights the broad applications of these AgNPs, demonstrating significant antimicrobial properties and potential anticancer activities. The nanoparticles showed a notable efficacy against a range of bacterial strains and exhibited promising anticancer properties in vitro, particularly tested against the HepG2 cell line. The AgNPs induce apoptosis and inhibit cellular proliferation, showcasing their potential as therapeutic agents in medical applications. This study substantiates the potential of biogenically synthesized AgNPs to serve as a safer, more efficient alternative to conventional nanoparticles, offering significant advantages in healthcare and beyond, owing to their tailored functionalities and reduced toxicity. The findings promote further exploration and development of green synthesis methods in nanoparticle production, ensuring sustainability and broad applicability in modern technology and medicine.

1. Introduction

Nanotechnology is an interdisciplinary research field responsible for the production of various nanomaterials. It is widely used in pharmaceuticals, drug and gene delivery, cosmetics, food, chemical, and aerospace industries (Mohamed S et al. 2014). Nanoparticles exhibit novel or enhanced properties on specific characteristics such as size, morphology, and distribution. New applications of nanoparticles are arising rapidly (Murphy, C 2008). They are an attractive resource for creating new structures and integrating them into biological systems. The excellent surface area-to-volume ratio ensures higher reactivity compared to larger particles (Foroozandeh, P and Aziz, A. A 2018). Recent studies have reported metal-based nanoparticles to have promising potential in biomedical applications. They have been used in biosensors, nano-catalysts, antimicrobial, pharmaceutical, and environmental applications due to their unique properties (Kumar, A et al. 2021; Cui, X et al. 2017; Mitchell, M et al. 2021). Nevertheless, the toxicological assessment has questioned the broad usage of these engineered products. Hence, developing sustainable, cost-effective, non-toxic, and productive synthesis routes for nanomaterials is being pursued. The green methodology allows for the production of nanomaterials with a well-defined shape and size in a manageable way (Rajasekhar, C. and Kanchi, S. 2019).

Nanoparticle biosynthesis is dynamic and depends on the plant species and the type of plant part (root, stem, leaf, seed, flower, etc.). Plants are reservoirs of important secondary metabolites such as alkaloids, flavonoids, terpenes, tannins, phenols, saponins, proteins, carbohydrates, and oils. These molecules have distinct therapeutic properties and antimicrobial activity (Jain, Devendra, H et al. 2009). Plant metabolites function as both reducing and stabilising agents throughout the process of synthesising nanoparticles. Plant-based nanoparticles have recently gained a high reputation in the biomedical field for being non-toxic and highly effective (Mohamed S et al. 2014).

Silver nanoparticles (AgNPs) have garnered significant research interest because of their physical and chemical properties and promising utilizations. AgNPs are known for their unique and diverse properties, such as optical properties, chemical stability, good electrical conductivity, catalytic activity, and thermal properties, enabling their incorporation into various products (Tsai, T et al. 2010; Lu, Y. C et al. 2010; Iravani, S et al. 2014). AgNPs have shown advantages in the fields of biolabeling, drug delivery, diagnostics, cytotoxicity, and as biocide for fighting microbial infections. Silver nanoparticles synthesized with various plant extracts have demonstrated powerful antibiofilm, antibacterial, antioxidant, anticancer, and other biochemical effects in green synthesis (Otari, S. V et al. 2019; Pelgrift, R. Y. and Friedman, A. J. 2013; harathi, D. and Bhuvaneshwari, V. 2014; Bharathi, D et al. 2018) Compared to traditional silver microparticles, AgNPs with large surface areas offer a better interface for interacting with bacteria. In the United States, nanosilver has been utilized as a biocidal agent for over a century (Nowack, B et al 2019) in the form of colloidal silver and silver nitrate. Both gram-negative and gram-positive bacteria strains are effectively eradicated by AgNPs (Chernousova, S. and Epple, M. 2018; Kędziora, A. et al. 2018; Taglietti, A. et al. 2019)

Cancer is a complex, multifactorial disease characterized by the uncontrolled growth and spread of abnormal cells. It is treated with various methods, including chemotherapy, hormone therapy, surgery, radiation, immunotherapy, and targeted therapy. Consequently, the goal is to find efficient, economical, and sensitive lead compounds with increased sensitivity and cell-targeted specificity. AgNPs have gained a considerable attention recently due to their medicinal uses as anticancer agents, diagnostics, and probing (Seigel, R. and Jemal, A 2015). Recent studies have highlighted the promising applications of AgNPs in oncology, particularly their ability to inhibit and potentially reverse tumor growth. Biogenically synthesized AgNPs, obtained using plant extracts, are gaining attention for their enhanced biocompatibility and reduced toxicity, which are crucial for clinical applications. For instance, AgNPs synthesized through green methods have shown significant anticancer activity against various cancer cell lines, suggesting their potential as an alternative therapeutic approach (Zhang, X. et al. 2020).

One of the pivotal mechanisms by which AgNPs exert their anticancer effects involves the production of reactive oxygen species (ROS), which can trigger apoptosis in cancer cells without causing harm to healthy cells (Ahn, E. and Park, Y 2015). This selective toxicity is particularly advantageous in cancer treatment, as it mitigates the side effects associated with traditional chemotherapy. In malignant cells, AgNPs attach to essential cellular components, disrupting cellular activities, blocking the cell cycle, and inducing apoptosis, which provides potential benefits in cancer treatment (Khan, M. S et al. 2021).

Moreover, the surface modification of AgNPs plays a crucial role in enhancing their interaction with tumor cells. Functionalization with specific ligands can direct these nanoparticles to tumour-specific markers, enhancing their selectivity and reducing potential harm to normal cells. Research has demonstrated that such targeted AgNPs can effectively localize within tumor tissues, boosting their anticancer efficacy while curbing systemic toxicity (Zhang, X. et al. 2020).

Furthermore, the green synthesis approach of AgNPs using plant extracts provides a sustainable method and introduces additional phytochemicals that may synergize with silver to enhance its anticancer activity. These phytochemical-coated AgNPs have been found to exhibit superior anticancer properties, including the ability to interfere with the tumour microenvironment and inhibit angiogenesis, which is crucial for tumour growth and metastasis (Ahn, E. and Park, Y 2015).

In this work, we present a method for synthesising AgNPs using a water-based leaf extract of *Alkanna tinctoria*, which is environmentally friendly and does not require the use of harmful chemicals. The results of AgNPs were characterized by X-ray Diffraction (XRD), UV-visible spectroscopy (UV-Vis), Fourier Transform Infrared spectroscopy (FT-IR), and Transmission electron microscopy (TEM). Moreover, with the HepG2 cell line, a study was carried out to investigate the antibacterial and cytotoxic effects of AgNPs.

2. Material and methods

Silver (II) nitrate and sodium hydroxide ($\text{NaOH} \geq 85.8\%$) were acquired from Fisher Scientific. All chemicals were used as received.

2.1. Preparation of alkanna tinctorial root extract

The *alkanna tinctorial* root was washed several times with double-distilled water to remove impurities. 2.5 g of *alkanna tinctorial* root was heated in 100 ml of double-ionized water at 60°C. The aqueous extract was filtered. The filtration was used as a reducing agent.

2.2. Silver nanoparticles synthesis

90 ml of 0.02 M AgNO_3 was mixed with 10 ml of *alkanna tinctorial* root extract. The mixture was heated at 70°C for 1 hr and the color changed from light brown to dark brown. The pH was maintained at 10 by adding 1 M of NaOH. The reaction was left to continue stirring for 3 hrs. The nanoparticles were collected using a Buckner funnel. The product was washed with DI water several times to remove the impurities. The resulting product was calcined at 200°C for 3 hrs.

2.3. Antibacterial activity of AgNPs:

The study included three human pathogenic microbial strains: *S. aureus*, *E. coli*, and yeast *Candida albicans* (*C. albicans*), as well as reference strains and *S. aureus* ATCC 29213, *E. coli* ATCC 25922, and *C.*

albicans ATCC 60193. All bacteria isolates were tested for antimicrobial susceptibility using a modified Kirby Bauer disc diffusion method and the Clinical and Laboratory Standards Institute (CLSI) guidelines.

2.4. Antimicrobial Assay of Plant Extracts.

Plant extract was tested for antimicrobial activity using the agar well diffusion method on Mueller Hinton Agar (MHA) plates. In order to modify the turbidity to 0.5 McFarland standards and achieve a final inoculum of 1.5×10^8 CFU/ml, organisms were inoculated in nutrient broth and potato dextrose broth and then incubated overnight at 37°C. Microbial culture broth that was standardized was used to culture MHA plates. Dimethyl sulfoxide (DMSO) dissolves plant extract at an 8 mg/ml concentration. The inoculated media contained two 6 mm discs. One disc was filled with a 41-plant extract, while the other was a negative/solvent control disc (DMSO). It was given about 30 minutes to diffuse at room temperature before being incubated for 18 to 24 hours at 37 C. After incubation, the test compounds' antibacterial activity was determined by looking at the plates for the development of a clear zone around the disc zone of inhibition was seen and measured in millimetres.

2.5. In vitro cell viability assay

The detection of cell viability and cell growth were performed using MTT Assay (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium Bromide, cat#475989-1GM, Sigma-Aldrich, Germany). Briefly, aliquots of 120 µl of the suspended cells (5×10^4 mL⁻¹) were given to 60 µL of a serial dilution of the AgNPs in a 96-well plate. After 3 days of incubations, 20 µl of MTT-solution were given to each well²⁴, and the cells further cultivated for an additional two hours. Formazan crystals were dissolved in isopropanol. The intensity of the resulting color was measured at 595 nm as described previously.

3. Result and Dissection

3.1. UV Vis

The UV-Vis spectra of the synthesized AgNPs have a distinct absorption peak at around 420 nm, indicative of the surface plasmon resonance (SPR) of silver nanoparticles (Fig. 2). The distinctness and location of the peak indicate that the AgNPs exhibit a high degree of uniformity in size and are evenly distributed throughout the solution. As the wavelength increases above 420 nm, the absorbance consistently drops without any notable absorption peaks at higher wavelengths. This suggests no major agglomeration or presence of bigger particles in the sample. The absorption peak observed around 420 nm is typical for spherical AgNPs and corresponds to the collective oscillation of conduction band electrons induced by the interaction with light, known as surface plasmon resonance. As per earlier reports, the peak's position can be influenced by factors such as the particle size, shape, and the dielectric environment around the nanoparticles (Zhang, X. et al. 2020; Jensen, T. R et al. 2000; Khatun, M et al. 2023). Typically, smaller nanoparticles would have their SPR peak at shorter wavelengths, whereas larger or aggregated nanoparticles would display a red shift towards longer wavelengths. The sharp and symmetric nature of the peak suggested that the synthesized nanoparticles are predominantly spherical

and have a narrow size distribution. This is favourable for most applications that require consistent particle behaviour and properties. In the context of potential applications, these characteristics suggested that the AgNPs could be effectively used in areas such as biological sensing, where particle uniformity is crucial for consistent sensor performance (Ali, H. et al. 2023; Naganthran, A. et al. 2022).

3.2. FT-IR spectrum of solid AgNPs

The spectral data presents multiple absorption peaks, suggesting various functional groups or molecular bonds in the analyzed AgNPs sample. The prominent peaks occur at approximately 500, 1000, 1500, and 3000 cm^{-1} , each indicative of different molecular interactions or structures. The sharpness and distinct positions of these peaks suggest that the sample has well-defined chemical structures (Fig. 3).

The peak at 500 cm^{-1} could be indicative of metal-ligand vibrations or lattice vibrations in inorganic compounds. Earlier studies by Durran et al (Durran, S. E. et al. 2021) have observed similar features in transition silver metal complexes. A peak at 1000 cm^{-1} typically, this peak might correspond to C-O stretching in ethers or esters and the exact assignment can depend significantly on the AgNPs sample (Bayu, A. et al. 2019). The peak at 1500 cm^{-1} is often characteristic of C = C stretching in aromatic compounds. The literature, including Embrechts et al Embrechts, H. et al. 2020) provides detailed correlations for these types of organic functionalities. Finally, peaks at 3000 cm^{-1} in this region are typically due to C-H stretching vibrations in alkanes, alkenes, or aromatic hydrocarbons. The peak's intensity and shape can provide insights into the carbon atoms' hybridization state (Hadjiivanov, K. I. et al. 2021; Viter, R., and Iatsunskyi, I. 2019).

3.3. XRD pattern of AgNPs

The XRD pattern displays several sharp peaks indicating the AgNPs were highly crystalline material. The prominent peaks at approximately 20°, 30°, 40°, 50°, and 60° 2 θ suggest specific crystalline phases. The intensity of the peaks suggests strong crystallinity, with the highest peak around 30° being particularly notable (Fig. 4a). The sharp peaks signified well-defined crystal planes, which indicate that the material has a high degree of crystalline purity. The peak positions are indexed to specific crystallographic planes known for metal oxides. The AgNPs were a common material like AgNO₃ in its anatase phase, the peak around 25.3° corresponds to the (101) plane. The absence of additional broad peaks or background hump suggests that there are no significant amounts of amorphous phases present. This indicates that the sample was predominantly crystalline [34]. Figure 4b shows a layered XRD plot with multiple datasets, indicating the presence of different crystalline phases in the AgNPs sample. The overlaid XRD patterns suggested the identification of silver (Ag), silver oxide (Ag₂O), and silver carbide (AgC₂), with distinct peaks corresponding to each material's crystallographic planes (Fig. 4b). It was characterized by prominent peaks at around 38°, 44°, and 64° (2 θ), corresponding to the (111), (200), and (220) planes of face-centered cubic (FCC) silver. The peaks at approximately 32° and 55° (2 θ), indicated that it matched the (111) and (311) planes and it suggested the presence of cubic silver(I) oxide. The less intense peaks at about 36° and 61° (2 θ) indicated compound was less commonly observed, and shown here matching

the (111) and (311) planes respectively (Szymanski, N. J. et al. 2024). The metallic silver along with its oxide and carbide forms could indicate a multi-step synthesis or reaction process where silver has undergone partial oxidation and carbonization. This might occur in environments where both carbon and oxygen are present during AgNPs synthesis. The presence of different phases of silver significantly affects the nano material's properties such as conductivity, catalytic activity, and chemical stability. Specifically, AgNPs are known for their high electrical conductivity, and antimicrobial properties, and are also used as a catalyst in some applications (Phuong, N. et al. 2023).

3.4. TEM images of AgNPs

The TEM micrographs demonstrated that the nanoparticles predominantly exhibited a nearly spherical morphology. Analysis of the TEM images indicated that the particle sizes ranged from 2 to 18 nm (Nabikhan, A. et al. 2010). The core size of the particles was analysed using the 8-bit bundled version of ImageJ software (Zhang S. et al. 2023) This size distribution is consistent with the typical range of silver nanoparticles reported in previous research (Rajan, R. et al 2015). Such a distribution falls well within the expected parameters for AgNPs, confirming the successful synthesis and stabilization of nanoparticles. The nearly spherical shape and size range suggest that the synthesis method employed was effective in controlling the particle morphology and preventing excessive growth or agglomeration, ensuring that the nanoparticles remained within the desired nanoscale dimensions. This consistency with prior studies reinforces the reliability and reproducibility of the synthesis process used in this study.

3.5. Antimicrobial activity of AgNPs

Figure 6 and Table 1 depict a disk diffusion test to assess the antimicrobial susceptibility of bacteria against AgNPs. This method involved employing antibiotic-impregnated disks on an agar plate inoculated with the specific bacteria and then observing the zones of inhibition (mm) around the disks. The three plates (Figs. 6a, 6b, and 6c) display varying responses to the antibiotics labeled as 'S' (AgNPs) and 'C' (Control). Figure 6a shows an inhibition zone around the AgNPs coated disk, indicating that the bacteria are highly susceptible. There is no zone of inhibition around the 'C' disk and no moderate susceptibility to control. Figure 6b exhibits a zone of inhibition around 'S' and no zone around 'C', indicating moderate susceptibility to AgNPs and control, respectively. Figure 6c shows a comparatively large inhibition zone around the 'S' disks, and no zone around the 'C' disk suggesting that the pathogen was susceptible to 'S' disks and resistant to 'C' disks. The large zone of inhibition in Fig. 6c for AgNPs was highly effective against the bacteria grown on the plate. Conversely, the smaller zone suggests lower efficacy, which may require higher doses or prolonged treatment. Figure 6b's uniform moderate zones indicate that AgNPs are moderately effective, potentially requiring higher concentrations to attain the desired therapeutic effect. The minimal inhibition zones in Fig. 6a are indicative of bacterial resistance to control and intermediate to AgNPs. The antimicrobial activity of AgNPs results was reported by numerous earlier studies (Salehi S. et al. 2016; Majoumouo, M. et al. 2019; Ropisah M. et al. 2022; Fernando, K. et al 2024).

Table 1
Antimicrobial activity AgNPs against selected microbial pathogens.

Microbial Pathogens	Zone of Inhibition (mm)	
	AgNPs ()	AgNPs ()
<i>S. aureus</i> ATCC 29213	12.00 ± 0.15	0.00
<i>E. coli</i> ATCC 25922	12.00 ± 1.00	0.00
<i>C. albicans</i> ATCC 60193	20.00 ± 0.25	0.00

3.6. Cytotoxic studies of AgNPs against HEPG2 cell lines

The microscopic examination of HEPG2 cancer cells treated with various concentrations of AgNPs revealed significant dose-dependent cytotoxic effects (Fig. 7). The untreated HEPG2 (Fig. 7D) cells served as the MeOH control group. These cells displayed normal morphology, characterized by a dense monolayer with a typical polygonal shape and clear cell boundaries. The high confluency and uniformity of the cells indicated healthy and actively proliferating cells. Treatment with high concentrations of AgNPs led to extensive cell death and very low residual cell density (Fig. 7A). The majority of the cells lost their adherence properties, becoming highly rounded or fragmented. The few remaining cells displayed extreme morphological distortions, indicating severe cytotoxic effects. This extensive cell death is likely due to overwhelming oxidative stress and direct physical interactions of AgNPs with cellular components, resulting in critical damage to cellular structures and functions. In Fig. 7B, exposure to moderate concentrations of AgNPs resulted in more pronounced cellular damage. The cells exhibited severe morphological alterations, including significant rounding and detachment from the substrate. There was a marked decrease in cell density, reflecting increased cell death and reduced proliferation. The extent of cellular damage at this concentration suggests that AgNPs induce substantial oxidative stress, leading to apoptosis or necrosis in a considerable fraction of the cell population. In Fig. 7C, the cells treated with a low concentration of AgNPs showed noticeable changes in morphology and cell density compared to the control. The treated cells began to lose their typical polygonal shape, becoming more rounded and irregular. There was also a slight reduction in cell density, indicating initial cytotoxic effects of AgNPs at lower concentrations. These morphological changes suggest the beginning of cellular stress and damage, possibly due to oxidative stress induced by AgNPs.

The dose-dependent response observed in HEPG2 cells upon AgNPs treatment aligns with the known cytotoxic mechanisms of nanoparticles. At low concentrations, AgNPs induce mild oxidative stress, leading to subtle morphological changes and reduced cell proliferation. As the concentration increases, oxidative stress becomes more severe, leading to substantial cellular damage, apoptosis, or necrosis. At high concentrations, the overwhelming oxidative stress and physical interactions of AgNPs with cellular components result in extensive cell death. The present study results corresponded with an earlier report by Morais et al (Morais, M. et al 2021) studying the cytotoxicity activity of AgNPs against prostate cancer cells.

These findings highlight the potential of AgNPs as an effective anticancer agent, capable of inducing significant cytotoxic effects in cancer cells. However, the concentration-dependent cytotoxicity also underscores the need for careful dosage optimization to maximize therapeutic efficacy while minimizing potential side effects. Further studies are warranted to elucidate the precise molecular mechanisms underlying AgNPs-induced cytotoxicity and to explore the potential of combining AgNPs with other therapeutic modalities for enhanced anticancer efficacy [Jensen, T. R. et al. 2000; Yuan, Y. et al. 2018; Ajaykumar, A. P. et al. 2023].

3.7. Determination of IC₅₀ value of AgNPs against HEPG2 cell lines

Figure 9 depicts a dose-response curve assessing the cytotoxic effect of AgNPs on the HEPG2 cell line, a commonly used human liver cancer cell model. This type of analysis is pivotal in evaluating the potential therapeutic index of AgNPs, mostly in cancer treatment. The Fig. 8 graph shows the association between the concentration of AgNPs and the viability of HEPG2 cells. The data points form a sigmoidal curve typical for dose-response analyses, where cell viability decreases with increasing concentrations of the AgNPs. At concentrations below 1 µg/ml, the AgNPs showed minimal cytotoxicity, as cell viability remains close to 100%. The half-maximal inhibitory concentration (IC₅₀), which is the concentration at which the AgNPs reduced cell viability by 50%, was determined to be $\approx 3.47 \pm 0.1$ µg/ml. This indicates the potency of the compound; the lower the IC₅₀ value, the more potent the AgNPs. Between 1 µg/ml and 10 µg/ml, there is a steep decline in cell viability, indicating a significant increase in the AgNPs cytotoxic effects within this concentration range. Elsewhere 10 µg/ml, the curve plateaus, suggesting that maximum cytotoxicity is achieved, and further increases in concentration do not significantly alter cell viability.

The IC₅₀ value is a critical parameter for assessing the therapeutic potential of anticancer agents. An IC₅₀ of 3.47 µg/ml suggested that the AgNPs have a moderate level of potency against HEPG2 cells, which could be advantageous if the AgNPs also exhibit selectivity towards cancer cells over normal cells. The current IC₅₀ concentration studies are associated with earlier reports by Lee et al (Lee, K. X. et al. 2023) and Venmani et al (Venmani, S. et al. 2023). The mode of action of AgNPs induce cytotoxicity could be due to apoptosis induction, cell cycle arrest, or interference with specific metabolic pathways essential for cell survival. Comparing the IC₅₀ value of these AgNPs with those of currently used chemotherapeutic agents could provide insights into their relative efficacy and potential advantages. The data suggested that the AgNPs hold promise as a therapeutic agent against liver cancer. However, pharmacokinetic properties such as absorption, distribution, metabolism, and excretion (ADME) need to be assessed to further understand its potential as a viable drug (Ropisah Me. Et al 2023; Peter Takác. Et al. 2023). Additionally, *in vivo* studies are crucial to evaluate the efficacy and safety of the AgNPs in a biological context that more closely resembles the clinical scenario.

In conclusion, the dose-response curve for the HEPG2 cell line provides valuable preliminary data on the cytotoxic potential of the AgNPs. These results lay the groundwork for more comprehensive studies to

evaluate the AgNPs' mechanism of action, therapeutic index, and potential for clinical application in cancer therapy.

4. Conclusion

This study underscores the significant potential of biogenically synthesized silver nanoparticles (AgNPs) as versatile agents in medical applications, particularly for their antimicrobial and anticancer properties. The AgNPs demonstrated substantial efficacy against various bacterial strains and showed promising results in inhibiting the proliferation of the HepG2 cancer cell line through the induction of apoptosis. These findings suggest that biogenic AgNPs can offer a safer and more efficient alternative to conventional nanoparticles, attributed to their specific functionalities and reduced toxicity. Furthermore, the study advocates for the continued exploration and refinement of green synthesis methods in nanoparticle production. This approach not only enhances the sustainability of nanoparticle synthesis but also broadens their applicability across modern technological and medical fields. The promising results presented here pave the way for future research and development, potentially revolutionizing therapeutic strategies and contributing to advancements in healthcare and beyond.

Declarations

CRedit authorship contribution statement

Rajeh Alotaibi: Writing – original draft, Supervision, Methodology, Formal analysis, Conceptualization, Funding acquisition. **Riyadh H. Alshammari:** Visualization, Writing – review & editing, data curation. **Sultan Almadhhi:** Investigation, Resources, Writing – review & editing. **Saad G. Alshammari:** Data curation, Formal analysis. **Ahmed S. Alobaidi:** Data curation, Formal analysis. **Ahmad Rady:** Data curation, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Figures

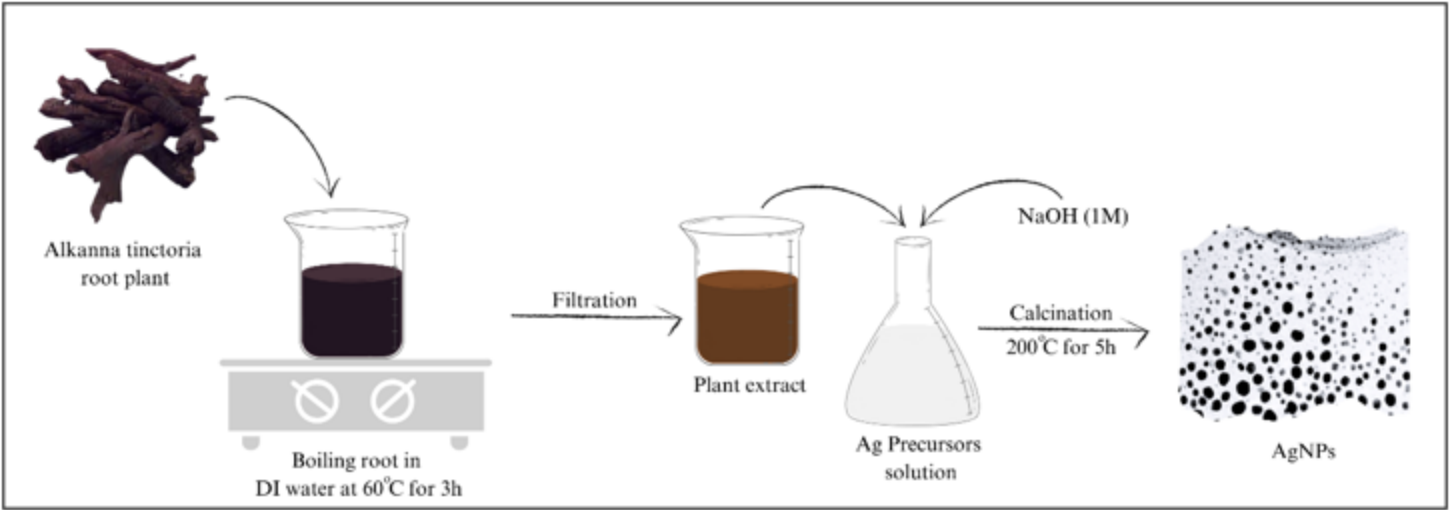


Figure 1

Schematic of the synthesis of silver nanoparticles with alkanna plant root extract.

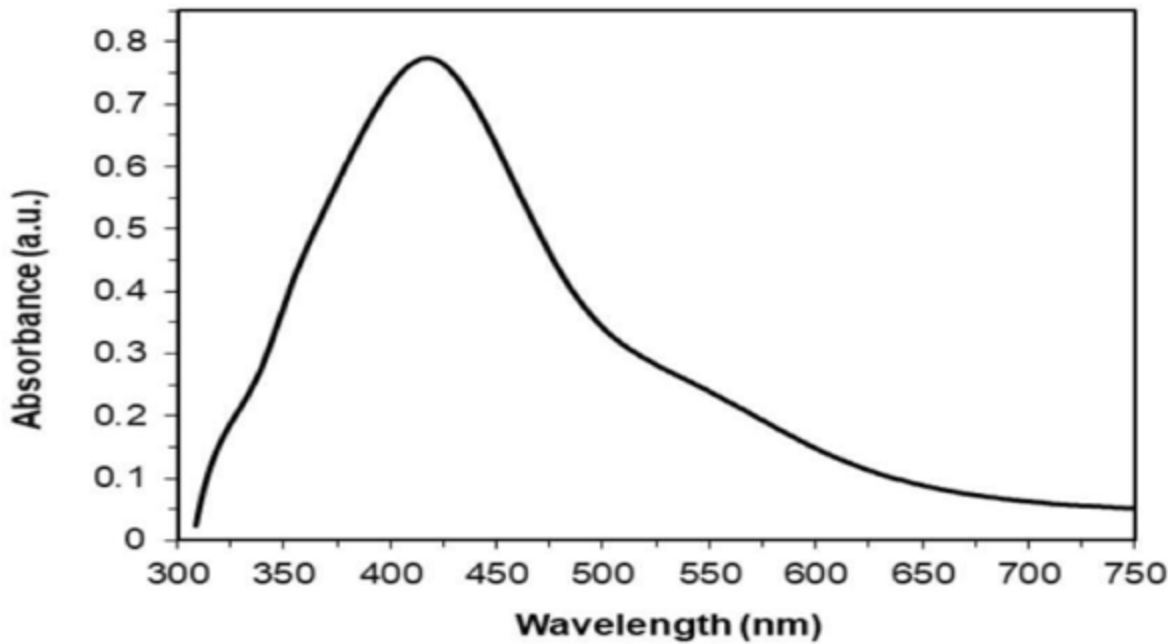


Figure 2

UV-Vis spectrum of AgNPs.

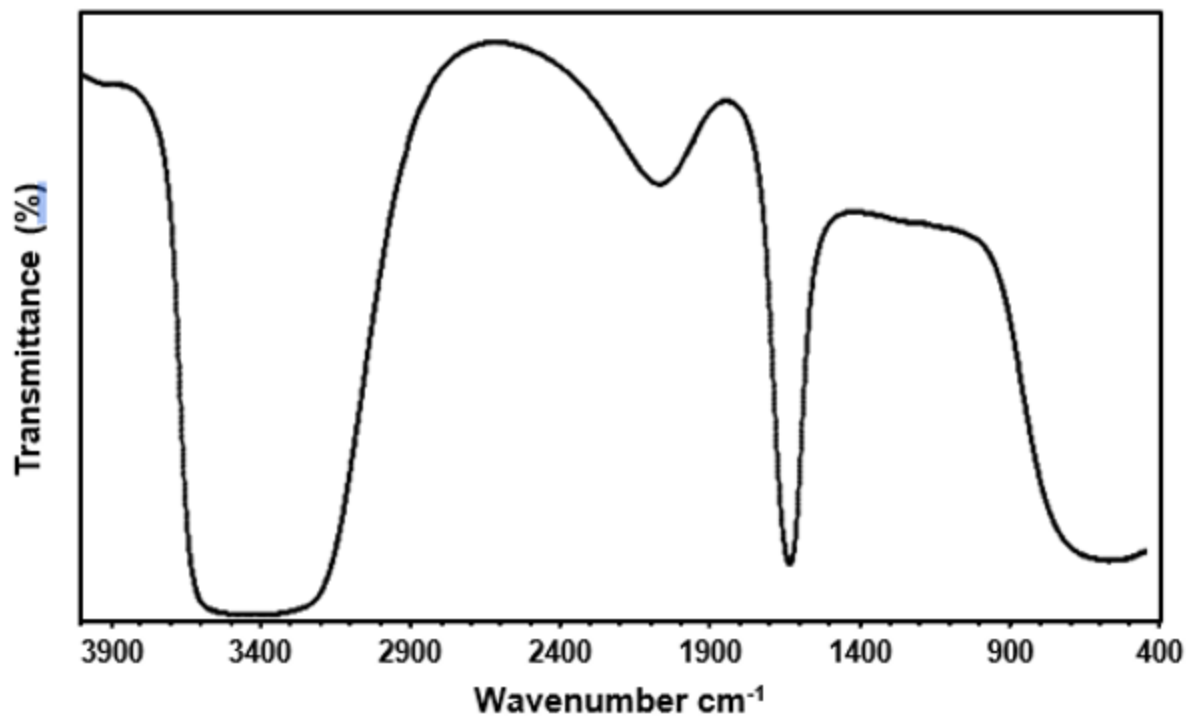


Figure 3

FT-IR spectrum of solid AgNPs.

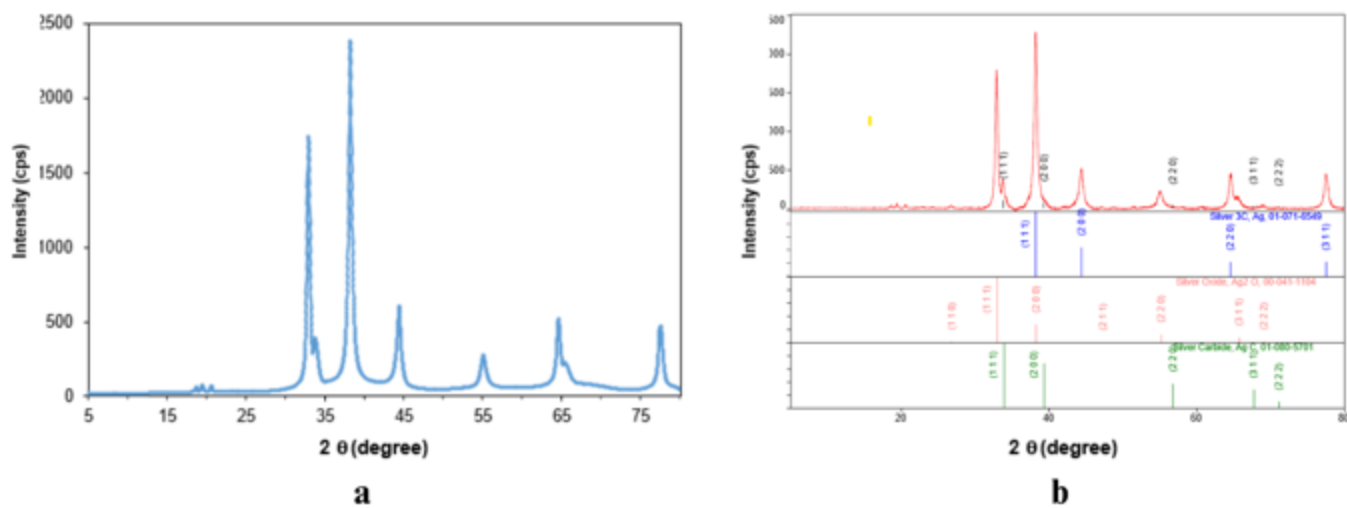


Figure 4

XRD pattern of AgNPs; a) XRD spectrum, b) Detailed XRD pattern of AgNPs.

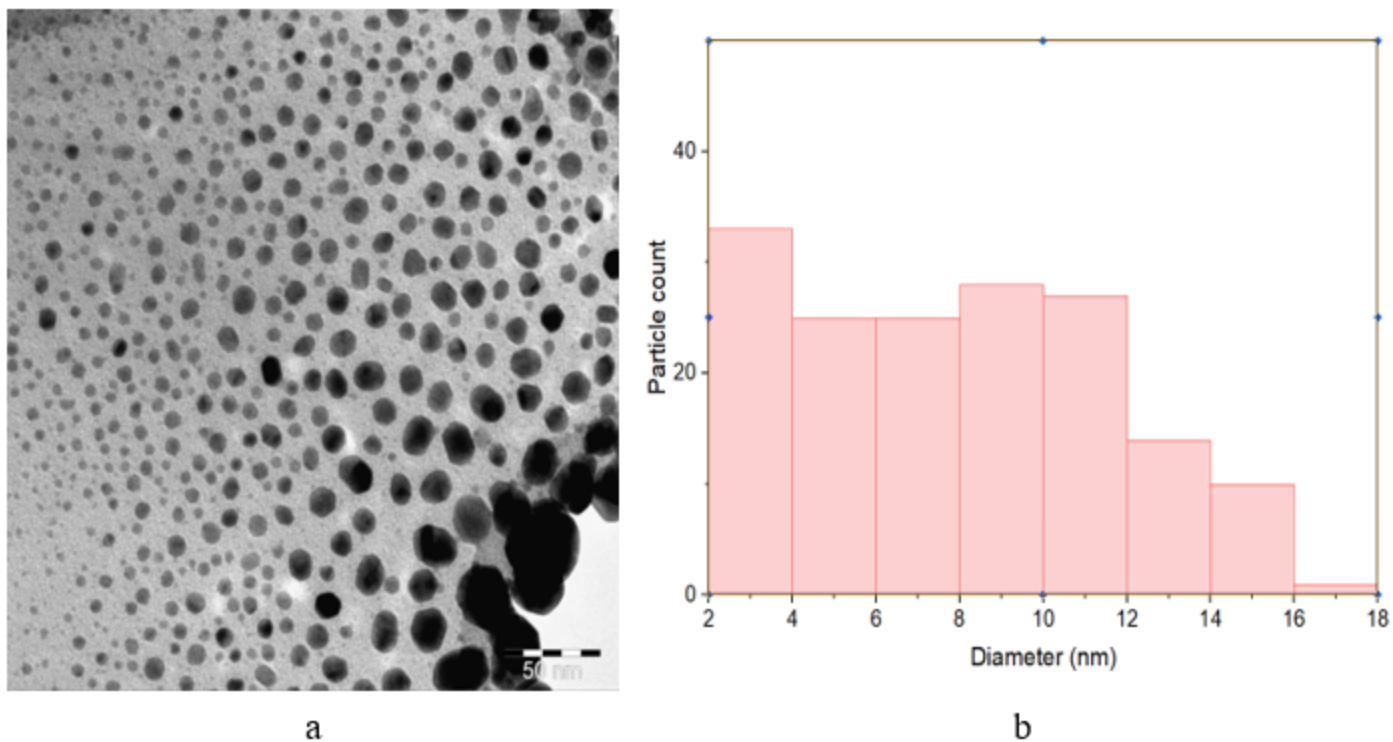


Figure 5

(a,b) TEM images and size distribution Histogram of AgNPs.

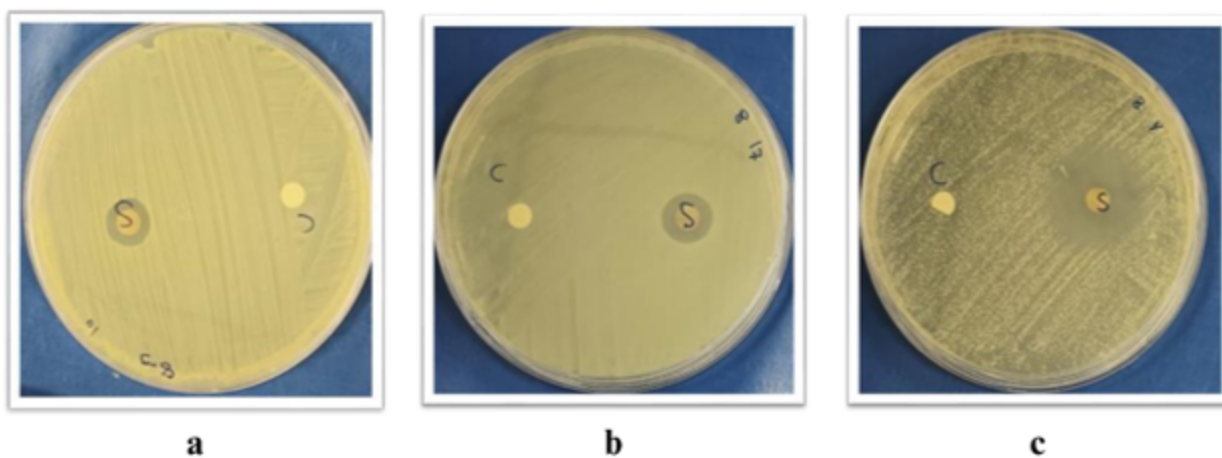


Figure 6

Antimicrobial activity of AgNPs against microbial pathogens; (A) *S. aureus*, (b) *E. coli*, (c) *C. albicans*.

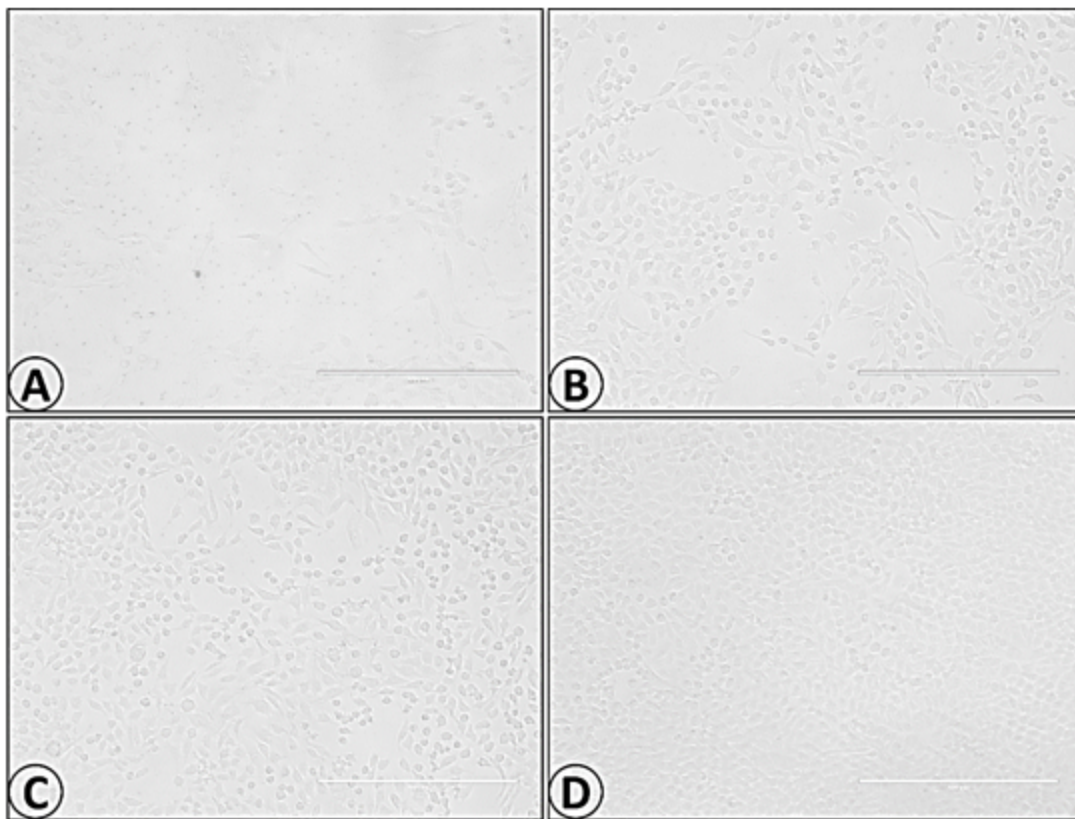


Figure 7

Microscopic examination of HEPG2 cancer cell line responses to different concentrations of AgNPs; (A) AgNPs $6.85 \mu\text{g ml}^{-1}$, (B) $2.28 \mu\text{g ml}^{-1}$, (C) $0.76 \mu\text{g ml}^{-1}$, and (D) MeOH control group.

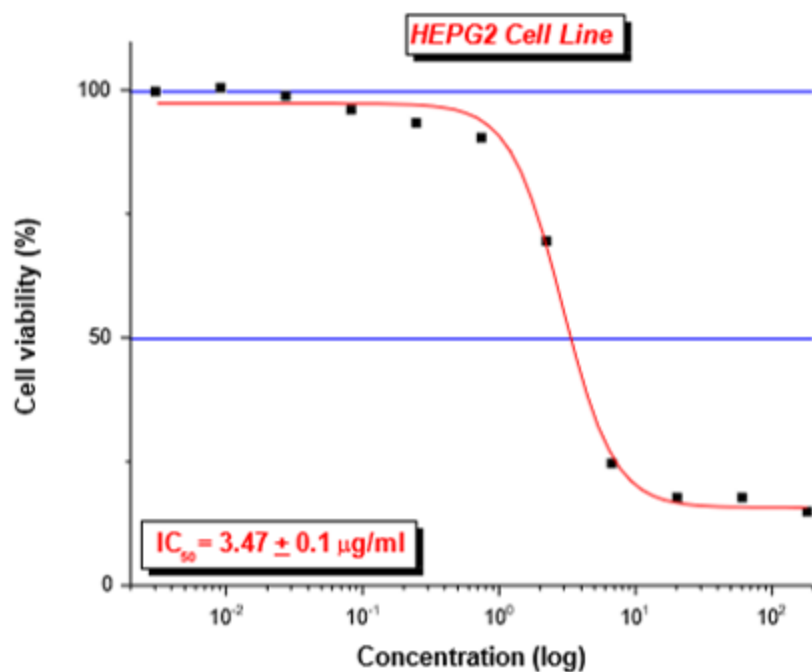


Figure 8

Assessment of IC₅₀ concentration of AgNPs against HEPG2 cell lines.