



ORIGINAL ARTICLE

Ecofriendly synthesis of silver nanoparticles using Kei-apple (*Dovyalis caffra*) fruit and their efficacy against cancer cells and clinical pathogenic microorganisms



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Abstract Green fabrication has become a safe approach for producing nanoparticles. Plant-based biogenic synthesis of silver nanoparticles (AgNPs) has emerged as a possible alternative to traditional chemical production. In this paper, we provide a low-cost, green synthesis of AgNPs utilizing using Kei-apple (*Dovyalis caffra*) fruit extract. Ultraviolet–visible (UV–Vis) spectroscopy, Fourier Transform Infrared (FTIR), Transmission Electron Microscopy (TEM), X-Ray Diffraction (XRD), Scanning-Electron Microscope (SEM), and Dynamic Light Scattering (DLS) analyses were used to characterize green produced AgNPs. The formation of AgNPs was shown to have a surface resonance peak of 415 nm in UV–visible spectra, and FTIR spectra verified the participation of biological molecules in Synthesis of AgNPs. The TEM revealed that the biosynthesized AgNPs were mostly spherical in form, with size range of 12–53 nm. XRD diffractogram was used to demonstrate the face cubic centre (fcc) character of AgNPs. Excellent anticancer activity of AgNPs was recorded where more than 80% of Prostate Cancer (PC-3) cell lines was inhibited by 100–150 µg/mL of AgNPs, while 38% only was recorded using AgNO₃ and 55.62% was recorded *D. caffra* fruit extract at 150 µg/mL. Destructions of PC-3 cell was observed as a result of exposed to AgNPs, followed by *D. caffra* fruit extract, while minor alterations were recorded as exposed to AgNO₃. The

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2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging using AgNPs was three fold using fruit extract at 100 µg/mL indicating good antioxidant activity. Excellent inhibitory activity of AgNPs was recorded against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus fumigatus* with inhibition diameter zone 28.22 ± 0.25 mm, 23.21 ± 0.35 mm, 27.25 ± 0.03 mm, 28.40 ± 0.15 mm, 29.23 ± 0.44 mm, and 9.52 ± 0.5 mm, respectively compared with AgNO₃. *D. caffra* fruits considered a promising and safe source for fabrication of AgNPs with multi-biological functions.

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1. Introduction

The unique properties of nanoparticles (NPs) have attracted the attention of researchers in various fields (Hashem and Salem, 2021, Salama et al., 2021, Salem and Fouda, 2021). Nanoparticles' unique properties, such as low melting points, optical and magnetic properties, high surface area to volume ratios, and excellent mechanical strength, have been documented in numerous scientific reports, making them useful not only in biological fields like medicine, diagnostics, drug delivery, agriculture, but also in industrial mechanical applications (Burduşel et al., 2018, Ganash et al., 2018, Mathur et al., 2018, Alsharif et al., 2020, Salem et al., 2020, Badawy et al., 2021, Abdelaziz et al., 2022, Salem, 2022). Among NPs, silver nanoparticles (AgNPs) have received some attention due to their unique physicochemical and biological properties (Abdelghany et al., 2018; Salem et al., 2022; Sharaf et al., 2022). The biological synthesis of NPs has several advantages, including reduced production of toxic by-products, increased stability, and reduced toxicity to healthy cells (Hashem et al., 2021). Furthermore, biogenic synthesis is low-cost, allowing for the rapid production of effective NPs (Hammad et al., 2022; Mohamed et al., 2021). Plant extracts, fungus, bacteria, and algae have all been proposed as safe sources for the synthesis of NPs in the prior (Ghany et al., 2013, Mohamed 2013, Shaheen et al., 2021). When compared to chemical procedures, green approaches allow for the synthesis of NPs in a variety of sizes and forms, each with its own set of characteristics (Salem et al., 2023; Salem and Fouda, 2021). Plants are a good and quick source of various NPs (Mittal et al., 2013). However, the world literature comprises very little information about the bio-fabrication of NPs using *Dovyalis caffra*. *D. caffra* (Kei-apple) belongs to family *Salicaceae* (Qanash et al., 2022). There is still a need for a cost-effective, commercially viable, and ecologically friendly method of synthesizing AgNPs from novel plant sources. Despite the fact that various studies have confirmed the production of AgNPs from a variety of plants (Abdelghany et al., 2018). Kei-apple fruit extract was used to investigate the production and cytotoxic effects of Ag, Au, and Au-Ag bimetallic nanoparticles, which exhibited anticancer potential against breast cancer MCF7 cell line (Adeyemi et al., 2019a,b). Synthesis of ZnONPs by *D.caffra* fruits extract, and its photocatalytic evaluations was also evaluated (Adeyemi et al., 2019a,b). From previous literature, it is evident that the formation of NPs by plant extract is enabled by the presence of phenolic acids, terpenoids, alkaloids, and polysaccharides within the plant extract that can act as reducing and capping agents for NPs formation (Kuppusamy et al., 2016, Aboyewa et al., 2021). They exhibited good reservoir of these bioactive ingredients. Numerous biological activities of NPs stabilized with plant extracts have been studied. Green AgNPs have been used to combat drug resistance in a variety of applications, including anticancer and antibacterial (Wypij et al., 2021). Among some documents, Abdelghany et al. (Abdelghany et al., 2018), have recorded good antimicrobial and anti-tumor effect of AgNPs mediated by green approaches. Zhang et al. (Zhang et al., 2019) studied the antibacterial activity of AgNPs from plants, and showed food-born and human-pathogenic bacteria. The

current research aimed to synthesis of AgNPs using *D. caffra* fruit extracts with studding their some biological activities including antitumor, antioxidant and antimicrobial activities.

2. Material and methods

2.1. Material

Silver nitrate (AgNO₃) used in this study was purchased from Sigma-Aldrich, Louis, USA. Other chemicals, culture media and reagents used in this study were purchased from Modern Lab Co., India in analytical grade without any purification required.

2.2. Preparation of *D. caffra* extract for AgNPs synthesis

During the months of January to August 2021, fresh, healthy fruits were picked from the *D. caffra* plant. *D. caffra* plant was collected from Menoufia Governorate, Egypt (30° 62'8014" N, 116° 31' 070334" E). The fruits were rinsed in a stream of running tap water to eliminate any dust, then pulped (skin and meat) in an electric mixer for additional extraction. The pulp was filtered using a 1 mm mesh to get smooth pulp free of skin and fibers. The purified extract was used to generate AgNPs. In a clean Erlenmeyer flask, 1 mL of aqueous *D. caffra* fruits extract and 9 mL of 1 mM AgNO₃ solution were combined to make the appropriate reaction mixture. On the other hand, as a control, the identical experimental setup containing 1 mL aqueous *D. caffra* fruits extract with 9 mL distilled water was used. Both flasks were incubated in the rotary shaker for 2–4 h at room temperature in the dark. The generated AgNPs were then separated and purified using pure water and rapid centrifugation (9000 rpm, 20 min at 10 °C) SiGMA 2 k15, Germany. The AgNPs was separated and dried at 80 °C for 48 h. For further analysis and bioactivity evaluation, the dried AgNPs were stored at 4 °C.

2.3. Characterization of silver nanoparticles

During the incubation time, the formation of AgNPs was visually monitored by following the changes in suspension colour. The creation of Ag colloids by *D. caffra* extract was also frequently examined using UV–Vis spectra (JENWAY 6305 Spectrophotometer) at wavelengths of 200–800 nm to identify a strong absorption peak linked to surface plasmonic excitation. *D. caffra* extract without silver nitrate was used to symbolize as blank. The formation of AgNPs in the absorbance

range 400–450 nm was suggested by a strong peak in the UV visible spectrum. To identify functional groups relevant for reduction, regulating, and encapsulating AgNPs, FT-IR analysis was done on *D. caffra* fruit extract and bio generated AgNPs. Using a FT-IR spectrometer (Agilent system Cary 630 FT-IR model) and the potassium bromide (KBr) pellet method, the assessment was completed. The frequencies were recorded at a resolution of 4 cm^{-1} in the $4000\text{--}400\text{ cm}^{-1}$ range. TEM (JEOL 1010, Tokyo, Japan) was utilized to characterize AgNPs in order to identify their size and shape. The sample was made by dropping the AgNPs solution onto a carbon coated Cu-grid and placing it into a specimen holder. The AgNPs were analyzed using an X-ray diffractometer X'Pert Pro (Philips, Eindhoven, Netherlands), which was fitted with a ratio detector of Ni-filter/Cu-K radiation ($=1.5405$) and maintained at a voltage of 40 kV and a current of 30 mA. The crystalline-nature of AgNPs was studied at temperatures ranging from 10° to 80°C . The collected peaks were allocated and compared to the Joint Committee on Powder-Diffraction Standards database (JCPDS). The surface morphology of Ag particles were confirmed by scanning-electron microscope (SEM) (JEOL- JSM.6360LA, Japan). DLS was used to study the dispersion of AgNPs in colloidal solution utilizing Zetasizer Nano equipment (Malvern Instruments, Worcestershire, UK). The AgNPs sample was re-suspended in purified water at 25 ppm and vortex-mixed to obtain a homogenous solution. Colloidal solution (1.5 mL) of AgNPs was then transferred to a square cuvette for detection. The measurements were done in the range of 0.1–1000 nm. Zetasizer software was used to examine the data of AgNPs sample.

2.4. Inhibitory activity of AgNPs against some bacteria and fungi

The inhibitory activity of AgNPs against bacteria and fungi was achieved using a disc-diffusion test as described previously [16] with some minor modifications. Four bacteria including *Bacillus Subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 90274), one unicellular fungus *Candida albicans* (ATCC 10221) and one filamentous fungus *Aspergillus fumigatus* (ATCC 10845), that provided from Regional center for mycology and biotechnology (RCMB), Al-Azhar University, Egypt. Sterile petri dishes containing 20 mL of Mueller–Hinton agar medium were inoculated by test bacteria (0.1 mL bacterial suspensions, 10^8 cfu mL^{-1}) via streaking method. Sterile petri dishes containing also 20 mL of Yeast Extract Peptone Dextrose agar were used as medium of fungi growth, streaked by 0.1 mL bacterial suspensions, 10^4 cfu mL^{-1} . Two dilutions including 15 and 30 mg/mL of AgNPs were prepared from the stock solution of 30 mg/mL of AgNPs using DMSO. Filter paper (Whatman filter paper-3) discs with diameter 6 mm radius under aseptic conditions were immersed in each dilution, then dried, placed on the surface of the inoculated growth media. The inoculated plates were kept in refrigerator for 40 min. for proper diffusion of AgNPs, followed by incubation at 30°C for 2–3 days for fungi and 37°C for 1 day for bacteria. The visualized inhibition zone around each disc was measured. Discs loaded with Gentamycin (0.1 mg/mL) as antibiotic, Ketoconazole (0.1 mg/mL) as antifungal, were used as a positive control. Discs loaded with DMSO and AgNO_3 (30 mg/

mL) was used as a negative control in separate streaked plates with tested organisms giving no any activity.

2.5. Antioxidant activity of AgNPs

To detect the antioxidant activity of AgNPs using DPPH free radical scavenging assay, 1 mL of AgNPs at different dilutions up to $800\text{ }\mu\text{g/mL}$ dissolved in 10 % DMSO was added to 1 mL of 0.3 mM DPPH (Abdelghany et al., 2021). The reaction mixture was kept in the dark for 30 min. At 540 nm, the absorbance was recorded as a result of reduction in color intensity of DPPH using UV–Vis spectrophotometer, model (UV-1800 Shimadzu). Ascorbic acid at different concentrations up to $40\text{ }\mu\text{g/mL}$ of was applied as reference. The following formula was used to calculate inhibition %.

$$\text{Inhibition\%} = \frac{\text{Blank absorbance} - \text{AgNPs absorbance}}{\text{Blank absorbance}} \times 100$$

2.6. Antitumor assay

PC-3 cell lines were obtained from Nawah Scientific Inc., Cairo, Egypt. Antibiotics (Penicillin 100 units/mL and streptomycin 100 mg/mL) were added to the Dulbecco's Modified Eagle Medium and 10% of heat-inactivated fetal bovine serum was used for cancer cell preservation at 37°C in humidified containing 5% (v/v) of CO_2 atmosphere. Viability of cell was detected via Sulforhodamine B (SRB) assay, briefly the cells suspension ($100\text{ }\mu\text{L}$) containing 5×10^3 cells was poured 96-well plates containing treated medium by different levels of samples, then incubated for 24 h. After exposure of cells to treatments, it fixed via substituting media by $150\text{ }\mu\text{L}$ of 10 % Trichloroacetic acid (TCA) and preserved for 1 h at 4°C , followed by TCA desiccant and washing the cells for 5 times by distilled water. Solution of TCA was detached, and the cells were washed 5 times using distilled water. The cells were immersed by $70\text{ }\mu\text{L}$ SRB solution (0.4% w/v) for 10 min in a dark at 25°C , then the plates were washed by 1% of acetic acid for 3 times, the dried in air for 12 h. Dissolve protein bound SRB stain was performed using $150\text{ }\mu\text{L}$ TRIS base solution (10 mM , pH 10.5), using a BMG LABTECH®- FLUOstar Omega microplate reader (Ortenberg, Germany) the absorbance was reading at 540 nm.

3. Result and discussion

3.1. Synthesis and characterization of AgNPs

Plants are widely available and may be used to manufacture metal nanoparticles (M–NPs), particularly AgNPs. Plants convert metal ions to M–NPs at a far quicker pace than microbes (Vanlalveni et al., 2021). The crude extract of ripe fruit Kei-apple (Fig. 1) was evaluated for synthesis of AgNPs. The dissolving of the *D. caffra* extract resulted in a quick shift in colour from yellow to dark brown after 4 h, demonstrating the rapid reduction of Ag^+ to Ag^0 in AgNO_3 solution (Fig. 2A), whereas the control samples showed no change. The primary and most visible confirmation for the creation of Ag NPs is the colour change in the solution caused by sur-



Fig. 1 Ripe fruit Kei-apple (orang arrows) and extract (E).

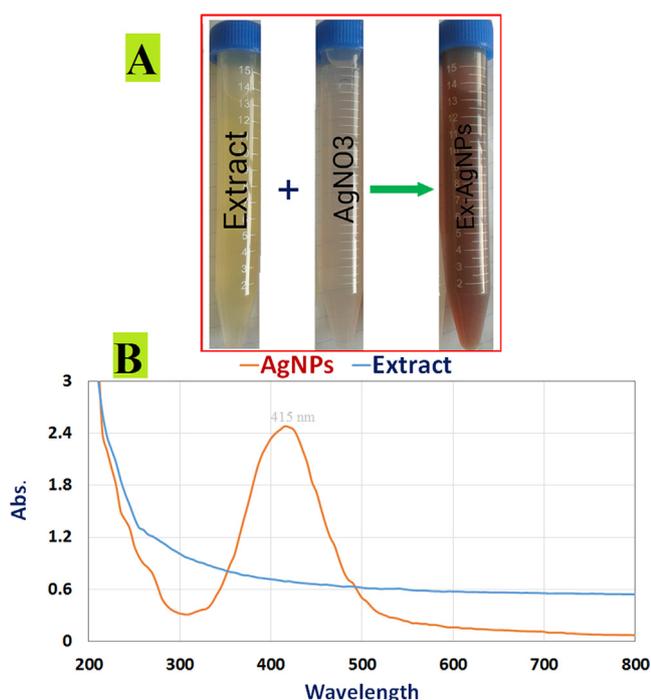


Fig. 2 Biosynthesis of AgNPs confirmed. (A) Color changes in *D. caffra* extract after adding 1 mM aqueous AgNO_3 solution for AgNP production; (B) UV-visible spectrum of AgNPs generated by *D. caffra* extract (Orange curve) and UV-visible spectrum of *D. caffra* extract.

face plasmonic excitation of AgNPs (Pirtarighat et al., 2019). Furthermore, UV-Visible optical findings validated the production of AgNPs in the mixture, with a surface plasmon-resonance (SPR) spectra at 415 nm of maximum absorption (Fig. 2B). The obtained results are analogous to those published in the literature, in which a *Datura stramonium* extract

was employed to produce silver nanoparticles, and UV-Visible absorbance spectra indicated that the SPR band for Ag particles was in the 400–450 nm range (Gomathi et al., 2017). The aqueous extract of a previously collected batch of plant was utilized to validate the repeatability of AgNPs synthesis, which produced AgNPs when the optimal ratio of plant extract to Ag^+ (1–9 mL) was applied (Gomathi et al., 2017, Aslam et al., 2021).

Fig. 3 also depicts the FTIR spectra of biogenic AgNPs produced from *D. caffra* extract following interaction with Ag^+ , as well as a *D. caffra* extract control without Ag^+ . As seen in Fig. 3, the FTIR data shows a slight change in the peak location of spectra. The amount of functional biological groups responsible for NPs stabilization, which serve as capping or stabilizing agents, is revealed by spectral analysis. Different absorption peaks were found in FTIR measurements based on AgNPs mediated by *D. caffra* extract at 3659 cm^{-1} , 3335 cm^{-1} , 3098 cm^{-1} , 2355 cm^{-1} , 2068 cm^{-1} , 1623 cm^{-1} , 669 cm^{-1} , 566 cm^{-1} , and 482 cm^{-1} . In the presence of phenols and alcohols with free $-\text{OH}$ and $-\text{NH}$ groups, absorption bands at 3659 cm^{-1} , 3335 cm^{-1} , and 3098 cm^{-1} emerge (Akinfenwa et al., 2021). The presence of symmetric stretching of $-\text{COO}$ and alkanes, $-\text{C}-\text{N}$ groups, is represented by the bands at 2355 cm^{-1} and 2068 cm^{-1} . The absorption band at 1629 cm^{-1} is corresponded to the $-\text{NH}$ (amide group) and $-\text{C}=\text{C}-$ in aromatic materials (Aref and Salem, 2020). The $-\text{OH}$ and $-\text{C}-\text{N}$ stretching vibrations of amines are attributed to bands at 1407 cm^{-1} , 1228 cm^{-1} , and 1054 cm^{-1} (Krithiga et al., 2015). In the spectrum of AgNPs, additional bands at 669 cm^{-1} , 566 cm^{-1} , and 482 cm^{-1} were discovered, which might allude to the binding of hydroxyl of a plant's organic metabolite with AgNPs (Masum et al., 2019). Proteins that contain free carboxylate sites can interact to AgNPs and stabilize them. The location of emission spectra in the FTIR spectrum of *D. caffra* extract and biosynthesized Ag NPs differed only little. The presence of *D. caffra* extract components in AgNP biosynthesis was verified by shifting peaks (Taher et al., 2018). As a result, *D. caffra* extract substances such as NH, CO, and OH groups play an important role in the reduction and stability of AgNPs (Pirtarighat et al., 2019, Eid et al., 2020).

The TEM image (Fig. 4A) clearly demonstrated that most AgNPs were significantly the spherical shape seems to be predominant, which was consistent with the SEM image (Fig. 4A). AgNPs were observed to have an average size of 12 nm to 53 nm. The brilliant circular spots in the area selected electron diffraction (SAED) pattern (Fig. 4B) indicated the (311), (220), (200), and (111) planes, as well as the crystalline structure of *D. caffra*'s Ag particles. The XRD pattern of AgNPs is seen in Fig. 4C. In the first stage, the indexing was completed, and miller indices were allocated to each peak (Vishwasrao et al., 2019). AgNPs with face-centered cubic symmetry accounted for the whole of the reflection peaks (Shah et al., 2021). The AgNPs were extremely crystalline, as evidenced by the high intensity of peaks. Peaks at 38.2° , 45.66° , 64.42° , and 77.74° in the diffraction pattern of AgNPs were attributed to 111, 200, 220, and 311, respectively, of face-centered cubic pure AgNPs (JCPDS 87-0720). It confirmed that Ag metal was the main ingredient of AgNPs. The peaks' line widening is mostly owing to the tiny particle size (Jain and Mehata 2017). The AgNPs generated by the reduction of Ag^+ ions by the *D. caffra* extract are crystalline in nature,

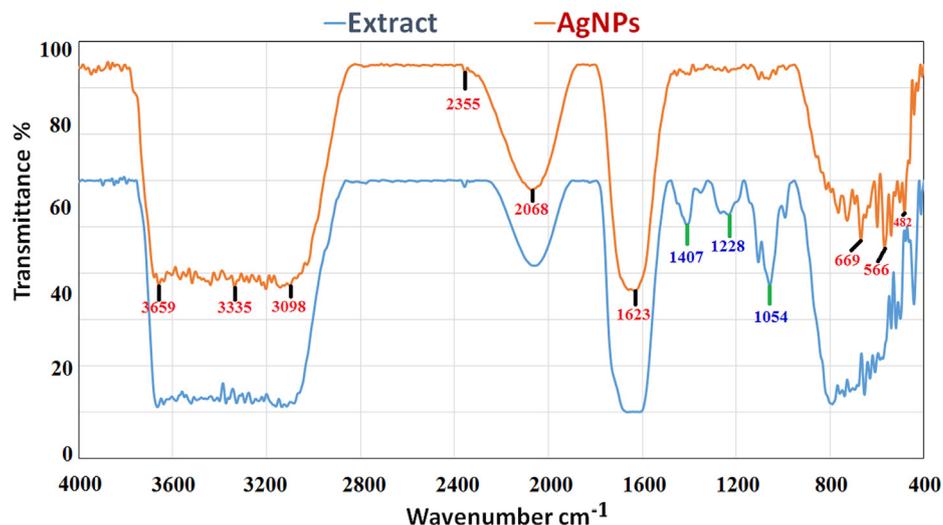


Fig. 3 Fourier-transform infrared spectrum of biosynthesized AgNPs and extract of *D. caffra*.

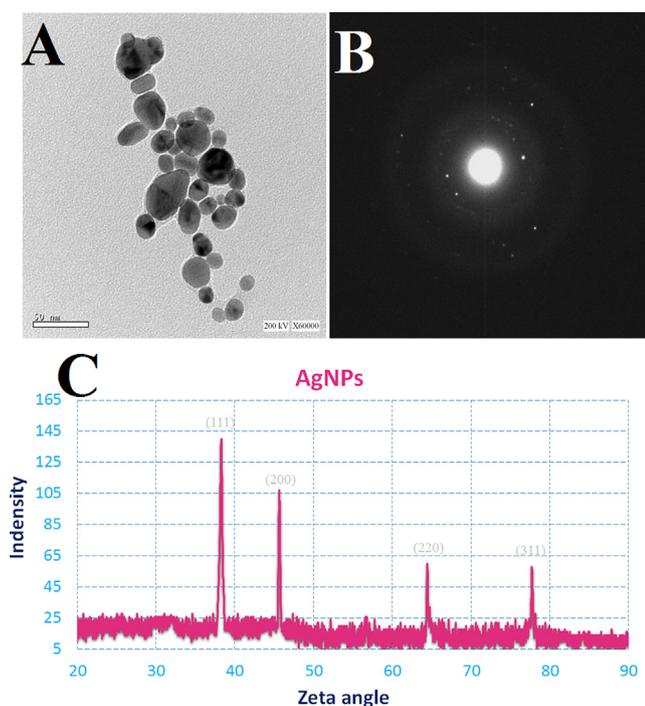


Fig. 4 TEM image (A); SAED pattern (B); and XRD (C) of the synthesized AgNPs by *D. caffra* extract.

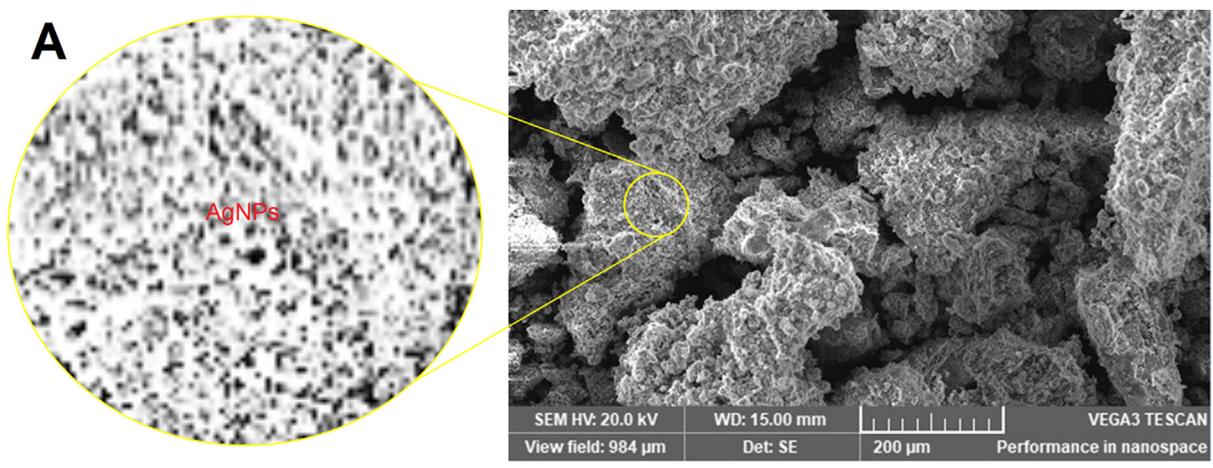
according to the X-ray diffraction observations. The results showed that the phyto-synthesized AgNPs were made up of high-purity crystalline Ag particles.

The AgNPs morphology was examined using a scanning electron microscope (SEM) image (Fig. 5A). The bulk of Ag particles were spherical in form, with a few oval AgNPs thrown in for good measure. The biosynthesized AgNPs had been evenly distributed throughout the solution. The findings of this study are consistent with prior research that found spherical AgNPs when synthesis was facilitated by plant

extract (Ansari and Alzohairy 2018, Pirtarighat et al., 2019). Dynamic light scattering (DLS) analysis was used to determine the average diameter of total Ag particles. The bulk of the AgNPs had a diameter of 90 nm with average diameter 170 nm (Fig. 5B). However, the size derived via DLS is modified by metabolites of *D. caffra* deposited on NP surface, such as organic molecules attached as stabilizers, as well as the metal core of Ag particles (Aref and Salem 2020). In the SEM image, the biomolecule coating of the biosynthesized Ag NPs can be seen. The function of *D. caffra* extract metabolites in the production and stabilization of biosynthesized AgNPs is confirmed by this layer. They are molecules found in plant extracts that attach to the edge of AgNPs and function as chelating agents, according to published data (Taruna et al., 2016).

3.2. Antimicrobial activity of AgNPs

To overcome the problems of antibiotic resistance bacteria and microbial infection development, discover or development safe antimicrobial compounds are necessary in the current days. The green synthesized AgNPs at two concentrations (15 mg/mL and 30 mg/mL) were tested against some bacteria and fungi using agar disc diffusion protocol compared with AgNO₃ and positive control. Good inhibitory activity of AgNPs was observed against tested microorganisms with inhibition diameter zone was 28.22 ± 0.25 mm, 23.21 ± 0.35 mm, 27.25 ± 0.03 mm, 28.40 ± 0.15 mm, 29.23 ± 0.44 mm, and 9.52 ± 0.5 mm against *B. Subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans* and *A. fumigatus*, respectively (Fig. 6). Via disc diffusion assay, the antibacterial activity of AgNPs was confirmed against *S. aureus*, *B. cereus* and *P. aeruginosa*, *E. coli* (Chandrasekharan et al., 2022). In another recent study, different diameter of inhibition zones 13 mm, 17 mm, 18 mm and 14 mm were recorded against *Listeria monocytogenes*, *E. coli*, *Shigella dysenteriae* and *Salmonella typhi*, respectively as a result of AgNPs activity (Sharma et al., 2022). The obtained results indicated that AgNPs showed weak antifungal activity against filamentous



B

Z-Average (nm): 170.0562 Derived Count Rate (kcps): 107130.287105...
 Standard Deviation: 0 Standard Deviation: 0
 %Std Deviation: 0 %Std Deviation: 0
 Variance: 0 Variance: 0

Size d.nm	Mean Number %	Std Dev Number %	Size d.nm	Mean Number %	Std Dev Number %	Size d.nm	Mean Number %	Std Dev Number %	Size d.nm	Mean Number %	Std Dev Number %
0.4000	0.0		5.615	0.0		78.82	20.0		1106	0.0	
0.4632	0.0		6.503	0.0		91.28	16.7		1281	0.0	
0.5365	0.0		7.531	0.0		105.7	11.9		1484	0.0	
0.6213	0.0		8.721	0.0		122.4	7.7		1718	0.0	
0.7195	0.0		10.10	0.0		141.8	4.8		1990	0.0	
0.8332	0.0		11.70	0.0		164.2	3.0		2305	0.0	
0.9649	0.0		13.54	0.0		190.1	1.9		2659	0.0	
1.117	0.0		15.69	0.0		220.2	1.1		3091	0.0	
1.294	0.0		18.17	0.0		255.0	0.6		3580	0.0	
1.499	0.0		21.04	0.0		295.3	0.3		4145	0.0	
1.736	0.0		24.36	0.0		342.0	0.1		4801	0.0	
2.010	0.0		28.21	0.0		396.1	0.0		5560	0.0	
2.328	0.0		32.67	0.0		458.7	0.0		6439	0.0	
2.696	0.0		37.84	0.0		531.2	0.0		7456	0.0	
3.122	0.0		43.82	0.0		615.1	0.0		8635	0.0	
3.615	0.0		50.75	2.8		712.4	0.0		1.000e4	0.0	
4.187	0.0		58.77	10.7		825.0	0.0				
4.849	0.0		68.06	18.4		955.4	0.0				

Statistics Graph (1 measurements)

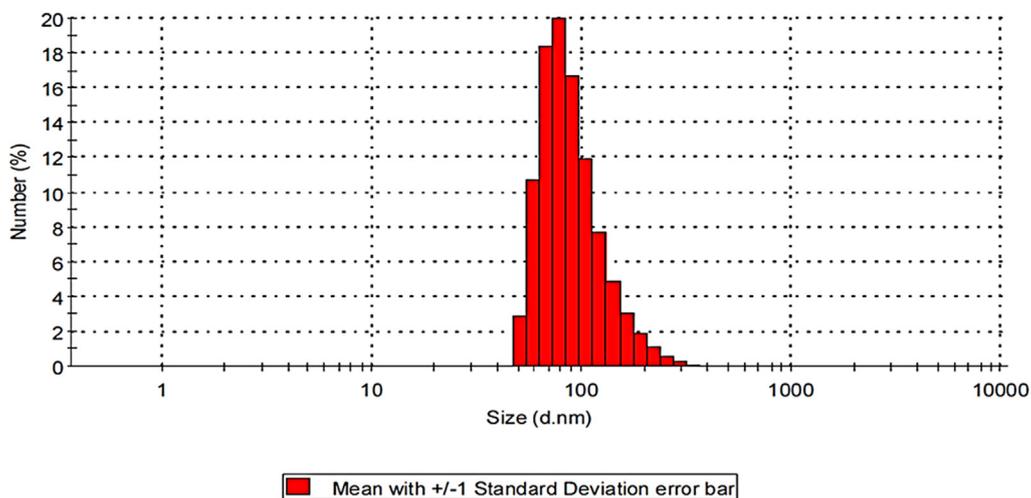


Fig. 5 SEM image (A) and DLS analysis (B) of AgNPs synthesized by *D. caffra* extract.

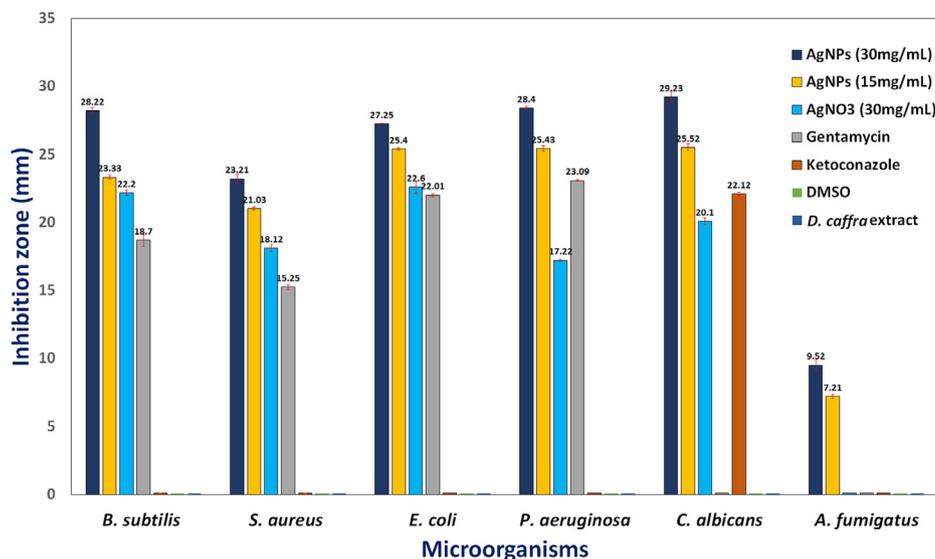


Fig. 6 Antimicrobial activity of AgNPs synthesized by *D. caffra* extract against some pathogenic strains.

fungus *A. fumigatus* unlike unicellular fungus *C. albicans*, but what supports the unique properties of AgNPs is that the AgNO₃ did not give any inhibition at the same used concentration of AgNPs (Fig. 6). These observations were partially agreement with the results Ahmad et al. (Ahmad et al., 2022), where who mentioned that AgNPs showed excellent antibacterial activity, moderate antifungal potential against *Alternaria alternata* and minor antifungal potential against *Fusarium gramineum*. The exact of the action mechanism of AgNPs against microorganisms still under researchers studies day up to day. Although some mechanisms were reported such as interference with microbial DNA, disruption of proteins (Abdel-Ghany et al., 2018, Sharma et al., 2022).

3.3. Antioxidant activity

Research for biological antioxidant compounds increase day by day, therefore the antioxidant characteristic of AgNPs was studied in some scientific papers. The obtained experiment reflected excellent antioxidant activity AgNPs followed by *D. caffra* fruit extract at the same utilized concentrations where the DPPH scavenging was 52.68 and 52.68 at 800 µg/ml. DPPH scavenging using AgNPs was three fold using fruit extract at 100 µg/ml (Fig. 7A). These results were compared to ascorbic acid as a potent antioxidant. Recently, Gecer et al. (Gecer et al., 2021) documented the antioxidant activity of AgNPs synthesized by *Echinacea purpurea* extract and recommended the application of AgNPs as additives in drugs and food. Antioxidant of *D. caffra* fruit was recorded in some studies due to the presence of phenolic and flavonoid compounds (Taher et al., 2018), and amino acids (Augustyn et al., 2018). DPPH scavenging % increment with increasing concentration AgNPs and fruit extract in dose depending manner. From the obtained results AgNPs displayed a promising antioxidant activity as compared to the standard, ascorbic acid (Fig. 7B). Good radical scavenging capability of AgNPs can be attributed to bioactive secondary metabolites present in the fungal cell filtrate which acts as a capping agent in nanoparticles.

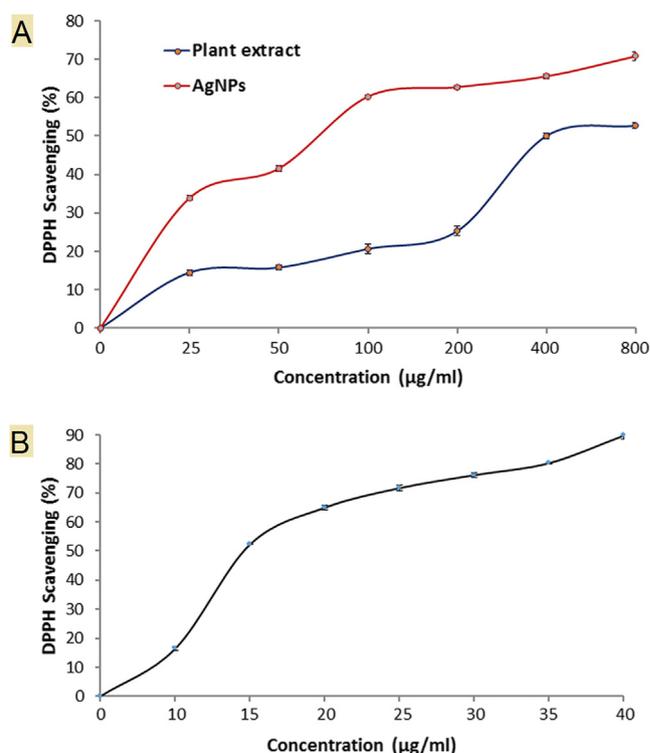


Fig. 7 DPPH Scavenging (%) of AgNPs, plant extract (A) and ascorbic acid (B).

3.4. Anticancer activity

Results of anticancer activity was documented against PC-3 cell lines at different concentrations of AgNPs compared with AgNO₃ and *D. caffra* fruit extract (Fig. 8). Inhibition of cell viability % was concentration dependent manner from 25 up to 150 µg/mL. AgNPs was more effective at all applied concentrations than *D. caffra* fruit extract followed by AgNO₃ and,

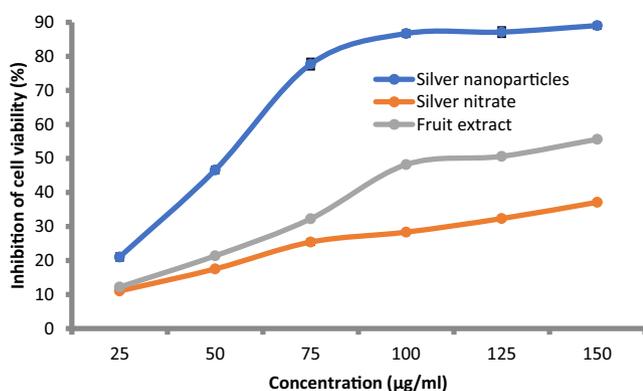


Fig. 8 Anticancer activity of AgNPs synthesized by *D. caffra* extract compared to *D. caffra* fruit extract and AgNO₃.

for example the inhibition of cell viability at 25 µg/mL was 21.03, 12.21 and 11.06 %, respectively. Sharply cell viability inhibition more than 80 % was observed at 100–150 µg/mL of AgNPs, while at the same concentration of AgNO₃, viability inhibition not reached to 38%. At 150 µg/mL of *D. caffra* fruit extract viability inhibition reached to 55.62%. Previous investigations have shown that biosynthesized AgNPs have a cytotoxic impact *in vitro* against PC-3, lung cancer cell lines, MCF-7, Hep-2 and HeLa cell lines (Raman et al., 2015, Al-Sheddi et al., 2018, Sangour et al., 2021, Chen et al., 2022). Although the anticancer potential of AgNPs was reported in numerous studies but the activity varies regarding size of AgNPs and source of synthesis. Rajawat et al. (Rajawat et al., 2016) observed that the proliferation of MCF-7 cancer cell lines were completely inhibited by 9 nm of AgNPs compared to 15 nm of AgNPs. Ovais et al. (Ovais et al., 2016) and Erdogan et al. (Erdogan et al., 2019) mentioned that the inhibitory mechanisms of AgNPs to cancer cells via mitochondria-mediated caspase apoptosis. The AgNPs showed cytotoxicity with IC₅₀ of 40 µg/mL against A549 cells (Akther et al., 2019). The differences of AgNPs toxicity dependent on its size and morphology beside cancer cell type. Morphological changes were observed on cells exposed to AgNPs, followed by *D. caffra* fruit extract, while negligible changes were recorded on AgNO₃ exposed

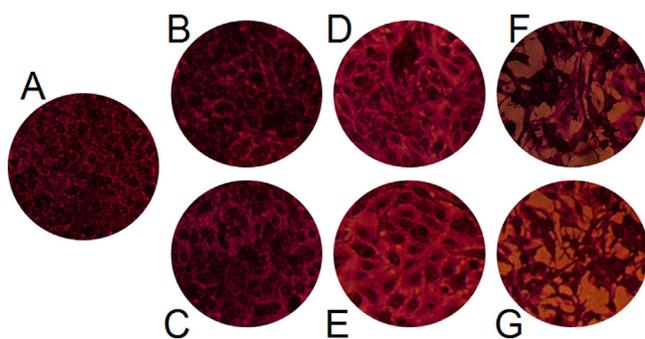


Fig. 9 Morphological alteration of treated PC-3. Control (A without treatment), 100 µg/mL (B) and 150 µg/mL (C) of AgNO₃; 100 µg/mL (D) and 150 µg/mL (E) of *D. caffra* fruit extract; 100 µg/mL (F) and 150 µg/mL (G) of AgNPs.

cells (Fig. 7). In the same line Firdhouse and Lalitha (Firdhouse and Lalitha 2013) noted that the apoptosis rate was highly of PC-3 treated by biosynthesized AgNPs than exposed to silver ions. There is no clear differentiation among control cells (Fig. 9A) and treated by 100 µg/mL (Fig. 9B) and 150 µg/mL (Fig. 9C) of AgNO₃. While relatively changes in the treated cells were observed including irregular clusters and floating cells using *D. caffra* fruit extract (Fig. 9D&E). On the other hand, membrane shrinkage, failure of cell adhesion, blebbing of cell membrane, lyses of cell membrane, appearance of unusual cellular crinkle and cell destruction were microscopically recorded at 100 µg/mL (Fig. 9F) and more observed at 150 µg/mL of AgNPs (Fig. 9G). The changes in the cells may be due to the interface of AgNPs with the functioning of PC-3 proteins. Most recognizable alterations in treated cells were consistent with recent report (Morais et al., 2021) viewing on efficacy of AgNPs against PC-3. Another mechanism reflect the efficacy of AgNPs through its induction to Fragmentation of PC-3 DNA that cause apoptosis in the cells (Zhang et al., 2019). The obtained finding demonstrated that the antiproliferative action of AgNPs mainly depends on the concentration, therefore, *D. caffra* fruit extract mediated AgNPs may offer as promising source for cancer treatment. Current finding suggested that the anticancer potential may be due to AgNPs may perhaps penetrate into the cell membrane of cancer cell leading to DNA damage as demonstrated by the DNA fragmentation (Kovács et al., 2022).

4. Conclusion

The created AgNPs were characterized using UV–Vis spectra, FT-IR analysis, TEM, XRD, SEM and DLS. All these characterization confirmed the synthesis of AgNPs. Through TEM analysis, the average size of AgNPs was 12 nm to 53 nm in spherical forms. The results of the FTIR spectra showed that the functional groups present in *D. caffra* extract play a major role in the formation of AgNPs. *D. caffra* fruit extract is a well-known producer of AgNPs, which have antioxidant, antibacterial, and anticancer properties, and may be used to solve a variety of medicinal, nutritional, and industrial problems.

CRedit authorship contribution statement

Aisha M.H. Al-Rajhi: Conceptualization, Writing – review & editing. **Salem S. Salem:** Conceptualization, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. **Asmaa A. Alharbi:** Writing – original draft. **T.M. Abdelghany:** Writing – original draft, Writing – review & editing.

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.

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