



Natural gum as bio-reductant to green synthesize silver nanoparticles: assessing the apoptotic efficacy on MCF-7 and SH-SY5Y cell lines and their antimicrobial potential

Mohammad Hashemabadi¹ · Hosseinali Sasan² · Mojdeh Amandadi³ ·
Reza Mohammadinejad⁴ · Gholamreza Farnoosh⁵ · Mostafa Azimzadeh^{6,7} ·
Ramezan Ali Taheri¹

Received: 11 December 2019 / Revised: 23 April 2020 / Accepted: 24 May 2020
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

In the present study, silver nanoparticles (AgNPs) were synthesized using *Prunus avium* gum extract and their physicochemical properties together with antibacterial and cytotoxic effects were evaluated. The characterization of these AgNPs was performed by UV–vis spectroscopy, transmission electron microscopy, scanning electron microscopy, and Fourier transforms infrared spectroscopy. The bio-performance of the nanoparticles was also evaluated. Our results exhibit the prominent antibacterial activity of AgNPs against both Gram-positive and Gram-negative bacteria. The MTT results indicate cytotoxicity of AgNPs on MCF-7 and SH-SY5Y cell lines at 50 and 10 $\mu\text{g mL}^{-1}$ concentrations, respectively. Moreover, gene expression analysis by real-time PCR represents the upregulation of Bax mRNA and downregulation of

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00289-020-03238-9>) contains supplementary material, which is available to authorized users.

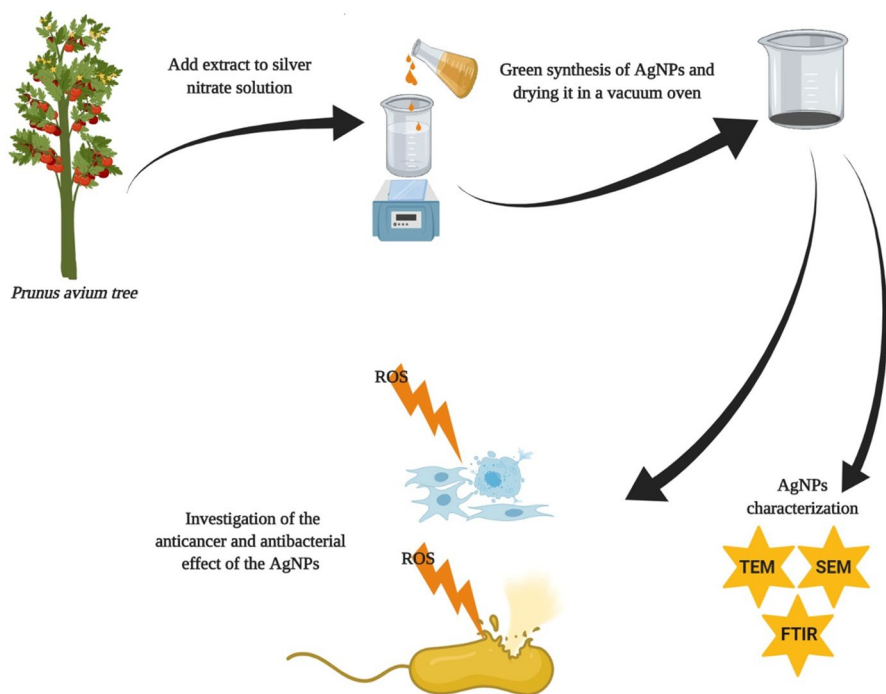
✉ Ramezan Ali Taheri
taheri@bmsu.ac.ir

- ¹ Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
- ² Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran
- ³ Department of Biochemistry, Faculty of Biological Science, Tarbiat Modares University, Tehran, Iran
- ⁴ Pharmaceutics Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran
- ⁵ Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
- ⁶ Medical Nanotechnology and Tissue Engineering Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, PO Box 89195-999, Yazd, Iran
- ⁷ Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, PO Box 89195-999, Yazd, Iran

Bcl-2 mRNA, demonstrates the induction of apoptosis after AgNPs treatment. These findings imply that green-synthesized AgNPs could be a promising candidate in biomedical applications such as battling cancer and infectious diseases.

Graphic abstract

The scheme of preparation of AgNPs and its effect on cell and bacteria.



Keywords *Prunus avium* gum · Silver nanoparticle · Cancer cell line · Cytotoxic activity · Antibacterial activity

Introduction

Nanoparticles are being defined as major building blocks of nanotechnology, and their awesome properties came from increasing contact surface due to diameter change to nano-size [1, 2]. Among nanomaterials, nanoscale solid support-based silver nanoparticles (AgNPs) as one of the significant members of metal nanoparticles have great medical and pharmaceutical applications due to good conductivity, biological compatibility, chemical stability, relative lower toxicity, antibacterial and anticancer activity and catalytic properties [3–6]. These unique and controllable characteristics lead to AgNPs utilize for developing medical and pharmaceutical systems such as therapeutic medicines, colorimetric sensors, drug delivery devices and bioimaging tools [7]. Moreover, AgNPs are one of the

promising nanoproducts commonly used as antimicrobial and anticancer agents against various bacteria, and cancer cells, respectively [8]. Ag^+ ions can readily diffuse into the cell and conduct to the nucleus through nuclear pore complexes, and create reactive oxygen species (ROS) which the high levels of these species lead to the DNA damage, chromosomal aberrations (genotoxicity) and eventually cell death [9]. Many studies purposely report the increased toxic activity of Ag^+ ions in comparison to AgNPs which show the toxicity levels of AgNPs are related to nanoparticle encapsulation agents [10–12]. Moreover, other reports show AgNPs cytotoxicity effect via the Trojan effect and RNA polymerase inhibition with a direct reciprocal interaction [13]. Hence, researchers are finding convenient and simple strategies for synthesizing AgNPs with potential for utilization as antimicrobial and anticancer agents.

The approaches for the synthesis of AgNPs are various, including ultrasound irradiation, electrosinning, and microemulsion [14–16]. The most common and easy method for AgNPs synthesis is chemical reduction by organic or inorganic reducing agents, which is based on a redox reaction [17–19]. The major problems with these methods are expensiveness, a requirement for sophisticated equipment and the ectopic release of toxic chemical materials which cause environmental pollutions [20]. Therefore, there is an unmet need for an inexpensive and biocompatible approach for the synthesis of AgNPs. The biogenic synthesis of AgNPs using cell-free extract [21], enzymes [22] and plant extracts [23], as the most important natural capping agents play a major role in biocompatible mechanism with less toxic waste [24]. Green synthesis of AgNPs opens up a new avenue toward therapeutics, antioxidant [25], antibacterial [26] and cytotoxicity [27] properties of these nanomaterials.

Gum secretion is a protective mechanism against water stress, insect attack, mechanical and chemical injuries. This phenomenon is the result of *Pseudomonas syringae* or *Stereum purpureum* infections in the cherry tree [28]. Spreading the antibiotic-resistant pathogens is very threatening for the successful treatment of microbial diseases. Therefore, it is essential to find an efficient substance with antibacterial properties [29]. Gum derived oligosaccharides and polysaccharides demonstrated to have remarkable activity in bactericidal and cytotoxicity mechanisms [30]. For instance, Kora and coworkers developed a green method for synthesizing AgNPs using gum ghatti (*Anogeissus latifolia*) and demonstrated the bactericidal and cytotoxic effect [31]. The antibacterial and cytotoxic efficacy of the biologically synthesized AgNPs against some multidrug-resistant bacteria and various cancer cell lines, respectively, were investigated [32]. For example, Nakkala et al. presented an eco-friendly method for synthesizing AgNPs using by *Ficus religiosa* leaf extract and represented their biological activity against *Pseudomonas fluorescens*, *Salmonella typhi*, *Bacillus subtilis* and *Escherichia coli*, as well as A549, Hep2, HeLa and SH-SY5Y cancer cell lines [33]. Moreover, AgNPs synthesized by *Dimocarpus longan* Lour. peels aqueous extract revealed significant antibacterial activity and cytotoxic effect against human prostate cancer PC-3 cells [34]. Recently, a study indicated the Sweet cherry (*Prunus avium*) gum has, on average, 75.14% carbohydrate [35]. These carbohydrates can use as reducing and stabilizing agents for the AgNPs synthesizing. Hence, in the present study, we aimed to achieve the biological synthesis of AgNPs using the *Prunus avium* (*P. avium*) gum.

Prunus avium is indigenous to the region between the Black and Caspian seas in Asia and then spread to other areas. The amount of its bioactive and phenolic components can't be ignored [36, 37]. Moreover, the antioxidant activity of anthocyanin from the *P. avium* was similar to common antioxidants like butylated hydroxytoluene and superior to vitamin E [38], as well as antimicrobial and anticancer properties of its extract were studied [39, 40]. Following this line of research, in this study, we synthesized AgNPs using the *P. avium* gum and characterized these AgNPs by UV–vis spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), and Fourier transform infrared (FTIR) spectroscopy. Moreover, we evaluated the bactericidal effect and anticancer mode of action of the synthesized AgNPs in Gram-positive and Gram-negative bacteria, as well as neuroblastoma (SH-SY5Y) and breast adenocarcinoma cell line (MCF-7), respectively.

Materials and methods

Materials

Prunus avium gum was collected from Kerman, Iran. Silver nitrate (AgNO_3) (99.98% purity) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St Louis, MO, USA). DMEM, FBS and antibiotics were bought from Biosera (East Sussex, United Kingdom). All other reagents were of analytical grade.

Biosynthesis of AgNPs

Prunus avium gum was selected as a reduction agent for the biosynthesis of AgNPs. Gums were dried in air and ground to a powder form. A total of 5.0 g of dried gums were immersed in 200 mL distilled water, gum extracts were produced under a magnetic stirrer bar for 24 h at 40 °C. Then, the solution was centrifuged for 10 min at 10,000 rpm and the supernatant was filtered using Whatman No. 1 filter paper (Whatman plc, Kent, UK). The crude extract was added drop by drop to AgNO_3 aqueous solution (1 mmol L^{-1}) at the ratio of 1:1 for 3 h at 45 °C to create AgNPs. After the reduction reaction, the mixture was centrifuged for 10 min at 10,000 rpm. The supernatant was decanted off and the obtained sediment was re-suspended with distilled water and again centrifuged for three times. Finally, the precipitation of AgNPs was dried in a vacuum oven at 40 °C for 12 h [41].

Characterization of AgNPs

The initial identification of silver reduction was carried out using UV–visible spectroscopy in the range of 300–800 nm. Lyophilized form of nanoparticle was made and the powder was used for further studies [39]. The existence of functional group and composition of AgNPs were confirmed by FTIR which were recorded in potassium bromide (KBr) pellets in the region of $4000\text{--}500 \text{ cm}^{-1}$ at a resolution of

4 cm^{-1} . In addition, for morphological studies, size examination was performed by transmission electron microscopy (TEM) (Leo906, Zeiss100KV model, Germany) at an acceleration voltage of 200 kV, and scanning electron microscope (SEM) ((FE-SEM, SIGMA; Carl Zeiss Meditec AG; Jena, Germany) under acceleration voltage of 25 kV. The DLS and zeta potential were applied to study the changing trend of particle size and its stability.

Cytotoxicity effect

In-vitro anticancer effect of green-synthesized AgNPs was evaluated against HFF cell line (Royan stem cell bank, RSCB0153) as the normal cell (control) and SH-SY5Y (human bone marrow neuroblastoma, C611, Pasteur Institute of Iran) and MCF-7 (human breast adenocarcinoma cell line, C135, Pasteur Institute of Iran) as tumor cell lines by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma, Germany)) assay (34). In brief, cells were cultured in DMEM supplemented with 10% fetal bovine serum, 100 mg mL^{-1} streptomycin and 100 units mL^{-1} penicillin at 37°C in a humid atmosphere with 5% CO_2 . After 24 h cells were detached from the bottom of the flask by 0.25% trypsin solution and centrifuged at 1500 rpm for 5 min, and the supernatant was removed. Then, the precipitate was re-suspended in medium and the presence of living cells was determined using a Neubauer chamber. Both cells were seeded in 96 well plates at a density of 1.0×10^4 and incubated for 24 h at 37°C . The cells were treated with various concentrations of AgNPs and incubated for 24 h at 37°C . After that 100 μL of fresh medium replaced by old medium and MTT was added to each well at a final concentration of 0.5 mg mL^{-1} . Cells were incubated at 37°C for 4 h at a humidified incubator. Then, their medium was removed and crystals of formazan formed were collected by adding 20 μL of DMSO followed by incubation for 30 min. Then, the optical absorbance was determined using an ELISA reader at 480 and 620 nm.

Bax and Bcl2 genes expression with real-time PCR

RNA extraction, cDNA synthesis and RT-PCR assay

At first, the SH-SY5Y cell line was treated with an effective concentration of AgNPs, [IC_{50} ($5 \mu\text{g mL}^{-1}$)] and incubated for 24 h. RNA extraction from the cell line was performed by using the guanidine isothiocyanate-phenol-chloroform method using RNX plus reagent (CinnaGen, Iran)). The quality of the extracted RNAs was evaluated by Nanodrop 1000 machine (NanoDrop, Wilmington, USA). For cDNA synthesis, each reaction consists of 250 ng μL^{-1} of template RNA and 50 pmol μL^{-1} of oligo dT in a final volume of 20 μL and was incubated in 70°C for 5 min, immediately cooled on ice for 3 min. Then, 2.0 μL reaction buffer, 1.0 μL dNTP (10 mmol L^{-1}) and 10 U RNazin were added, followed by incubation at 37°C for 5 min. RNA samples were reverse transcribed for cDNA synthesis by adding reverse transcriptase enzyme and incubated at 42°C for 60 min and then inactivated at 70°C for 10 min. The reaction product was kept at -20°C . RT-PCR for Bax,

Bcl2 and GAPDH genes was done with using 2.0 μL from the RT-reaction mixture in a total volume of 20 μL of reagent PCR which containing 2.0 μL PCR buffer, 2.5 mmol L^{-1} MgCl_2 , 200 mmol L^{-1} of each dNTP, 0.2 mmol L^{-1} of each primer (listed in Table 1) and 2.5 U of Taq DNA polymerase (Cinnagan-Iran). The reactions were performed in a DNA thermocycler as follows: 39 cycles of pre-denaturation at 95 °C for 8 min and denaturation at 95 °C for 20 s, annealing at 58 °C for 25 s, and elongation at 72 °C for 15 s.

Real-time PCR

The expression level of Bax and Bcl2 genes, as well as endogenous control (GAPDH gene), was determined by real-time PCR. Each reaction was included of 10 μL SYBR Green PCR Master Mix (SMObio, Taiwan), 1 μL of cDNA template, 1 μL of each primer (listed in Table 1) were added and final volume of reaction was adjusted to 20 μL with double distilled water. The reactions were carried out using qPCR system (Analytica gena, Germany) with an optimized temperature program include pre-denaturation at 95 °C for 8 min, followed by 30 cycles of denaturation at 95 °C for 20 s, the annealing at 58 °C for 25 s and the extension at 72 °C for 15 s.

Antimicrobial assay

The bactericidal activity of AgNPs was tested against the Gram-positive bacteria (*Bacillus cereus* ATCC:14579, *Staphylococcus aureus* ATCC:23235) and Gram-negative bacteria (*Acinetobacter baumannii* ATTC:43498, *Klebsiella pneumonia* ATCC: BAA1705, and *Escherichia coli* ATCC:25922) by using agar diffusion method. The agar plates were inoculated with 50 μL suspensions of each of these bacteria and different form of extract and nanoparticle (*P. avium* gum extract (PAGE), the synthesized AgNPs (PAG-AgNPs), and trypton-AgNPs (the AgNPs synthesized by trypton as the reducing agent) as control) were placed on the agar plate away from the antibiotic (ampicillin) and incubated at 37 °C for 20 h.

Statistical analysis

Statistical analysis was conducted by SPSS statistics version 24. Comparisons among different cells were conducted by one-way ANOVA. Data were represented

Table 1 Primers used in this study

Primers	Sequences (5'–3')	Length (nt)
F-Bax	TTTTCAGGGTTTCATCCAG	125
R-Bax	ACTTCTTGGTGGACGCATC	
F-Bcl2	ATGTGTGTGGAGAGCGTCAA	172
R-Bcl2	AGGAGAAATCAAACAGAGGC	
F-GAPDH	AGGCAACTAGGATGGTGTGG	112
R-GAPDH	TTGATTTTGGAGGGATCTCG	

as mean $\pm$ SE and a P value of <0.05 was considered as the criterion for statistical significance.

Results and discussion

Characterization of the synthesized AgNPs

Visual observation and UV–vis spectral studies

In this work, we synthesized AgNPs by mixing silver nitrate (AgNO_3) solution with the fresh extract of *P. avium* gum in a 1:1 ratio at 45°C . Change in the solution color from yellow to radish brown indicates the reduction of Ag^+ to Ag^0 , the formation of AgNPs and the surface plasmon resonance (SPR) effect (Fig. 1a). Figure 1b depicts the UV–vis absorption spectrum of *P. avium* gum extract (PAGE) and the synthesized AgNPs (PAG-AgNPs). The UV–vis spectra for the synthesized AgNPs showed a single peak with a maximum around 450 nm, which attributes to the surface plasmon resonance (SPR) of conducting electrons from the surface of AgNPs. This result is consistent with the previous studies [42, 43]. The mechanism of AgNPs biosynthesis by the gum extract is not fully understood. It is investigated that the gum of plants acts as reducing and stabilizing agents for nanoparticle synthesizing. The *Prunus avium* gum has, on average, 75.14% carbohydrate, 11.3% uronic acids, 1.11% protein, 7.53% moisture content

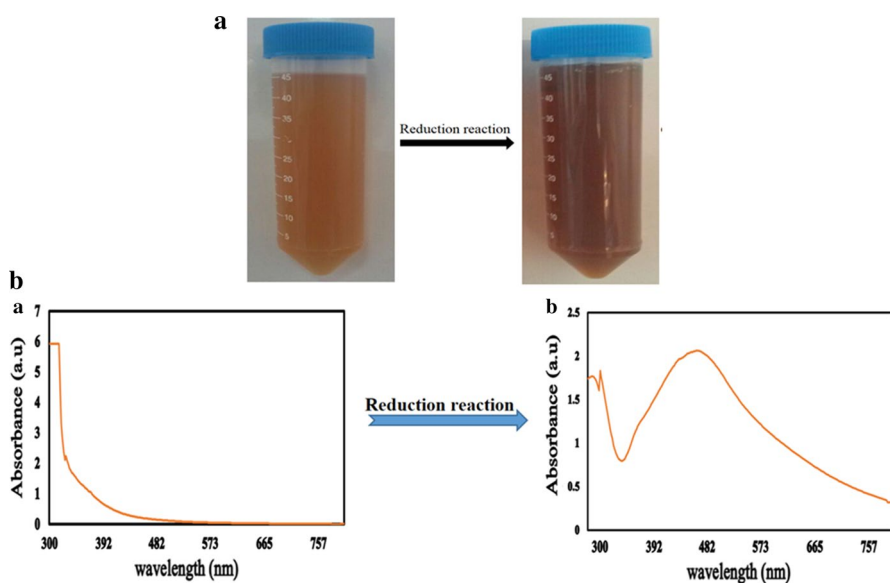


Fig. 1 **a** Color change of the mixture to dark brown during the reaction indicates synthesis of silver nanoparticles. **b** UV–vis spectra of AgNO_3 solution after adding extract. Increasing in the peak intensity (450 nm) shows the reduction reaction

and 3.12% ash. It consists of carbohydrate polymers, including mannose, arabinose, galactose xylose, and uranic acid, as well as functional groups such as carboxyl and hydroxyl groups [35]. Generally, a gum is an anionic functional group with a great charge density. This negative charge attracts the positively charged of Ag^+ ions to the polymeric chain. Subsequently, these Ag^+ get reduced to Ag atoms by oxidizing the hydroxyl groups to carboxyl group. Moreover, oxidation of the existing hydroxyl groups occurs by the dissolved air and carbonyl ($-\text{CHO}$ and $-\text{COOH}$) groups. In turn, these powerful reducing groups reduce further Ag^+ ions to Ag atoms. Additionally, the polysaccharides present in the *Prunus avium* gum caps and stabilizes the synthesized Ag nanoparticles [44]. The optimization condition for percentage of the gum extract, concentration of the silver precursor, and the effect of pH and temperature is shown in Fig. 2.

Characterization of AgNPs

To investigate the morphological features and size of synthesized AgNPs, TEM and SEM studies were conducted and images are shown in Fig. 3a, b, respectively. According to the TEM image, the synthesized AgNPs represented any physical contact and were almost monodispersed spherical nanoparticles. The size of these particles was in the range of 14–34 nm which is appropriate for medical applications. Moreover, the SEM micrograph demonstrated the spherical shape of AgNPs and revealed a similar diameter range for these particles. The

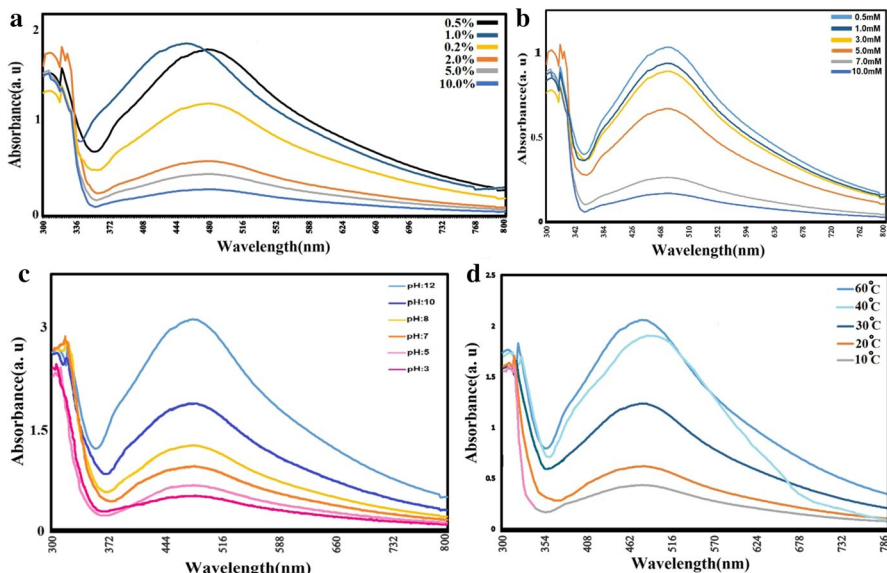


Fig. 2 Optimization condition for percentage of the gum extract (a), concentration of silver precursor (b), and the effect of pH (c) and temperature (d)

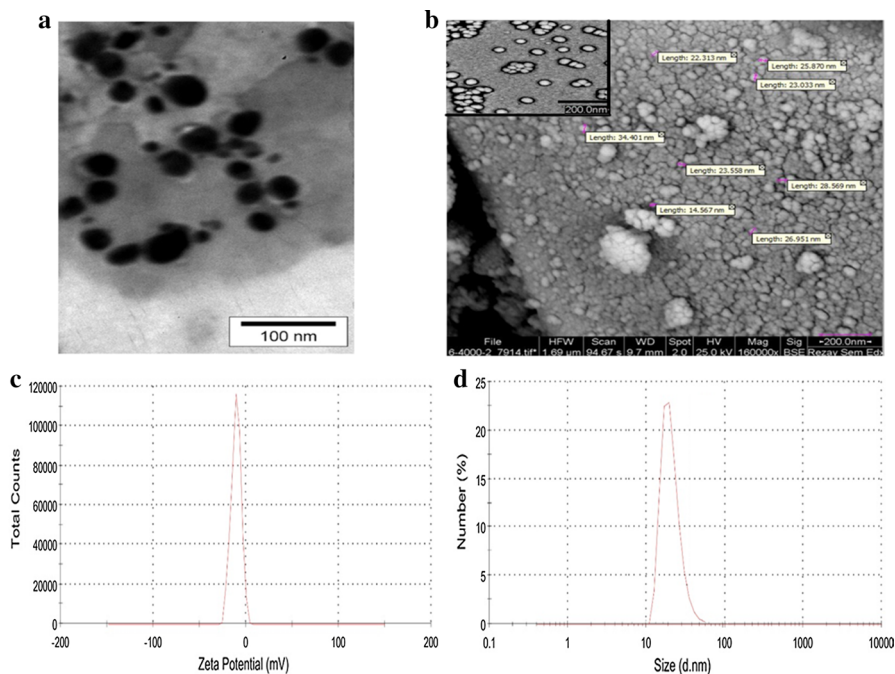


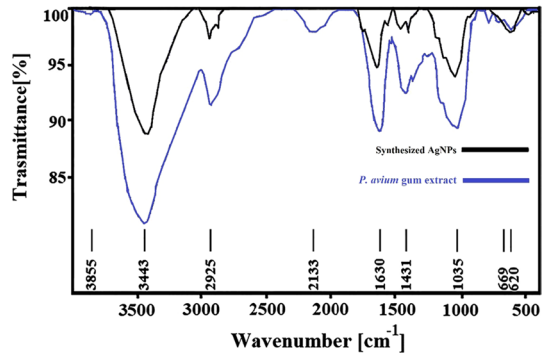
Fig. 3 **a** Transmission electron microscopy (TEM) and **b** scanning electron microscopy (SEM) imaging of the green-synthesized silver nanoparticles. **c** The DLS characterization which show particle size distribution **d** Zeta potential of the synthesized AgNPs after 20 days

maximum particle size distribution with DLS was 30 nm as shown in Fig. 3c, and Zeta potential of the synthesized AgNPs after 20 days is shown in Fig. 3d that demonstrate the stability of the AgNPs.

FTIR analysis

FTIR analysis was used to identify possible functional groups present in *P. avium* gum extract that contributed to the reduction of metal salts and stabilization of synthesized AgNPs. The IR spectrums of *P. avium* gum extract and synthesized AgNPs are shown in Fig. 4. The extract represented vibrational stretches at 3425 cm^{-1} , 2919 cm^{-1} , 1632 cm^{-1} , 1434 cm^{-1} , 1384 cm^{-1} , 1029 cm^{-1} , 576 cm^{-1} , and 429 cm^{-1} . The broadband at 3425 cm^{-1} could be assigned to hydrogen-bonded O H group in *P. avium* gum [45]. The band at 2919 cm^{-1} represents symmetric stretching of vibration of $-\text{CH}_3$ group [46]. The peaks at 1632 cm^{-1} , 1434 cm^{-1} and 1384 cm^{-1} attribute vibrational stretches of carbonyl group, stretching vibrations of aromatic ($\text{C}=\text{C}$) and deformation (bending) vibration $-\text{CH}_3$ groups, respectively [47, 48]. The peaks at 1029 cm^{-1} , 576 cm^{-1} , and 429 cm^{-1} were due to the C–N (alkyl) stretching, C–C bending, and the bending vibration mode of Si–O–Si bond, respectively [49, 50]. Synthesized AgNPs represented vibrational peaks at

Fig. 4 FTIR spectra of *Prunus avium* gum extract before and after forming NPs from 500 to 4000 cm^{-1}



3855 cm^{-1} , 3443 cm^{-1} , 2925 cm^{-1} , 2136 cm^{-1} , 1630 cm^{-1} , 1431 cm^{-1} , 1035 cm^{-1} , 669 cm^{-1} , and 620 cm^{-1} . In the FTIR spectrum of AgNPs, the band shift observed from 3425 to 3443 cm^{-1} , 1632 to 1630 cm^{-1} and 2919 to 2925 cm^{-1} suggested that the hydroxyl and carbonyl groups might have been involved in bio-reduction of Ag^+ ions to AgNPs.

Antibacterial activity

Recently, utilizing the organic antimicrobial agents has attracted much attention for making powerful antibiotic agents against microorganisms [51]. The antibacterial effect of AgNPs on Gram-positive and Gram-negative bacteria is different, but we can prove the superiority of one over the other. The antibacterial activity of *P. avium* gum extract (PAGE) and the synthesized AgNPs (PAG-AgNPs) were examined on some human nosocomial and food pathogenic bacteria, including *A. baumannii*, *K. pneumonia* and *E. coli*, as Gram-negative bacteria, and *B. cereus* and *S. aureus*, as Gram-positive bacteria and their efficacy was shown by the inhibition zone. Whereas PAGE did not possess any activity against tested bacteria (except for *A. baumannii* which its zone of inhibition (ZOI) is 4.0 mm), PAG-AgNP represented growth inhibition around the disks against these bacteria. The zone of inhibition of around 14, 12 and 11 mm diameter was observed for the tested Gram-negative bacterial strains *E. coli*, *A. baumannii* and *K. pneumonia*, respectively. In the case of Gram-positive bacteria, *B. cereus* and *S. aureus*, the detected ZOI was 9.0 and 12 mm, respectively (Table 2 and Fig. 5). This result is in good agreement with previous studies, demonstrated that Gram-positive bacteria have greater resistance to AgNPs than Gram-negative bacteria [52, 53]. This can be explicated by considering the presence of peptidoglycans in the cell wall of Gram-positive bacteria and their ability for production of pigments against reactive oxygen species (ROS) which makes this class of bacteria more resistant against the antibacterial effect of AgNPs [54]. As expected, the positive control, tryptone-AgNP showed antimicrobial activity, but the ZOI values for all tested bacterial strains with PAG-AgNP are larger than the tryptone-AgNP. Therefore, it can be concluded that the synthesized AgNPs had a significant antibacterial effect against both Gram-positive and Gram-negative bacteria which is comparable with previous methods for AgNPs synthesizing (Table 3)

Table 2 Zone of inhibition for *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *B. cereus*, *E. coli* on exposure to PAGE (*Prunus avium* gum extract), Tryptone-AgNP, PAG-AgNP (*Prunus avium* gum AgNP) and standard antibiotic ampicillin

Test compound		Zone of inhibition (mm)				
		<i>K. pneumoniae</i>	<i>A. baumannii</i>	<i>S. aureus</i>	<i>B. cereus</i>	<i>E. coli</i>
1	PAGE	0	4	0	0	0
2	Tryptone-AgNP	8	10	9	7	7
3	PAGNP	11	12	12	9	14
4	Ampicillin	8	28	13	19	29

[55–58]. The mechanism of bacterial activity of AgNPs could be through the positive charge of Ag^+ ions that interact with the negative charge on the bacterial cell wall which changes cell wall morphology and permeability [59, 60]. AgNPs have great affinity for interaction with phosphorous and, sulfur-containing biomolecules such as membrane and intracellular proteins and DNA which affect cell proliferation, respiration and the survival of the cell. It is investigated that the Ag^+ can inhibit and disrupt structure of proteins through thiol and amino groups binding [61]. This may be responsible for the induction of reactive oxygen species (ROS), which results in the inhibition of respiratory enzymes and, finally cell death [62, 63]. Moreover, Ag^+ ions have bactericidal activity through interaction with the peptidoglycan cell wall and plasma membrane [64]. By interfering with sulfhydryl groups in protein, they also prevent DNA replication in bacteria (Fig. 5f) [65].

Anticancer activity

The cytotoxicity of the synthesized AgNPs (PAG-AgNPs) on MCF-7 and SH-SY5Y cancerous cell lines and HFF cells as the normal cell line (control) at 1.0, 3.0, 10 and $50 \mu\text{g mL}^{-1}$ concentrations of synthesized nanoparticles was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. As shown in Fig. 6, HFF cells were slightly inhibited by increasing PAG-AgNPs concentration. The results revealed that PAG-AgNPs inhibited the cell viability of both cancerous cell lines in a concentration-dependent manner after 24 h treatment while being nontoxic toward the normal cell line, HFF (Fig. 6). The highest cytotoxicity of synthesized AgNPs is observed in $50 \mu\text{g mL}^{-1}$ for MCF-7 and SH-SY5Y, which were statistically significant compared to their controls. It may be the result of the entrance of AgNPs in different manners such as endocytosis, pinocytosis or phagocytosis and then interaction with cell constituent and production of free oxygen radicals which can induce the DNA fragmentation and apoptosis. Also, the IC50 value (the concentration of the synthesized AgNPs that is required for 50% inhibition) of PAG-AgNPs was estimated at $10.8 \mu\text{g mL}^{-1}$ for MCF-7 cells and $8.82 \mu\text{g mL}^{-1}$ for SH-SY5Y cells at 24 h. Recent studies have been demonstrated the cytotoxicity effects of green-synthesized nanoparticles on cancerous cell lines (Table 4) [34, 66, 67]. The highest cytotoxicity of synthesized AgNPs on the viability of MCF-7, SH-SY5Y cells, and HFF as normal cell line is shown in (Fig. 7).

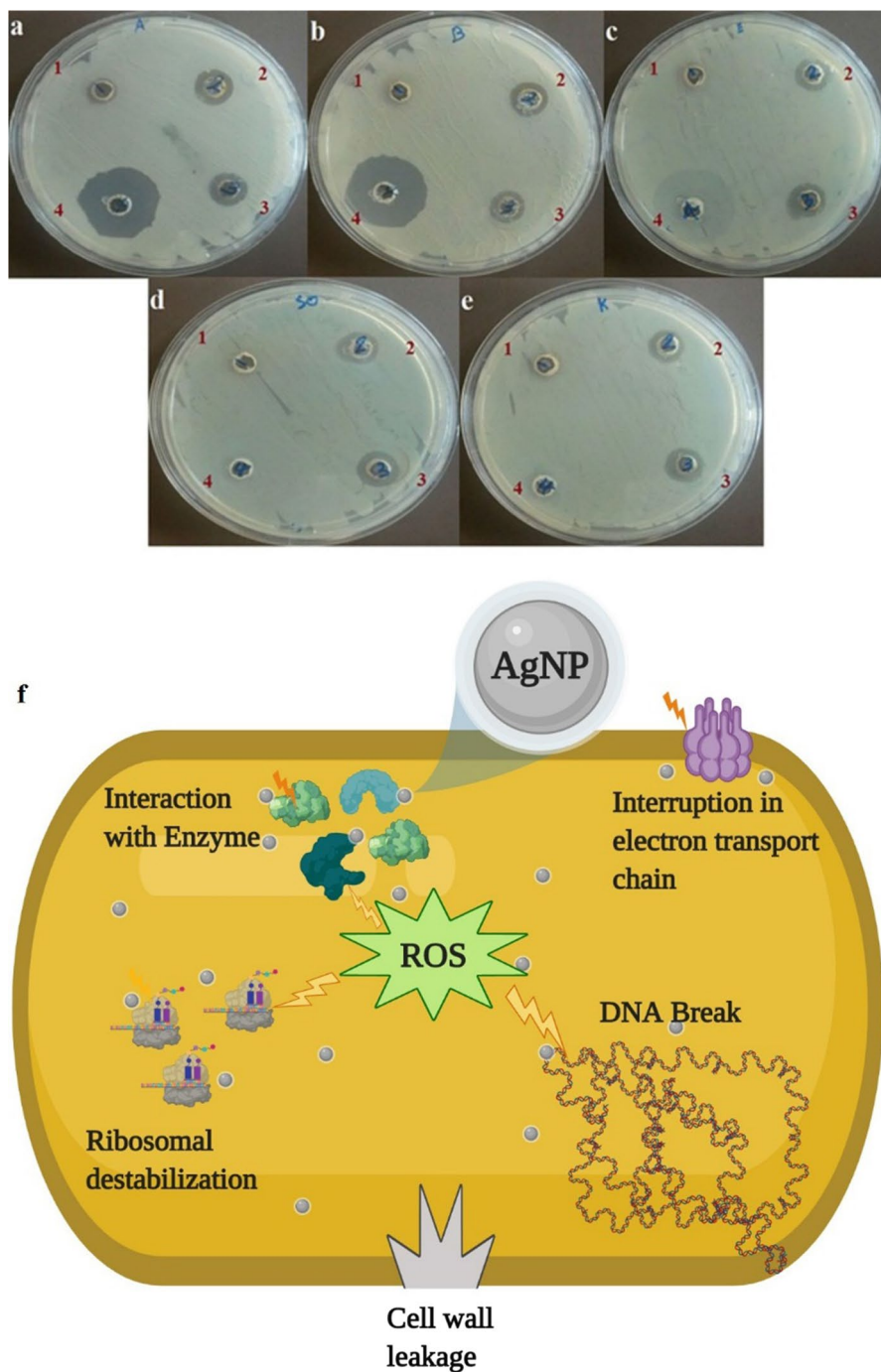


Fig. 5 Antimicrobial efficacy of AgNPs against strains of **a** *A. baumannii*, **b** *B. cereus*, **c** *E. coli*, **d** *S. aureus*, and **e** *K. pneumoniae*, after exposure to (1) PAGE (2) Tryptone-AgNP (3) PAG-AgNP (4) Ampicillin, and their antimicrobial effects. Schematic diagram and plausible mechanisms for the antibacterial activity of AgNPs (**f**)

Table 3 Recent studies for green synthesis of AgNPs

Plant name	Reducing agent	Size (nm)	Shape	Antibacterial activity	References
<i>Azadirachta indica</i>	Water extract of gum	12.09–29.65	Spherical	<i>S. enteritidis</i> , <i>B. cereus</i>	[55]
<i>Clitoria ternatea</i>	Extract of leaves	20	Spherical	<i>Bacillus subtilis</i> , <i>S. aureus</i> , <i>Streptococcus pyogenes</i> , <i>E. coli</i> , <i>Pseudomonas</i>	[56]
<i>Diospyros paniculata</i>	Extract of root	14–28	Spherical	<i>B. subtilis</i> , <i>S. aureus</i> , <i>S. pyogenes</i> , <i>K. pneumoniae</i> , <i>P. vulgaris</i> , <i>E. coli</i> , and <i>P. aeruginosa</i>	[57]
<i>Emblca officinalis</i>	Extract of fruit	10–70	Spherical	<i>S. aureus</i> , <i>B. subtilis</i> , <i>E. coli</i> , <i>K. pneumoniae</i>	[58]
<i>Prunus avium</i>	Gum extract	14–34	Spherical	<i>B. cereus</i> , <i>S. aureus</i> , <i>A. baumannii</i> , <i>K. pneumoniae</i> , <i>E. coli</i>	The present study

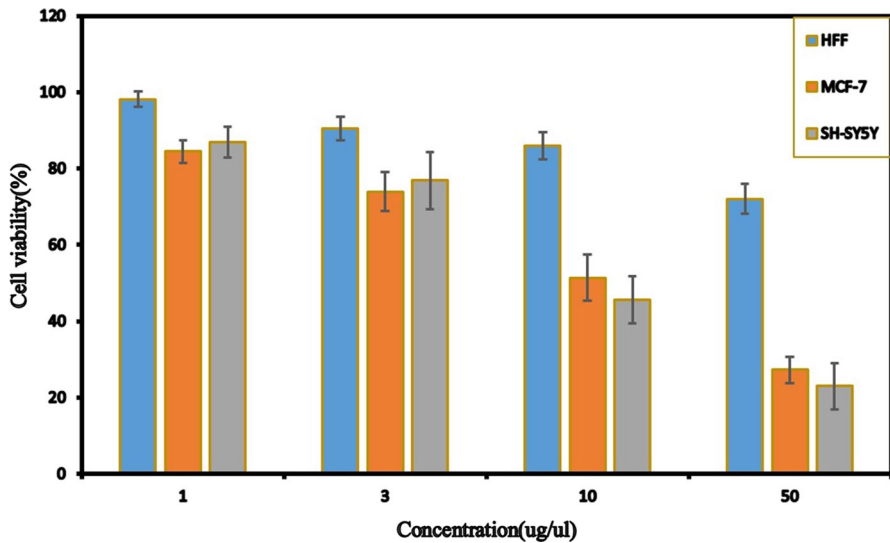


Fig. 6 Cytotoxicity evaluation of AgNPs on MCF-7 and SH-SY5Y cells as cancer cell line. Highest cytotoxicity of synthesized AgNPs is observed in $50 \mu\text{g ml}^{-1}$ for both of cell lines. HFF cell line is as control (normal cell)

Table 4 Comparison of anticancer activity of the study with previous studies

Plant name	Size (nm)	Cell lines	IC ₅₀ ($\mu\text{g mL}^{-1}$)	References
<i>Anthemis atropatana</i>	38.89	HT29	4.88	[34]
		L929	15.46	
<i>Dimocarpus longan</i> Lour.	8–22	H1299	5.33	[66]
		VCaP	87.33	
		BxPC-3	38.9	
<i>Dimocarpus longan</i> Lour.	9–32	PC-3	10	[67]
<i>Prunus avium</i>	14–34	MCF-7	10.8	The present study

Effect of synthesized AgNPs on the apoptosis-related genes expression

The results of RT-PCR for primers optimization was shown in (Fig. 8) and the expression levels of apoptosis-related genes, Bax and Bcl-2, at mRNA level in MCF-7 cells after 24 h treatment with PAG-AgNPs have been evaluated by real-time PCR. After AgNPs treatment, the level of Bax was upregulated (4.65%, $P < 0.05$), whereas the Bcl-2 level was found to be downregulated (0.38, $P < 0.05$) (Fig. 9). These results show a crucial role of synthesized AgNPs for inducing apoptosis in MCF-7 cells. It is well known that the downregulation of anti-apoptotic genes, Bcl-2 is crucial in activating caspases and inducing programmed cell death [68]. Recent studies have demonstrated that the expression ratio of pro- to

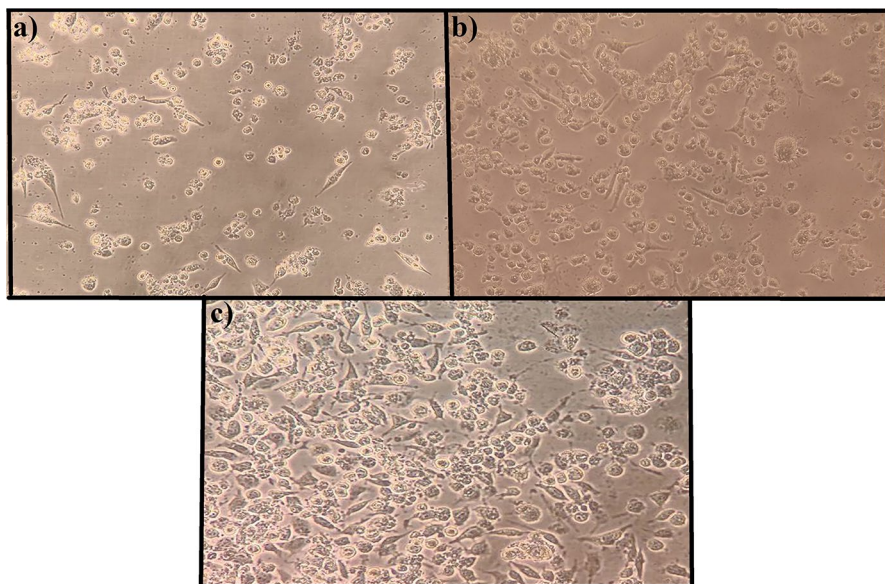


Fig. 7 Image of cell viability of SH-SY5Y (a), MCF-7 (b) and HFF cell line (c) treated by synthesized AgNPs with concentration of $50 \mu\text{g ml}^{-1}$

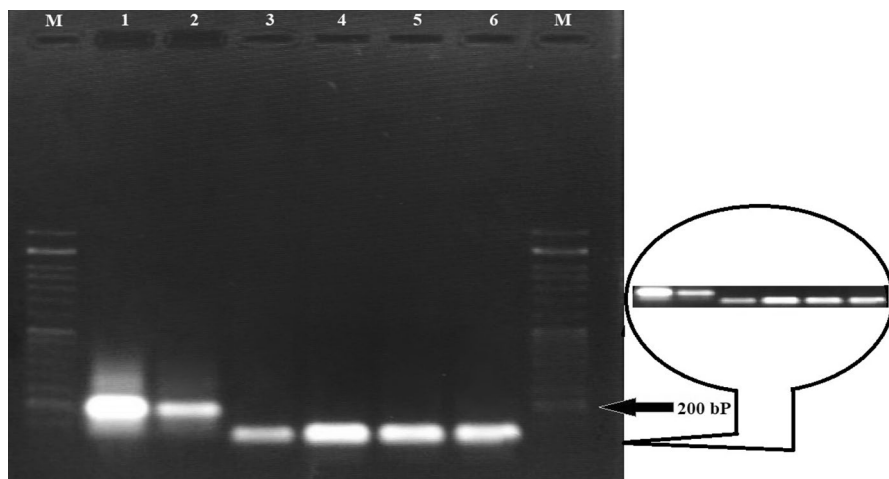


Fig. 8 The result of RT-PCR assay for apoptosis-related genes, Bax and Bcl-2 as well as GAPDH as the reference gene on 1% agarose gel. M is the marker, 1, 3 and 5 are Bcl2, Bax and GAPDH genes without any treatment, respectively. 2, 4 and 6 are Bcl2, Bax and GAPDH genes treated by the highest concentration of AgNPs respectively

anti-apoptotic proteins represents susceptibility of cells to a death signal. In fact, in the downregulation of Bcl-2 expression and upregulation of Bax expression, heterodimerization of Bcl-2 with Bax is decreased and homodimers of Bax will

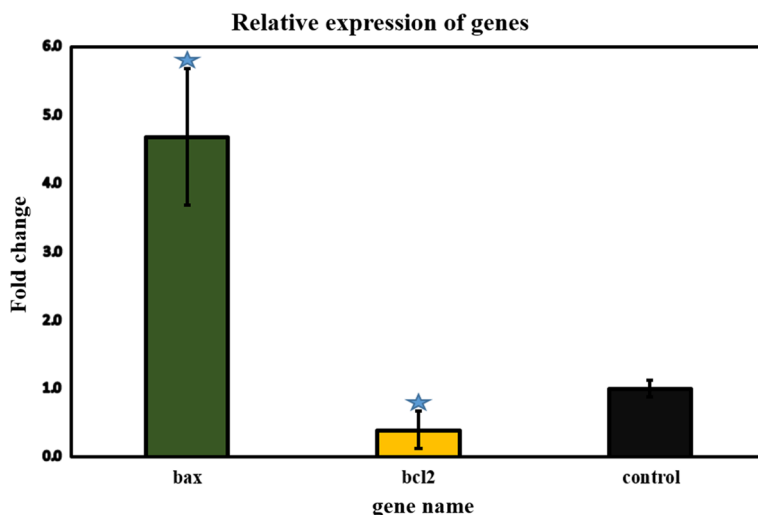


Fig. 9 Gene expression of apoptosis-related genes, Bax and Bcl2 which show increase in Bax gene expression and decrease in Bcl2 gene expression

be formed which will stimulate apoptosis [69]. Previous studies also have been demonstrated that AgNPs can affect the expression levels of pro- and anti-apoptotic genes in other cancer cell lines and induce apoptosis [66].

Conclusion

In this study, *P. avium* gum extract was used as the reducing and stabilizing agent for simple, cost-effective, eco-friendly and green synthesizing of AgNPs. These biogenic AgNPs exhibited reliable properties and biological activities. The synthesized AgNPs proved to possess bactericidal effects against Gram-positive and Gram-negative bacteria. Moreover, these nanoparticles had prominent cytotoxic effects on the MCF-7 and SH-SY5Y cancer cell lines. The significant biological effect of green-synthesized AgNPs makes these nanoparticles as a potential active candidate for biomedical applications, especially anticancer therapy.

Acknowledgements The authors acknowledge the financial support for this investigation by the Research Council of Baqiyatallah University of Medical Sciences (Tehran, Iran) and Shahid Bahonar University (Kerman, Iran).

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

References

- Nadimi AE, Ebrahimipour SY, Afshar EG, Falahati-Pour SK, Ahmadi Z, Mohammadinejad R, Mohamadi M (2018) Nano-scale drug delivery systems for antiarrhythmic agents. *Eur J Med Chem* 157:1153–1163
- Mohammadinejad R, Dadashzadeh A, Moghassemi S, Ashrafizadeh M, Dehshahri A, Pardakhty A, Sassan H, Sohrevardi S-M, Mandegary A (2019) Shedding light on gene therapy: carbon dots for the minimally invasive image-guided delivery of plasmids and noncoding RNAs-A review. *J Adv Res* 18:81–93
- Pandey S, Do JY, Kim J, Kang M (2020) Fast and highly efficient catalytic degradation of dyes using κ -carrageenan stabilized silver nanoparticles nanocatalyst. *Carbohydr Polym* 230:115597
- Ahmadi Z, Mohammadinejad R, Ashrafizadeh M (2019) Drug delivery systems for resveratrol, a non-flavonoid polyphenol: emerging evidence in last decades. *J Drug Deliv Sci Technol* 51:591–604
- Khatami M, Zafarnia N, Bami MH, Sharifi I, Singh H (2018) Antifungal and antibacterial activity of densely dispersed silver nanospheres with homogeneity size which synthesized using chicory: an in vitro study. *Journal de mycologie medicale* 28:637–644
- Khatami M, Varma RS, Zafarnia N, Yaghoobi H, Sarani M, Kumar VG (2018) Applications of green synthesized Ag, ZnO and Ag/ZnO nanoparticles for making clinical antimicrobial wound-healing bandages. *Sustain Chem Pharm* 10:9–15
- Pandey S, Goswami GK, Nanda KK (2018) Green synthesis of biopolymer–silver nanoparticle nanocomposite: an optical sensor for ammonia detection. *Int J Biol Macromol* 51:583–589
- Pandey S, Ramontja J (2016) Sodium alginate stabilized silver nanoparticles–silica nanohybrid and their antibacterial characteristics. *Int J Biol Macromol* 93:712–723
- AshaRani P, Low Kah Mun G, Hande MP, Valiyaveetil S (2009) Cytotoxicity and genotoxicity of silver nanoparticles in human cells. *ACS Nano* 3:279–290
- Ordzhonikidze C, Ramaiyya L, Egorova E, Rubanovich A (2009) Genotoxic effects of silver nanoparticles on mice in vivo. *Acta Naturae* 1:99–101
- Kim JS, Sung JH, Ji JH, Song KS, Lee JH, Kang CS, Yu IJ (2011) In vivo genotoxicity of silver nanoparticles after 90-day silver nanoparticle inhalation exposure. *Saf Health Work* 2:34–38
- Panda KK, Achary VMM, Krishnaveni R, Padhi BK, Sarangi SN, Sahu SN, Panda BB (2011) In vitro biosynthesis and genotoxicity bioassay of silver nanoparticles using plants. *Toxicol In Vitro* 25:1097–1105
- Wang Z, Liu S, Ma J, Qu G, Wang X, Yu S, He J, Liu J, Xia T, Jiang G-B (2013) Silver nanoparticles induced RNA polymerase–silver binding and RNA transcription inhibition in erythroid progenitor cells. *ACS Nano* 7:4171–4186
- Bagwe RP, Khilar KC (1997) Effects of the intermicellar exchange rate and cations on the size of silver chloride nanoparticles formed in reverse micelles of AOT. *Langmuir* 13:6432–6438
- Husein MM, Rodil E, Vera JH (2005) A novel method for the preparation of silver chloride nanoparticles starting from their solid powder using microemulsions. *J Colloid Interface Sci* 288:457–467
- Bai J, Li Y, Li M, Wang S, Zhang C, Yang Q (2008) Electrospinning method for the preparation of silver chloride nanoparticles in PVP nanofiber. *Appl Surf Sci* 254:4520–4523
- Wu ZG, Munoz M, Montero O (2010) The synthesis of nickel nanoparticles by hydrazine reduction. *Adv Powder Technol* 21:165–168
- Bin Ahmad M, Lim JJ, Shameli K, Ibrahim NA, Tay MY (2011) Synthesis of silver nanoparticles in chitosan, gelatin and chitosan/gelatin bionanocomposites by a chemical reducing agent and their characterization. *Molecules* 16:7237–7248
- Vimala K, Mohan YM, Sivudu KS, Varaprasad K, Ravindra S, Reddy NN, Padma Y, Sreedhar B, MohanaRaju K (2010) Fabrication of porous chitosan films impregnated with silver nanoparticles: a facile approach for superior antibacterial application. *Colloids Surf B* 76:248–258
- Mohammadinejad R, Moosavi MA, Tavakol S, Vardar DÖ, Hosseini A, Rahmati M, Dini L, Hussain S, Mandegary A, Klionsky DJ (2019) Necrotic, apoptotic and autophagic cell fates triggered by nanoparticles. *Autophagy* 15:4–33
- Narayanan KB, Sakthivel N (2010) Biological synthesis of metal nanoparticles by microbes. *Adv Coll Interface Sci* 156:1–13
- Schneidewind H, Schöler K, Strelau KK, Weber K, Cialla D, Diegel M, Mattheis R, Berger A, Möller R, Popp J, Beilstein (2012) The morphology of silver nanoparticles prepared by enzyme-induced reduction. *J Nanotechnol* 3:404–414

23. Hossain MA, AL-Raqmi KAS, AL-Mijizy ZH, Weli AM, Al-Riyami Q (2013) Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. *Asian Pac J Trop Biomed* 3:705–710
24. Mohammadinejad R, Shavandi A, Raie DS, Sangeetha J, Soleimani M, Hajibehzad SS, Thangadurai D, Hospet R, Popoola JO, Arzani A (2018) Plant molecular farming: production of metallic nanoparticles and therapeutic proteins using green factories. *Green Chem* 21:1845–1865
25. Lim YY, Murtijaya J (2007) Antioxidant properties of *Phyllanthus amarus* extracts as affected by different drying methods. *Food Sci Technol* 40:1664–1669
26. Sathishkumar M, Sneha K, Won S, Cho C-W, Kim S, Yun Y-S (2009) Cinnamon *zeylanicum* bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity. *Colloids Surf B* 73:332–338
27. Safaeipour M, Shahverdi AR, Shahverdi HR, Khorramizadeh MR, Gohari AR (2018) Green synthesis of small silver nanoparticles using geraniol and its cytotoxicity against fibrosarcoma-wehi 164. *Avicenna J Med Biotechnol* 1:111
28. Boothby D (1983) Gummosis of stone-fruit trees and their fruits. *J Sci Food Agric* 34:1–7
29. Himejima M, Kubo I (1991) Antibacterial agents from the cashew *Anacardium occidentale* (Anacardiaceae) nut shell oil. *J Agric Food Chem* 39:418–421
30. Gurav SS, Gulkari VD, Duragkar NJ, Patil AT (2008) Antimicrobial activity of *Butea monosperma* Lam. gum. *Iran J Pharmacol Ther* 7:21–24
31. Kora AJ, Beedu SR, Jayaraman A (2012) Size-controlled green synthesis of silver nanoparticles mediated by gum ghatti (*Anogeissus latifolia*) and its biological activity. *Org Med Chem Lett* 2:1–10
32. Azizi S, Mohamad R, Rahim RA, Mohammadinejad R, Ariff AB (2017) Hydrogel beads bio-nano-composite based on Kappa-Carrageenan and green synthesized silver nanoparticles for biomedical applications. *Int J Biol Macromol* 104:423–431
33. Nakkala JR, Mata R, Sadras SR (2017) Green synthesized nano silver: synthesis, physicochemical profiling, antibacterial, anticancer activities and biological in vivo toxicity. *J Colloid Interface Sci* 499:33–45
34. He Y, Du Z, Ma S, Cheng S, Jiang S, Liu Y, Li D, Huang H, Zhang K, Zheng X (2016) Biosynthesis, antibacterial activity and anticancer effects against prostate cancer (PC-3) cells of silver nanoparticles using *Dimocarpus Longan* Lour. peel extract. *Nanoscale Res Lett* 11:300
35. Shabani H, Askari G, Jahanbin K, Khodaeian F (2016) Evaluation of physicochemical characteristics and antioxidant property of *Prunus avium* gum exudates. *Int J Biol Macromol* 93:436–441
36. Jakobek L, Šeruga M, Novak I, Medvidović-Kosanović M (2007) Flavonols, phenolic acids and antioxidant activity of some red fruits. *Dtsch Lebensm-Rundsch* 103:369
37. Jakobek L, Šeruga M, Šeruga B, Novak I, Medvidović-Kosanović M (2009) Phenolic compound composition and antioxidant activity of fruits of *Rubus* and *Prunus* species from Croatia. *Int J Food Sci Technol* 44:860–868
38. Seeram NP, Momin RA, Nair MG, Bourquin LD (2001) Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries. *Phytomedicine* 8:362–369
39. Ahn S-M, Ryu H-Y, Kang D-K, Jung I-C, Sohn H-Y (2009) Antimicrobial and antioxidant activity of the fruit of *Prunus avium* L. *Microbiol Biotechnol Lett* 37:371–376
40. Serra AT, Seabra IJ, Braga ME, Bronze M, de Sousa HC, Duarte CM (2010) Processing cherries (*Prunus avium*) using supercritical fluid technology. Part 1: recovery of extract fractions rich in bio-active compounds. *J Supercrit Fluids* 55:184–191
41. Indana MK, Gangapuram BR, Dadigala R, Bandi R, Guttena V (2016) A novel green synthesis and characterization of silver nanoparticles using gum tragacanth and evaluation of their potential catalytic reduction activities with methylene blue and Congo red dyes. *J Anal Sci Technol* 7:19
42. Moosa AA, Ridha AM, Allawi MH (2015) Green synthesis of silver nanoparticles using spent tea leaves extract with atomic force microscopy. *Int J Curr Eng Technol* 5:3233–3241
43. George BPA, Kumar N, Abrahamse H, Ray SS (2018) Apoptotic efficacy of multifaceted biosynthesized silver nanoparticles on human adenocarcinoma cells. *Sci Rep* 8:1–14
44. Madhusudhan A, Reddy GB, Krishana IM (2019) Green synthesis of gold nanoparticles by using natural gums. *Nanomater Plant Potential* 1:111–134
45. Chen J, Li L, Zhou X, Sun P, Li B, Zhang X (2018) Preliminary characterization and antioxidant and hypoglycemic activities in vivo of polysaccharides from *Huidouba*. *Food Funct* 9:6337–6348
46. Tyagi P, Tyagi R (2011) Synthesis of bisphosphodiester surfactants derived from tetradecanol and different methylene chains as a spacer derived from α - ω -alkyl dibromides. *Tenside, Surfactants, Deterg* 48:293–300

47. Shivashankar M, Mandal BK (2013) Design and evaluation of chitosan-based novel pH sensitive drug carrier for sustained release of cefixime. *Trop J Pharm Res* 12:155–161
48. Shahab S, Filipovich L, Kumar R, Darroudi M, Borzehandani MY, Gomar M, Hajikolaee FH (2015) Photochromic properties of the molecule Azure A chloride in polyvinyl alcohol matrix. *J Mol Struct* 1101:109–115
49. Whitehead R, Handy N (1975) Variational calculation of vibration-rotation energy levels for triatomic molecules. *J Mol Spectrosc* 55:356–373
50. Martín-Lara M, Blázquez G, Ronda A, Pérez A, Calero M (2013) Development and characterization of biosorbents to remove heavy metals from aqueous solutions by chemical treatment of olive stone. *Ind Eng Chem Res* 52:10809–10819
51. Ahmed S, Ahmad M, Swami BL, Ikram S (2016) A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. *J Adv Res* 7:17–28
52. Khorrami S, Zarrabi A, Khaleghi M, Danaei M, Mozafari M (2018) Selective cytotoxicity of green synthesized silver nanoparticles against the MCF-7 tumor cell line and their enhanced antioxidant and antimicrobial properties. *Int J Nanomed* 13:8013
53. Barbinta-Patrascu M, Badea N, Pirvu C, Bacalum M, Ungureanu C, Nadejde P, Ion C, Rau I (2016) Multifunctional soft hybrid bio-platforms based on nano-silver and natural compounds. *Mater Sci Eng, C* 69:922–932
54. Rizzello L, Pompa PP (2014) Nanosilver-based antibacterial drugs and devices: mechanisms, methodological drawbacks, and guidelines. *Chem Soc Rev* 43:1501–1518
55. Velusamy P, Das J, Pachaiappan R, Vaseeharan B, Pandian K (2015) Greener approach for synthesis of antibacterial silver nanoparticles using aqueous solution of neem gum (*Azadirachta indica* L.). *Ind Crops Prod* 66:103–109
56. Narayanaswamy K, Rajalakshmi A, Jayachitra A (2015) Green Synthesis of silver nanoparticles using leaf extracts of *Clitoria ternatea* and *Solanum nigrum* and study of its antibacterial effect against common nosocomial pathogens. *J Nanosci* 1:1–8
57. Rao NH, Lakshmidhevi N, Pammi S, Kollu P, Ganapathy S, Lakshmi P (2016) Green synthesis of silver nanoparticles using methanolic root extracts of *Diospyros paniculata* and their antimicrobial activities. *Mater Sci Eng, C* 62:553–557
58. Ramesh P, Kokila T, Geetha D (2015) Plant mediated green synthesis and antibacterial activity of silver nanoparticles using *Emblica officinalis* fruit extract. *Spectrochim Acta Part A Mol Biomol Spectrosc* 142:339–343
59. Patil SV, Borase HP, Patil CD, Salunke BK (2012) Biosynthesis of silver nanoparticles using latex from few euphorbian plants and their antimicrobial potential. *Appl Biochem Biotechnol* 167:776–790
60. Dibrov P, Dzioba J, Gosink KK, Häse CC (2002) Chemiosmotic mechanism of antimicrobial activity of Ag⁺ in *Vibrio cholerae*. *Antimicrob Agents Chemother* 46:2668–2670
61. Choi O, Deng KK, Kim N-J, Ross L Jr, Surampalli RY, Hu Z (2008) The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. *Water Res* 42:3066–3074
62. Holt KB, Bard AJ (2005) Interaction of silver (I) ions with the respiratory chain of *Escherichia coli*: an electrochemical and scanning electrochemical microscopy study of the antimicrobial mechanism of micromolar Ag⁺. *Biochemistry* 44:13214–13223
63. Diamond DM, Campbell AM, Park CR, Halonen J, Zoladz PR (2007) The temporal dynamics model of emotional memory processing: a synthesis on the neurobiological basis of stress-induced amnesia, flashbulb and traumatic memories, and the Yerkes-Dodson law. *Neural Plast* 2007:1–33
64. Radzig M, Nadochenko V, Koksharova O, Kiwi J, Lipasova V, Khmel I (2013) Antibacterial effects of silver nanoparticles on gram-negative bacteria: influence on the growth and biofilms formation, mechanisms of action. *Colloids Surf B* 102:300–306
65. Seth D, Choudhury SR, Pradhan S, Gupta S, Palit D, Das S, Debnath N, Goswami A (2011) Nature-inspired novel drug design paradigm using nanosilver: efficacy on multi-drug-resistant clinical isolates of tuberculosis. *Curr Microbiol* 62:715–726
66. Dehghanizade S, Arasteh J, Mirzaie A (2018) Green synthesis of silver nanoparticles using *Anthemis atropatana* extract: characterization and in vitro biological activities. *Artif Cells Nanomed Biotechnol* 46:160–168
67. He Y, Du Z, Ma S, Liu Y, Li D, Huang H, Jiang S, Cheng S, Wu W, Zhang K (2016) Effects of green-synthesized silver nanoparticles on lung cancer cells in vitro and grown as xenograft tumors in vivo. *Int J Nanomed* 11:1879

68. Jin H-O, Seo S-K, Woo S-H, Lee H-C, Kim E-S, Yoo D-H, Lee S-J, An S, Choe T-B, Kim J-I (2018) A combination of sulindac and arsenic trioxide synergistically induces apoptosis in human lung cancer H1299 cells via c-Jun NH2-terminal kinase-dependent Bcl-xL phosphorylation. *Lung Cancer* 61:317–327
69. Baharara J, Namvar F, Ramezani T, Mousavi M, Mohamad R (2015) Silver nanoparticles biosynthesized using *Achillea biebersteinii* flower extract: apoptosis induction in MCF-7 cells via caspase activation and regulation of Bax and Bcl-2 gene expression. *Molecules* 20:2693–2706

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.