



Characterization and antioxidant-antimicrobial activity of silver nanoparticles synthesized using *Punica granatum* extract

A. Aygün¹ · S. Özdemir² · M. Gülcan³ · M. S. Yalçın⁴ · M. Uçar⁵ · F. Şen¹ 

Received: 3 May 2020 / Revised: 9 December 2020 / Accepted: 1 March 2021
© Islamic Azad University (IAU) 2021

Abstract

In this study, low-cost, simple, and eco-friendly green synthesis of silver nanoparticles was performed using pomegranate extract (Ag NPs/PG). *Punica granatum* extract acts as a reducing agent and capping agent in the synthesis of Ag NPs. The presence of Ag NPs/PG was analyzed using characterization methods such as UV–Vis absorption spectroscopy (UV–Vis), Fourier transform infrared spectrophotometer (FTIR), X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD), and transmission electron microscopy (TEM). XRD, TEM, and XPS analysis showed the presence elements, surface morphology, various shapes, and the presence of Ag NPs in the sample. The Ag NPs/PG particle sizes were found to be 5–45 nm in TEM analysis. By using the XRD technique, Ag NPs were determined to be a cubic crystal structure with face-centered. Furthermore, the antioxidant and antibacterial properties of Ag NPs/PG were also investigated. The enhanced biological activity of *Punica granatum*-mediated green synthesized Ag NPs was investigated based on antioxidant, antimicrobial, and DNA cleavage studies. The Ag NPs showed 91.6% DPPH (2,2-diphenyl-1-picrylhydrazyl) radical inhibition rate activity at the highest concentration (500 mg mL⁻¹). It exhibited significant chemical nuclease activity. Ag NPs/PG had significant antibacterial activity against gram-positive and gram-negative bacteria, but gram-negative bacteria more susceptible than gram-positive. Biogenic synthesis of Ag NPs with the help of *Punica granatum* extract is a useful and environmentally safe synthesis technique. It is thought that Ag NPs obtained using *Punica granatum* may have potential use in the fields of nanomedicine, nanobiosensor, and nanobiotechnology in the near future.

Keywords Antimicrobial · Antioxidant · DNA cleavage · Silver nanoparticle · *Punica granatum*

Editorial responsibility: Samareh Mirkia.

✉ F. Şen
Fatihsen1980@gmail.com

- ¹ Sen Research Group, Biochemistry Department, Faculty of Arts and Science, Dumlupinar University, Evliya Celebi Campus, 43100 Kutahya, Turkey
- ² Food Processing Programme, Technical Science Vocational School, Mersin University, Yenisehir, TR-33343 Mersin, Turkey
- ³ Chemistry Department, Faculty of Science, Van Yüzüncü Yıl University, Zeve Campus, 65080 Van, Turkey
- ⁴ Department of Chemical and Chemical Processing Technologies, Technical Science Vocational School, Mersin University, Yenisehir, TR-33343 Mersin, Turkey
- ⁵ Chemistry Department, Faculty of Arts and Science, Afyon Kocatepe University, 03200 Afyon, Turkey

Introduction

Recently, nanotechnology is a science where comprehensive studies have been carried out to develop and use new materials at the nanoscale level and continuous improvements have been made. In addition, it provides an understanding of the unique properties of nanomaterials and learning how to make usage of them in various fields of applications (Ghodake and Lee 2011; Heera and Shanmugam 2015). Various synthesis methods such as physical, chemical, and biological are used for nanoparticle synthesis (Iravani et al. 2014). Due to the toxic effects on human health, wide applications of chemical and physical methods are limited (Lalitha et al. 2013). However, the biological method in the synthesizing of nanoparticles is known as a clean, non-toxic, and environmentally friendly method (Mittal et al. 2013). Because of these advantages, biologically synthesized nanoparticles, especially green synthesis has gained prominence recently. The green synthesis utilizes a sort of biological



products, including microorganisms, marine algae, plant tissue, plant extracts, and fruits (Parveen et al. 2016). Phenolic compounds in plant extracts are involved in the decrease of metal salts. Also, these biomolecules in the extract provide stabilization of nanoparticles.

Various parts of plants can be used to synthesize inorganic metal nanoparticles like gold, platinum, titanium, silver, nickel, palladium, copper, magnetite, and zinc. Among them, silver nanoparticles (Ag NPs) attract more attention due to their widespread application in the areas of therapeutics, catalysis, microelectronics, biomolecular detection, and diagnostics (Raj et al. 2018). Furthermore, reported studies in the literature indicated that the medicinal use of Ag NPs includes antibacterial, antifungal, anti-diabetic, anti-inflammatory, antioxidant agents, also cancer treatment and diagnosis (Sondi and Salopek-Sondi 2004; BarathManiKanth et al. 2010; Justin Packia Jacob et al. 2012; Kuppusamy et al. 2016).

As a result of scientific studies in the literature, it is reported that the content of *Punica granatum* fruit contains plenty of antioxidants, polyphenolic substances, and vitamins (Lebaka et al. 2018; Bonesi et al. 2019). Thus, biomolecules, which are abundant in the content of pomegranate fruit, provide a great advantage in reducing metal salts. Furthermore, studies have reported that pomegranate fruit has antibacterial, antiviral, antioxidant, anti-inflammatory, antihypertensive, anticancer, and antiatherogenic effects (Viana et al. 2010).

In this study, Ag NPs were obtained by using the green synthesis method using *Punica granatum* extract. The synthesized Ag NPs were characterized using various characterization techniques (XRD, TEM, XPS, and FTIR, UV-Vis), and their therapeutic potential was evaluated as an antioxidant, DNA cleavage ability, and antimicrobial agents.

Materials and methods

Chemicals

All chemicals used in the experiments were purchased from Sigma Aldrich. The *Punica granatum* used in the experiments in this study was obtained from the city of Antalya in Turkey.

Extraction of procedure

Punica granatum fruit skins were dried and pulverized. 5 gr of dried *Punica granatum* shell powder was added in 100 mL of alcohol, and the extract was obtained with the aid of a microwave oven. The resulting extract was filtered through filter paper.

The synthesis silver nanoparticles procedure from *Punica granatum*

20 mL of *Punica granatum* extract in the balloon joje was added to 80 mL of distilled water and 100 mL volume was completed. Then, 16 mg of silver nitrate (AgNO_3) metal salt was added to the balloon joje. The *Punica granatum* extract was allowed on a magnetic stirrer for 2 days at 200 rpm at 90 °C to reduce the AgNO_3 metal salt. The initially colorless liquid turned into a brown color after 2 days, which indicates that the AgNO_3 metal salt was reduced to Ag (0). In addition to the visual examination, it was confirmed that Ag NPs were obtained using different characterization methods. Also, to remove unreacted phytochemicals, the liquid from which Ag NPs are collected was washed with alcohol and centrifuged at 4000 rpm.

DPPH activity of synthesized Ag NPs/PG

The free radical scavenging capacity of *Punica granatum*-mediated green synthesized Ag NPs (Ag NPs/PG) was examined using the 2,2-diphenyl-1-picrylhydrazylhydrate (DPPH) method (Salih Ağırtaş et al. 2015). 500 μL of different concentrations of Ag NPs/PG were added in test tubes in the presence of 2.0 ml of (0.004%) DPPH. DPPH was allowed to stand for 30 min in a dark environment at 25 °C, and then the absorbance spectra of DPPH were recorded by monitoring the absorption band at 517 nm. The same experiment was applied using Trolox and ascorbic acid as a positive control. DPPH radical scavenging activity as a percentage of DPPH color change was calculated using the formula below:

$$\% \text{ Inhibition Activity} = \frac{[A_0 - A_1]}{A_0} \times 100,$$

A_0 = control absorbance and $A_1 = A_1$ is the absorbance value of the solution containing *Punica granatum*-mediated green synthesized Ag nanoparticles and DPPH after 30 min.

The ability of DNA cleavage of synthesized Ag NPs/PG

The ability of DNA cleavage of Ag NPs/PG samples was examined by conducting agarose gel electrophoresis. pBR322 DNA molecules and two different concentrations (200 $\mu\text{g} \cdot \text{mL}^{-1}$ and 500 $\mu\text{g} \cdot \text{mL}^{-1}$) Ag NPs/PG were incubated at 37 °C for 30 min and 90 min. After that, 50% glycerol and 0.25% bromophenol blue mixture were added as loading dye into the reaction solution. These solutions were electrophoresed for 1.5 h at 50 V by using 0.8% agarose gel. DNA-containing agarose gel electrophoresis was monitored under UV light, and the gel electrophoresis image was photographed.



Antimicrobial activity of synthesized Ag NPs/PG

The antimicrobial properties of Ag NPs/PG samples were assessed using a broth microdilution method. A single colony of various microorganisms such as *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus*, *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 10536), *Enterococcus hirae* (ATCC 10541), *Candida albicans*, and *Legionella pneumophila* subsp. *pneumophila* (ATCC 33152) was used for this purpose. The growth media for bacterial and fungal species were inoculated with $2.9 \cdot 10^8$ CFU/mL and incubated for 24 h to propagate inoculated cultures. Two-fold serial dilutions of Ag NPs/PG were prepared at 2048 $\mu\text{g/mL}$ and diluted from 1024 to 1 $\mu\text{g/mL}$ in 96-well plates. The bacterial culture with Ag NPs/PG added was incubated for 1 day at 37 ± 2 °C. After 1 day, the minimum inhibitory concentration (MIC) values were calculated. The lowest drug concentration that prevents the growth of microorganisms is expressed as the MIC value. In this study, the lowest concentration of Ag NPs preventing the growth of fungal and bacterial species was determined as MIC value.

Results and discussion

Morphological and chemical characterizations of the synthesized Ag NPs were examined by the UV–Vis spectrum, TEM, XPS, and XRD analysis. The reduction of Ag^+ ion to Ag^0 during the reaction with the ingredients in *Punica granatum* extract was observed by UV–Vis spectroscopy. At first, the colorless AgNO_3 solution became a light brown color after a few hours, while it was observed that it turned dark brown at the end of the synthesis. The brown color observed

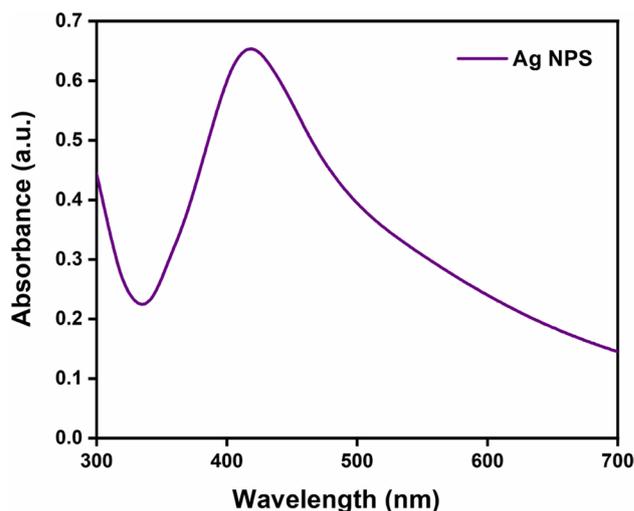
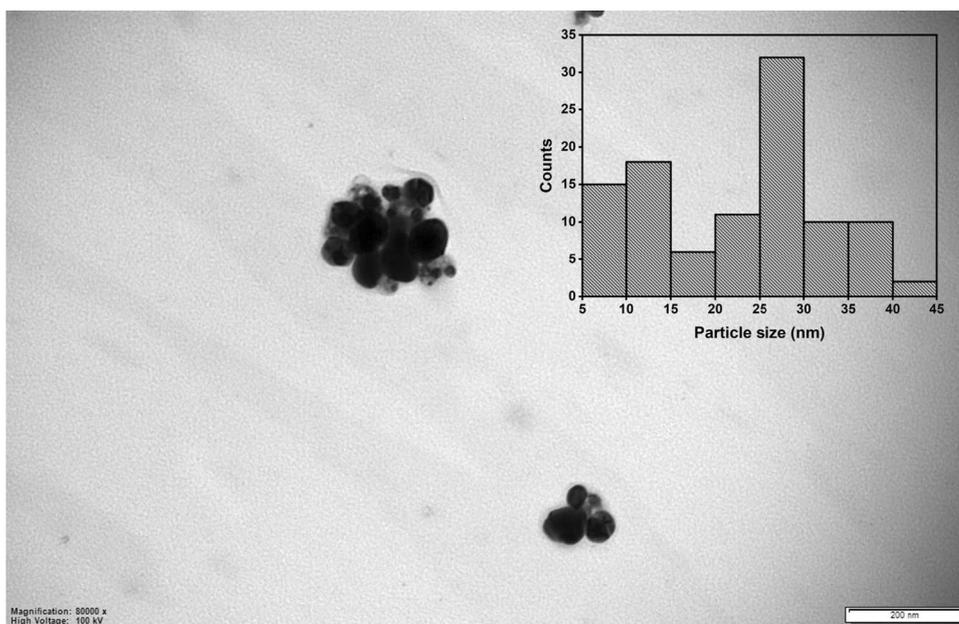


Fig. 1 The UV–Vis analysis image of Ag NPs synthesized using *Punica granatum* extract

at the end of the synthesis is thought to occur due to the stimulation of surface plasmon vibrations specific to silver nanoparticles (Krishnaraj et al. 2010). Figure 1 shows the UV–Vis spectrum analysis of the synthesized Ag NPs/PG. Ag NPs/PG showed an absorbance band at 418 nm wavelength in the UV–Vis spectrum. The absorbance value at 418 nm has the characteristic SPR of silver nanoparticles and indicates that the spectrum Ag NPs obtained have a small particle size (Rodríguez-Luis et al. 2016). The results of the UV–Vis analysis of the synthesized Ag NPs/PG were observed to be compatible with the literature (Aygiün et al. 2020).

TEM analysis is shown in Fig. 2. Morphological properties and particle size of green synthesized Ag NPs/PG were

Fig. 2 a The TEM analysis and b particle size image of Ag NPs synthesized using *Punica granatum* extract



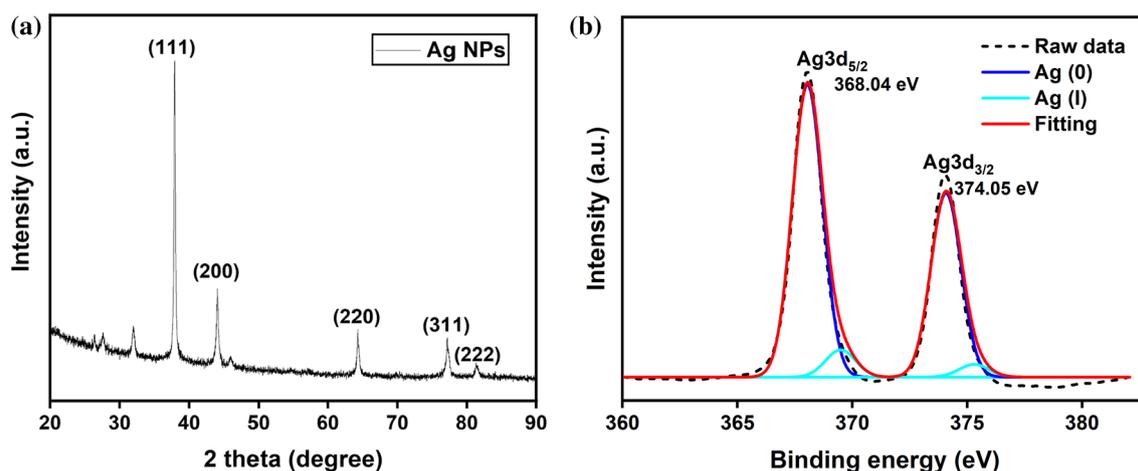


Fig. 3 **a** The XRD analysis and **b** XPS analysis image of Ag NPs synthesized using *Punica granatum* extract

examined by the TEM characterization method. According to TEM analysis, it was observed that Ag NPs/PG has a spherical structure, and the particle size is between 5–45 nm. However, Ag NPs/PG was observed to be agglomerated in some regions. The particle size of the Ag NPs/PG synthesized is consistent with the literature.

The structure of the crystal of the synthesized Ag NPs/PG was examined utilizing XRD analysis. Figure 3a shows the XRD diffraction of Ag NPs/PG. The peaks seen at 2θ in the XRD analysis correspond to 37.89° , 44.07° , 64.21° , 77.13° and 81.53° , (111), (200), (220), (311) and (222) lattice plane clusters. These planes indicate that Ag NPs/PG is crystalline and fcc (face-centered cubic). The XRD values we obtained are consistent with the XRD analysis of Ag NPs/PG in the literature (Aygün et al. 2019). The results of the XPS analysis of Ag NPs/PG are shown in Fig. 3b. The 3d spectrum region of Ag NPs/PG was analyzed by XPS characterization method. In the Ag 3d spectrum, the binding energies corresponding to Ag $3d_{5/2}$ and Ag $3d_{3/2}$ regions were observed two peaks at 368.04 eV and 374.05 eV values. Also observed in Fig. 3b are Ag (I) (369.48/375.30 eV) peaks that may be related to the non-reduced and/or oxidized species of Ag.

Biomolecules involved in the synthesis of Ag NPs were determined using FTIR analysis. FTIR analysis results of *Punica granatum* extract and Ag NPs are shown in Fig. 4. It was observed that both spectra showed a band between 3300 cm^{-1} and 3500 cm^{-1} and shortening occurred in the spectrum containing Ag NPs (Roy et al. 2015). This situation confirms that Ag NPs are reduced. The bands at 2918 cm^{-1} and 2849 cm^{-1} are due to the presence of aldehydic C–H stretch and it is observed that Ag-NPs shift to a lower frequency with the formation (Idrees et al. 2019). The observed band at 1635 cm^{-1} expresses amide I vibrations and it is thought that the reason for the shift of this band to 1631 cm^{-1} is the proteins to bind to Ag-NPs via amine

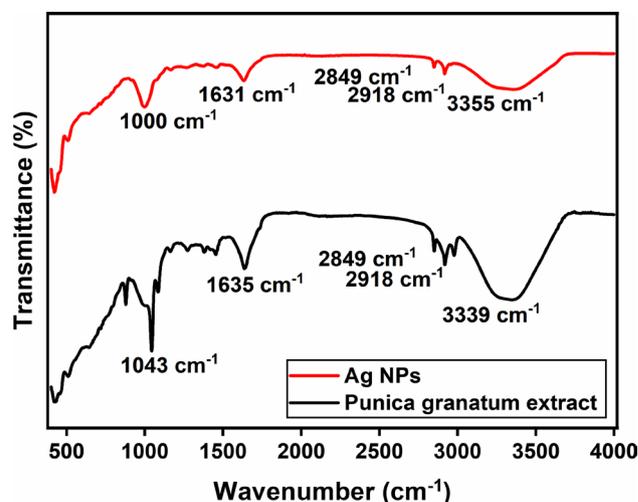


Fig. 4 The FTIR analysis image of Ag NPs synthesized using *Punica granatum* extract

groups (Shekhawat et al. 2013). The other band at 1041 cm^{-1} represents the –C–O–C– stretch and the shear observed in this band reveals that Ag-NPs are synthesized (Batool et al. 2017). As a result of FTIR analysis, it is clearly shown that biomolecules in plant extract play a role in the synthesis of Ag NPs (Idrees et al. 2019).

DPPH radical scavenging activity

There are many analytical methods to determine the antioxidant capacity of the compounds, and the DPPH method is one of the most commonly used methods (Garcia et al. 2012). The free DPPH radical is reduced when it reacts with hydrogen donors. During this reduction, the color of the radical alters from purple to yellow by the decrease in

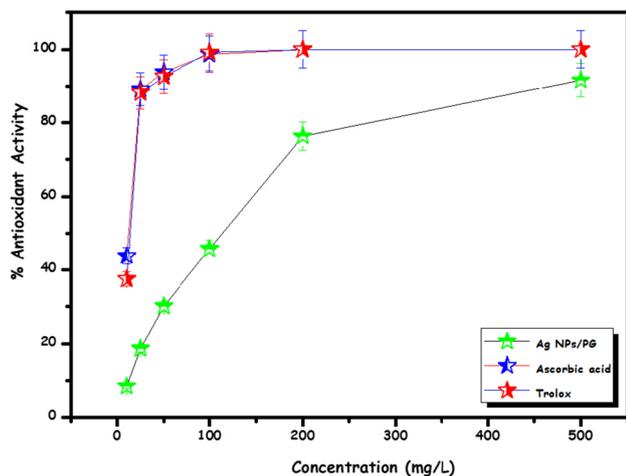


Fig. 5 The DPPH scavenging activity of Ag NPs synthesized using *Punica granatum* extract

absorbance at 517 nm. So, DPPH radical scavenger analysis was used to determine the antioxidant activity of Ag NPs/PG. Figure 5 shows the percentage of antioxidant activity of Ag NPs/PG in comparison with ascorbic acid and Trolox. The antioxidant activity of Ag NPs/PG has raised in a dose-dependent manner. Ag NPs/PG showed a minimum of 8.4% of the scavenging activity against DPPH radicals at 10 mg/L, while ascorbic acid and Trolox showed 43.8% and 37.5% scavenging activity, respectively, at 10 mg/L. The maximum scavenging activity (91.6%) was determined at 500 mg/L, while the scavenging activity of ascorbic acid and Trolox was determined as 100%. Palanisamy et al. (Palanisamy et al. 2017) reported that the green synthesis of Ag NPs with the help of *Sargassum polycystum* aqueous extract showed 78.2% DPPH scavenging activity at 500 mg/L. Also, Khoshnamvand et al. (Khoshnamvand et al. 2019) indicated that Ag NPs/PG synthesized with the help of *Allium ampeloprasum* L. leaf extract exhibited an 82.4% DPPH inhibition activity. Ag NPs/PG demonstrated higher antioxidant activity than their green route synthesized Ag NPs.

The activity of DNA cleavage

The ability of DNA cleavage of green synthesized Ag NPs by means of *Punica granatum* extract was investigated by gel electrophoresis. Plasmid pBR322 DNA was utilized as the target. When supercoiled form plasmid DNA was conducted on agarose gel electrophoresis, it was found to progress faster than supercoiled circular conformation (Form I). If a single strand is cleaved, the conformation of the supercoil will relax to generate a slower-moving nicked circular conformation (Form II). As a result of the splitting of both strands, the linear conformation (Form III) will migrate among Form II and Form I (Kumar et al. 2011). Figure 6

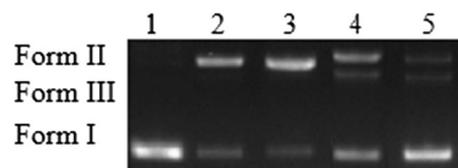


Fig. 6 The DNA cleavage of Ag NPs synthesized of Ag NPs synthesized using *Punica granatum* extract. pBR 322 DNA (Lane 1); 200 $\mu\text{g mL}^{-1}$ Ag NPs/PG + pBR 322 DNA (Lane 2) (30 min incubation); 500 $\mu\text{g mL}^{-1}$ Ag NPs/PG + pBR 322 DNA (Lane 3) (30 min incubation); 200 $\mu\text{g mL}^{-1}$ Ag NPs/PG + pBR 322 DNA (Lane 4) (90 min incubation); 500 $\mu\text{g mL}^{-1}$ Ag NPs/PG + pBR 322 DNA (Lane 5) (90 min incubation)

Table 1 The MIC values of Ag NPs synthesized using *Punica granatum* extract

Microorganisms	MIC values ($\mu\text{g mL}^{-1}$)
<i>E. hirae</i>	128
<i>E. coli</i>	16
<i>P. aeruginosa</i>	32
<i>S. aureus</i>	64
<i>L. pneumophila</i> subsp. <i>Pneumophila</i>	16
<i>B. cereus</i>	128
<i>C. albicans</i>	512

displays gel electrophoretic separation of pBR322 DNA after 30 and 90 min incubation with 200 and 500 $\mu\text{g mL}^{-1}$ concentrations of the Ag NPs/PG. The results showed that pBR322 plasmid DNA was broken when it interacted with 200 $\mu\text{g mL}^{-1}$ concentrations of the Ag NPs/PG Form I and increased Form II DNA intensity. Also, higher concentration (500 $\mu\text{g mL}^{-1}$) and incubation time (90 min) led to the conversion of Form I into Form III. According to the results obtained from the DNA cleavage study, we can say that Ag NPs synthesized green by means of *Punica granatum* show significant chemical nuclease activity. It may be used as an alternative cancer drug after further investigations.

Antimicrobial activity

The antimicrobial activity of Ag NPs/PG against gram-negative (*E. coli*, *L. pneumophila*, *P. aeruginosa*) and gram-positive (*E. hirae*, *B. cereus*, *S. aureus*) bacteria was determined using the broth microdilution method. In addition, the antifungal activity of Ag NPs/PG was tested against the *C. albicans* fungal strain. Table 1 shows the MIC values of Ag NPs/PG. The MIC values of *Punica granatum*-mediated green synthesized Ag NPs were determined as 128 $\mu\text{g mL}^{-1}$, 16 $\mu\text{g mL}^{-1}$, 64 $\mu\text{g mL}^{-1}$, 16 $\mu\text{g mL}^{-1}$, 32 $\mu\text{g mL}^{-1}$, 128 $\mu\text{g mL}^{-1}$, and 512 $\mu\text{g mL}^{-1}$



against to *E. hirae*, *E. coli*, *S. aureus*, *L. pneumophila*, *P. aeruginosa*, *B. cereus*, and *C. albicans*, respectively. According to results, Ag NPs/PG exhibited a strong inhibitory effect against the selected strains. However, it was observed that the MIC values of gram-negative bacteria had lower values than gram-positive bacteria. Similar results were reported Geethalakshmi and Sarada, (Geethalakshmi and Sarada 2013) and Wang et al. (Pakravan et al. 2013). The gram-negative bacteria are more sensitive because of the easy diffusion of Ag NPs through the cellular membrane than gram-positive bacteria which has a thick outer murein layer. So, Ag NPs can even permeate the cytoplasm, interact with sulfur-containing enzymes and proteins, and interfere with the replication of DNA (Durán et al. 2016; Ravichandran et al. 2016). In a study in the literature, Ag NPs were synthesized using *Malus domestica* extract. The antibacterial activity of the synthesized Ag NPs was evaluated, and MIC values were determined. MIC values of the synthesized Ag NPs were 34.17 µg/mL and 27.47 µg/mL for *E. coli* and *P. aeruginosa*, respectively (Mariadoss et al. 2019). In another study, Ag NPs were obtained with the help of *Cestrum nocturnum* plant. Antibacterial and antioxidant activities of biogenic Ag NPs were determined. The MIC value of biogenic Ag NPs for *E. coli* bacteria is determined

as 16 µg/mL (Keshari et al. 2020). Nouri et al., produced Ag NPs by ultrasonic-assisted green synthesis using *Mentha aquatica* leaf extract. The catalytic and antibacterial activities of the synthesized Ag NPs were tested. MIC values of ultrasonic-assisted synthesized Ag NPs against *S. aureus* and *E. coli* bacteria were calculated as 198 µg/mL and 58 µg/mL, respectively (Nouri et al. 2020). In another study, one-step and one-minute biogenic synthesis of Ag NPs was reported using commercial green tea extract (*Camellia sinensis*). Antibacterial activity of biogenic nanoparticles was investigated against gram-negative *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC 700603), *P. aeruginosa* (ATCC 27853), and gram-positive *S. aureus* (ATCC 29213) bacteria. MIC values of Ag NPs were calculated as 15 µg/mL, 250 µg/mL, 30 µg/mL, and 250 µg/mL for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus* bacteria, respectively (Rolim et al. 2019). Suresh et al. reported their green synthesis by reducing Ag ions to Ag NPs using the aqueous root extract of *Delphinium denudatum*. Antibacterial activities of the synthesized Ag NPs were determined for gram-positive and gram-negative bacterial strains by the micro-broth dilution method. MIC value of Ag NPs against *B. cereus* bacteria was found as 500 µg/mL (Suresh et al. 2014). Table 2 shows

Table 2 MIC values of Ag NPs synthesized in green using different plants against pathogenic bacteria

Studied bacteria	Biosource of extract	Size of Ag NPs (nm)	MIC values (µg/L)	References
<i>E. hirae</i>	<i>Rosa canina</i>	13.9–20.57	256	Gulbagca et al. (2019)
	<i>Artemisia annua L</i>	20–90	150	Aghajanyan et al. (2020)
<i>E. coli</i>	<i>Mentha aquatica</i>	8	58	Nouri et al. (2020)
	<i>Allium cepa</i>	10–23	312	Gomaa (2017)
	<i>Malus domestica</i>	50–107.3	34.17	Mariadoss et al. (2019)
	<i>Camellia sinensis</i>	34.68	15	Rolim et al. (2019)
	<i>Allium ampeloprasum</i>	8–50	37.5	Jalilian et al. (2020)
	<i>Delphinium denudatum</i>	≤85	500	Suresh et al. (2014)
	<i>P. aeruginosa</i>	<i>Malus domestica</i>	50–107.3	27.47
<i>P. aeruginosa</i>	<i>Camellia sinensis</i>	34.68	30	Rolim et al. (2019)
	<i>Allium cepa</i>	10–23	156	Gomaa (2017)
	<i>Delphinium denudatum</i>	≤85	250	Suresh et al. (2014)
	<i>S. aureus</i>	<i>Mentha aquatica</i>	8	198
<i>S. aureus</i>	<i>Camellia sinensis</i>	34.68	250	Rolim et al. (2019)
	<i>Delphinium denudatum</i>	≤85	250	Suresh et al. (2014)
	<i>Allium cepa</i>	10–23	312	Gomaa (2017)
<i>L. pneumophila subsp. pneumophila</i>	<i>Rosa canina</i>	13.9–20.57	16	Gulbagca et al. (2019)
	<i>Reishi mushroom</i>	15–22	16	Aygiin et al. (2019)
<i>B. cereus</i>	<i>Delphinium denudatum</i>	≤85	500	Suresh et al. (2014)
	<i>Allium cepa</i>	10–23	625	Gomaa (2017)
<i>C. albicans</i>	<i>Allium cepa</i>	10–23	625	Gomaa (2017)
	<i>Tollens reaction</i>	25	<i>C. albicans I:420</i> <i>C. albicans II:210</i>	Panáček et al. (2009)



the comparison of the antibacterial activities of Ag NPs obtained by green synthesis with the literature.

Conclusion

As a result of this study, Ag NPs were obtained by using low cost, easy, and non-toxic green synthesis method by using *Punica granatum* shell extract. Biomolecules (phenolic compounds, enzymes, flavonoids, etc.) in *Punica granatum* shell extract were used as reducing and stabilizing agents in the green synthesis of Ag NPs. Ag NPs/PG was found to be spherical and have a particle size between 5 and 45 nm. In addition, XRD analysis showed that the green synthesized Ag NPs/PG had a fcc crystal structure. The data achieved in this work showed that Ag NPs synthesized using *Punica granatum* extract were revealed to exhibit significant activities in biological assays of antimicrobial, antioxidant, and DNA cleavage which could be useful in nanomedicine applications.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13762-021-03246-w>.

Acknowledgements The authors declare that there are no competing interests.

Author contributions AA, SÖ, MG, MSY, MU, and FS participated in the study design and coordination, conducted experimental studies, and prepared the manuscript. All authors have read and approved the last article.

Declarations

Funding LB is supported by a project [SR/WOS-A/LS-45/2018 (G)] granted by the Department of Science and Technology, Government of India under the DST Women Scientist Scheme.

Acknowledgements Authors thank Bose Institute for the necessary facilities. We acknowledge the technical assistance of Ms. Tanima Modak Dhar at Bose Institute in handling the Atomic Absorption Spectrophotometer.

Conflict of interest No conflict of interest.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Availability of data and material Not applicable.

Code availability Not applicable.

References

- Aghajanyan A, Gabrielyan L, Schubert R, Trchounian A (2020) Silver ion bioreduction in nanoparticles using *Artemisia annua* L. extract: characterization and application as antibacterial agents. 10:66. <https://doi.org/10.1186/s13568-020-01002-w>
- Aygün A, Özdemir S, Gülcan M et al (2019) Synthesis and characterization of reishi mushroom-mediated green synthesis of silver nanoparticles for the biochemical applications. J Pharm Biomed Anal. <https://doi.org/10.1016/j.jpba.2019.112970>
- Aygün A, Gülbağça F, Nas MS et al (2020) Biological synthesis of silver nanoparticles using Rheum ribes and evaluation of their anticarcinogenic and antimicrobial potential: a novel approach in phytonanotechnology. J Pharm Biomed Anal. <https://doi.org/10.1016/j.jpba.2019.113012>
- BarathManiKanth S, Kalishwaralal K, Sriram M et al (2010) Antioxidant effect of gold nanoparticles restrains hyperglycemic conditions in diabetic mice. J Nanobiotechnol 8:16. <https://doi.org/10.1186/1477-3155-8-16>
- Batool S, Idrees M, Hussain Q, Kong J (2017) Adsorption of copper (II) by using derived-farmyard and poultry manure biochars: efficiency and mechanism. Chem Phys Lett 689:190–198. <https://doi.org/10.1016/j.cplett.2017.10.016>
- Bonesi M, Tundis R, Sicari V, Loizzo MR (2019) The juice of pomegranate (*Punica granatum* L.): Recent studies on its bioactivities. In: Quality Control in the Beverage Industry: Volume 17: The Science of Beverages. Elsevier, pp 459–489
- Durán N, Durán M, de Jesus MB et al (2016) Silver nanoparticles: a new view on mechanistic aspects on antimicrobial activity. Nanomed Nanotechnol Biol Med 12:789–799
- Garcia EJ, Cadarin Oldoni TL, de Alencar SM et al (2012) Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. Braz Dent J 23:22–27. <https://doi.org/10.1590/S0103-64402012000100004>
- Geethalakshmi R, Sarada DVL (2013) Characterization and antimicrobial activity of gold and silver nanoparticles synthesized using saponin isolated from *Trianthema decandra* L. Ind Crops Prod 51:107–115. <https://doi.org/10.1016/j.indcrop.2013.08.055>
- Ghodake G, Lee DS (2011) Green synthesis of gold nanostructures using pear extract as effective reducing and coordinating agent. Korean J Chem Eng 28:2329–2335. <https://doi.org/10.1007/s11814-011-0115-4>
- Gomaa EZ (2017) Antimicrobial, antioxidant and antitumor activities of silver nanoparticles synthesized by *Allium cepa* extract: a green approach. J Genet Eng Biotechnol 15:49–57. <https://doi.org/10.1016/j.jgeb.2016.12.002>
- Gulbagca F, Ozdemir S, Gulcan M, Sen F (2019) Synthesis and characterization of Rosa canina-mediated biogenic silver nanoparticles for anti-oxidant, antibacterial, antifungal, and DNA cleavage activities. Heliyon. <https://doi.org/10.1016/j.heliyon.2019.e02980>
- Heera P, Shanmugam S (2015) Review article nanoparticle characterization and application: an overview
- Idrees M, Batool S, Kalsoom T et al (2019) Biosynthesis of silver nanoparticles using *Sida acuta* extract for antimicrobial actions and corrosion inhibition potential. Environ Technol 40:1071–1078. <https://doi.org/10.1080/09593330.2018.1435738>
- Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B (2014) Synthesis of silver nanoparticles: chemical, physical and biological methods. Res Pharm Sci 9:385–406
- Jalilian F, Chahardoli A, Sadrjavadi K et al (2020) Green synthesized silver nanoparticle from *Allium ampeloprasum* aqueous extract: characterization, antioxidant activities, antibacterial and cytotoxicity effects. Adv Powder Technol 31:1323–1332. <https://doi.org/10.1016/j.appt.2020.01.011>



- Justin Packia Jacob S, Finub JS, Narayanan A (2012) Synthesis of silver nanoparticles using Piper longum leaf extracts and its cytotoxic activity against Hep-2 cell line. *Colloids Surfaces B Biointerfaces* 91:212–214. <https://doi.org/10.1016/j.colsurfb.2011.11.001>
- Keshari AK, Srivastava R, Singh P et al (2020) Antioxidant and antibacterial activity of silver nanoparticles synthesized by *Cestrum nocturnum*. *J Ayurveda Integr Med* 11:37–44. <https://doi.org/10.1016/j.jaim.2017.11.003>
- Khoshnamvand M, Huo C, Liu J (2019) Silver nanoparticles synthesized using *Allium ampeloprasum* L. leaf extract: characterization and performance in catalytic reduction of 4-nitrophenol and antioxidant activity. *J Mol Struct* 1175:90–96. <https://doi.org/10.1016/J.MOLSTRUC.2018.07.089>
- Krishnaraj C, Jagan EG, Rajasekar S et al (2010) Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. *Colloids Surfaces B Biointerfaces* 76:50–56. <https://doi.org/10.1016/j.colsurfb.2009.10.008>
- Kumar LS, Prasad KS, Revanasiddappa HD (2011) Synthesis, characterization, antioxidant, antimicrobial, DNA binding and cleavage studies of mononuclear Cu(II) and Co(II) complexes of 3-hydroxy-*N'*-(2-hydroxybenzylidene)-2-naphthohydrazide. *Eur J Chem* 2:394–403. <https://doi.org/10.5155/eurjchem.2.3.394-403.232>
- Kuppusamy P, Yusoff MM, Maniam GP, Govindan N (2016) Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications—an updated report. *Saudi Pharm J* 24:473–484
- Lalitha A, Subbaiya R, Ponmurugan P (2013) Green synthesis of silver nanoparticles from leaf extract *Azhadirachta indica* and to study its anti-bacterial and antioxidant property
- Lebaka VR, Wee YJ, Narala VR, Joshi VK (2018) Development of New Probiotic Foods—A Case Study on Probiotic Juices. In: *Therapeutic, Probiotic, and Unconventional Foods*. Elsevier, pp 55–78
- Mariadoss AVA, Ramachandran V, Shalini V et al (2019) Green synthesis, characterization and antibacterial activity of silver nanoparticles by *Malus domestica* and its cytotoxic effect on (MCF-7) cell line. *Microb Pathog* 135:103609. <https://doi.org/10.1016/j.micpath.2019.103609>
- Mittal AK, Chisti Y, Banerjee UC (2013) Synthesis of metallic nanoparticles using plant extracts. *Biotechnol Adv* 31:346–356
- Nouri A, Tavakkoli Yarak M, Lajevardi A et al (2020) Ultrasonic-assisted green synthesis of silver nanoparticles using *Mentha aquatica* leaf extract for enhanced antibacterial properties and catalytic activity. *Colloids Interface Sci Commun* 35:100252. <https://doi.org/10.1016/j.colcom.2020.100252>
- Pakravan P, Kashanian S, Khodaei MM, Harding FJ (2013) Biochemical and pharmacological characterization of isatin and its derivatives: from structure to activity. *Pharmacol Rep* 65:313–335. [https://doi.org/10.1016/S1734-1140\(13\)71007-7](https://doi.org/10.1016/S1734-1140(13)71007-7)
- Palanisamy S, Rajasekar P, Vijayaprasath G et al (2017) A green route to synthesis silver nanoparticles using *Sargassum polycystum* and its antioxidant and cytotoxic effects: an in vitro analysis. *Mater Lett* 189:196–200. <https://doi.org/10.1016/j.matlet.2016.12.005>
- Panáček A, Kolář M, Večeřová R et al (2009) Antifungal activity of silver nanoparticles against *Candida* spp. *Biomaterials* 30:6333–6340. <https://doi.org/10.1016/j.biomaterials.2009.07.065>
- Parveen K, Banse V, Ledwani L (2016) Green synthesis of nanoparticles: Their advantages and disadvantages. In: *AIP Conference Proceedings*. American Institute of Physics Inc.
- Raj S, Chand Mali S, Trivedi R (2018) Green synthesis and characterization of silver nanoparticles using *Enicostemma axillare* (Lam.) leaf extract. *Biochem Biophys Res Commun* 503:2814–2819. <https://doi.org/10.1016/j.bbrc.2018.08.045>
- Ravichandran V, Vasanthi S, Shalini S et al (2016) Green synthesis of silver nanoparticles using *Atrocarpus altilis* leaf extract and the study of their antimicrobial and antioxidant activity. *Mater Lett* 180:264–267. <https://doi.org/10.1016/j.matlet.2016.05.172>
- Rodríguez-Luis O, Hernandez-Delgadillo R, Sánchez-Nájera R et al (2016) Green synthesis of silver nanoparticles and their bactericidal and antimycotic activities against oral microbes. *J Nanomater*. <https://doi.org/10.2174/1574891x10666150529150526>
- Rolim WR, Pelegrino MT, de Araújo LB et al (2019) Green tea extract mediated biogenic synthesis of silver nanoparticles: characterization, cytotoxicity evaluation and antibacterial activity. *Appl Surf Sci* 463:66–74. <https://doi.org/10.1016/j.apsusc.2018.08.203>
- Roy K, Sarkar CK, Ghosh CK (2015) Photocatalytic activity of biogenic silver nanoparticles synthesized using yeast (*Saccharomyces cerevisiae*) extract. *Appl Nanosci* 5:953–959. <https://doi.org/10.1007/s13204-014-0392-4>
- Salih Ağırtaş M, Karataş C, Özdemir S (2015) Synthesis of some metallophthalocyanines with dimethyl 5-(phenoxy)-isophthalate substituents and evaluation of their antioxidant-antibacterial activities. *Spectrochim Acta Part A Mol Biomol Spectrosc* 135:20–24. <https://doi.org/10.1016/j.saa.2014.06.139>
- Shekhawat MS, Manokari M, Kannan N, et al (2013) Synthesis of silver nanoparticles using *Cardiospermum halicacabum* L. leaf extract and their characterization
- Sondi I, Salopek-Sondi B (2004) Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J Colloid Interface Sci* 275:177–182. <https://doi.org/10.1016/j.jcis.2004.02.012>
- Suresh G, Gunasekar PH, Kokila D et al (2014) Green synthesis of silver nanoparticles using *Delphinium denudatum* root extract exhibits antibacterial and mosquito larvicidal activities. *Spectrochim Acta Part A Mol Biomol Spectrosc* 127:61–66. <https://doi.org/10.1016/j.saa.2014.02.030>
- Viana GSB, Menezes SMS, Cordeiro LN, Matos FJA (2010) Biological effects of pomegranate (*Punica granatum* L.), especially its antibacterial actions, against microorganisms present in the dental plaque and other infectious processes. In: *Bioactive Foods in Promoting Health*. Elsevier Inc., pp 457–478

