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# GREEN SYNTHESIS OF METAL NANOPARTICLES USING FRUIT EXTRACTS: EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES

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**Abstract**

*The increased need to obtain sustainable and environmentally friendly approaches to the fabrication of nanomaterial has stimulated studies on biogenic synthesis pathways. The current paper presents the green synthesis of silver nanoparticles (AgNPs) by using fruit extract of Punica granatum (pomegranate) as a natural reducing and capping agent in aqueous solutions. The best synthesis was done at pH 9, 60 C, and extract-salt ratio of 1:5 which was confirmed by a typical surface plasmon resonance (SPR) peak at 432 nm. X-ray diffraction (XRD) showed face-centred cubic (FCC) crystalline AgNPs with an average crystallite size of 18.4 ± 2.1 nm, which is in line with the transmission electron microscopy (TEM) measurements (mean diameter 20.3 ± 3.6 nm). Fourier-transform infrared spectroscopy (FTIR) was used to identify the phenolic hydroxyl (O -H, -3310 cm) and carbonyl (C -O, -1635 cm) stretches as the main functional groups that were the cause of reduction and stabilization. Colloidal stability was confirmed using dynamic light scattering (DLS) which gave a zeta potential of -31.4 mV. The produced AgNPs exhibited a high antioxidant activity (DPPH IC50 = 28.6 + 1.4 -1mL; ABTS IC50 = 24.1 + 1.2 -1mL), which is much higher than the crude extract itself. Agar well diffusion and broth microdilution assays of antimicrobials against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans demonstrated minimum inhibitory concentration (MIC) values of 12.550 µg/mL, with Gram-positive bacteria being more susceptible. These results make pomegranate-mediated AgNPs a scalable, non-toxic nanomaterial with potential biomedical uses.*

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## Introduction

Nanotechnology has become one of the most radical sciences of new science with the ability to precisely engineer materials at the atomic and molecular level. Metal nanoparticles have been studied with significant interest in the biomedical, catalytic, agricultural, and environmental fields due to their outstanding optical, electronic, and biological properties, which are attributed to high surface-area-to-volume ratio and quantum confinement effects (Akter et al., 2022; Santhosh et al., 2023). Silver nanoparticles (AgNPs) are the most fully investigated of these, with a broad-spectrum antimicrobial effect, well-defined surface chemistry, and versatility to a wide variety of functionalization approaches.

Traditional physicochemical synthesis techniques, such as chemical reduction, sol-gel processing, laser ablation, and sputtering, usually involve the use of hazardous reducing agents (such as sodium borohydride) and stabilizing agents (such as polyvinylpyrrolidone (PVP)) which produce toxic by-products and require high-energy processing conditions (Doan et al., 2022). Such restrictions have triggered a paradigm shift to sustainable biogenic strategies. Green synthesis uses living organisms or their extracts rich in metabolites to catalyze nanoparticle nucleation and stabilization in ambient conditions without the use of synthetic chemicals and minimizing the environmental footprint (Gour & Jain, 2019; Mathew et al., 2023).

Fruit extracts are one of the most popular biological matrices to synthesize green NPs because they contain extremely high levels of polyphenols, flavonoids, ascorbic acid, tannins, and terpenoids (Rahman et al., 2023). These phytochemicals can act as both reducing agents (donating electrons to reduce Ag + to Ag 0 ) and capping agents (binding to nascent NP surfaces to inhibit agglomeration). Ellagitannins, punica granatum (pomegranate) is especially abundant in ellagic acid, anthocyanins, and punica granatum (pomegranate) is a particularly effective bioreductor of NPs (Al-Thabaiti et al., 2022; Noman et al., 2023).

The pathogenesis of many chronic conditions such as cancer, diabetes, cardiovascular disorders, and neurodegenerative conditions is based on the presence of oxidative stress as a result of the disproportion between the production of reactive oxygen species (ROS) and antioxidant defence (Hussain et al., 2022). Bio-synthesised AgNPs are also reported to have a high free-radical scavenging capacity, which can be attributed to both the remaining surface-bound phytochemicals and AgNP-mediated free-radical redox chemistry (Javed et al., 2023). At the same time, the growing issue of antimicrobial resistance (AMR) in the international setting (estimated to kill 10 million people every year by 2050) is driving the need to develop new therapeutic agents. AgNPs interfere with microbial cell membranes, prevent DNA replication, and produce intracellular ROS in a thermodynamically expensive way compared to conventional antibiotics, and thus pathogens incur significant costs to acquire resistance (Srikar et al., 2022; WHO, 2023).

Although there is a significant literature on the use of plants in the synthesis of NP, there are few systematic studies that have linked the phytochemical makeup of particular fruit extracts to the physicochemical characteristics and subsequent bioactivities of the synthesized NPs. This gap is filled by the present study through (i) optimization of the synthesis parameters of pomegranate-mediated AgNPs, (ii) extensive characterization of the products, (iii) quantitative assessment of antioxidant activity, (i.e. DPPH, ABTS, FRAP), and (iv) the determination of antimicrobial activity, in relation to clinically relevant Gram-positive,

## Literature Review

### Green Synthesis: Principles and Mechanisms

The philosophy of biogenic NP synthesis is based on the concept of green chemistry, enshrined in the twelve principles of Anastas and Warner. The process takes place in three steps:

(1) activation, when metal ions react with bioactive compounds;

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(2) nucleation, when the metal ion is reduced to create initial NP nuclei; and (3) termination, when surface-adsorbed biomolecules prevent further growth (Doan et al., 2022). Extract concentration, pH, temperature, and redox potential of phytochemicals are the factors that regulate the kinetics of each stage (Mathew et al., 2023).

Ahmad et al. (2023) showed that polyphenolic compounds with ortho-dihydroxy (catechol) moieties are the main electron donors of metal ion reduction process, and their oxidation to quinone analogs fuels Ag + to Ag - reduction. Flavonoids also play a role due to their aromatic ring systems that stabilise nascent NP surfaces by means of the interaction of π-electrons clouds (Chen et al., 2022). Such dual functionality implies that fruit extracts containing a larger amount of phenols will always result in smaller, monodisperse NPs with better colloidal stability (Rahman et al., 2023).

**Characterization of Biosynthesized Silver Nanoparticles**

Non-destructive and fast confirmation of the formation of AgNPs using UV-visible spectroscopy of the formation is based on the characteristic SPR peak as a result of collective oscillation of conduction band electrons. Information regarding the size of the particles and size distribution is encoded by the peak position (which is usually 400450 nm in the case of spherical AgNPs) and half-width, respectively (Al-Thabaiti et al., 2022). A red-shift of the SPR peak to longer wavelengths is a sign of larger particles or an anisotropic morphology like triangular nanoplates, e.g. when using mango peel to stabilize AgNPs (λmax = 447 nm, mean diameter 38 nm) reported by Santhosh et al. (2023).

XRD patterns of biosynthesised AgNPs characteristically display diffraction peaks at 2θ ≈ 38.1°, 44.3°, 64.5°, and 77.4°, corresponding to the (111), (200), (220), and (311) planes of FCC metallic silver (JCPDS Card No. 04-0783).

The computed crystallite sizes through the Debye-Scherrer equation (D = K -1541 nm 1/2cos57; K = 0.9, 1/2cos57) are usually between 10-35nm in the case of fruit-extract-mediated particles (Noman et al., 2023).

The OH stretching (˜32003450 cm -1), C=O stretching (˜16001750 cm -1), C-O stretching (˜10001300 cm -1) have always been considered the signature bands in the FTIR analysis, which confirms the presence of hydroxyl and carbonyl groups in the surface capping (Javed et al

**Antioxidant Activity of Metal Nanoparticles**

AgNPs have been observed to free radical scavenge in a variety of assay systems. Hussain et al. (2022) identified the action of agNPs prepared with the extracts of Citrus limon and Citrus sinensis to be 22.4 -45.6 µg/mL and ascribed the activity to remaining surface-bound ascorbate and flavonoid molecules. Comparative analyses always show that biosynthesised AgNPs are superior to respective crude extracts in antioxidant assays, presumably because of the catalytic amplification of the electron-transfer capacity of the metallic core (Chen et al., 2022). The FRAP assay that determines the ability to reduce Fe 3+ to Fe 2 + in low-pH conditions gives a complementary mechanistic view of reducing power that is independent of radical scavenging (Mathew et al., 2023).

**Antimicrobial Mechanisms and Activity**

AgNPs have a complex antimicrobial effect, with several mechanisms operating simultaneously: (i) electrostatic adhesion of positively charged Ag + ions to the negatively charged bacterial membrane, disrupting its integrity; (ii) intracellular ROS generation leading to oxidative DNA damage and protein denaturation; (iii) inhibition of the ATP synthesis by blocking respiratory chain enzymes; and ( Gram-positive, which have a thicker peptidoglycan cell wall, but do not have an outer membrane, exhibit counterintuitive greater susceptibility to AgNPs in most studies - due to the better penetration of the ion through the homogeneous cell wall (Ahmad et al., 2023).

Recently there have been investigations of synergistic interactions between AgNPs and traditional antibiotics. Wang et al. (2023) showed that sub-MICs of pomegranate-AgNPs four-fold decreased MIC of ampicillin against methicillin-resistant S. aureus (MRSA), indicating potential potential uses in combination therapy. On the same note, Noman et al. (2023) claimed that biosynthesised ZnO NPs of guava extract had lower

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MIC values of 6.25 µg/mL than bulk ZnO (>500 µg/mL).

**Materials and Methods**

**Materials and Reagents**

Pomegranate (Punica granatum L., Wonderful cultivar) fruits were purchased at a local certified organic market. Silver nitrate ($AgNO_3$, ≥99.0%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), Folin-Ciocalteu phenol reagent, gallic acid, quercetin, sodium carbonate ($Na_2CO_3$), iron(III) chloride ($FeCl_3$), and ferrous sulphate ($FeSO_4$) were all purchased from Sigma-Aldrich (St. Louis, MO, USA) at analytical grade. Mueller-Hinton Broth (MHB) and Mueller-Hinton Agar (MHA) were obtained from Oxoid (UK). Throughout, distilled and deionized water (18.2 M o cm cm, Milli-Q, Merck) was used.

**Fruit Extract Preparation**

The pericarp was manually removed, washed three times with distilled water and freeze-dried at 50C to prepare the arils of pomegranate. Laboratory milled the dried product to a fine powder, which was sieved with a 250 µm mesh. Aqueous extraction was done by suspending 10 g of powder in 100 mL of Milli-Q water, stirring continuously at 60 0 C, 30 minutes and successively filtering using Whatman No. 1 filter paper and a 0.45 m nylon membrane syringe filter. The resulting clear extract was stored at 4 °C and used within 48 h.

**Phytochemical Screening and Quantification**

The Folin-Ciocalteu method was used to estimate the total phenolic content (TPC) using gallic acid as the standard curve (0 0 400 µg/mL, R 2 = 0.9987) and measuring the absorbance at 765 nm after 30 minutes incubation. Findings are reported in mg gallic acid equivalent/gram dry weight (mg GAE/g DW). The total flavonoid content (TFC) was measured by the method of aluminum chloride colorimetry using quercetin as a standard (0-200 ug/mL) at 510 nm and in the form of mg quercetin equivalents/gram dry weight (mg QE/g DW). The 2,6-dichlorophenolindophenol (DCPIP) titration method was used to identify the content of ascorbic acid (Rahman et al., 2023).

The AgNPs synthesized using the silver nitrate reduction method are optimized by means of energy and time.

The dropwise addition of 5 mL pomegranate extract to 45 mL of 1 mM AgNO 3 solution (extract: salt ratio 1:9 v/v initially) was used to initiate synthesis. A clean Erlenmeyer flask reaction mixtures were prepared and incubated on a temperature-controlled magnetic stirrer. The effect of four synthesis parameters was investigated systematically using a one-factor-at-a-time (OFAT) approach: (i) pH (4, 7, 9, 11) adjusted with 0.1 M NaOH and HCl; (ii) temperature (25, 40, 60, 80 °C); (iii) extract-to-salt ratio (1:1, 1:5, 1:10); and (iv) reaction time (15, 30, 60, 120 min). AgNPs were formed visually when the yellowish colour change to dark brown occurred and observed spectrophotometrically (UV-1900, Shimadzu) at 300-700 nm. The best setting was selected by maximizing SPR peak absorbance with minimum half-bandwidth.

**Characterization**

UV-Visible absorption spectra were measured using a Shimadzu UV-1900 spectrophotometer with a wavelength of 300 to 700 nm. The patterns were recorded as XRD patterns by a Bruker D8 Advance diffractometer (CuK alpha radiation, 20 -80 o, 20 o step 0.02 o/s). The Debye-Scherrer equation was used to determine the crystallite size. FTIR spectra of NP pellets that had been dried by lyophilization (KBr pellets) were obtained using a PerkinElmer Frontier FTIR spectrometer (4000400 cm -1 32 scans 4 cm -1 resolution). TEM on a JEOL JEM-2100Plus (200 kV) was carried out on drop-casted NP suspension on carbon-coated copper grids. ImageJ software was used to measure particle size based on TEM images (n ≥ 100 particles). Dynamic light scattering (DLS) was used to measure hydrodynamic size, polydispersity index (PDI), and zeta potential on a Malvern Zetasizer Nano ZS.

**Antioxidant Assays**

The DPPH radical scavenging activity was measured by using 100 µL of AgNP suspension (or extract) in different concentrations (10-200 ug/mL) at room temperature in the dark by adding 3.9 mL of freshly prepared 0.1 mM DPPH in methanol and incubating at room temperature over 30 min and measuring the absorbance at The results were presented as percent inhibition = [(A control-A sample)/A control) x 100] and IC 50 were

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interpolated using sigmoid dose response curves which were fitted in GraphPad Prism 10. The radical cation decolorisation process was carried out in the presence of ABTS+ and Trolox was used as a reference to perform the experiment at 734 nm (Chen et al., 2022). At 593 nm, the reducing power of the antioxidants was determined using FeSO 4 as the standard, and this was in moles of Fe 2 + gram. The experiments were done thrice (n = 3) and ascorbic acid was used as a positive control.

**Antimicrobial Assays**

Their antimicrobial activity was tested against four reference strains, including Staphylococcus aureus (ATCC 25923), Bacillus subtilis (ATCC 6633), Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), and Candida albicans (ATCC Bacterial inocula were standardized to 0.5 McFarland (~1.5 × $10^8$ CFU/mL) in 0.9% saline. In the case of agar well diffusion 20 mL of MHA was poured into each of the Petri plates and left to solidify before inoculation through uniform distribution. Wells (6 mm diameter) were prepared and 50 µL of AgNP suspension was placed in them at two concentrations (50 and 100 µg/mL). Ampicillin (10 µg/disc, Gram-positive), gentamicin (10 µg/disc, Gram-negative) and fluconazole (25 µg/disc, C. albicans) were used as positive controls; the negative control was sterile distilled water. Plates were left to incubate at 37 o C, 24 (bacteria) or 48 h (fungi) and zones of inhibition (ZOI) quantified in millimeters. The broth microdilution on 96-well plates (CLSI M07-A10 guidelines) was decided on with a twofold serial dilution range of 3.125-200 µg/mL, which defines the MIC (Srikar et al., 2022).

**Statistical Analysis**

Any quantitative data are given as mean (SD) of three or more independent measurements. To compare the means of groups, one-way analysis of variance (ANOVA) and Tukey honestly significant difference (HSD) post-hoc test were used. Phytochemical composition and biological activity were analyzed through Pearson correlation analysis of relationships. The statistical significance was considered p below 0.05. All calculations were done in IBM SPSS Statistics v29.0 (IBM Corp., Armonk, NY) and GraphPad Prism 10.

**Results**

**Phytochemical Profile of Pomegranate Extract**

Phytochemical screening established the presence of phenolics, flavonoids, tannins, terpenoids and saponins in the pomegranate aqueous extract. The quantitative analysis showed that the total phenolic content was 148.6 + 4.3 mg GAE/g DW, total flavonoid content was 62.4 + 2.8 mg QE/g DW and ascorbic acid was 18.2 + 0.9 mg/100 g DW (Table 1). These values coincide with those obtained in other nanomaterial studies with pomegranate extracts (Al-Thabaiti et al., 2022; Noman et al., 2023).

**Table 1.** ***Phytochemical composition of pomegranate (Punica granatum) aqueous extract.***

| Phytochemical Parameter | Value (mean ± SD) | Method / Standard |
|---|---|---|
| Total Phenolic Content (TPC) | 148.6 ± 4.3 mg GAE/g DW | Folin-Ciocalteu / Gallic acid |
| Total Flavonoid Content (TFC) | 62.4 ± 2.8 mg QE/g DW | $AlCl_3$ colorimetric / Quercetin |
| Ascorbic Acid | 18.2 ± 0.9 mg/100 g DW | DCPIP titration |
| Tannins | 34.8 ± 1.6 mg TAE/g DW | Tannic acid equivalent |
| Saponins | Present | Froth / foam test |

*Values represent mean ± SD (n = 3). GAE = gallic acid equivalents; QE = quercetin equivalents; TAE = tannic acid equivalents; DW = dry weight.*

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**Synthesis and Optimization of AgNPs**

The development of AgNPs was evidenced by a colour change of pale yellow (AgNO 3 solution) to dark brown which was as a result of SPR excitation in metallic silver. Optimised AgNPs AgNPs had a distinct, symmetrical SPR peak at 432 nm (Figure 1) in the UV-Vis absorption spectrum.

Optimisation experiments indicated that pH 9 generated the sharpest and strongest SPR peak that was in line with an increased accessibility of phenolate anions as an electron donor in alkaline solutions. The highest absorbance was obtained at 60 C and 60 min reaction time whereas an extract-to-salt ratio of 1:5 provided enough phytochemical availability with the necessary concentration of AgNO 3 (Table 2).

**Table 2.** ***Optimisation of synthesis parameters for pomegranate-mediated AgNPs.***

| Parameter | Levels Tested | Optimal Value | SPR Absorbance |
|---|---|---|---|
| pH | 4 \| 7 \| 9 \| 11 | 9 | 1.84 ± 0.04 |
| Temperature (°C) | 25 \| 40 \| 60 \| 80 | 60 | 1.84 ± 0.06 |
| Reaction time (min) | 15 \| 30 \| 60 \| 120 | 60 | 1.84 ± 0.03 |
| Extract:Salt ratio (v/v) | 1:1 \| 1:5 \| 1:10 | 1:5 | 1.84 ± 0.05 |

*SPR absorbance measured at 432 nm. Values represent mean ± SD (n = 3).*

***TEM Morphology and DLS***

TEM micrographs showed the existence of mostly spherical AgNPs with an average diameter of 20.3 +/- 3.6 nm with a small size distribution (Figure 4). Elemental silver was identified as the dominant component (Ag peak at 3.0 keV) in EDS. DLS measurements provided a hydrodynamic diameter of 28.7 +-4.2 nm, which is a bit bigger than the TEM values because of the hydration shell. The PDI of 0.218 was a moderately monodisperse suspension. Strong electrostatic repulsion between NPs and sufficient colloidal stability to conduct bioactivity measurements were confirmed by the zeta potential of -31.4 mV (Table 3)..

**Table 3.** ***Physicochemical characterisation summary of synthesised AgNPs.***

| Property | Result | Technique / Notes |
|---|---|---|
| SPR peak position | 432 nm | UV-Vis spectroscopy |
| Crystallite size | 18.4 ± 2.1 nm | XRD / Scherrer equation |
| TEM particle size | 20.3 ± 3.6 nm | TEM; n = 100 particles |
| Hydrodynamic diameter | 28.7 ± 4.2 nm | DLS (includes hydration shell) |
| PDI | 0.218 | < 0.3 = monodisperse |
| Zeta potential | −31.4 mV | < −25 mV = colloidally stable |
| Crystal structure | FCC metallic Ag | JCPDS card 04-0783 |
| Primary capping groups | O–H, C=O, C–O | FTIR; ~3289, 1612, 1056 $cm^{-1}$ |

*Values represent mean ± SD (n = 3). PDI = polydispersity index; FCC = face-centred cubic.*

**Antioxidant Activity**

Table 4 sums up the antioxidant actions of AgNPs, crude pomegranate extract, and ascorbic acid (positive control). AgNPs also showed significantly better free-radical scavenging ability than the crude extract in all three tests. DPPH $IC_{50}$ values were 28.6 ± 1.4, 42.3 ± 2.1, and 8.4 ± 0.6 µg/mL for AgNPs, extract, and ascorbic acid, respectively. ABTS $IC_{50}$ values followed a similar trend (24.1 ± 1.2 vs 36.8 ± 1.9 µg/mL for AgNPs vs extract). FRAP values were 412.4 ± 18.6 µmol $Fe^{2+}$ eq/g for AgNPs, significantly higher than the crude extract

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(289.3 ± 14.2 µmol $Fe^{2+}$ eq/g; $p < 0.05$, Tukey's test). The analysis of Pearson correlation showed that TPC and DPPH scavenging had a significant positive correlation ($r = 0.943$, $p < 0.01$), which validated phenolics as the most contributing factors to antioxidant activity.

**Table 4.** ***Antioxidant activity comparison of AgNPs, pomegranate extract, and ascorbic acid.***

| Sample | DPPH $IC_{50}$ (µg/mL) | ABTS $IC_{50}$ (µg/mL) | FRAP (µmol $Fe^{2+}$ eq/g) |
|---|---|---|---|
| Pomegranate-AgNPs | 28.6 ± 1.4 a | 24.1 ± 1.2 a | 412.4 ± 18.6 a |
| Crude pomegranate extract | 42.3 ± 2.1 b | 36.8 ± 1.9 b | 289.3 ± 14.2 b |
| Ascorbic acid (standard) | 8.4 ± 0.6 c | 9.2 ± 0.8 c | — |

*Values represent mean ± SD (n = 3). Different superscript letters within each column indicate significant differences ($p < 0.05$, Tukey's HSD test). Lower $IC_{50}$ = stronger activity.*

**Antimicrobial Activity**

Agar well diffusion assays showed distinct zones of inhibition (ZOI) with all the tested organisms at both AgNP concentrations (Table 5). The largest ZOI was recorded against S. aureus at 100 µg/mL (19.4 ± 0.8 mm), followed by B. subtilis (17.8 ± 0.7 mm). Gram-negative bacteria demonstrated less, yet significant, ZOI (E. coli: 15.2 ± 0.6 mm; P. aeruginosa: 12.6 ± 0.9 mm at 100 µg/mL). C. albicans was intermediate susceptible (ZOI = 13.8 ± 0.5 mm). Negative controls did not exhibit any inhibition. Representative Petri plates pictures.

**Table 5.** ***Zone of inhibition (mm) of pomegranate-AgNPs against test organisms.***

| Test Organism | Type | AgNPs 50 µg/mL | AgNPs 100 µg/mL | Crude extract | (+) Control | (—) |
|---|---|---|---|---|---|---|
| S. aureus ATCC 25923 | G+ | 15.6 ± 0.6 | 19.4 ± 0.8 | 10.2 ± 0.4 | 22.0 ± 1.0 | 0 |
| B. subtilis ATCC 6633 | G+ | 13.8 ± 0.7 | 17.8 ± 0.7 | 9.4 ± 0.5 | 20.5 ± 0.8 | 0 |
| E. coli ATCC 25922 | G— | 11.4 ± 0.5 | 15.2 ± 0.6 | 7.8 ± 0.6 | 18.2 ± 0.9 | 0 |
| P. aeruginosa ATCC 27853 | G— | 9.2 ± 0.8 | 12.6 ± 0.9 | 6.2 ± 0.7 | 16.8 ± 1.1 | 0 |
| C. albicans ATCC 10231 | Fungus | 10.6 ± 0.6 | 13.8 ± 0.5 | 7.2 ± 0.5 | 19.6 ± 0.8 | 0 |

*ZOI values (mm) include well diameter (6 mm). Values represent mean ± SD (n = 3). G+ = Gram-positive; G— = Gram-negative. Positive controls: Ampicillin (G+), Gentamicin (G—), Fluconazole (fungi).*

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**Discussion**

As shown in the current research, biogenic production of crystalline AgNPs was achieved through the help of pomegranate fruit extract, which was effective in obtaining physicochemical properties that are suitable in biomedical applications. The SPR peak of 432 nm aligns with spherical AgNPs (15-25 nm diameter) and is consistent with TEM measurements (20.3 +3.6 nm), and is within the range of 425-438 nm found by Al-Thabaiti et al. (2022), who used pomegranate-mediated NPs with 4

The significant effect of pH on the efficiency of synthesis is worth mentioning. The formation of NPs was greatly enhanced under alkaline conditions (pH 9) which can be explained by the deprotonation of phenolic hydroxyl groups to phenolate anions - forms with much higher reducing potential compared to their protonated counterparts (Ahmad et al., 2023). This pH-dependence is typical of polyphenol-mediated syntheses and has been verified by Chen et al. (2022) independently of citrus-derived AgNPs. The temperature optimum of 60 °C indicates an Arrhenius-controllable acceleration of the reaction kinetics in the absence of degradation of sensitive phytochemicals like ascorbic acid that starts to degrade at 70 o C (Rahman et al., 2023).

OH and C=O evidence on FTIR changes in the position of the O-H and C=O peaks on the formation of NP gives a mechanistic understanding of the duality of pomegranate polyphenols. The recorded red shift of the C=O band (˜25 cm -1 ) can be ascribed to the coordination of carbonyl oxygen to Ag surface atoms, which is a well-established phenomenon in phenolic-AgNPs (Javed et al., 2023). The zeta potential of -31.4 mV proves that adsorbed anionic phenolates onto the surface create enough repulsive electrostatic force to stop NP aggregation - a key factor in storage stability and biological activity (Mathew et al., 2023).

Two complementary mechanisms can be attributed to the higher antioxidant effect of AgNPs as compared to the crude extract (DPPH IC 50: 28.6 vs 42.3 50 g/mL; p = out of 0.05). First, AgNP core serves as a reservoir of electrons, which contributes to electron diffusion to DPPH radicals in a heterogeneous catalytic process (Hussain et al., 2022). Second, the 20 nm AgNPs have a high surface area (approximately 60 $m^2$ g1 -1 in the case of spherical particles) which exposes a high number of surface-bound phenolic antioxidants in accessible conformations. This amplification effect has been reported in various fruit-AgNP systems with an average IC50 of 30-50% compared with the parent extract (Chen et al., 2022; Javed et al., 2023).

This antimicrobial susceptibility pattern Gram-positive bacteria (S. aureus MIC = 12.5 µg/mL) are four-fold more susceptible than P. aeruginosa (MIC = 50 µg/mL) which is consistent with the mechanistic expectations. The outer membrane of Gram-negative bacteria is rich in lipopolysaccharides, which provides a selective barrier to permeability that suppresses the penetration of AgNP and lowers the levels of $Ag^+$ in the cell (Srikar et al., 2022). The intermediate antifungal effect on C. albicans (MIC = 25 µg/mL) could be explained by the effect of disrupting the ergosterol-membrane and inhibiting budding, which is not an antibacterial act (Wang et al., 2023). Though AgNP MIC values are higher than those of reference antibiotics, their multi-target mechanism of action results in cross-resistance acquisition by pathogens being thermodynamically unfavorable, which is a crucial asset in the AMR situation (WHO, 2023).

The current research has a number of limitations. Laboratory Characterization had been characterized by synthesis; scale-up reproducibility needs to be verified. Although in vitro antioxidant and antimicrobial data have a high level of indiction, they need to be tested in cellular and animal models before clinical translation. A fundamental requirement is cytotoxicity profiling against mammalian cell lines (e.g., HeLa, HEK-293) to be used in biomedical applications. Also, long-term colloidal stability under physiological conditions (pH 7.4, 37 C, ionic strength) needs to be studied systematically.

**Conclusion**

This research has managed to develop an optimized one-pot green synthesis procedure of crystalline AgNPs with Punica granatum fruit extract. FCC metallic silver, spherical, approximately 20 nm, colloidal ally stable (zeta

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potential -31.4 mV), and capped with pomegranate-derived polyphenols were extensively characterized as the synthesized NPs. The major findings are: (i) enhanced antioxidant action better than the parent extract (DPPH IC 50 = 28.6 µg/mL), which is due to synergistic metallic-core and surface-phenolic action; (ii) high broad-spectrum antimicrobial action (MIC 12.5-50 µg/mL) against clinically relevant path The pomegranate-AgNP system presented in the current paper is an environmentally friendly, scalable nanomaterial platform with a high potential to find application in wound care, food preservation, and nanocomposite antimicrobial surfaces. Future research must consider in vivo biocompatibility, synergistic antibiotic combinations and development of controlled-release formulations.

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