Research Article

Green-Synthesized Silver Nanoparticles from *Panax ginseng* Root Extract Against Ciprofloxacin-Resistant Clinical *Pseudomonas aeruginosa*: Antibacterial Activity and Combined Effects with Ciprofloxacin and Povidone-Iodine

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Abstract

Green synthesized silver nanoparticles (AgNPs) are potential antibacterial agents against drug resistant microorganisms. In this study, AgNPs were synthesized from aqueous Panax ginseng root extract and their antibacterial efficacy and synergistic activity with ciprofloxacin and povidone-iodine (PVP-I) were assessed against ciprofloxacin resistant clinical Pseudomonas aeruginosa isolates. The AgNPs were characterized by FTIR, UV–Visible spectroscopy, SEM and XRD and evaluated by broth microdilution. Characterization indicated creation of primarily quasi-spherical, crystalline AgNPs with distinctive UV-Visible peak at 424 nm. All the three isolates showed MIC values of 3.1 µg/mL with AgNPs. In combination experiments, AgNPs lowered PVP-I MIC to 300 µg/mL ($P < 0.001$), but did not influence ciprofloxacin MIC. These results confirm the antibacterial efficacy of P. ginseng-mediated AgNPs and their ability to improve PVP-I action against ciprofloxacin-resistant P. aeruginosa.

Keywords: Silver nanoparticles; *Panax ginseng*; Green synthesis; *Pseudomonas aeruginosa*; Ciprofloxacin resistance; Povidone–iodine; Combination effect.

1. Introduction

Pseudomonas aeruginosa is an opportunistic a major source of health care-associated infections especially in immunocompromised and critically patients (1–3). Its persistence and difficulty to treat are related to the innate and acquired resistance mechanisms, including decreased outer membrane permeability, multidrug efflux pumps, β-lactamase production and biofilm formation(7–4) .

Ciprofloxacin resistance is mostly connected with mutations at target site, overproduction of efflux pumps, reduction of permeability and biofilm associated responses (6–9). (AgNPs) have been identified as potent antibacterial agents due to their several mechanisms of action such as membrane disruption (10,11).

Plant-mediated green approach is a sustainable approach in which the phytochemicals are acting as reducing and stabilizing agents. (11,12). Green synthesis and stabilization of AgNPs by using Panax ginseng root extract was done successfully (12–15). Although several studies have been done on AgNPs, there is little information on P. ginseng mediated AgNPs against ciprofloxacin resistant clinical P. aeruginosa (10,16,17). Hence, the present work was designed to synthesize and characterize P. ginseng-mediated AgNPs by FTIR, UV–Visible spectroscopy, SEM, and XRD and to assess their antibacterial activity and combination effects with ciprofloxacin and povidone–iodine (PVP-I).

2. Materials and Method

2.1 Sample Collection, Identification, and Isolate Selection

Three hundred clinical samples comprising burn, wound, urine, sputum, blood and ear samples were aseptically obtained from patients with probable Pseudomonas aeruginosa infections at hospitals in Babylon Governorate, Iraq. Samples were transported under proper circumstances and processed within 2–4 h. The isolates were grown on cetrimide agar, and incubated aerobically at 37 °C for 24–48 h. Presumptive P. aeruginosa isolates were identified based on colony shape, Gramme staining, pigment synthesis, and traditional biochemical testing (18,19). Twenty-nine P. aeruginosa isolates were obtained. The 29 isolates were tested for antimicrobial susceptibility using the VITEK® (bioMérieux, France) (20). Of them, 20 isolates were antibiotic resistant and nine were sensitive. Three ciprofloxacin-resistant isolates with wide antibiotic resistance profiles were chosen for further assessment of antibacterial activity of AgNP and combination tests with ciprofloxacin and povidone–iodine (PVP-I).

2.2 Preparation of the Aqueous *Panax ginseng* Root Extract

The aqueous extract of Panax ginseng root was produced using Soxhlet extraction technique with modification according to the previously published aqueous extraction processes (21,22). Briefly, 5 g of the pulverized roots were extracted with 100 mL of distilled water at 100°C for 2 h. Filtered using Whatman No. 1 filter paper and kept in sterile amber vials at 4°C until used.

2.3 Preparation of Silver Nanoparticles (AgNPs)

The aqueous extract of P. ginseng root was used as reducing and stabilizing agent (14,22,23). Briefly, 5 mL of 0.02 M $AgNO_3$ was added to 90 mL of deionized water and heated at 90°C for 15 min. Then, 5 mL of P. ginseng extract was added dropwise and the reaction was maintained at 90°C for 60 min with continuous stirring .The appearance of reddish-brown color showed the formation of AgNP. The suspension was chilled and stored at 4 °C pending further investigation.

2.4 Characterization of Silver Nanoparticles (AgNPs)

FTIR spectra were obtained in the range 4000–400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans to determine the functional groups involved in the production and stabilization of nanoparticles (24,25). The distinctive surface plasmon resonance (SPR) band (24,26) was analyzed by recording UV–Visible spectra in the range of 200–1100 nm using deionized water as blank. SEM was used to characterize the morphology, surface properties and aggregation of AgNPs (24,25). XRD was used to analyses the crystalline structure and phase characteristics of AgNPs.(24,25)

2.5 Determination of the Minimum Inhibitory Concentration (MIC)

Determination of the minimum inhibitory concentrations (MICs) of silver nanoparticles (AgNPs), ciprofloxacin (CIP) and povidone–iodine (PVP-I) against three clinical Pseudomonas aeruginosa isolates was performed by broth microdilution in sterile 96-well microtiter plates according to standard MIC procedures (27–29), except for modifications for nanoparticle evaluation (16,30). AgNPs, CIP and PVP-I working solutions were produced at 100, 25 and 5000 µg/mL respectively, then serially diluted two-fold in Mueller–Hinton broth. The bacterial inoculum (≈1 × 10^6 CFU/mL) was added at an equal amount to a final volume of 200 µL/well to provide a final inoculum of ≈5 × 10^5 CFU/mL. Final concentrations were 50.0-0.8 µg/mL for AgNPs, 12.50-0.39 µg/mL for CIP and 2500-78.13 µg/mL for PVP-I. Plates were incubated at 37 °C for 18-24 h with positive and negative controls. The MIC was established as the lowest concentration that fully prevented observable bacterial growth. All experiments were conducted independently in three replicates.

2.6 Combination of AgNPs with PVP-I and CIP

Synergistic effects of AgNPs with PVP-I and CIP on three clinical P. aeruginosa isolates aeruginosa isolates were tested using a broth microdilution-based combination assay with minor modifications (16,28). Each isolate was examined separately in triplicate. In brief, each antimicrobial solution (50 µL) was mixed with standardized bacterial inoculum containing AgNPs (100 µL) at a predetermined final concentration of 1 µg/mL to provide a final volume of 150 µL/well. The final test values were 1250, 875, 612.5, 428.7, 300.1 and 210 µg/mL for PVP-I and 12.5, 9.4, 7.0, 5.3, 4.0 and 3.0 µg/mL for CIP. Wells lacking AgNPs. Plates were incubated at 37°C for 18–24 h and the MIC was recorded as the lowest antimicrobial dose that totally inhibited apparent bacterial growth(28). The impact of each combination was evaluated by comparing the MICs in the presence and absence of AgNPs (16).

2.7 Statistical Analysis

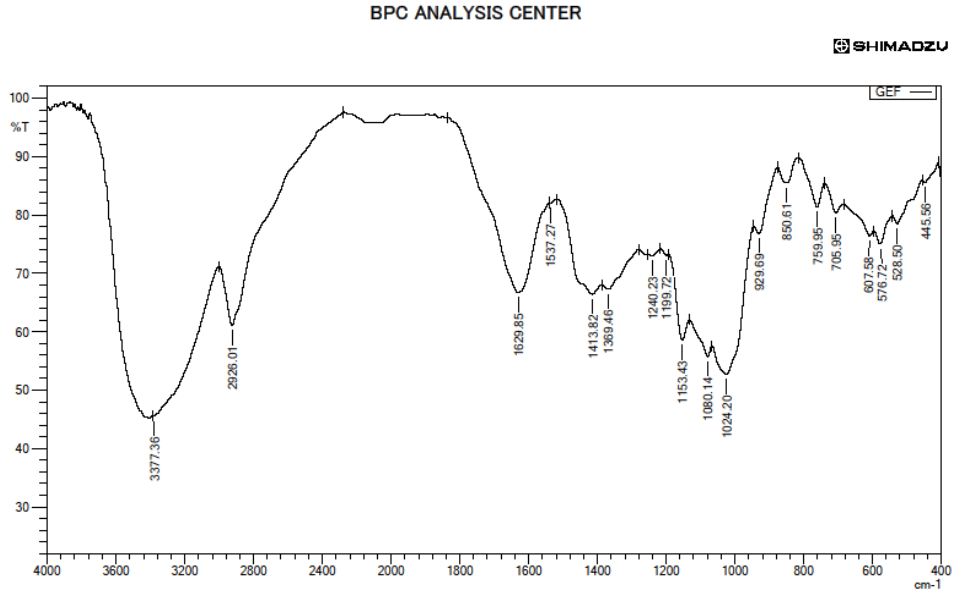
The data were analyzed using IBM SPSS Statistics and Microsoft Excel. Results were given as mean ± standard deviation (SD) wherever appropriate. Statistical significance was considered at $P < 0.05$. Microsoft Excel was used to organize and graphically portray data.

3 Results

3.1. FTIR Analysis

FTIR analysis indicated spectrum variations between P. ginseng extract and AgNPs (Figure 1) with prominent band shifts from 3377.36 to 3437.15 cm^{-1} and from 1629.85 to 1627.92 cm^{-1} and variations in the fingerprint area.

A



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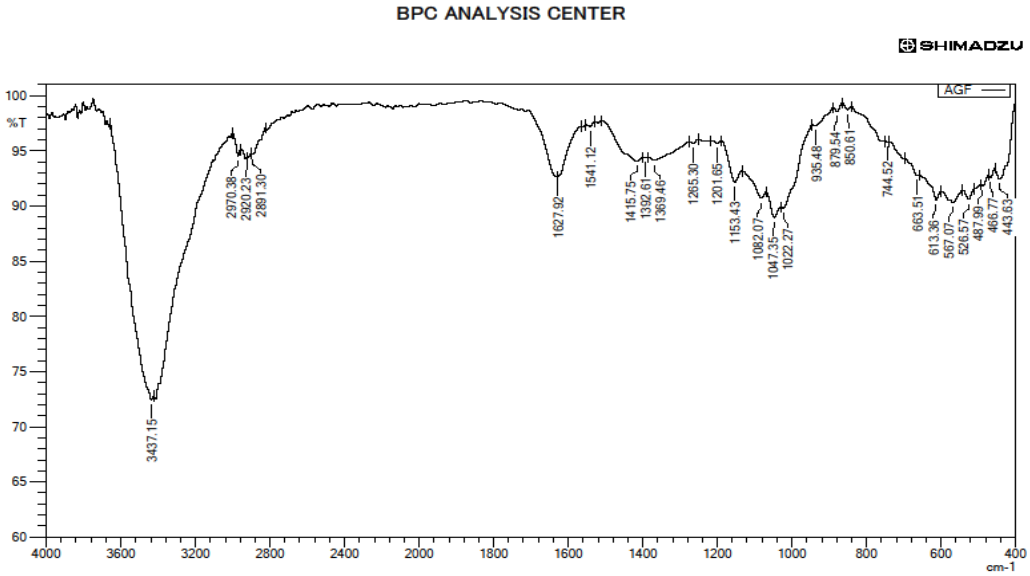
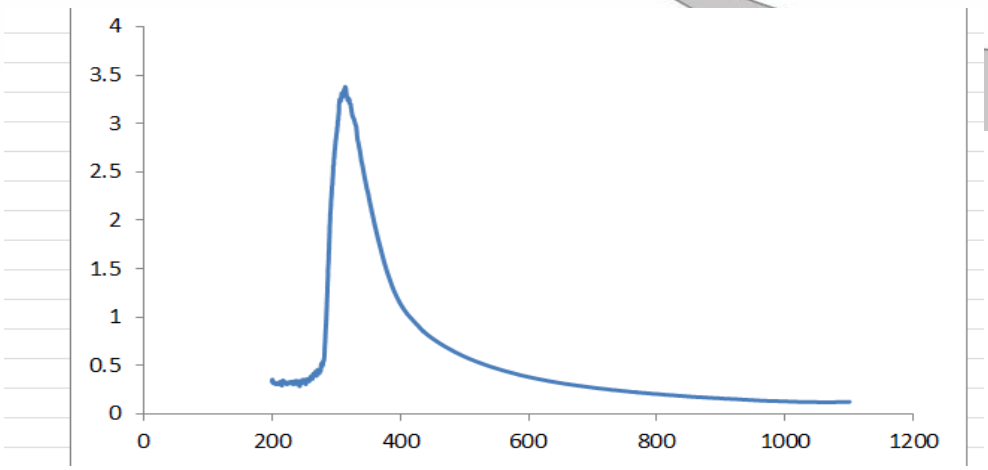


Figure 1. FTIR spectra of (A) *Panax ginseng* root extract and (B) green-synthesized AgNPs.

3.2 UV–Vis analysis

The aqueous extract of P. ginseng root displayed a maximum absorption peak at 314 nm with an absorbance of around 3.38, while the synthesized AgNPs showed a definite absorption peak at 424 nm with an absorbance of about 2.63 (Figure 2).



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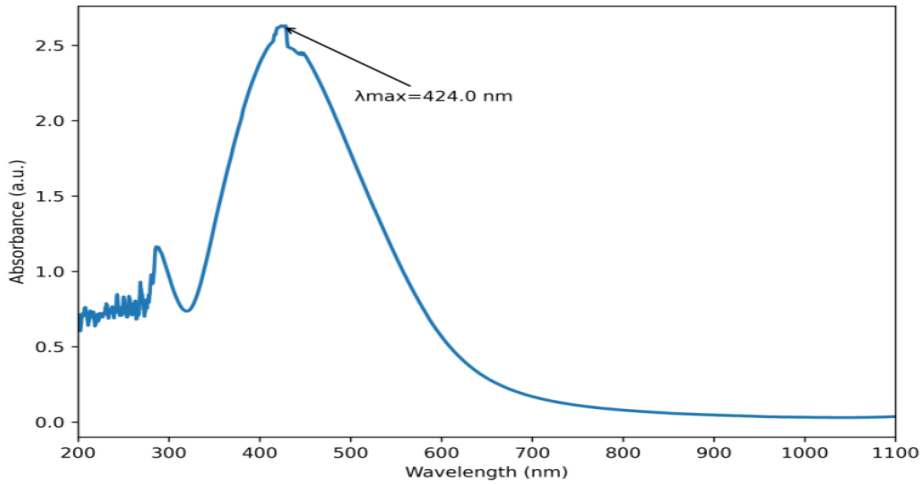


Figure 2. UV–Vis absorption aqueous P. ginseng root extract (λmax = 314 nm) and (B) synthesized AgNPs (λmax = 424 nm).

3.3 Scanning Electron Microscopy (SEM) Analysis

SEM indicated mostly quasi-spherical AgNPs with a very uniform distribution and minor localized aggregation. The particle size was found to be in the range of 31.41–42.90 nm (Figure 3).

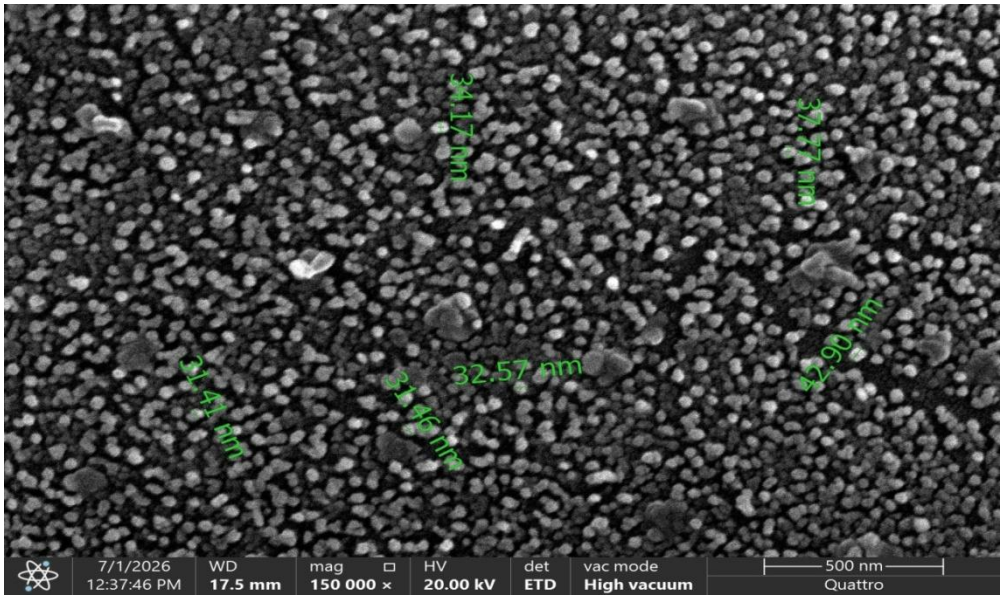


Figure 3. SEM image of synthesized AgNPs showing particle sizes ranging from 31.41 to 42.90 nm.

3.4 X-ray Diffraction (XRD) Analysis

XRD examination revealed a dominant diffraction peak at 2θ = 37.60° along with minor peaks at about 44°, 64°, and 77° confirming the crystalline structure of the synthesized AgNPs (Figure 4).

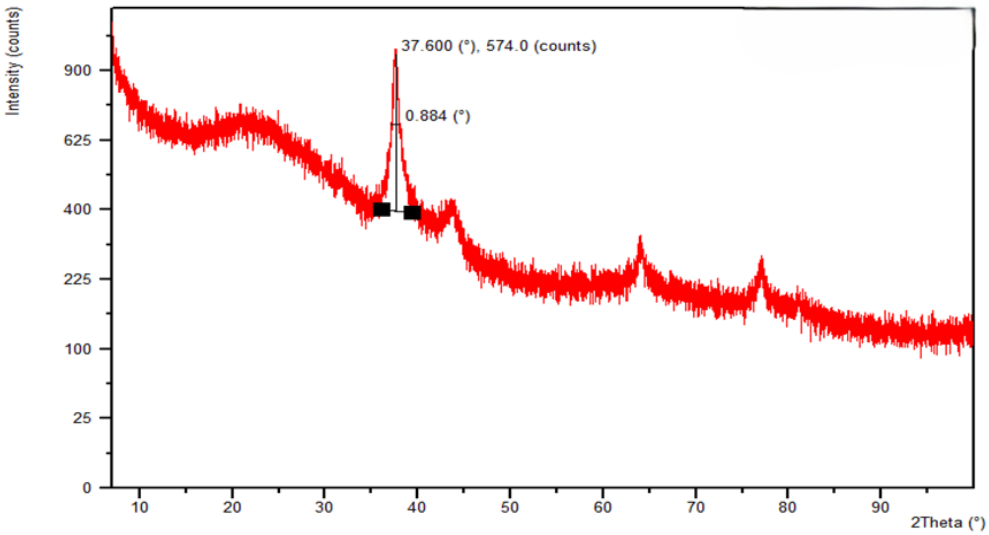


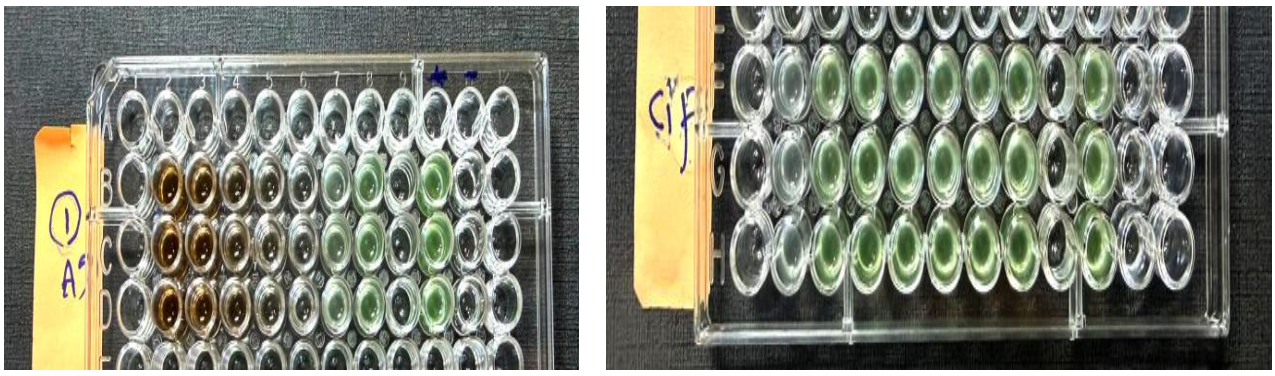
Figure 4. XRD pattern of the synthesized AgNPs showing a prominent diffraction peak at 2θ = 37.60°.

3.5 MIC of Silver Nanoparticles, Povidone–Iodine, and Ciprofloxacin Against *Pseudomonas aeruginosa*

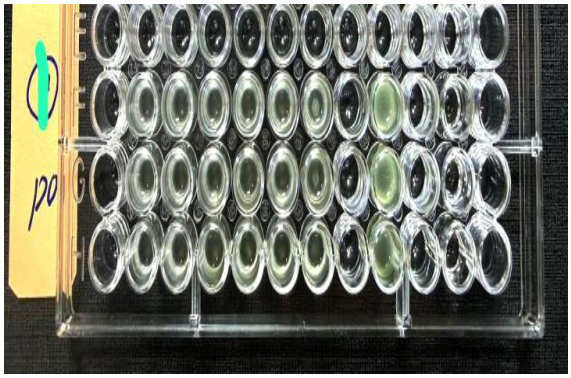
AgNPs showed a MIC of 3.1 µg/mL against all the three P. aeruginosa isolates while ciprofloxacin demonstrated a consistent MIC of 12.5 µg/mL. The PVP-I MICs for isolate 1 were 625 µg/mL and for isolates 2 and 3 were 1250 µg/mL (Table 1; Figure 5).

Table 1.MIC values of silver nanoparticles (AgNPs), ciprofloxacin (CIP), and povidone–iodine (PVP-I) against three clinical *Pseudomonas aeruginosa* isolates

Isolates	POV mic (ug/ml)	cip mic (ug/ml)	ag mic (ug/ml)
1	625	12.5	3.1
2	1250	12.5	3.1
3	1250	12.5	3.1



(A) AgNPs **(B) Ciprofloxacin**



(C) PVP-I (Isolate 1)



(D) PVP-I (Isolate 3)

Figure 5 Representative broth microdilution plates used for MIC determination against *Pseudomonas aeruginosa*: (A) AgNPs, (B) ciprofloxacin, (C) PVP-I, Isolate 1 (MIC = 625 µg/mL), and (D) PVP-I, Isolate 3 (MIC = 1250 µg/mL)

3.6 Effect of AgNPs on PVP-I and Ciprofloxacin MICs

The MIC of PVP-I was lowered from 625–1250 µg/mL to 300 µg/mL in all the three isolates when combined with AgNPs, which was statistically significant ($\chi^2 = 70.1$, $P < 0.001$). However, the ciprofloxacin MIC was not affected (12.5 µg/mL) in conjunction with AgNPs with no significant difference (Table 2;3; Figures 6 and 7).

Table 2. Effect of AgNPs on PVP-I and ciprofloxacin MICs against three clinical P. aeruginosa isolates.

Isolate	*PVP-I*	*PVP-I + AgNPs*	*CIP*	*CIP + AgNPs*
1	*625*	*300*	*12.5*	*12.5*
2	*1250*	*300*	*12.5*	*12.5*
3	*1250*	*300*	*12.5*	*12.5*

Table 3. Effect of silver nanoparticles (AgNPs) on the MIC of povidone–iodine (PVP-I) against *Pseudomonas aeruginosa* isolates.

Isolate	Pov	Pov+ag	Chi	P value
1	625	300	**70.1**	**0.001**
2	1250	300		
3	1250	300		

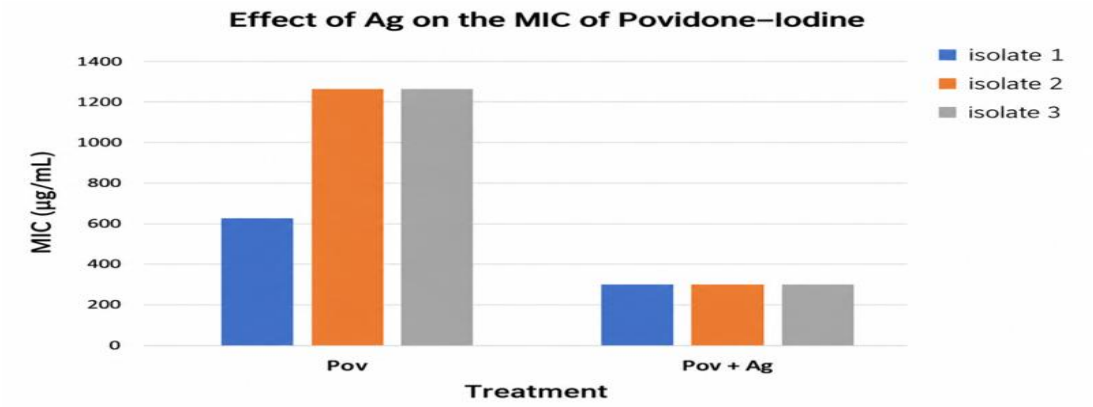


Figure 6. Effect of AgNPs on the MIC of povidone–iodine (PVP-I) against three clinical Pseudomonas aeruginosa isolates.

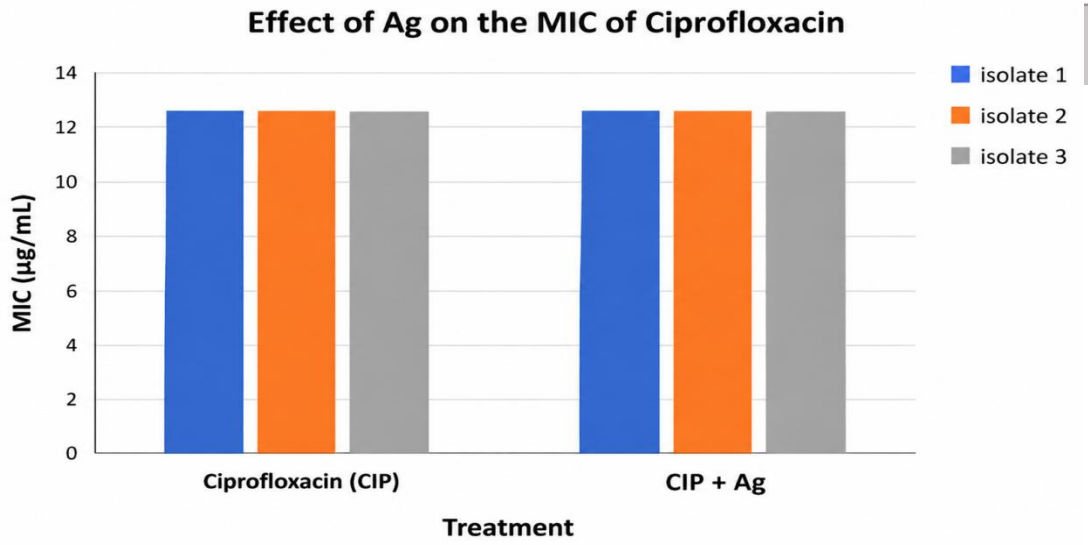


Figure 7. Effect of AgNPs on the MIC of ciprofloxacin (CIP) against three clinical Pseudomonas aeruginosa isolates.

Discussion

The current study showed effective green production of silver nanoparticles (AgNPs) utilizing aqueous Panax ginseng root extract. The FTIR study indicated shifts from 3377.36 to 3437.15 cm-1 and from 1629.85 to 1627.92 cm-1 and modifications in the fingerprint area. These variations confirm the participation of plant derived functional groups in production of AgNPs and their surface interactions. Biomolecules from P. ginseng have been previously involved in the production of AgNPs (14,21,22,31). Differences in the extract content, extraction conditions and interaction between biomolecules and nanoparticles could be related to the changes in FTIR bands. Thus, the observed alterations suggest the participation of functional groups without attributing them to specific phytochemicals.

The UV–Vis examination indicated the greatest absorption at 314 nm for the P. ginseng extract and a unique AgNP peak at 424 nm, which is characteristic of the surface plasmon resonance of silver nanoparticles. Similar SPR absorption as seen here has been observed for AgNPs mediated by P. ginseng and prepared from leaves and roots(14,21,31,32). Differences in the location of the SPR may be due to differences in the particle size, shape, aggregation, surface chemistry and synthesis circumstances.

SEM indicated mostly quasi-spherical AgNPs with a little amount of localized agglomeration and particle sizes between 31.41 and 42.90 nm. Similar spherical nanoparticles have been generated using P. ginseng although their size varies in different synthesis techniques (21–23,31). These discrepancies may be ascribed to plant material, precursor content, extract composition, reaction circumstances and characterization technique analysis showed a prominent diffraction peak at 2θ = 37.60°, accompanied by weaker peaks near 44°, 64°, and 77°. Similar crystalline patterns have been reported for P. ginseng-mediated AgNPs (21,31,33). Since crystallite size was not calculated in the present study, these findings are interpreted only as evidence of crystallinity and not as an estimate of crystallite size.

The synthesised AgNPs showed significant antibacterial activity against all the three ciprofloxacin-resistant with a MIC value of 3.1 µg/mL. The obtained value is within the range 2.65–21.25 µg/mL reported for AgNPs against MDR/XDR P. aeruginosa isolates(16) and is similar to the 1.406–5.625 µg/mL range reported against drug-resistant P. aeruginosa(34). The variation in MIC values may be due to the size of nanoparticles, surface properties, production technique, bacterial strain, inoculum and experimental conditions (11). Membrane disruption, oxidative stress, Ag^{+} release, DNA damage, and interference with protein synthesis and intracellular activities have all been linked to AgNP activity (11,35). However, these pathways were not explicitly evaluated in the current investigation.

The clinical significance of these results is linked to the capacity of P. aeruginosa to combine resistance to antibiotics with features associated with biofilm (7). AgNPs also showed antibiofilm and antivirulence activities against resistant P. aeruginosa, including suppression of biofilm formation, motility, protease activity, and pyocyanin production (16,35–37). However, the AgNPs investigated by (36) were produced using P. aeruginosa pyoverdine, while the nanoparticles produced in the current investigation were produced using P. ginseng

extract. These findings, then, confirm the overall antibiofilm and anti-virulence potential of AgNPs but do not prove that the same effects did occur in the current formulation. Furthermore, the current investigation did not directly assess biofilm formation and virulence variables.

All isolates showed ciprofloxacin MIC of 12.5 µg/mL which was not affected by the combination with 1 µg/mL AgNPs showing no enhancement under the circumstances studied. In contrast, a 3–10-fold decrease in ciprofloxacin MIC was documented when AgNPs were employed at 0.5× MIC (16). Conversely, (38) the report of no action of AgNPs in combination with ciprofloxacin against P. aeruginosa supported the current conclusion. In the current investigation, 1 µg/mL was roughly 0.32× the AgNP MIC of 3.1 µg/mL. The conflicting results may be attributed to the differences in AgNP concentration, nanoparticle properties, experimental methodology and bacterial strains. Therefore, the lack of augmentation at the tested dose does not eliminate a potential interaction at other concentrations.

PVP-I combined with silver nanoparticles (AgNPs) lowered the minimum inhibitory concentrations (MICs) from 625 µg/mL in isolate 1 and 1250 µg/mL in isolates 2 and 3 to around 300 µg/mL for all isolates, which is a reduction of approximately 52% and 76%, respectively. This implies that PVP-I is more effective under the conditions tested. PVP-I has showed clear antimicrobial activity against P. aeruginosa (39), while AgNPs have shown antibacterial and antibiofilm activity against clinical isolates of multidrug- and highly drug-resistant P. aeruginosa (16). In agreement with the present findings, (40) reported increased antibacterial effectiveness of AgNP–PVP-I nanocomplexes against P. aeruginosa, which is in keeping with the increased activity of the AgNP–PVP-I mixture in the present study. However, the differences in the nanoparticle characteristics, formulations, bacterial strains and experimental conditions should be considered for interpretation of these data.

The differential reactions to ciprofloxacin and PVP-I show an antimicrobial-agent-dependent impact. The AgNPs activity and their effects in combinations might be dependent on the nanoparticle characteristics, the concentration, bacterial strain and the companion antimicrobial (11,16). As the PVP-I result in the current work used a fixed dose of AgNPs rather than a full checkerboard/FICI test, it should be termed as increased antibacterial activity or MIC decrease rather than verified synergism. Furthermore, these results need be validated with a larger number of isolates, as only three selected clinical isolates were investigated.

Conclusion*:*

The study demonstrated the successful environmentally friendly generation of AgNPs using Panax ginseng root extract. The AgNPs showed antibacterial activity against ciprofloxacin resistant P. aeruginosa with MIC of 3.1 µg/mL and also increased the efficacy of PVP-I. However, the minimal inhibitory concentration of ciprofloxacin remained same. Therefore, the P. ginseng mediated AgNPs exhibited substantial antibacterial efficacy against resistant P. aeruginosa.

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