https://doi.org/10.17221/39/2024-CJFS

Harnessing nature's secrets: Silver nanoparticles from *Withania coagulans* fruit and root extracts unveil exceptional antioxidant and antimicrobial properties

Farwa Iftikhar[1], Rahmatullah Qureshi[1], Ayesha Siddiqa[1], Khurshid Anwar[1], Fizza Arshad[1], Zia-ur-Rehman Mashwani[1], Aayesha Riaz[2], Safir Ullah Khan[3], Amir Ali[1], Shahzad Iqbal[4], Ajaz Ahmad[5], Melissa Danae Bejarano Gómez[6]*

[1]*Department of Botany, Phir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan*
[2]*Department of Parasitology and Microbiology, Phir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan*
[3]*Department of Zoology, Faculty of Sciences, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan*
[4]*Faculty of Applied Energy System, Jeju National University, Jeju, South Korea*
[5]*Department of Clinical Pharmacy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia*
[6]*National School of Medicine and Homeopathy (ENMyH), National Polytechnic Institute, Mexico City, Mexico*

**Corresponding author: rahmatullahq@uaar.edu.pk, rahmatullahq@yahoo.com*

Citation: Iftikhar F., Qureshi R., Siddiqa A., Anwar K., Arshad F., Mashwani Z.R., Riaz A., Khan S.U., Ali A., Iqbal S., Ahmad A., Bejarano Gómez M.D. (2024): Harnessing nature's secrets: Silver nanoparticles from *Withania coagulans* fruit and root extracts unveil exceptional antioxidant and antimicrobial properties. Czech J. Food Sci., 42: 192–206.

Abstract: Nanotechnology, an emerging field, holds significant promise with applications across diverse sectors, including medicine, agriculture, and the biological sciences. To address environmental concerns, the green biosynthesis of silver nanoparticles (AgNPs) using plant extracts is favoured. This study focuses on the formulation and characterisation of AgNPs using extracts from *Withania coagulans* (Stocks) Dunal, a medicinal plant that holds a unique phytochemical profile. The AgNPs derived from *W. coagulans* root (WcAgNPR) and fruit (WcAgNPF) extracts were characterised using ultraviolet and visible light (UV-Vis) spectral analysis, scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), and Fourier-transform infrared spectroscopy (FTIR) spectroscopy. The findings reveal that both WcAgNPR and WcAgNPF exhibit substantial antioxidant potential, with robust iron reducing capabilities and potent 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. Additionally, they demonstrate strong hydrogen peroxide scavenging abilities. Notably, WcAgNPR outperforms WcAgNPF in the phosphomolybdate assay for antioxidant potential. Both AgNPs display remarkable antimicrobial efficacy, with minimal inhibitory concentrations (*MIC*) below 10 μg·mL^{-1} against gram-positive bacteria (*Staphylococcus aureus*) and noteworthy activity against gram-negative *Escherichia coli* (WcAgNPF with a *MIC* of 30 μg·mL^{-1} and WcAgNPR with a *MIC* of 60 μg·mL^{-1}). These findings highlight the silver nanoparticles' significant antioxidant and antimicrobial potential, suggesting their potential for *in vivo* use as antimicrobial agents with minimal oxidative damage.

Keywords: *W. coagulans*; nanotechnology; antibacterial; antioxidative

Supported by the Researchers Supporting Project Number (RSP2024R350), King Saud University, Riyadh, Saudi Arabia.

© The authors. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0).

https://doi.org/10.17221/39/2024-CJFS

Nanotechnology has been explored in various fields of research, including medicine and agriculture (Baker et al. 2017; McNamara and Tofail 2017). This technique has been employed in various medical applications ranging from biomedical imaging (for diagnosis and biochemical studies) to drug delivery for disease cure and prevention in living systems with much specificity and efficacy (Han et al. 2019; Manzano and Vallet-Regí 2020). Nanoparticles have been used recently in various research fields by the scientific community due to their nanosize ranging from 1 to 100 nm (Khan et al. 2019; Alharbi et al. 2022). Among all metallic nanoparticles, silver nanoparticles (AgNPs) possess unique properties and are commonly exploited in experimental researches (Rai et al. 2009). The current state of research on the green synthesis of AgNPs involves progresses in environmentally friendly synthesis methods and their expanding applications diverse medical fields (Rambaran and Schirhagl 2022). Silver nanoparticles have been studied for several therapeutic purposes as they are less toxic and have increased biodegradability and bioavailability. There are several methods for the synthesis of AgNPs. However, due to phytochemicals' natural antimicrobial and antioxidant potential, nanoparticles synthesised using plant extracts are reported to have more enhanced antimicrobial (Anwar et al. 2019) and antioxidant properties (Ahn et al. 2019).

Withania coagulans (Stocks) Dunal is a well-known medicinal plant of the family *Solanaceae* mainly found in South Asia (Khodaei et al. 2012). Whole plants and their parts, especially fruits, are used for medicinal purposes. The plant is most popular for its antidiabetic potential. Still, it has many other important benefits of medicinal importance as it has anti-hypercholesterolemic, neuroprotective, anti-inflammatory, antitumor and anticancer, cytotoxic, antioxidant, antimicrobial and renal and hepatoprotective effects (Khan et al. 2021). Phytochemical profile *W. coagulans* has withanolides- steroidal lactones as found in *W. sominifera*, making it highly valuable for pharmacognostic benefits (Khan et al. 2021; Gaurav et al. 2023). Withanolides is a group of steroidal lactones with a γ-lactone containing a basic skeleton of ergostane type compound (Zhang et al. 2012). Ergostane ($C_{28}H_{50}$) is a 4-ringed triterpene, and withanolides are its polyoxygenated γ-lactone containing derivatives (Mirjalili et al. 2009). Hence, plants like *W. coagulans* and *W. sominifera* have enzymes that oxidise steroids (like ergostane) in such a way that a γ-lactone ring forms as a result of interactions between oxidised carbons at position 22 and 26 (Mirjalili et al. 2009). Thus, the plant has a very rich phytochemical profile, having many different kinds of withanolides, a class of steroidal lactones, glucosides, fatty acids, etc. (Mirjalili et al. 2009). Previously, methanolic fruit extracts of *W. coagulans* have been reported for antibacterial activity against various bacterial strains (Peerzade et al. 2018). Similarly, AgNPs from *W. coagulans* leaf extract have been reported to have medicinal properties (Tripathi et al. 2019). This plant is extensively used in various diseases by employing fruits and roots. Thus, the present study was carried out to evaluate the antioxidant and antimicrobial activities of root and fruit extract of *W. coagulans*.

MATERIAL AND METHODS

Sample preparation and green synthesis of silver nanoparticles. Aqueous extracts of *W. coagulans* root and fruit were prepared by soaking shade-dried roots and fruits in distilled water after 12 h. The infused solutions were heated at 70–90 °C for 25 min. The extracts [5 g·(100 mL)$^{-1}$] were twice filtered using filter paper without any additional solvents. *W. coagulans* root and fruit extracts were utilised for AgNPs synthesis, adapting Tripathi et al. (2019) by mixing 1 mM silver nitrate ($AgNO_3$) with extracts at a 1:5 ratio, determined optimal for uniform AgNPs size, evident from surface plasmon resonance (SPR) shifts in ultraviolet and visible light (UV-Vis) spectral analysis (Tripathi et al. 2019) (Table 1 and Figure 1). In addition, the *W. coagulans* AgNP fruit (WcAgNPF) were washed with diluted ethanol 3 times more than *W. coagulans* AgNP root (WcAgNPR) due to their

Table 1. Experimental layout of the study

Treatment groups	Treatments
Antioxidant analysis	
T_0	ascorbic acid
T_1	WcAgNPR
T_2	WcAgNPF
Antimicrobial (a-antibacterial) analysis	
T_0	streptomycin
T_1	WcAgNPR
T_2	WcAgNPF

T_0 – standard group; T_1 – test group 1; T_2 – test group 2; WcAgNPR – *Withania coagulans* silver nanoparticle root; WcAgNPF – *Withania coagulans* silver nanoparticle fruit

https://doi.org/10.17221/39/2024-CJFS

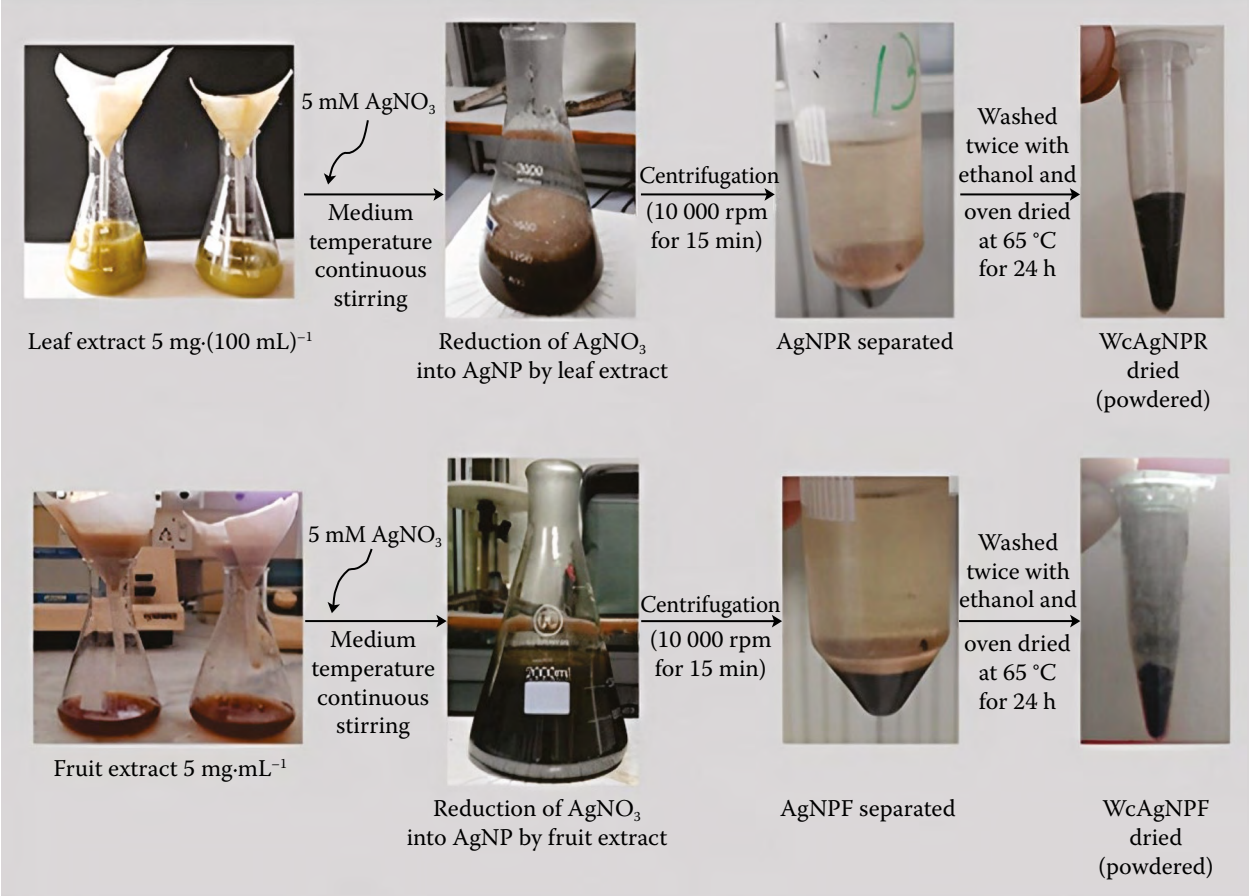

Figure 1. Different steps of the synthesis of WcAgNPR and WcAgNPF

WcAgNPR – *Withania coagulans* silver nanoparticle root; WcAgNPF – *Withania coagulans* silver nanoparticle fruit; $AgNO_3$ – silver nitrate; AgNP – silver nanoparticles

sticky consistency and coagulance, and later dried to fine powder at 65 °C for 24 hours.

Characterisation of silver nanoparticles. The synthesised AgNPs' characteristics were assessed through UV-Vis spectral analysis, Fourier-transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and energy dispersive X-ray (EDX) spectroscopy, focusing on uniformity, functional groups, and morphology.

Antioxidant analysis. The antioxidant potential of green-synthesised AgNPs (WcAgNPR and WcAgNPF) was evaluated in triplicate against ascorbic acid, determining the half maximal inhibitory concentration (IC_{50}) values across several assays.

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity. The antioxidative capacity was measured by DPPH radical colour change in methanolic solutions, with extracts tested at 10–200 μg·mL^{-1} as per the previous study with slight modifications (Khoshnamvand et al. 2019).

Hydrogen peroxide scavenging assay. Adapted from Rajoka et al. (2020), extracts and ascorbic acid (10–200 μg·mL^{-1}) were incubated with H_2O_2, assessing inhibition at 230 nm (Rajoka et al. 2020).

Ferric reducing power assay. Utilising Keshari et al. (2020)'s method, the reducing power of samples (10–200 μg·mL^{-1}) was determined post-incubation with potassium ferrocyanate and trichloroacetic acid (TCA), measuring optical density (*OD*) at 700 nm (Keshari et al. 2020).

Phosphomolybdate antioxidant activity. Following Moonmun et al. (2017) with slight modifications, samples (10–200 μg·mL^{-1}) were assessed in phosphomolybdate reagent, incubated at 95 °C in the dark, measuring *OD* at 765 nm (Moonmun et al. 2017).

Antimicrobial activity analysis. Disc diffusion and reference methods (Shahid et al. 2013; Peerzade et al. 2018) assessed WcAgNPs against *E. coli* and *S. aureus*, with streptomycin as a control (Hasan et al. 2020).

https://doi.org/10.17221/39/2024-CJFS

RESULTS

Characterisation of silver nanoparticles

UV visible spectroscopy. The synthesis of AgNPs was confirmed by recording the UV visible spectrum spectrophotometer (BMS UV-1602; Shimadzu, Japan) located in Fatima Jinnah Women University (FJWU), Rawalpindi, Pakistan. The surface plasmon resonance (SPR) peak of WcAgNPR and WcAgNPF was observed at 460 nm (Figure 2A) and 425 nm, respectively (Figure 2B).

Fourier transform infrared spectroscopy. FTIR analysis was performed using FTIR-5480 (Schimadzu, Japan). FTIR analysis of WcAgNPF showed strong peaks at 2 924.18, 2 854.74, 1 743.71, 1 635.69, 1 460.16, 1 375.29, 1 315.50, 1 261.49, 1 163.11, 1 097.53, 800.49, 719.47, and 468.72 cm^{-1} which indicated sp3 (one s orbital and three p orbitals hybridisation) C-H stretching, O-H bond stretching, C=O, C-C, C-N, C-O, C-Cl, and M-O bond stretching. Peaks at the wavelength of 719.47, 1 163.11, 1 460.16, 2 854.74, 2 924.18 cm^{-1} indicated presence of monosubstituted aromatic rings, tertiary alcohols, aromatic compounds, aldehydes, alkanes (Figure 3A) and are previously reported (Peerzade et al. 2018) from methanolic fruit extract of *W. coagulans*. FTIR analysis of WcAgNPR showed strong peaks at 3 354.32, 2 924.18, 2 850.88, 1 722.49, 1 639.55, 1 558.54, 1 537.32, 1 384.94, 1 261.49, 1 095.60, 1 026.16,

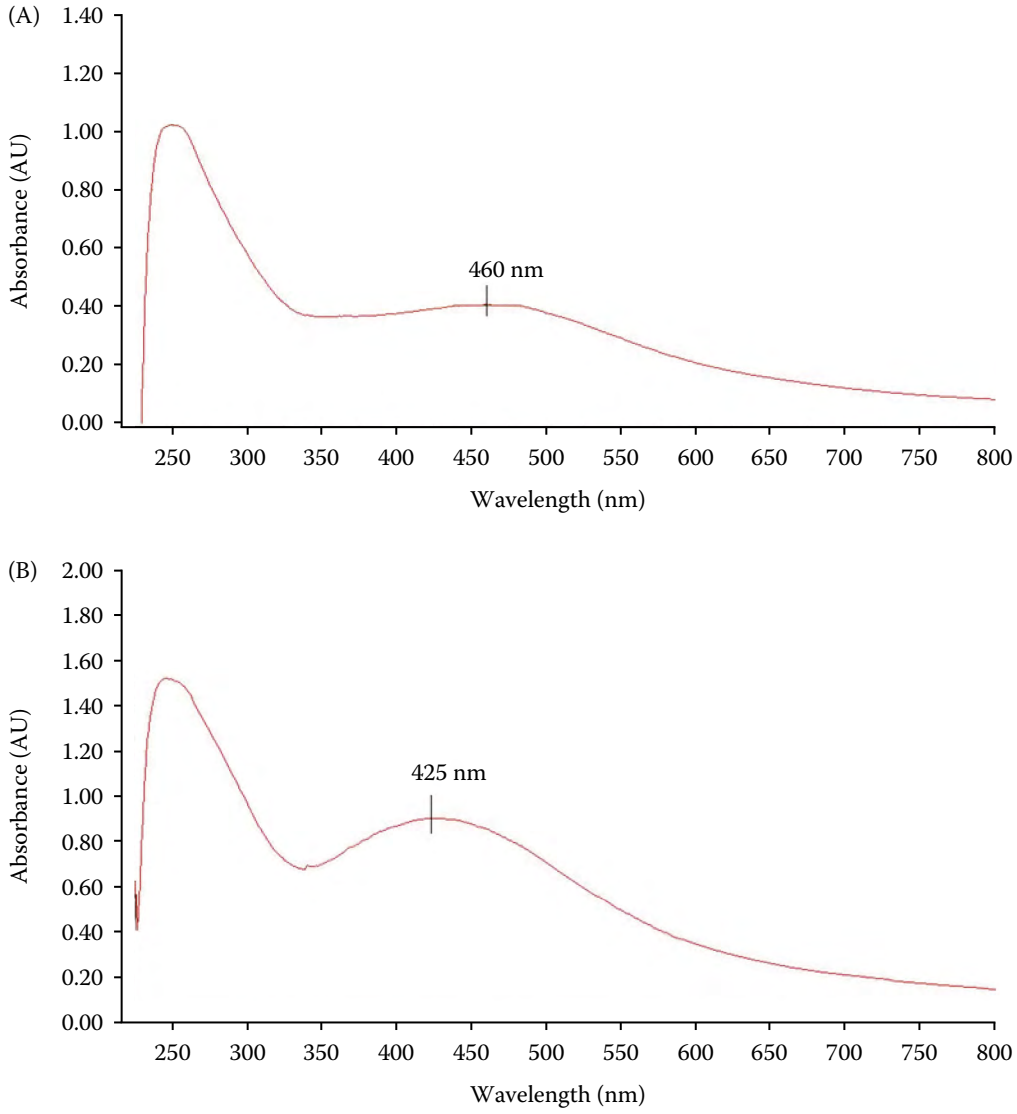

Figure 2. UV spectrum of green synthesised silver nanoparticles (A) WcAgNPR and (B) WcAgNPF

WcAgNPR – *Withania coagulans* silver nanoparticle root; WcAgNPF – *Withania coagulans* silver nanoparticle fruit; AU – absorbance unit

https://doi.org/10.17221/39/2024-CJFS

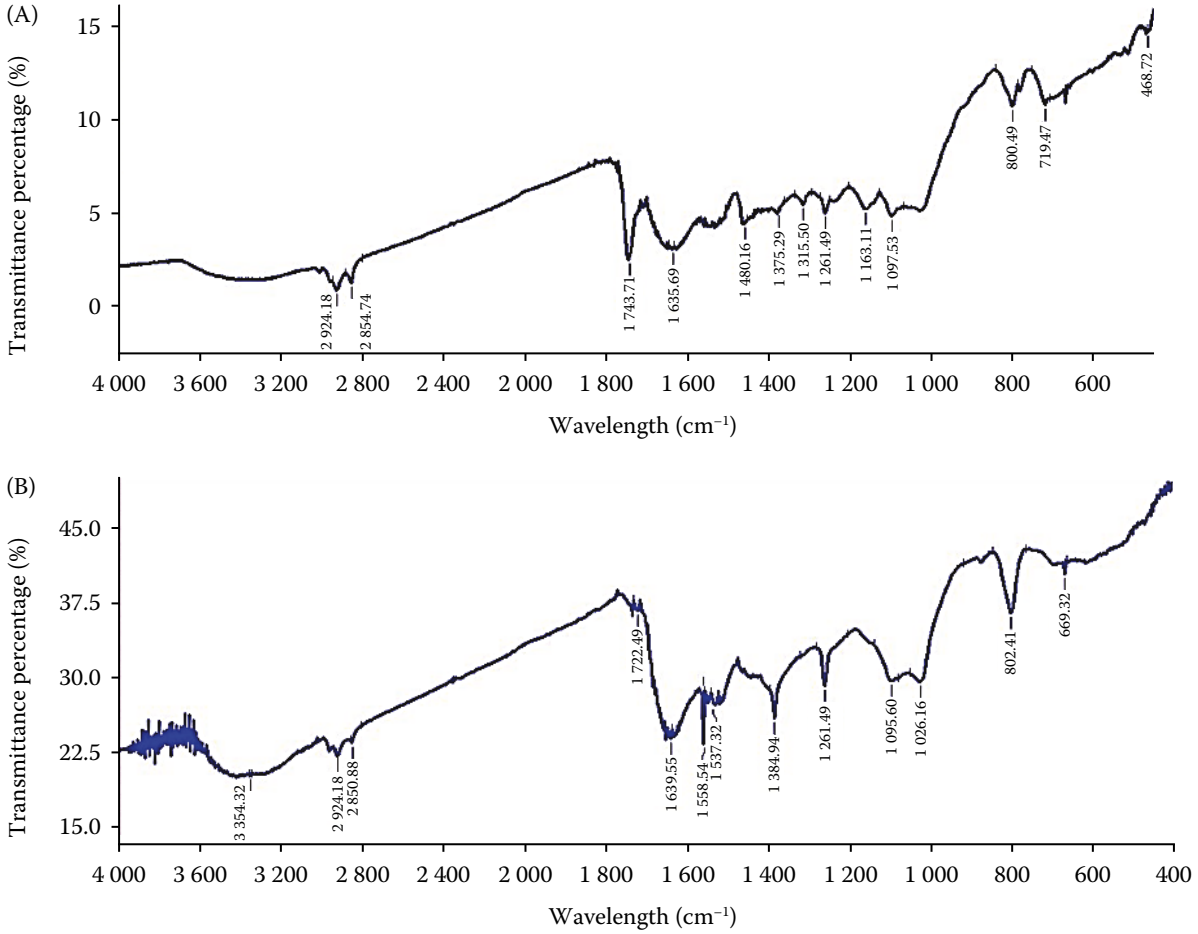

Figure 3. Fourier-transform infrared spectroscopy of green synthesised silver nanoparticles (A) WcAgNPF and (B) WcAgNPR

WcAgNPR – *Withania coagulans* silver nanoparticle root; WcAgNPF – *Withania coagulans* silver nanoparticle fruit

802.41, and 669.32 cm^{-1} which indicated O-H bond, C-H, C=O stretching, N-H bending, N-O asymmetric stretching, N-H wag, C-N and C-X stretching (Figure 3B).

Energy dispersion X-ray. For elemental analysis, silver nanoparticles were observed by EDX analysis using SEM with EDX Model JSM-5910 (JEOL, Japan) in the University of Peshawar (UOP). The highest peaks of silver were at 2.8 to 3.4 for WcAgNPR and WcAgNPF (Figure 4A and B).

X-ray diffraction. XRD analysis (Model JDX-3532; JEOL, Japan) showed WcAgNPR and WcAgNPF possess a cubic crystalline structure, aligning with the sodium chloride (NaCl) halite reference (JCPDS card No. 00-005-0628). Peaks at 32.07 (200) and 45.86 (220) for WcAgNPF and additional indices for WcAgNPR, including 27.57 (111) (Miller indices in the parentheses), indicated a face-centred cubic silver crystal structure. Both samples exhibited an unidentified peak around 38°, suggesting a characteristic feature of the nanoparticles (Figure 4C and D), similar to previous findings (Jemal et al. 2017). The same peak at 38° was observed in cobalt oxide nanoparticles (Co_3O_4NPs) synthesised using methanolic, hexane and methanolic + hexane extracts of *W. coagulans* (Hasan et al. 2020). Since the shape and crystalline nature of all three Co_3O_4NPs differed, the 38° peak could result from the capping agent covering the nanoparticle surface, thus equally fabricating the AgNPs synthesised by *W. coagulans*.

Scanning electron microscopy. SEM analysis by using SEM with EDX revealed spherical nanoparticles with an average diameter of ~ 40 nm for both WcAgNPR and WcAgNPF, though their size distributions varied. WcAgNPF particles ranged from 16 to 63 nm, mostly around 31 and 47 nm (Figure 5A). WcAgNPR showed a narrower size range of 25 to 60 nm, predominantly around 39 nm (Figure 5B), displaying a more uniform and numerous appearance compared to WcAgNPF.

https://doi.org/10.17221/39/2024-CJFS

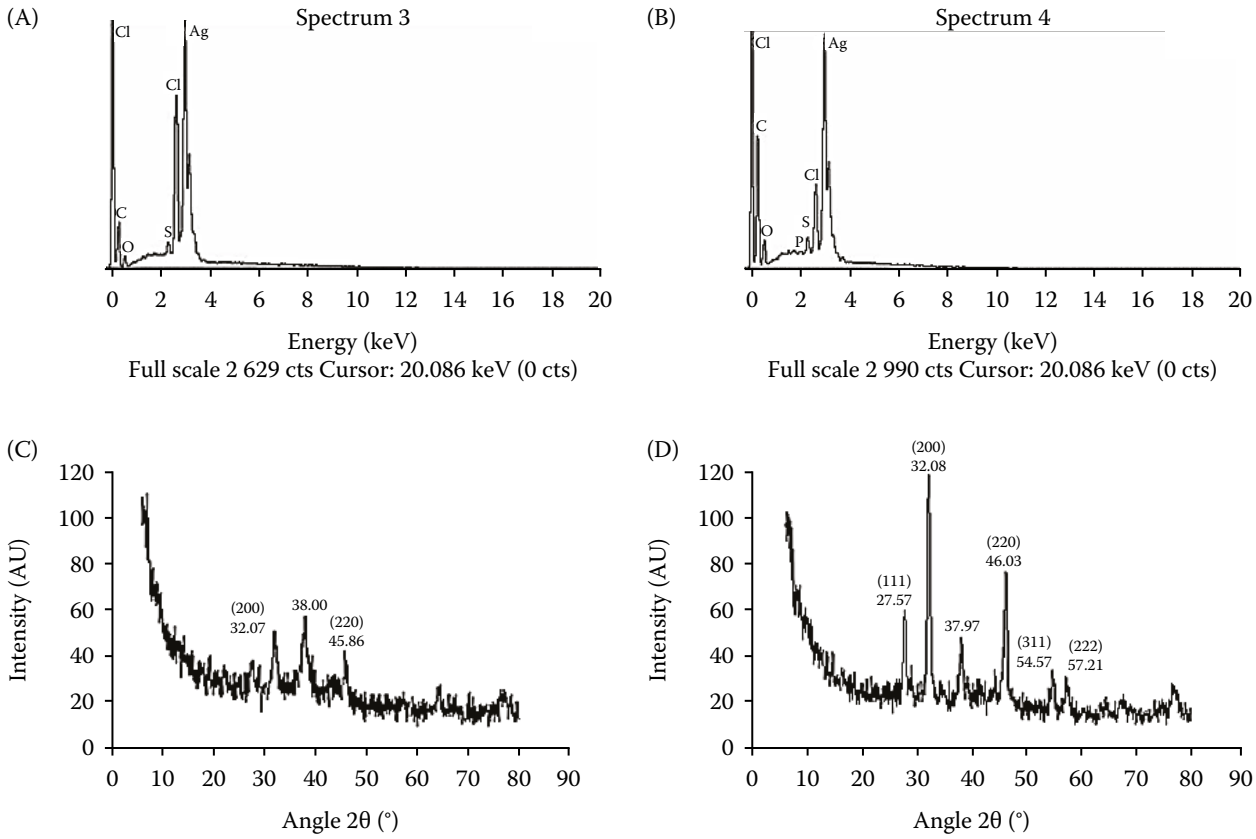

Figure 4. Energy dispersive X-ray of green synthesised silver nanoparticles: (A) WcAgNPR, (B) WcAgNPF, (C) XRD graph of WcAgNPR, and (D) XRD graph of WcAgNPF

WcAgNPR – *Withania coagulans* silver nanoparticle root; WcAgNPF – *Withania coagulans* silver nanoparticle fruit; XRD – X-ray diffraction; 2θ – angle between transmitted beam and reflected beam; AU – absorbance unit

Antioxidant activity analysis

DPPH free radical scavenging activity analysis. In the DPPH scavenging activity assay, WcAgNPR and WcAgNPF were tested in triplicates across 10 to 200 μg·mL^{-1} concentrations *vs.* ascorbic acid. At 200 μg·mL^{-1}, WcAgNPR and WcAgNPF exhibited

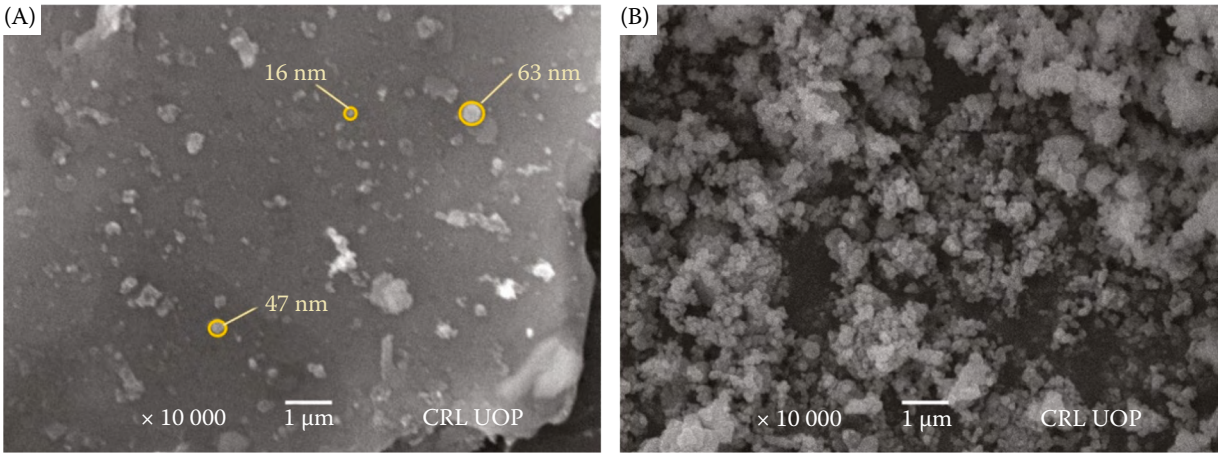

Figure 5. Scanning electron micrograph of silver nanoparticles: (A) WcAgNPF and (B) WcAgNPR

WcAgNPF – *Withania coagulans* silver nanoparticle fruit; WcAgNPR – *Withania coagulans* silver nanoparticle root; CRL UOP – Centralized Resource Laboratory University of Peshawar

https://doi.org/10.17221/39/2024-CJFS

inhibitions of 43.3 ± 0.02% and 42.5 ± 0.01%, respectively, with ascorbic acid at a significant 92.1 ± 0.01% (Figures 6A and B), highlighting ascorbic acid's superior antioxidant capacity. Despite WcAgNPR marginally outperforming WcAgNPF at this concentration, both showed notable scavenging potential. For concentrations of 150 and 100 µg·mL^{-1}, inhibitions were 37.8 ± 0.02% and 39.5 ± 0.01% for WcAgNPR, and 37.8 ± 0.01% and 38.3 ± 0.01% for WcAgNPF, respectively, showcasing consistent performance across these concentrations. Ascorbic acid demonstrated pronounced antioxidant activity, with inhibitions of 91.3 ± 0.01% and 70.6 ± 0.02% at these concentrations. At 60 µg·mL^{-1}, inhibition was slightly lower with WcAgNPR at 37.3 ± 0.01% and WcAgNPF at 36.3 ± 0.02%, while ascorbic acid maintained a higher inhibition rate of 68.9 ± 0.02%. The IC_{50} values, indicating the concentration required for 50%

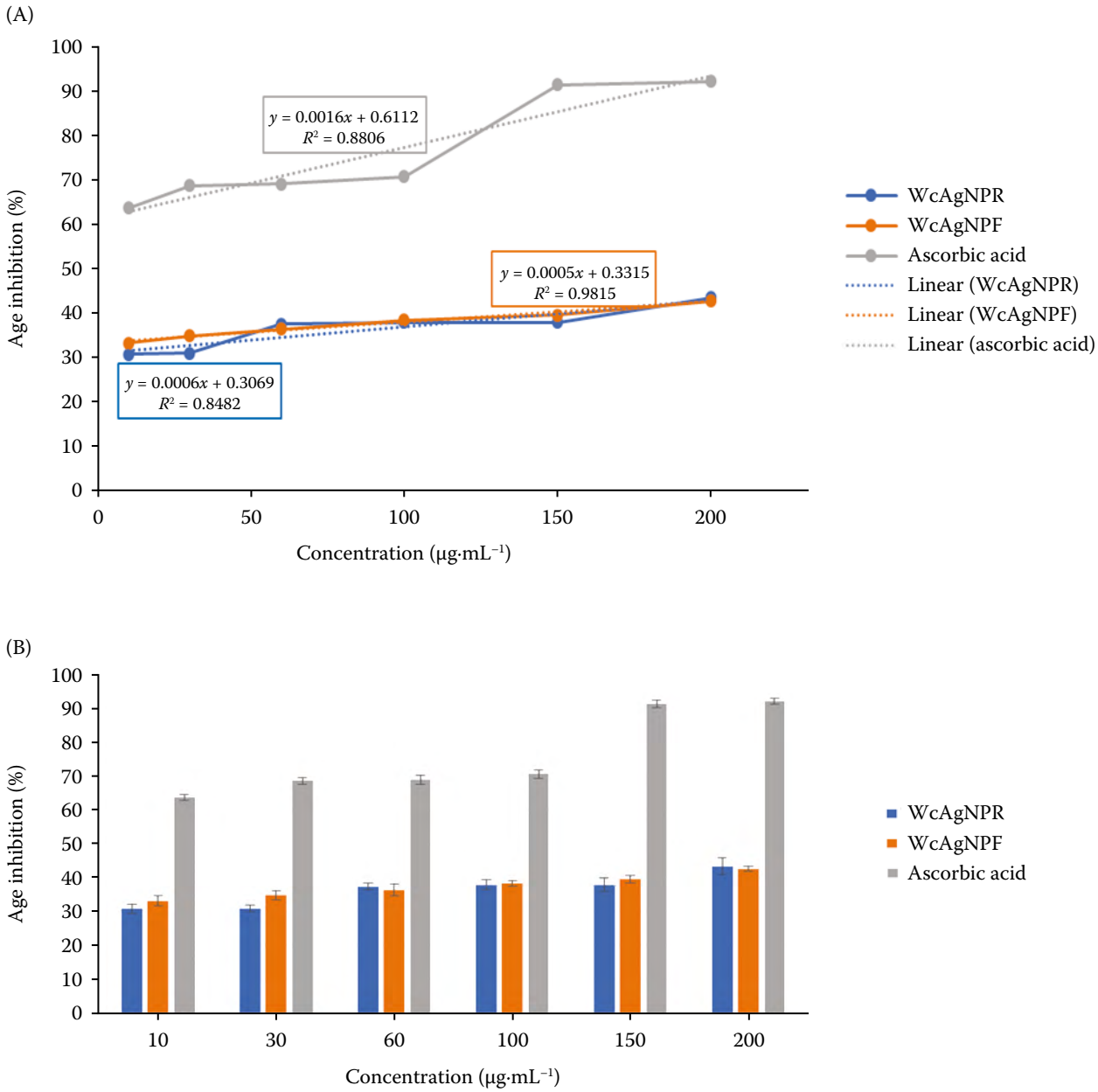

Figure 6. (A) Calibration curve of silver nanoparticles and ascorbic acid for 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity analysis, and (B) DPPH free radical scavenging activity of silver nanoparticles and ascorbic acid at different concentrations

WcAgNPF – *Withania coagulans* silver nanoparticle fruit; WcAgNPR – *Withania coagulans* silver nanoparticle root

https://doi.org/10.17221/39/2024-CJFS

inhibition, were determined to be 321 μg·mL^{-1} for WcAgNPR and 337 μg·mL^{-1} for WcAgNPF (Figures 6A and B), reflecting their scavenging effectiveness relative to ascorbic acid.

Hydrogen peroxide scavenging activity analysis. Antioxidant capacities of WcAgNPR and WcAgNPF, across concentrations from 10 to 1 000 μg·mL^{-1}, were compared with ascorbic acid. At 1 000 μg·mL^{-1}, scavenging rates were 58.1 ± 0.01% for WcAgNPR and 57.3 ± 0.01% for WcAgNPF, against 97.9 ± 0.01% for ascorbic acid. At 200 μg·mL^{-1}, WcAgNPR's inhibition (38.6 ± 0.01%) slightly surpassed WcAgNPF's (35.8 ± 0.01%), while ascorbic acid achieved 83.8 ± 0.01%. Concentrations of 150 μg·mL^{-1} and 100 μg·mL^{-1} saw WcAgNPR inhibitions at 36.8 ± 0.02% and 28.9 ± 0.01%, and WcAgNPF at 31.9 ± 0.02% and 25.0 ± 0.02%, respectively, with ascorbic acid presenting 78.8 ± 0.01% inhibition at 100 μg·mL^{-1}. Lower concentrations (10, 30, 60 μg·mL^{-1}) showed gradual increases in scavenging from 14.1 ± 0.01% to 25.3 ± 0.02% for WcAgNPR, and 13.4 ± 0.01% to 21.9 ± 0.01% for WcAgNPF, compared to ascorbic acid's 60.3 ± 0.01% to 72.4 ± 0.01%. The IC_{50} values were 754 μg·mL^{-1} for WcAgNPR and 764.15 μg·mL^{-1} for WcAgNPF (Figures 7A and B).

Ferric reducing antioxidant power analysis. Ferric-reducing antioxidant power assays for WcAgNPR and WcAgNPF were conducted in triplicates across 10 to 200 μg·mL^{-1} concentration, compared against

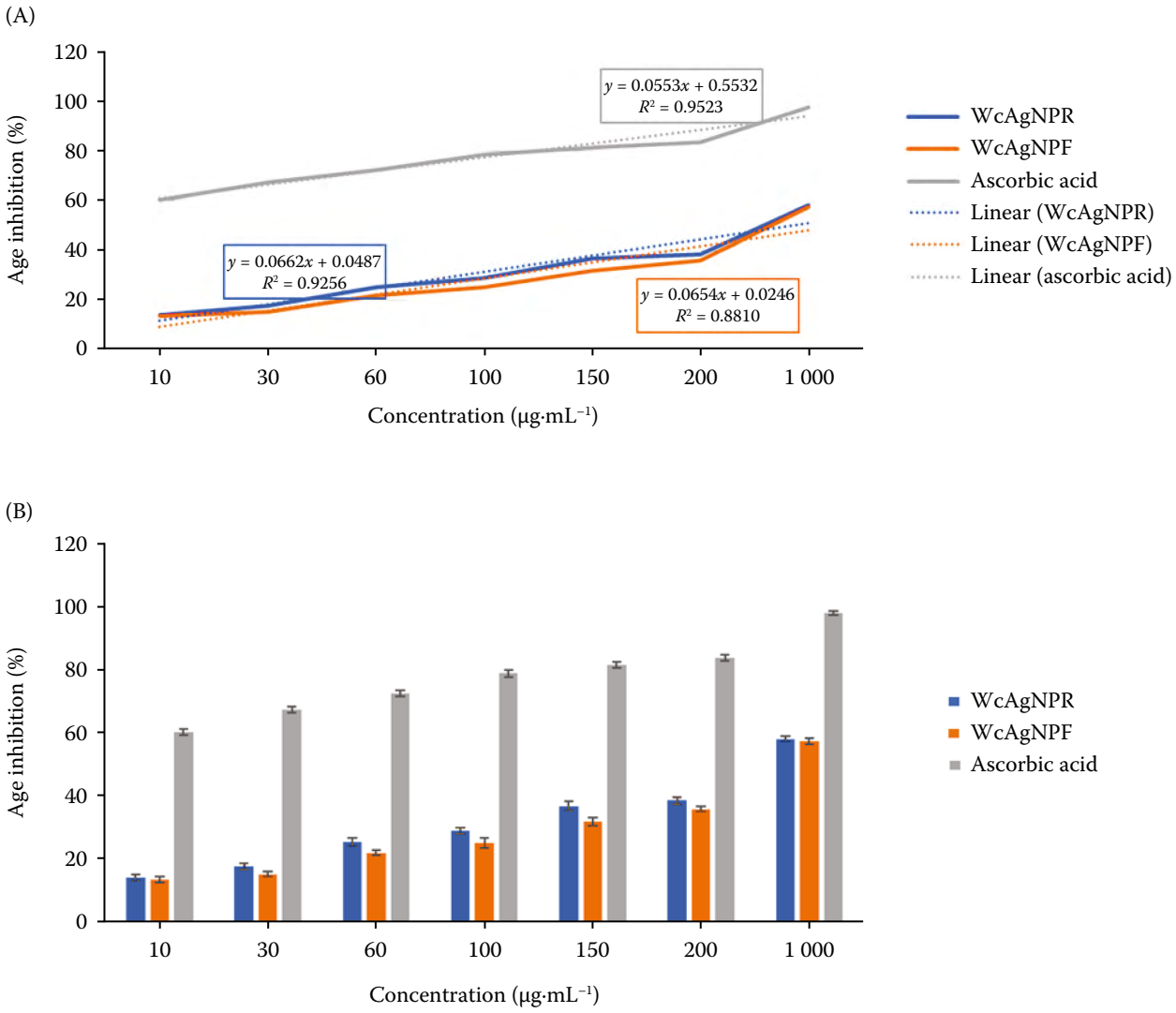

Figure 7. (A) Calibration curve of silver nanoparticles and ascorbic acid for hydrogen peroxide (H_2O_2) antioxidant activity analysis, and (B) hydrogen peroxide scavenging activity of silver nanoparticles and ascorbic acid at different concentrations

WcAgNPF – *Withania coagulans* silver nanoparticle fruit; WcAgNPR – *Withania coagulans* silver nanoparticle root

https://doi.org/10.17221/39/2024-CJFS

ascorbic acid. At 200 μg·mL^{-1}, inhibition rates were 71.5 ± 0.02% for WcAgNPR and 83.5 ± 0.01% for WcAgNPF, with ascorbic acid at 91.5 ± 0.01%. For 150 μg·mL^{-1} and 100 μg·mL^{-1}, WcAgNPR showed 70.5 ± 0.01% and 67.0 ± 0.02% inhibition, WcAgNPF had 80.1 ± 0.01% and 77.7 ± 0.01%, against ascorbic acid's 88.7 ± 0.02% and 78.5 ± 0.01%. At 60 μg·mL^{-1}, WcAgNPF and WcAgNPR exhibited 73.6 ± 0.01% and 64.2 ± 0.01% inhibition respectively (Figures 8A and B). At 30 μg·mL^{-1}, WcAgNPR's 56.9 ± 0.01% inhibition outperformed WcAgNPF's 49.9 ± 0.01%, with ascorbic acid at 67.1 ± 0.01%. Lastly, at 10 μg·mL^{-1}, WcAgNPR significantly inhibited more at 55.3 ± 0.01% compared to WcAgNPF's 27.6 ± 0.01%, and ascorbic acid showed 60.5 ± 0.01% inhibition (Figures 8A and B).

Phosphomolybdate antioxidant activity analysis. Phosphomolybdate antioxidant activity was assessed for WcAgNPR and WcAgNPF across 10 to 200 μg·mL^{-1} methanolic concentrations, juxtaposed with ascorbic acid. At 200 μg·mL^{-1}, WcAgNPR's inhibition (41.6 ± 0.01) surpassed WcAgNPF's (28.4 ± 0.01), against ascorbic acid's 70.6 ± 0.01%. For 150 μg·mL^{-1}, inhibitions were 17.4 ± 0.01 for WcAgNPR and 21.6 ± 0.01 for WcAgNPF (Figures 9A and B). At 100 μg·mL^{-1}, WcAgNPR and WcAgNPF exhibited 16.0 ± 0.02 and 19.2 ± 0.01% inhibition, respectively, with ascorbic acid at 67.7 ± 0.01%. At 60 μg·mL^{-1}, WcAgNPF had a 16.9 ± 0.01% inhibition, higher than WcAgNPR's 12.7 ± 0.01%. Lower concentrations showed WcAgNPR at 7.9 ± 0.01 (30 μg·mL^{-1}) and 6.1 ± 0.01 (10 μg·mL^{-1}),

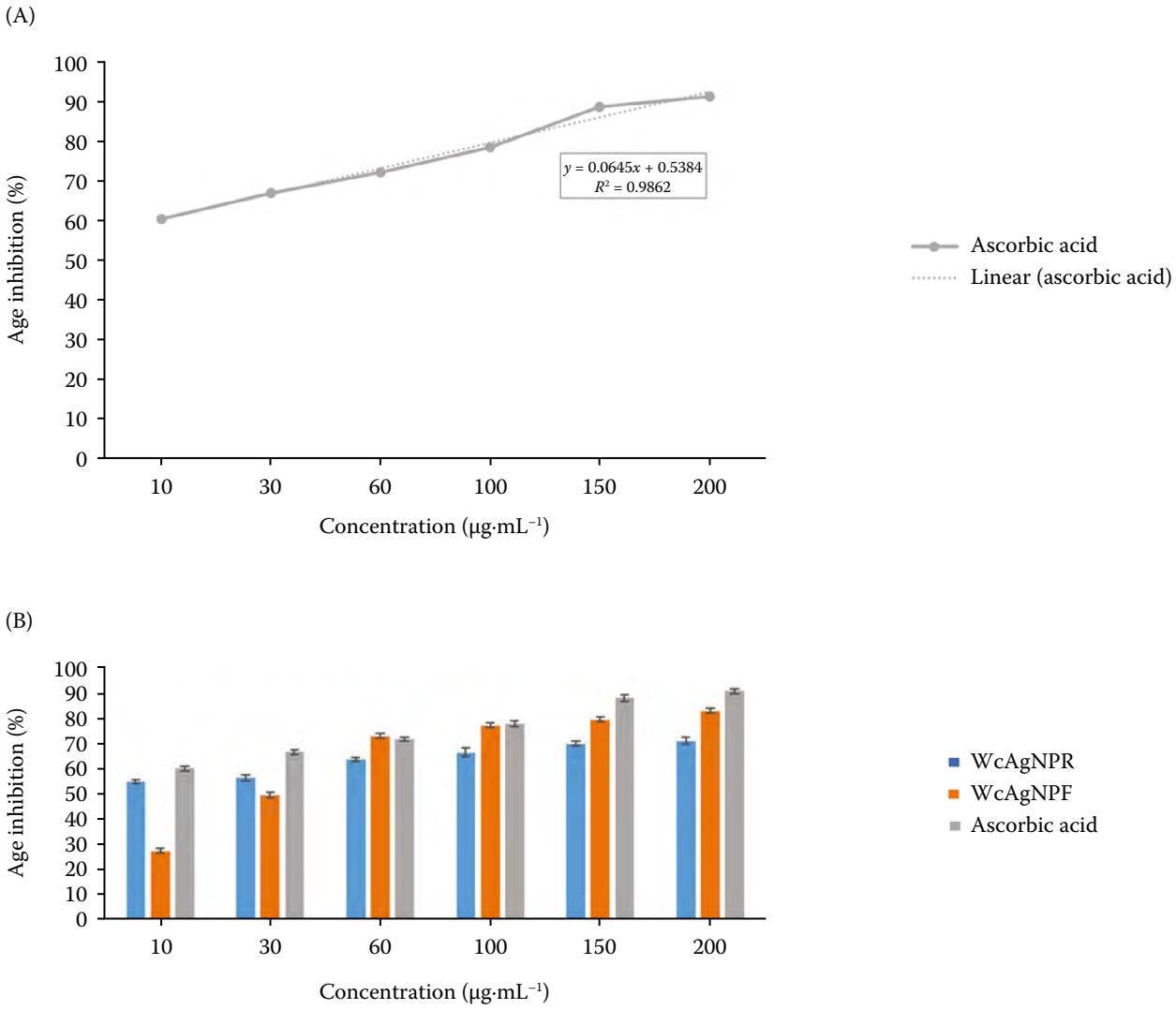

Figure 8. (A) Calibration curve of ascorbic acid for ferric reducing antioxidant power assay, and (B) ferric reducing power activity of silver nanoparticles and ascorbic acid at different concentrations

WcAgNPF – *Withania coagulans* silver nanoparticle fruit; WcAgNPR – *Withania coagulans* silver nanoparticle root

https://doi.org/10.17221/39/2024-CJFS

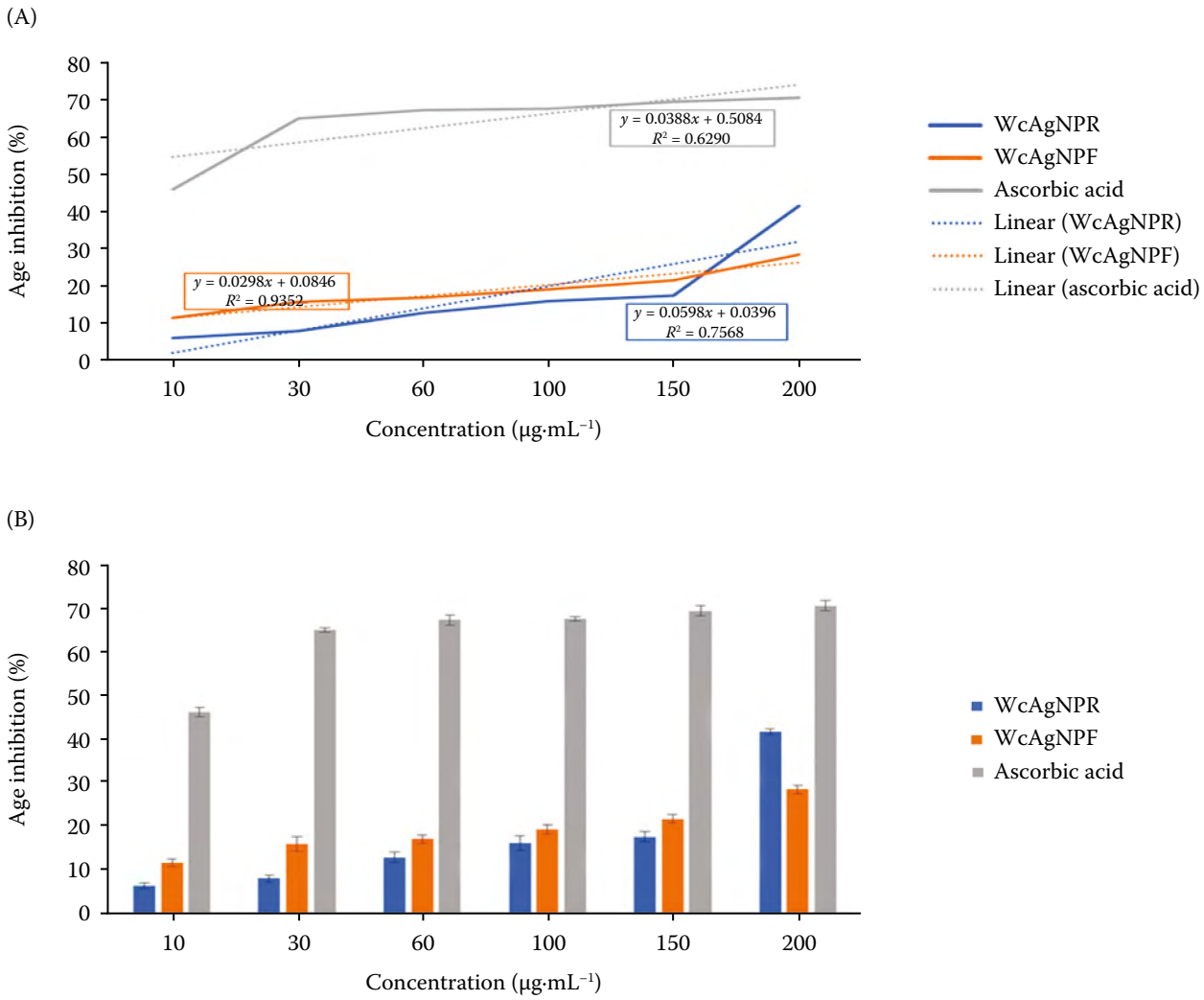

Figure 9. (A) Calibration curve of silver nanoparticles and ascorbic acid for phosphomolybdate antioxidant activity analysis, and (B) phosphomolybdate antioxidant activity of silver nanoparticles and ascorbic acid at different concentrations

WcAgNPF – *Withania coagulans* silver nanoparticle fruit; WcAgNPR – *Withania coagulans* silver nanoparticle root

while WcAgNPF demonstrated 11.5 ± 0.01 and 15.8 ± 0.02% at 10 and 30 µg·mL^{-1}. The IC_{50} values revealed WcAgNPR (836.7 µg·mL^{-1}) as more potent than WcAgNPF (1 675.01 µg·mL^{-1}) (Figures 9A and B).

Antimicrobial activity analysis

This experiment was undertaken by using the Disk diffusion method (also known as the Kirby-Bauer test), and bacterial strains of *E. coli* and *S. aureus* were cultured and tested by using agar plates (Figure 10). In *E. coli*, the antimicrobial efficacy of WcAgNPR increased with concentration, showing maximum zone of inhibition (ZI) at 200 µg·mL^{-1} (10.0 ± 0.17), followed by decrements at 150 µg·mL^{-1} (9.0 ± 0.12), 100 µg·mL^{-1} (8.0 ± 0.10), and 60 µg·mL^{-1} (7.0 ± 0.07) (Table 2, Figure 11A). WcAgNPF exhibited its highest activity at 1 000 µg·mL^{-1} (17.5 ± 0.13), with subsequent decreases at 200 µg·mL^{-1} (12.0 ± 0.50), and further reduced to 30 µg·mL^{-1} (7.0 ± 0.04). Streptomycin used as a control, displayed its peak activity at 100 µg·mL^{-1} (19.4 ± 0.10), with decreasing activity at higher and lower concentrations, showing a broad range of effectiveness against *E. coli* (Figure 11A). Against *S. aureus*, WcAgNPR's antimicrobial action peaked at 1 000 µg·mL^{-1} (15.0 ± 0.11), with consistent activity at 150 and 200 µg·mL^{-1} (10.0 ± 0.08 to 10.0 ± 0.22), and a notable reduction at the lowest concentration of 10 µg·mL^{-1} (7.5 ± 0.09). WcAgNPF also reached its maximum at 1 000 µg·mL^{-1} (15.0 ± 0.05), maintaining high activity at 200 µg·mL^{-1} (15.0 ± 0.04), with a gradual decrease to 10 µg·mL^{-1} (8.5 ± 0.01), where the lowest activity was recorded. Streptomycin

https://doi.org/10.17221/39/2024-CJFS

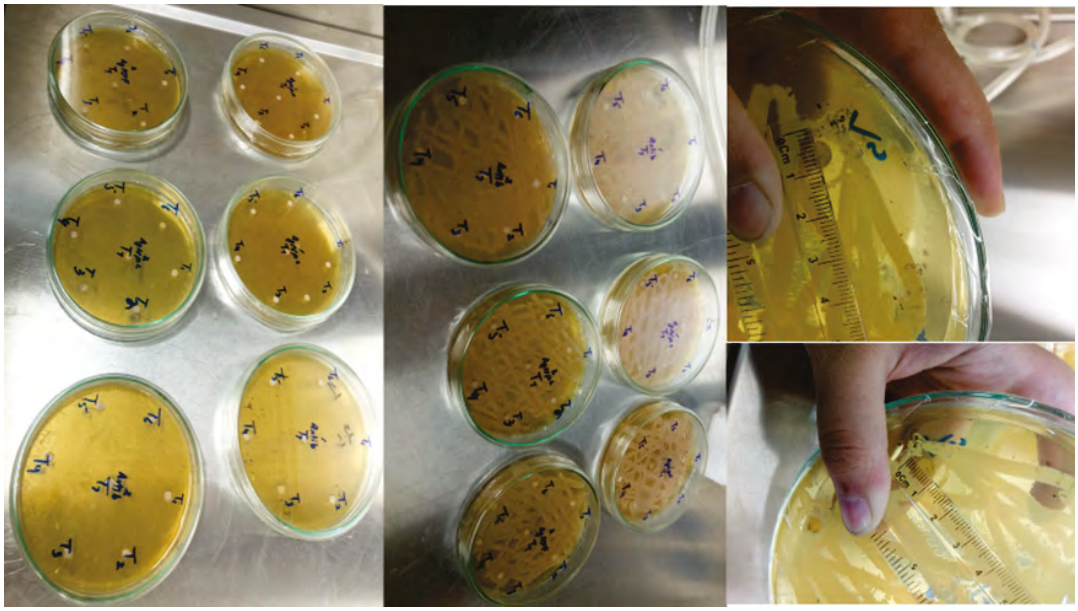

Figure 10. Performing antimicrobial activity using the disk diffusion method

Bacterial strains of *Escherichia coli* and *Staphylococococcus aureus* were cultured and tested by using agar plates

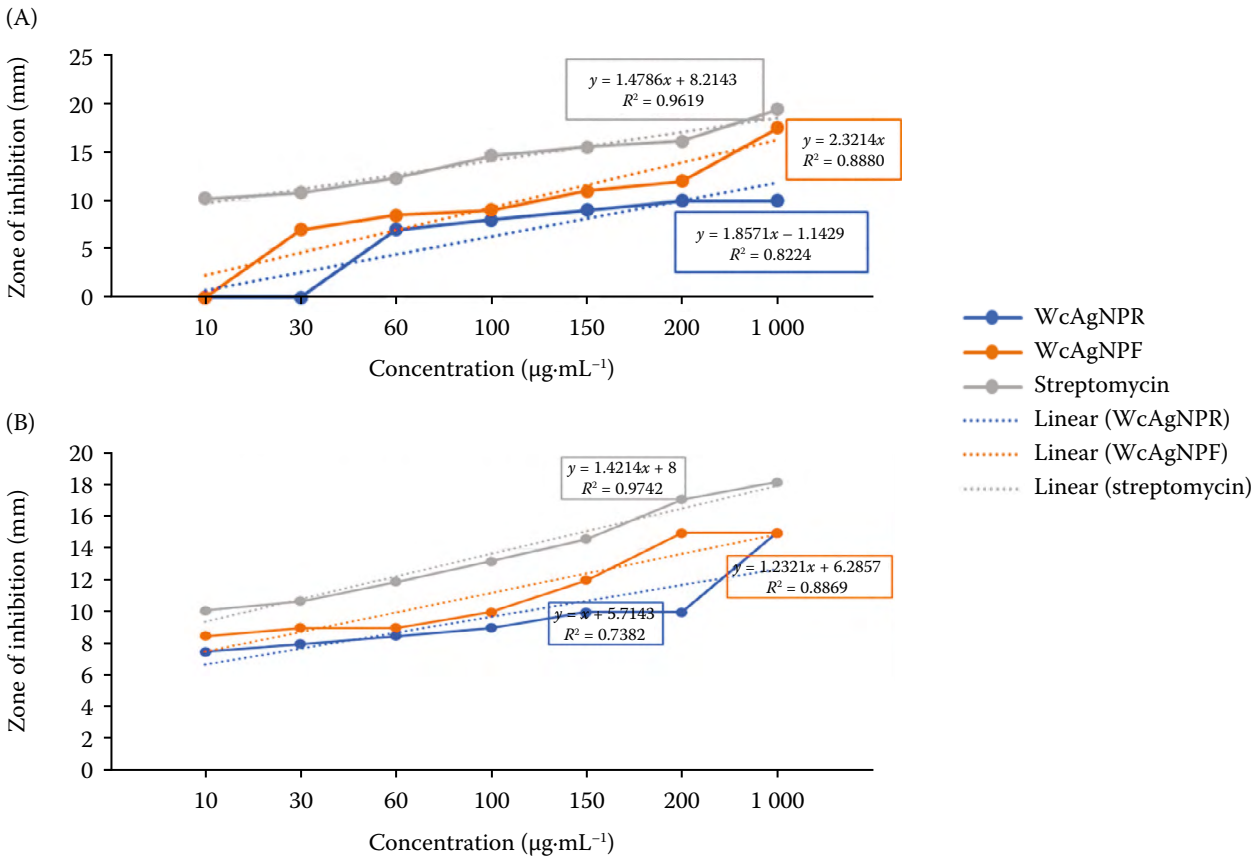

Figure 11. (A) Antimicrobial activity of silver nanoparticles (AgNPs) and streptomycin against *Escherichia coli*, and (B) antimicrobial activity of AgNPs and streptomycin against *Staphylococcus aureus*

WcAgNPF – *Withania coagulans* silver nanoparticle fruit; WcAgNPR – *Withania coagulans* silver nanoparticle root

https://doi.org/10.17221/39/2024-CJFS

Table 2. Antimicrobial activity (mm) of silver nanoparticles and streptomycin against *Escherichia coli* and *Staphylococcus aureus*

Concentration ($\mu g \cdot mL^{-1}$)	*Escherichia coli*			*Staphylococcus aureus*		
	WcAgNPR	WcAgNPF	streptomycin	WcAgNPR	WcAgNPF	streptomycin
10	Nil	Nil	10.2 ± 0.03	7.5 ± 0.09	8.5 ± 0.01	10.1 ± 0.05
30	Nil	7.0 ± 0.04	10.8 ± 0.06	8.0 ± 0.07	9.0 ± 0.06	10.7 ± 0.18
60	7.0 ± 0.07	8.5 ± 0.41	12.3 ± 0.10	8.5 ± 0.50	9.0 ± 0.10	11.9 ± 0.08
100	8.0 ± 0.10	9.0 ± 0.11	14.6 ± 0.56	9.0 ± 0.59	10.0 ± 0.08	13.2 ± 0.13
150	9.0 ± 0.12	11.0 ± 0.01	15.5 ± 0.41	10.0 ± 0.08	12.0 ± 0.12	14.6 ± 0.06
200	10.0 ± 0.17	12.0 ± 0.50	16.1 ± 0.05	10.0 ± 0.20	15.0 ± 0.04	17.1 ± 0.20
1 000	10.0 ± 0.15	17.5 ± 0.13	19.4 ± 0.10	15.0 ± 0.11	15.0 ± 0.05	18.2 ± 0.10

WcAgNPR – *Withania coagulans* silver nanoparticles root; WcAgNPF – *Withania coagulans* silver nanoparticles fruit

showed superior antimicrobial effectiveness across the board, with its activity spanning from 18.2 ± 0.10 at 1 000 $\mu g \cdot mL^{-1}$ to 10.1 ± 0.05 at 10 $\mu g \cdot mL^{-1}$, outperforming both WcAgNPR and WcAgNPF against *S. aureus* (Table 2, Figure 11B).

DISCUSSION

W. coagulans, a medicinally important wild plant, has been studied for its antioxidative and antimicrobial properties through various extracts and its use in synthesising silver nanoparticles, aiming to validate its significant potential in both antioxidant and antimicrobial activities. From our findings, the antioxidant potential of silver nanoparticles synthesised by using root extract (WcAgNPR) and fruit extract (WcAgNPF) was very significant. From DPPH scavenging activity analysis, the percentage inhibition of WcAgNPR was 37.8 ± 0.01 at the concentration of 100 $\mu g \cdot mL^{-1}$, which is greater than the percentage (26.5%) for the leaf extract of *W. coagulans* at the same concentration (Azhar et al. 2020). Moreover, the percentage scavenging showed by WcAgNPR at the concentration of 200 $\mu g \cdot mL^{-1}$ was 43.3 ± 0.02, which is comparable to the antioxidant activity shown by the root extract of *W. coagulans* at a much higher concentration of 800 ppm (parts per million) (Azhar et al. 2020). From ferric reducing antioxidant power assay findings, it is evident that present synthesised AgNPs have extremely significant antioxidants, which could be very useful in biological systems as fluorescence recovery after photobleaching (FRAP) is considered more reliable in terms of biological relevance as compared to other antioxidant measurements (Benzie and Strain 1996). Similarly, in the present research, maximum antioxidant activity was reported in the ferric-reducing power assay of both nanoparticles, with the percentage inhibition of WcAgNPF slightly higher than that of WcAgNPR at higher concentrations. These results are comparable to the previous findings in which *W. coagulans* fruit extract increased the ferric-reducing potential of diabetic rats (Shukla et al. 2012). Various studies have highlighted the potential of biocompatible and non-toxicity of green synthesised AgNPs that pose antibacterial and anticancer, apoptotic, antidiabetic and antioxidant properties (Mousavi et al. 2018; Geremew et al. 2024; Talib et al. 2024). In the present research, WcAgNPs can be an effective advancement in medicine as the synthesised WcAgNPF and WcAgNPR show antimicrobial as well as antioxidant potential, which is contrary to other AgNPs that either show antimicrobial or antioxidant potential. The reason behind this lies in the antimicrobial mechanism, as the AgNPs generally cause reactive oxidative species (ROS) production, hence halting microbial growth. Hydrogen peroxide is a strong free radical which is commonly produced in living organisms as a side product of various metabolic reactions for which peroxidases are biologically produced to scavenge hydrogen peroxide free radicals. These are natural antioxidants which scavenge hydrogen peroxide and hence prevent oxidative damage (Rudtanatip et al. 2022). Since these antioxidants are naturally found in biosystems, plant extracts and other samples of antioxidant potential are estimated via hydrogen peroxide scavenging activity analysis. In the hydrogen peroxide scavenging assay, the antioxidant potential of WcAgNPR was higher than WcAgNPF at varying concentrations ranging from 10 to 1 000 $\mu g \cdot mL^{-1}$, and optimum activity was observed at the highest concentration. These results are comparable with findings (Ahsan et al. 2020) where the silver nanoparticles synthesised from *Helicteresi-*

https://doi.org/10.17221/39/2024-CJFS

sora root extract showed optimum hydrogen peroxide (H_2O_2) scavenging activity at highest dose.

Phosphomolybdate assay is also employed to investigate an overall antioxidant potential. It has previously been used to examine the antioxidant activity of medicinal plants. It is based upon the reduction reaction of molybdenum Mo (VI) to Mo (V) by antioxidant molecules which is examined by the formation of green coloured phosphate/Mo complex (Khan et al. 2012). Our results indicate that ascorbic acid was more effective as its absorbance value is from 0.334 to 0.182 at the concentration of 10 to 200 μg·mL^{-1}. At the concentration of 200 μg·mL^{-1}, the percentage inhibition of WcAgNPR (41.6 ± 0.01) was higher than WcAgNPF (28.4 ± 0.01), whereas the percentage inhibition of ascorbic acid was 70.6 ± 0.01. Percentage inhibition of WcAgNPR and WcAgNPF is augmented by an increase in concentration, which is in accordance with the previous study (Madhusudhan et al. 2015). The antibacterial activity of WcAgNPs may primarily be due to the cytotoxicity of Ag^+ ions, which exhibit significant activity against *S. aureus*. However, SEM findings by Keshari et al. (2020) suggest that the WcAgNPs penetrate the bacterial cell wall, creating pores. This action is potentially linked to phytochemicals, such as withanolides and coagulansin A, that cap the AgNPs. The antimicrobial potential of WcAgNPF was slightly greater than WcAgNPR, and the minimal inhibitory concentrations (*MIC*) value of WcAgNPF was also much lower than that of WcAgNPR against *E. coli*, indicating that the antimicrobial potential of WcAgNPF was more than WcAgNPR. Overall, gram-positive bacteria *S. aureus* was more susceptible than gram-negative *E. coli*, which is in accordance with the previous results (Noreen et al. 2016) in which acetone seed extract of *W. coagulans* showed a minimum zone of inhibition against *E. coli* in comparison to other microbes including *S. aureus*. At the concentration of 10 μg·mL^{-1}, WcAgNPF showed an inhibition zone (8.5 ± 0.01) greater than WcAgNPR (7.5 ± 0.09), which is equal to the zone reported by Tripathi et al. (2019) for leaf-synthesised silver nanoparticles at the same concentration. Finally, WcAgNPF posed more antimicrobial potential than WcAgNPR.

CONCLUSION

The present findings demonstrate that green-synthesised silver nanoparticles (WcAgNPs) using *W. coagulans* exhibit substantial antimicrobial properties against gram-positive *S. aureus* and gram-negative *E. coli*. These nanoparticles, particularly WcAgNPF, showed greater efficacy against both bacteria types than WcAgNPR, with significantly higher activity against *S. aureus*. Additionally, the silver nanoparticles possess notable antioxidant capabilities, especially in ferric-reducing assays, suggesting the involvement of iron-reducing phytochemicals in their synthesis. The promising IC_{50} values from DPPH scavenging assays indicate their potential safe use in *in vivo* applications for pathogenic issues. These findings highlight the dual functionality of WcAgNPs in medical, agricultural, and environmental applications, underscoring the need for further detailed studies to explore their mechanisms and optimise synthesis parameters for enhanced effectiveness.

Acknowledgement. The authors are grateful to the staff of Department of Botany, Phir Mehr Ali Shah Arid Agriculture University Rawalpindi, for their always stimulating support to these studies. The authors would also like to extend their sincere appreciation to the Researchers Supporting Project Number (RSP2024R350), King Saud University, Riyadh, Saudi Arabia.

REFERENCES

Ahn E.-Y., Jin H., Park Y. (2019): Assessing the antioxidant, cytotoxic, apoptotic and wound healing properties of silver nanoparticles green-synthesized by plant extracts. Materials Science and Engineering: C, 101: 204–216.

Ahsan A., Farooq M.A., Ahsan Bajwa A., Parveen A. (2020): Green synthesis of silver nanoparticles using *Parthenium hysterophorus*: Optimization, characterization and *in vitro* therapeutic evaluation. Molecules, 25: 3324.

Alharbi N.S., Alsubhi N.S., Felimban A.I. (2022): Green synthesis of silver nanoparticles using medicinal plants: Characterization and application. Journal of Radiation Research and Applied Sciences, 15: 109–124.

Anwar A., Masri A., Rao K., Rajendran K., Khan N.A., Shah M.R., Siddiqui R. (2019): Antimicrobial activities of green synthesized gums-stabilized nanoparticles loaded with flavonoids. Scientific Reports, 9: 1–12.

Azhar M.F., Naseer U., Aziz A., Zafar S., Qadir I., Farooq M., Ahmad I., Anjum K. (2020): Antioxidant and phytochemical composition of leaves, stem and root extracts of *Withania coagulans* and *Withania somnifera*. Zeitschrift Arznei-Gewurzpflanzen, 25: 27–30.

Baker S., Volova T., Prudnikova S.V., Satish S., Prasad N. (2017): Nanoagroparticles emerging trends and future prospect in modern agriculture system. Environmental Toxicology and Pharmacology, 53: 10–17.

https://doi.org/10.17221/39/2024-CJFS

Benzie I.F., Strain J.J. (1996): The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay. Analytical Biochemistry, 239: 70–76.

Gaurav H., Yadav D., Maurya A., Yadav H., Yadav R., Shukla A.C., Sharma M., Gupta V.K., Palazon J. (2023): Biodiversity, biochemical profiling, and pharmaco-commercial applications of *Withania somnifera*: A Review. Molecules, 28: 1208.

Geremew A., Gonzalles J. 3rd, Peace E., Woldesenbet S., Reeves S., Brooks N. Jr., Carson L. (2024): Green synthesis of novel silver nanoparticles using *Salvia blepharophylla* and *Salvia greggii*: Antioxidant and antidiabetic potential and effect on foodborne bacterial pathogens. International Journal of Molecular Sciences, 25: 904.

Han X., Xu K., Taratula O., Farsad K. (2019): Applications of nanoparticles in biomedical imaging. Nanoscale, 11: 799–819.

Hasan M., Zafar A., Shahzadi I., Luo F., Hassan S.G., Tariq T., Zehra S., Munawar T., Iqbal F., Shu X. (2020): Fractionation of biomolecules in *Withania coagulans* extract for bioreductive nanoparticle synthesis, antifungal and biofilm activity. Molecules, 25: 3478.

Jemal K., Sandeep B., Pola S. (2017): Synthesis, characterization, and evaluation of the antibacterial activity of *Allophylus serratus* leaf and leaf derived callus extracts mediated silver nanoparticles. Journal of Nanomaterials, 2017: 1–11.

Keshari A.K., Srivastava R., Singh P., Yadav V.B., Nath G. (2020): Antioxidant and antibacterial activity of silver nanoparticles synthesized by *Cestrum nocturnum*. Journal of Ayurveda and Integrative Medicine, 11: 37–44.

Khan R.A., Khan M.R., Sahreen S., Ahmed M. (2012): Assessment of flavonoids contents and *in vitro* antioxidant activity of *Launaea procumbens*. Chemistry Central Journal, 6: 43.

Khan I., Saeed K., Khan I. (2019): Nanoparticles: Properties, applications and toxicities. Arabian Journal of Chemistry, 12: 908–931.

Khan M.I., Maqsood M., Saeed R.A., Alam A., Sahar A., Kieliszek M., Miecznikowski A., Muzammil H.S., Aadil R.M. (2021): Phytochemistry, food application, and therapeutic potential of the medicinal plant (*Withania coagulans*): A review. Molecules, 26: 6881.

Khodaei M., Jafari M., Noori M. (2012): Remedial use of withanolides from *Withania coagolans* (Stocks) Dunal. Advancements in Life Sciences, 2: 6–19.

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. Journal of Molecular Structure, 1175: 90–96.

Madhusudhan D., Agsar D., Sulochana M. (2015): Water soluble melanin of *Streptomyces lusitanus* DMZ3 persuade synthesis of enhanced bio-medically active silver nanoparticles. Journal of Cluster Science, 26: 1077–1089.

Manzano M., Vallet-Regí M. (2020): *Mesoporous silica* nanoparticles for drug delivery. Advanced Functional Materials, 30: 1902634.

McNamara K., Tofail S.A. (2017): Nanoparticles in biomedical applications. Advances in Physics: X, 2: 54–88.

Mirjalili M.H., Moyano E., Bonfill M., Cusido R.M., Palazon J. (2009): Steroidal lactones from *Withania somnifera*, an ancient plant for novel medicine. Molecules, 14: 2373–2393.

Moonmun D., Majumder R., Lopamudra A. (2017): Quantitative phytochemical estimation and evaluation of antioxidant and antibacterial activity of methanol and ethanol extracts of *Heliconia rostrata*. Indian Journal of Pharmaceutical Sciences, 79: 79–90.

Mousavi S.M., Hashemi S.A., Ghasemi Y., Atapour A., Amani A.M., Savar Dashtaki A., Babapoor A., Arjmand O. (2018): Green synthesis of silver nanoparticles toward bio and medical applications: Review study. Artificial Cells, Nanomedicine, and Biotechnology, 46: S855–S872.

Noreen H., Zaman B., Rahman U.A., Hassan W. (2016): Antioxidant and antimicrobial efficacies of *Withania coagulans* seed extract against pathogenic bacteria and fungi. The International Journal of Biotechnology, 5: 45–51.

Peerzade N., Sayed N., Das N. (2018): Antimicrobial and phytochemical screening of methanolic fruit extract of *Withania coagulans* L. Dunal for evaluating the antidiabetic activity. Pharma Innovation Journal, 7: 197–204.

Rai M., Yadav A., Gade A. (2009): Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances, 27: 76–83.

Rajoka M.S.R., Mehwish H.M., Zhang H., Ashraf M., Fang H., Zeng X., Wu Y., Khurshid M., Zhao L., He Z. (2020): Antibacterial and antioxidant activity of exopolysaccharide mediated silver nanoparticle synthesized by *Lactobacillus brevis* isolated from Chinese koumiss. Colloids and Surfaces B: Biointerfaces, 186: 110734.

Rambaran T., Schirhagl R. (2022): Nanotechnology from lab to industry – A look at current trends. Nanoscale Advances, 4: 3664–3675.

Rudtanatip T., Pariwatthanakun C., Somintara S., Sakaew W., Wongprasert K. (2022): Structural characterization, antioxidant activity, and protective effect against hydrogen peroxide-induced oxidative stress of chemically degraded *Gracilaria fisheri* sulfated galactans. International Journal of Biological Macromolecules, 206: 51–63.

Shahid W., Durrani R., Iram S., Durrani M., Khan F.A. (2013): Antibacterial activity *in vitro* of medicinal plants. Sky Journal of Microbiology Research, 1: 5–21.

https://doi.org/10.17221/39/2024-CJFS

Shukla K., Dikshit P., Shukla R., Gambhir J.K. (2012): The aqueous extract of *Withania coagulans* fruit partially reverses nicotinamide/streptozotocin-induced diabetes mellitus in rats. Journal of Medicinal Food, 15: 718–725.

Talib H., Mehmood A., Amjad M.S., Mustafa A., Khan M.A.R., Raffi M., Khan R.T., Ahmad K.S., Qureshi H. (2024): Antibacterial, antioxidant, and anticancer potential of green fabricated silver nanoparticles made from *Viburnum grandiflorum* leaf extract. Botanical Studies, 65: 4.

Tripathi D., Modi A., Narayan G., Rai S.P. (2019): Green and cost effective synthesis of silver nanoparticles from endangered medicinal plant *Withania coagulans* and their potential biomedical properties. Materials Science and Engineering: C, 100: 152–164.

Zhang H., Samadi A.K., Cohen M.S., Timmermann B.N. (2012): Anti-proliferative withanolides from the *Solanaceae*: A structure-activity study. Pure and Applied Chemistry, 84: 1353–1367.

Received: March 1, 2024
Accepted: May 24, 2024
Published online: June 18, 2024