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Green biosynthesis of silver nanoparticles using *Prunus cerasifera pissardii nigra* leaf and their antimicrobial activities against some food pathogens

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Abstract: In this study, silver nanoparticles (AgNPs) were synthesised using the *Prunus cerasifera pissardii nigra* (PC) leaf extract in an easy, low-cost and environmentally friendly way. According to the ultraviolet-visible (UV-vis) spectrophotometer analysis data, the nanocrystals demonstrated a characteristic peak at 456 nm. Scanning electron microscopy (SEM), field emission scanning electron microscopy (FE-SEM), transmission electron microscopy (TEM) and energy-dispersive X-ray (EDX) spectroscopy analyses revealed that the morphological structures of the biosynthesised AgNPs were mostly spherical. According to the results of X-ray diffraction (XRD) analysis, it was determined that the crystal structures of AgNPs were cubic. The size of the nanoparticles was calculated as 23.60 nm using the Debye-Scherrer equation. The zeta potential of the synthesised nanomaterial was measured as –15.5 mV. The minimum inhibitory concentration (MIC) values of AgNPs on *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 11774, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231 were determined to be 0.062, 0.250, 0.125, 0.500 and 0.125 μg mL^{-1}, respectively.

Keywords: AgNPs; field emission scanning electron microscopy; inhibitory concentration; Fourier transform infrared; transmission electron microscopy

Nanotechnology deals with the production, characterisation and manipulation of nano-sized materials between 1 nm and 100 nm (Sarfraz et al. 2021). In recent years, parallelly to the developments in nanotechnology, nanoparticles have become an important study topic due to their extensive applications in various fields such as antimicrobial agents, diagnostics, biomarkers, drug delivery, cell labelling, cancer therapy and water purification (Mousavi et al. 2018; Kumari et al. 2019; Kowsalya et al. 2021). Nanoparticles can be produced by many methods such as chemical reduction, physicochemical reduction, photochemical reduction, electrochemical reduction, radiolysis and thermal evaporation (Iravani et al. 2014; Yadi et al. 2018). However, recently, instead of traditional methods, nanoparticles have started to be produced by low-cost 'green synthesis' procedures that do not use any toxic solvents and do not pollute the environment (Hussain et al. 2016; Bandeira et al. 2020, Hatipoğlu 2021a). For this purpose, nanoparticles are synthesised using biological sources such as plants, fungi, bacteria and algae (Parial et al. 2012; Singh et al. 2016; Acay et al. 2019; Chellamuthu et al. 2019; Hatipoğlu 2021b). Secondary plant metabolites utilised in nanoparticle production act as a capping agent that reduces metal ions and stabilises the shape and size of the nanoparticles produced. The most significant of these plant metabolites are polyphenols, which are highly concentrated in the environment and have strong reducing properties. Proteins, carbohydrates, terpenoids and alkaloids are other plant metabolites that show similar effects (Swilam and Nematallah 2020).

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Metals frequently used in nanoparticle studies are silver (Zhang and Jiang 2020), gold (Keskin et al. 2022), zinc (Thema et al. 2015) and platinum (Thirumurugan et al. 2016). Especially silver (Ag) is known to have a significant suppressive effect on the growth and survival of bacteria. The 'Ag' ion can prevent cell division and deoxyribonucleic acid (DNA) replication (Ramya and Subapriya 2012). Due to their relatively small size and large surface area, silver nanoparticles (AgNPs) exhibit strong antimicrobial activity against microorganisms such as bacteria, viruses and fungi (Loo et al. 2018; Hatipoğlu 2022). AgNPs bind to cell membrane proteins and catalyse the formation of reactive oxygen species in bacterial cells. Thus, they cause cell death due to oxidative stress (Hoseinnejad et al. 2017; Alkhalaf et al. 2020). It also damages DNA and inhibits cell proliferation by inducing apoptosis (Li et al. 2020).

In the study, AgNPs were obtained from *Prunus cerasifera pissardii nigra* (PC) leaf by green synthesis. As far as we know, there is no document that AgNPs were obtained from the plant in question until now. PC, also known as the cherry plum, is a plant that is native to Southeastern Europe (Balkan Peninsula, Crimea) and Western and Central Asia (Caucasus, Iran, Iraq). PC is a thorny shrub tree with sphere-shaped, yellow/red/purple-coloured fruits. Its young leaves are deep purple. When ripe, the leaves turn a dark green colour (Horvath et al. 2008; Kalyoncu et al. 2016; Popescu and Caudullo 2016; Huang et al. 2019).

In this study, AgNPs were synthesised by reducing $AgNO_3$ (Ag^{+1} to Ag^0) with PC plant leaf extract to produce plant-compatible nanoparticles. The nanomaterial obtained was characterised, and its antimicrobial activities against several foodborne pathogens were demonstrated.

MATERIAL AND METHODS

Material. The green leaves of PC were collected from Mardin Province in southeast Turkey. Silver nitrate ($AgNO_3$, 99.98% purity), vancomycin, fluconazole and colistin were purchased from Sigma Aldrich (Germany). *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 11774 and *Candida albicans* ATCC 10231 were also used in the study for the antimicrobial activities of PC-AgNPs.

Extraction of PC leaf. The green leaves of the PC were washed thoroughly with distilled water and dried under room conditions. Dried leaves (25 g) were taken, mixed with 250 mL of distilled water and boiled. The extract was cooled to room temperature. Then, it was filtered through Whatman No. 1 filter paper (United Kingdom) and stored in the refrigerator for the synthesis of PC-AgNPs.

Synthesis of PC-AgNPs. For the synthesis of PC-AgNPs, 1 mM $AgNO_3$ aqueous solution was prepared using solid $AgNO_3$. $AgNO_3$ solution (200 mL) and plant extract (200 mL) were allowed to react stably at room temperature. The formation of PC-AgNPs was determined by ultraviolet-visible (UV-vis) spectroscopy wavelength scanning (CARY 60; Agilent, USA) at different time intervals (15, 30, 45, 60, and 120 min) depending on the colour change (Pugazhendhi et al. 2018). After the reaction, the dark solution was centrifuged (14 000 rpm, 10 336 g, 25 min) (Ohaus FC5706; Hanna, USA). The solid fraction obtained at the end of centrifugation was washed several times with distilled water, and the final residue (PC-AgNPs) was dried in an oven at 80 °C for 48 h (UN 55; Memmert, Germany). Then the dry part was ground into powder using a mortar and pestle.

Characterisation techniques of PC-AgNPs. UV-vis spectra of PC-AgNPs were defined on a spectrophotometer (CARY 60 UV-visible spectrophotometer; Agilent, USA) within the wavelength range of 300–800 nm. The morphology, size, crystal structure and surface distributions of PC-AgNPs were analysed by scanning electron microscopy (SEM) (EVO 40 LEQ; Carl Zeiss AG, Germany), field emission scanning electron microscopy (FE-SEM) (FEG 240; Quanta, USA), transmission electron microscopy (TEM) (JEM-1010; JEOL, USA), X-ray diffraction (XRD) (Rad B-DMAX II; Rigacu, Japan), energy-dispersive X-ray (EDX) (FEG 240; Quanta, USA) and Zetasizer (Malvern Ins. Ltd., United Kingdom). The crystal size of PC-AgNPs was calculated according to the Debye-Scherrer equation ($D = K\lambda/\beta\cos\theta$; where: D – particle size; K = 0.90; λ– X-ray wavelength value; θ – Bragg diffraction angel; β – experimental full width at the half maximum in radians) (Aktepe and Baran 2021). Moreover, Fourier transform infrared (FT-IR) spectroscopy (Cary 630; Agilent, USA) was used to determine the functional groups in the plant extract and the functional groups responsible for the reduction at the end of the reaction with the silver salt.

Antimicrobial activity of PC-AgNPs. Minimum inhibitory concentrations (MICs) of PC-AgNPs on gram-negative (*P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922) and gram-positive (*S. aureus* ATCC 29213, *B. subtilis* ATCC 11774) bacteria and *C. albicans* ATCC 10231 were determined by microdilution using a 96-well microtiter plate. Mueller Hinton Broth for

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bacteria (Merck, Germany) and Roswell Park Memorial Institute (RPMI) medium (growth medium used in cell culture; Sigma-Aldrich, Germany) were added to the wells, 100 µL (16 ppm: initial concentration) of each medium. Then, 100 µL (16 ppm) of PC-AgNPs were added to the first well and diluted by transferring 100 µL each time to the next well. After that, McFarland 0.5 turbidity solution was prepared separately for each microorganism. 100 µL of these solutions were added to each well. It was incubated at 37 °C for 24 h (UN 55; Memmert, Germany). After incubation, the lowest concentration without growth was determined as the MIC value (Baran and Keskin 2020). In addition, 128 ppm of vancomycin, colistin and fluconazole and 1 mM $AgNO_3$ solution each were used to compare the antimicrobial effects of PC-AgNPs on *S. aureus, E. coli, B. subtilis, P. aeruginosa* and *C. albicans*.

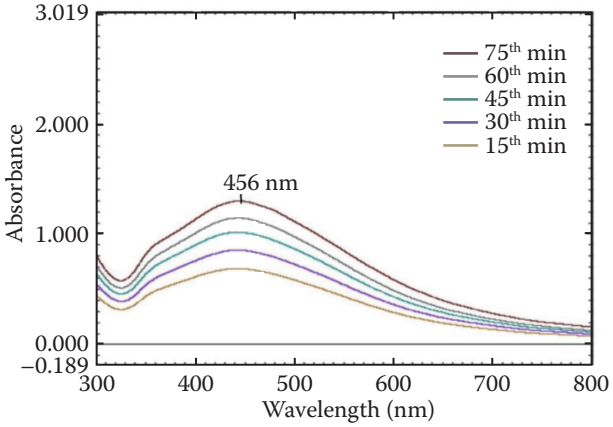

Figure 1. Ultraviolet-visible (UV-vis) absorption spectra of *Prunus cerasifera pissardii nigra*-silver nanoparticles (PC-AgNPs)

RESULTS AND DISCUSSION

It is seen that the UV-vis spectra of PC-AgNPs change from light yellow to dark purple (starting at 15 min and ending at 75 min) (Figure 1). UV-vis spectroscopy analysis revealed that PC-AgNPs peaked at the specific absorbance value of approximately 456 nm. Similarly, some researchers reported that the absorption spectrum of AgNPs is between 425 nm and 461 nm due to surface plasmon resonance (Udayasoorian et al. 2011; Swamy et al. 2015; Eren and Baran 2019a).

The presence of pure silver was also demonstrated in the EDX analysis of PC-AgNPs (Figure 2). It is understood that silver has an elemental structure in the EDX pattern. Moreover, the EDX analysis of PC-AgNPs shows that they contain 80.75% silver, 10.35% carbon and 8.90% oxygen (Table 1). Weak signals such as oxygen and carbon in the EDX profile may originate from organic biomolecules in PC leaf pulp adsorbed on the surface of the nanoparticles. Due to surface plasmon resonance, AgNPs exhibit a typical optical absorption peak at about 3 kiloelectron-volts (keV). Akintelu et al. (2019) and Fatema et al. (2019) also showed EDX silver peaks in their studies.

The results of SEM, FE-SEM and TEM images show that PC-AgNPs mostly have a spherical form (Figures 3–5). AgNPs were also reported to be spheri-

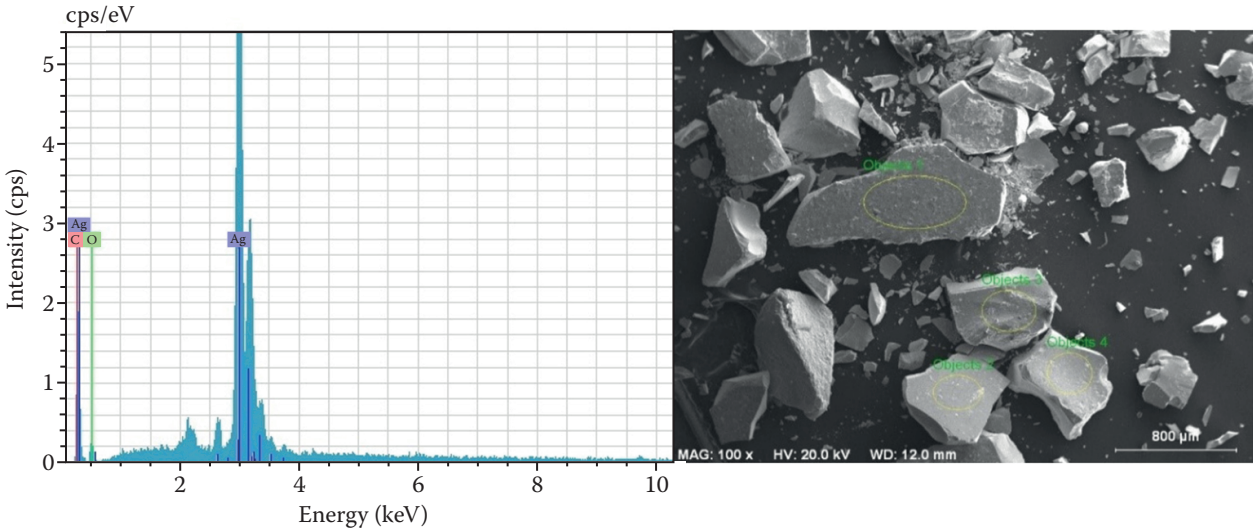

Figure 2. Energy-dispersive X-ray (EDX) surface analysis of *Prunus cerasifera pissardii nigra*-silver nanoparticles (PC-AgNPs)

keV – kiloelectron-volts; cps – counts per second; MAG – magnification; HV – high voltage; WD – working distance

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Table 1. Elemental composition of *Prunus cerasifera pissardii nigra*-silver nanoparticles (PC-AgNPs) by the energy-dispersive X-ray (EDX) analysis (%)

Element	Weight	Atomic
Ag (*L*-series)	80.75	34.55
C (*K*-series)	10.35	39.78
O (*K*-series)	8.90	25.67
Total	100.00	100.00

L-series – light-medium elements; *K*-series – light elements

cal in similar studies by other researchers (Lopes and Courrol 2018; Pallela et al. 2018; Baran 2019).

AgNPs have sizes between 2 nm and 95 nm (Becaro et al. 2015; Kedi et al. 2018; Nguyen et al. 2018). The sizes of PC-AgNPs in this study were measured in the range from 5.55 nm to 13.19 nm, with an average of 10.50 nm (Figure 5).

It is determined which functional groups are involved in plant-induced reduction using FT-IR spectroscopy. Separate infrared spectroscopy was performed on the plant leaf extract used in the reduction and the mix-

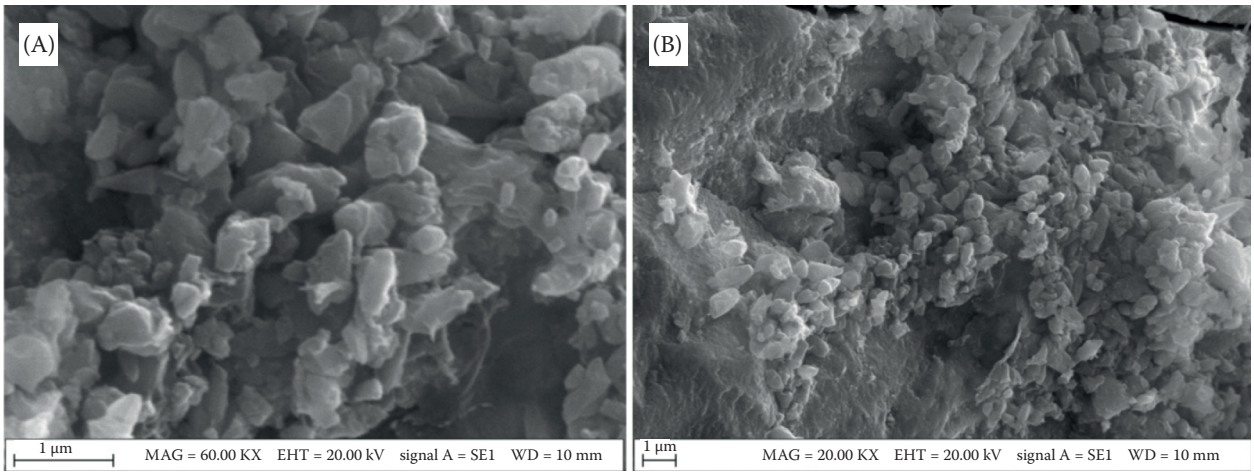

Figure 3. Scanning electron microscopy (SEM) images of *Prunus cerasifera pissardii nigra*-silver nanoparticles (PC-AgNPs): (A) 60.00 KX, (B) 20.00 KX

KX – magnification coefficient; MAG – magnification; EHT – energy high tension; WD – working distance; SE1 – secondary electron

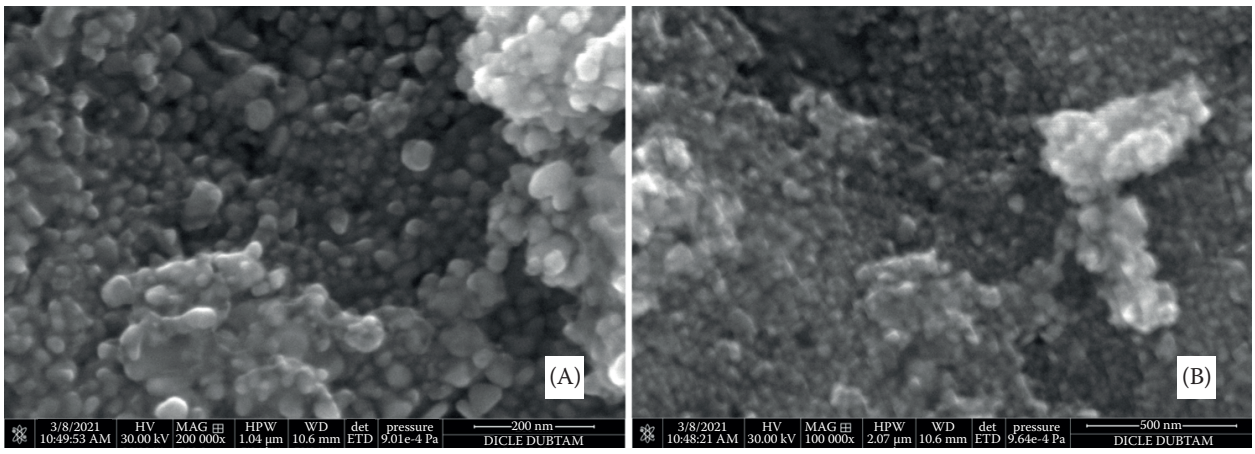

Figure 4. Field emission scanning electron microscopy (FE-SEM) images of *Prunus cerasifera pissardii nigra*-silver nanoparticles (PC-AgNPs): (A) 200 nm, (B) 500 nm

HV – high voltage; MAG – magnification; HPW – highest particle width; WD – working distance; det – detector; ETD – Everhardt-Thornley detector

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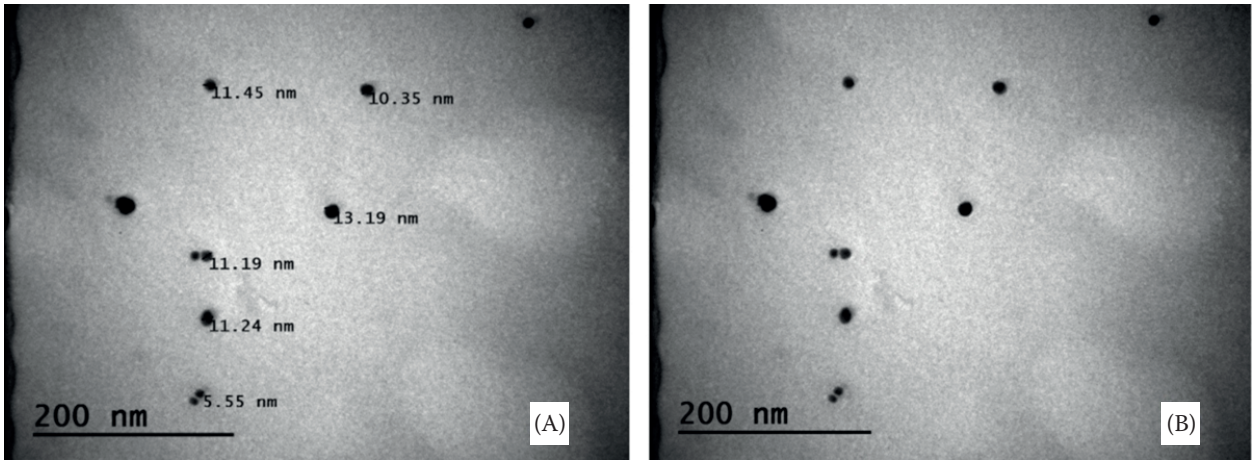

Figure 5. Transmission electron microscopy (TEM) images of *Prunus cerasifera pissardii nigra*-silver nanoparticles (PC-AgNPs): (A) sized, (B) unsized

ture at the end of the reaction (Figure 6). In comparison, it is observed that the obvious peaks participating in the reduction belong to the bonds -OH, C=O and -C-C (Smith and Meissl 2007; Muthusamy et al. 2017). The shifts at 3 336, 2 119, and 1 635 cm^{-1} in the figure indicate that the groups -OH, -CN and C=O, respectively, are involved in the reduction.

In XRD analysis results, peaks 111, 200, 220, and 311°, which coincide with 38.018, 44.32, 64.52, and 77.48 at 2θ, respectively, are sharp peaks representing the face-centred cubic crystal structure of silver (Figure 7) (Eren and Baran 2019b, Aktepe 2021). Other researchers also reported that peaks at 111, 200, 220, and 311° at 2θ belong to silver (Agarwal et al. 2018; Huang et al. 2019; Rajoka et al. 2020). The crystal size of the obtained AgNPs was calculated as 23.60 nm by the Debye-Scherrer equation (Aktepe and Baran 2021).

The zeta potential is the electric charge on the surface of the material surrounded by the medium. The zeta potential is also a measure of stability. Substances with high zeta potential are not prone to cementing or clustering together. Nanoparticles with a significantly lower negative charge, on the other hand, can enter the cell more easily (Tavakol et al. 2016; Maddinedi et al. 2017). In this study, the zeta potential of PC-AgNPs was measured to be –5.5 mV (Figure 8). This value indicates that PC-AgNPs are stable and uniformly distributed. Different zeta potential values of AgNPs synthesised from various materials have been reported (Maillard et al. 2018; Patil et al. 2018; Jebril et al. 2020; Thirumagal and Jeyakumari 2020).

As microorganisms develop resistance to antibiotics, the antimicrobial properties of AgNPs become increasingly important. PC-AgNPs appear to be effec-

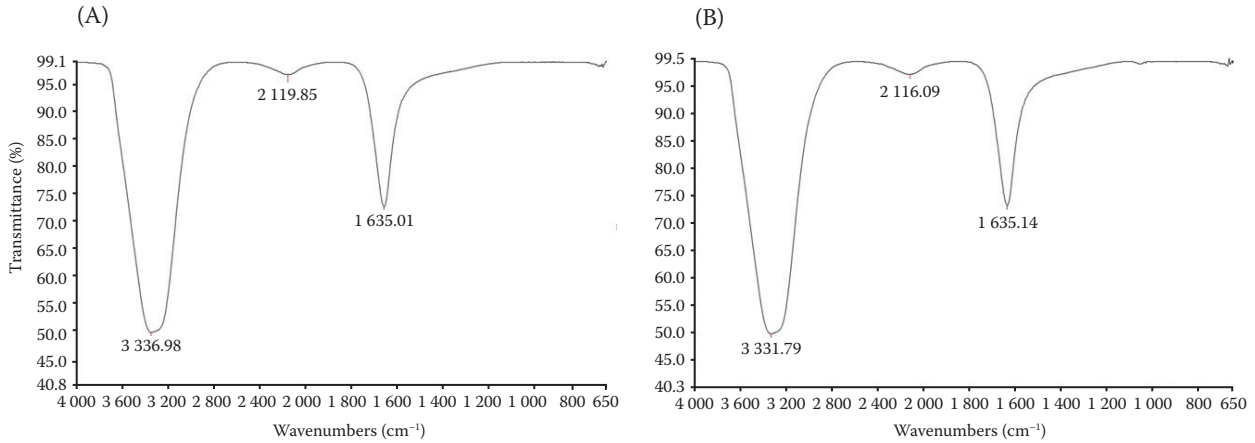

Figure 6. Fourier transform infrared (FT-IR) spectra of (A) *Prunus cerasifera pissardii nigra* (PC) leaf extract, (B) PC--silver nanoparticles (PC-AgNPs)

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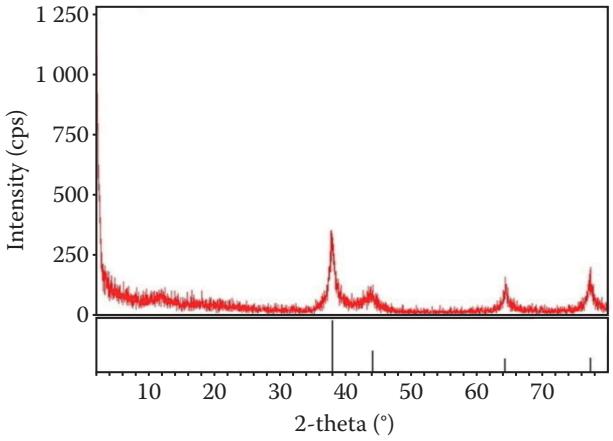

Figure 7. X-ray diffraction (XRD) patterns of *Prunus cerasifera pissardii nigra*-silver nanoparticles (PC-AgNPs)

cps – counts per second

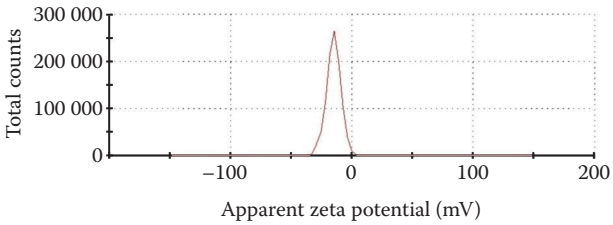

Figure 8. Zeta potential of *Prunus cerasifera pissardii nigra*-silver nanoparticles (PC-AgNPs)

tive against food pathogens, according to the findings of this study (Table 2). In comparison with $AgNO_3$ and antibiotics, PC-AgNPs demonstrated significant antimicrobial effects on microorganisms at lower concentrations. This study demonstrated that PC-AgNPs are effective not only at lower concentrations but also across a broader range of bacteria. The results of the present study are consistent with other researchers' results that AgNPs synthesised from different plants have similar effects on these microorganisms (Otari et al. 2014; Moodley et al. 2018; Garibo et al. 2020)

Table 2. Minimum inhibitory concentration (MIC) values of *Prunus cerasifera pissardii nigra*-silver nanoparticles (PC-AgNPs), $AgNO_3$ and antibiotics (μg mL^{-1})

Microorganisms	PC-AgNPs	$AgNO_3$	Standard antibiotics
Staphylococcus aureus ATCC 29213	0.062	2.65	2
Bacillus subtilis ATCC 11774	0.125	1.32	1
Escherichia coli ATCC 25922	0.250	0.66	2
Pseudomonas aeruginosa ATCC 27853	0.500	1.32	4
Candida albicans ATCC 10231	0.125	0.66	2

CONCLUSION

In this study, biocompatible nanoparticles were successfully synthesised using a PC leaf extract. The synthesis of AgNPs using the PC leaf extract as a reducing agent was carried out at a low cost, environmentally friendly, and in a simple and fast way. Conversely, neither toxic nor hazardous substances were used in the biosynthesis. UV-vis absorption, EDX, and XRD analyses all validated the production of PC-AgNPs. SEM, FE-SEM, and TEM analyses demonstrated that AgNPs were spherical. This study concluded that AgNPs, even at very low concentrations, had an inhibitory effect on *S. aureus* ATCC 29213, *E. coli* ATCC 25922, *B. subtilis* ATCC 11774, *P. aeruginosa* ATCC 27853 and *C. albicans* ATCC 10231, as an alternative to antibiotics. It was concluded that the smaller the size of nanoparticles, the greater their antimicrobial effectiveness; additional research should be carried out to determine the toxic effect of PC-AgNPs on other microorganisms.

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