

Evaluation of Antioxidant Activities of Silver Nanoparticles Synthesized by Aqueous Extract of *Maclura pomifera* (Raf.) Schneid. Fruit

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Abstract. Plant-based nanoparticles (NPs) synthesis has gained increasing attention due to its cost-effectiveness, rapidity, and environmental friendliness. In this study, the green synthesis of silver nanoparticles (MP-AgNPs) using *Maclura pomifera* fruit extract and their antioxidant activities were investigated. The synthesized MP-AgNPs were characterized by Ultraviolet-Visible (UV-Vis) spectroscopy, Scanning Electron Microscopy (SEM), Energy-Dispersive X-ray Spectroscopy (EDX), Fourier Transform Infrared (FTIR) spectroscopy, and X-ray Diffraction (XRD) analysis. According to the EDX results, the elemental composition by mass was 22.72% carbon, 13.89% oxygen, and 57.74% silver. SEM analysis revealed that the MP-AgNPs were predominantly spherical, with an average particle size of 39.02 nm. The zeta potential of MP-AgNPs was measured as -34.6 mV, indicating good nanoparticle stability and electrostatic repulsion among particles. In antioxidant assays, the ABTS radical scavenging activity showed IC_{50} values of 6.36 ± 0.02 $\mu\text{g/mL}$ for MP-AgNPs, 7.27 ± 0.00 $\mu\text{g/mL}$ for BHT, and 10.58 ± 0.01 $\mu\text{g/mL}$ for the extract. Furthermore, FRAP assay results demonstrated increased antioxidant activity in the order of extract (3.55 ± 0.00 $\mu\text{mol TE/mg}$), BHT (5.33 ± 0.01 $\mu\text{mol TE/mg}$), and MP-AgNPs (5.39 ± 0.02 $\mu\text{mol TE/mg}$).

Introduction

Nanotechnology encompasses the synthesis of nanoparticles (NPs), the variability in their shape, size, and chemical composition, as well as the characterization of their morphology-dependent magnetic, electrical, and optical properties. Green synthesis of NPs, utilizing bioactive compounds and metal oxides, has found increasing applications across various fields, including energy, biomedicine, pharmaceuticals, cosmetics, food, and agriculture [1–3].

This synthesis approach offers several advantages, as it does not require chemical reducing agents, stabilizers, or toxic solvents [1, 4–5]. Additionally, it is cost-effective, safe, and suitable for the large-scale production of NPs [1,6]. In green synthesis, molecules such as phenolic acids, flavonoids, and alkaloids present in plant extracts serve as bioreducing agents [7]. Furthermore, when nanoparticles synthesized using plant extracts are employed, a synergistic effect is often observed due to the combined activities of both the plant extract and the metal nanoparticles (MNPs) [8].

Maclura pomifera (MP), a member of the Moraceae family, is a hardwood species widely distributed in the southwestern United States and Turkey. Numerous studies have reported various biological activities associated with MP and its constituents. These include antiproliferative [9], antioxidant [10], enzymatic and antibacterial activities [11], hepatoprotective effects [12], insect-repellent or

insecticidal properties [13], anticancer potential [14], and antimicrobial activity [15–16]. MP is rich in secondary metabolites such as isoflavones [17–18], flavonoids [19], and, triglycerides and triterpenes [19–20]. Notably, MP contains significant amounts of flavonoids, particularly isoflavones such as osajin and pomiferin [18,21], which have been reported to exhibit antioxidant properties [22]. Additionally, 5-O-caffeoylquinic acid, pentacosane, and palmitic acid have been identified as major compounds in the dichloromethane extract of MP [23].

In this study, the green synthesis of silver nanoparticles (MP-AgNPs) using *Maclura pomifera* fruit extract and their antioxidant activities were investigated through DPPH•, ABTS•+, and FRAP assays. The synthesized Mp-AgNPs were characterized by UV-Vis spectroscopy, Scanning Electron Microscopy (SEM), Energy-Dispersive X-ray Spectroscopy (EDX), Fourier Transform Infrared (FTIR) spectroscopy, zeta potential analysis, and X-ray Diffraction (XRD). Plant secondary metabolites play a crucial role in the reduction of silver ions to silver metal during the synthesis of AgNPs. These bio-reducing compounds not only facilitate nanoparticle formation but also impart functional properties, such as antioxidant activity, which may have significant applications in the food industry.

Material and Methods

Chemicals

α,α -Diphenyl- β -picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and silver nitrate (AgNO_3) were purchased from Sigma-Aldrich.

Biosynthesis of silver nanoparticles using *Maclura pomifera*

Maclura pomifera fruits were separated, dried, and ground into powder. A total of 100 g of the powder was placed in an Erlenmeyer flask, and 0.5 L of distilled water was added. The mixture was heated at 80 °C for two hours. After heating, the resulting solution was filtered to obtain the plant extract. A silver nitrate (AgNO_3) solution (2.5 g in 200 mL of distilled water) was added dropwise to the plant extract under continuous stirring, and the reaction mixture was refluxed at 70 °C for two hours. During the reaction, a color change from yellow to brown was observed, indicating the reduction of Ag^+ ions to metallic Ag^0 nanoparticles (Fig. 1). Upon completion, the synthesized Mp-AgNPs were collected by centrifugation at 5000 rpm for 20 minutes, washed thoroughly with distilled water, and subsequently dried by lyophilization [24].

Characterization methods

Surface morphology of Mp-AgNPs was examined by Quanta Feg450 Scanning Electron Microscopy (SEM), and ultraviolet–visible (UV–Vis) spectra were recorded with Hitachi U-2900 spectrophotometer. UV–Vis wavelength scanning was performed between 300 and 800 nm. XRD analysis was carried out by Malvern Panalytical diffractometer with HighScore Plus software. The elemental composition of Mp-AgNPs was determined by energy-dispersive X-ray spectroscopy (EDX). Zeta potential measurement was carried out by Zetasizer Nano ZSP (Malvern) instrument. Fourier transform infrared (FTIR) spectra were obtained by Jasco FT/IR 4700 spectrometer.

Antioxidant Activity

DPPH• free radical scavenging activity

The activities of the samples were evaluated following the method described by Sahin Yaglioglu et al. [25], and the results were expressed as the half maximal inhibitory concentration (IC_{50}).

ABTS•+ scavenging activity

The activities of the samples were assessed according to the methodology described by Guzel et al. [26], and the results were expressed as the half-maximal inhibitory concentration (IC_{50}).

Ferric reducing antioxidant power (FRAP)

The activities of the samples were assessed according to the method of Oyaizu [27] with some modifications as described by Sahin Yaglioglu et al. [25].

Statistical analysis

Statistical analyses were performed using SPSS version 11.05 for Windows with one-way ANOVA. Results are expressed as mean \pm standard deviation, and differences were considered statistically significant at $P < 0.05$.

Results and Discussion

MP-AgNPs were synthesized using an aqueous extract of *Maclura pomifera* fruit and a silver nitrate (AgNO_3) solution. The reduction of Ag^+ ions during the reaction was evidenced by a color change from light yellow to dark brown, confirming the formation of MP-AgNPs (Fig. 1).

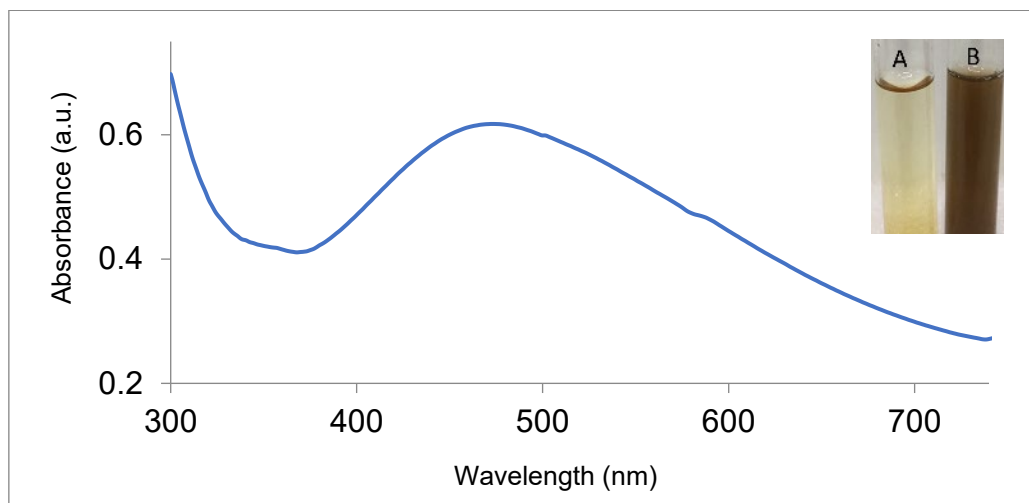


Fig. 1 UV-Vis spectrum of *Maclura pomifera* fruit extract (A) and *Maclura pomifera* fruit extract'silver nanoparticles (MP-AgNPs) (B)

A color change from light yellow to brown was observed, indicating the reduction of Ag^+ ions to Ag^0 . This change was confirmed by UV-Vis analysis, which showed a characteristic peak at 475 nm supported the successful synthesis of Mp-AgNPs (Fig. 1).

X-ray diffraction (XRD) analysis confirmed the crystalline nature of Mp-AgNPs (Fig. 2). The diffraction peaks observed at 2θ values of 38.25° , 44.42° , 64.59° , and 77.43° correspond to the (111), (200), (220), and (311) Bragg reflections, respectively, characteristic of the face-centered cubic (fcc) crystalline structure of silver (JCPDS No. 87-0720) [24]. The average crystallite size of Mp-AgNPs was calculated using the Debye–Scherrer formula, and the Rietveld refinement results are presented in Fig. 2. Crystallite sizes were estimated using the Scherrer equation ($D = K\lambda / \beta \cos \theta$), with a shape factor $K=0.90$ and Cu $K\alpha$ wavelength $\lambda=1.5406 \text{ \AA}$. Based on an FWHM value of approximately 0.234° (radians) for each peak, the calculated crystallite sizes are 35.91 nm, 36.65 nm, 40.14 nm, and 43.48 nm for the (111), (200), (220), and (311) planes, respectively, giving an average crystallite size of $\sim 39.02 \text{ nm}$. These results confirm the formation of well-crystalline silver nanoparticles with sizes consistent with XRD peak broadening.

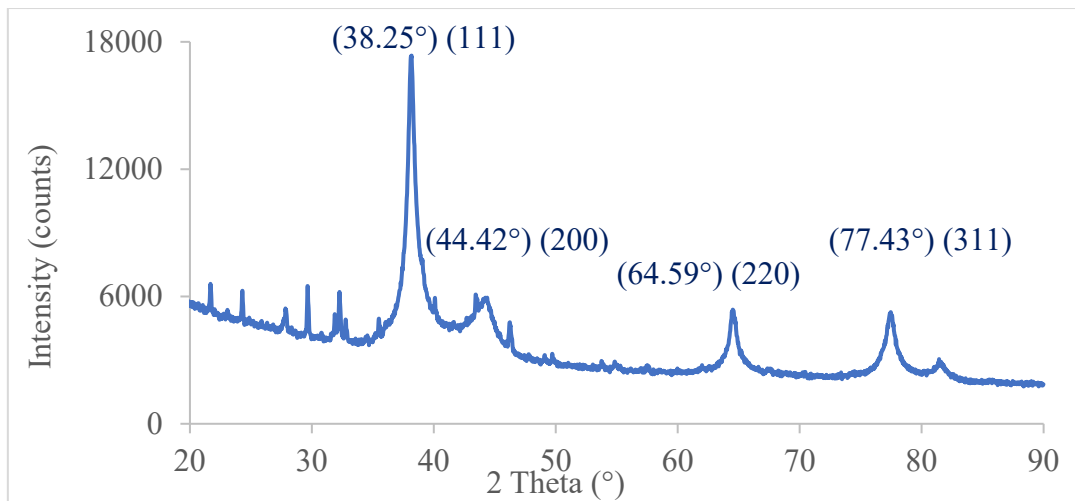


Fig. 2 XRD pattern spectrum of *Maclura pomifera* fruit silver nanoparticles (MP-AgNPs)

The energy-dispersive X-ray (EDX) spectrum and the quantitative elemental analysis of Mp-AgNPs are shown in Fig. 3 and Table 1, respectively.

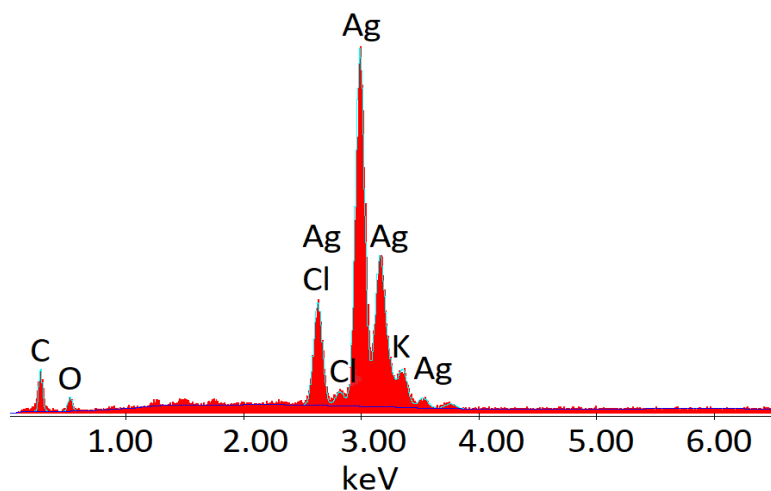


Fig. 3 EDX spectrum of *Maclura pomifera* fruit extract'silver nanoparticles (MP-AgNPs)

The morphology and size of MP-AgNPs were analyzed by Scanning Electron Microscopy (SEM) (Fig. 4), revealing an average particle size of 39.02 nm. The formation of Mp-AgNPs was further confirmed by energy-dispersive X-ray (EDX) analysis. As shown in Table 1, the elemental composition by mass was 22.72% carbon, 13.89% oxygen, and 57.74% silver.

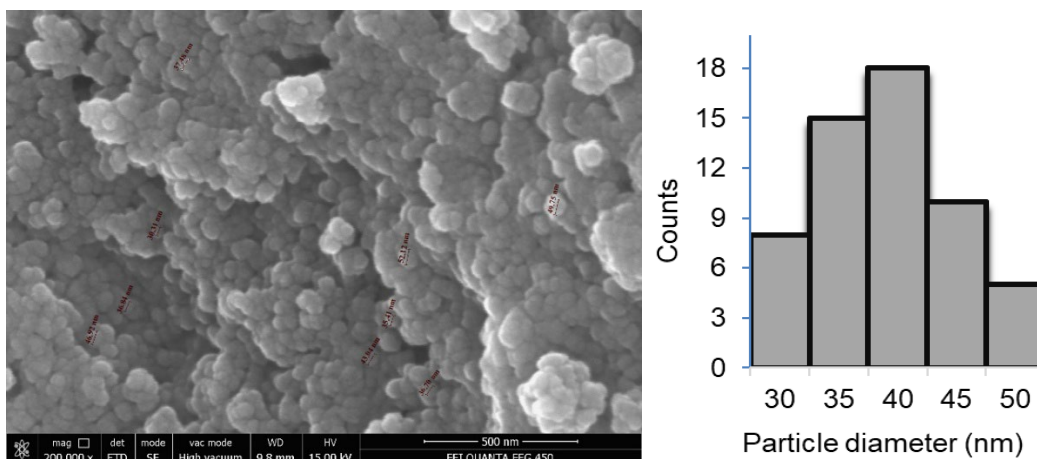


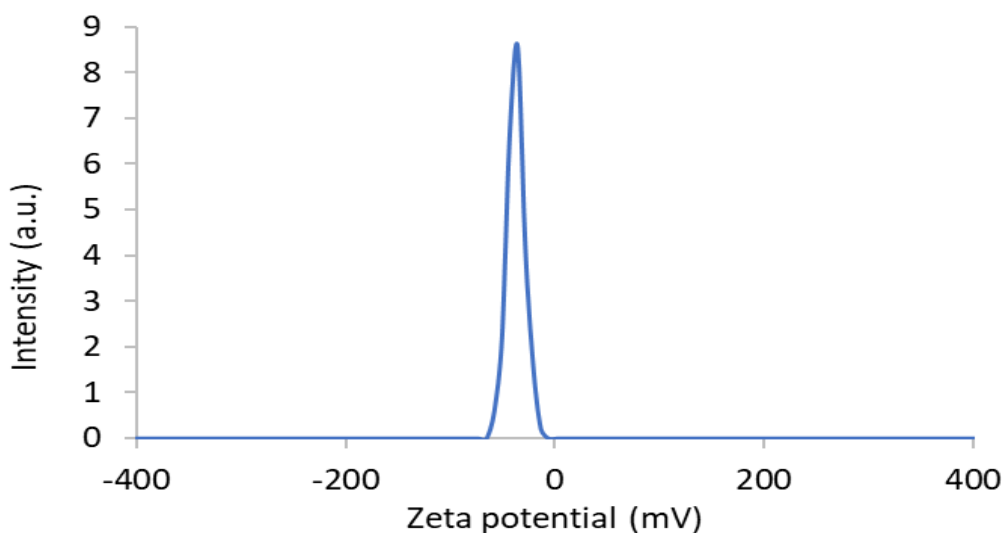
Fig. 4 SEM image of *Maclura pomifera* fruit extract'silver nanoparticles (MP-AgNPs) and particle size distribution.

Table 1 The EDX quantitative results (%) of *Maclura pomifera* fruit extract'silver nanoparticles (MP-AgNPs)

EDS Quantitative Results		
Element	Wt%	At%
CK	22.72	54.80
OK	13.89	25.15
CIK	5.34	4.34
AgL	57.74	15.50
KK	0.31	0.21

Zeta potential refers to the electric potential at the slipping plane between the particle surface's inner Helmholtz layer and the surrounding bulk fluid. It reflects the surface charge of particles and provides an indication of the colloidal system's stability. Several parameters—such as temperature, pH, reaction time, the concentration of the metal salt, and the volume of plant extract—substantially affect the green synthesis of nanoparticles. The interplay of these factors plays a decisive role in controlling the morphology and size of the resulting nanoparticles. Variations in plant extract composition can significantly influence the yield, size, morphology, distribution, and aggregation behavior of phyto-mediated nanoparticles (phyto-MNPs). Lower extract concentrations generally favor the formation of larger nanoparticles, whereas higher concentrations promote the synthesis of smaller, spherical, isotropic, well-dispersed, and aggregation-free phyto-MNPs [28]. While increasing the extract concentration can enhance AgNP production, agglomeration occurs beyond a certain threshold concentration. Agglomeration of nanoparticles can lead to a decrease in their functional properties and economic value [29].

Particles with high positive or negative zeta potential values repel each other, preventing aggregation. Thus, zeta potential provides insight into the surface charge-related properties of the nanoparticles. The zeta potential of MP-AgNPs was measured as -34.6 mV, indicating good nanoparticle stability due to electrostatic repulsion between particles (Fig. 5).

**Fig. 5** Zeta potential of *Maclura pomifera* fruit extract'silver nanoparticles (MP-AgNPs)

Fourier Transform Infrared (FTIR) analysis was conducted to identify the functional groups present in the aqueous extract of *Maclura pomifera* fruit (Fig. 6). These functional groups play crucial roles as reducing and/or stabilizing agents during nanoparticle synthesis. The characteristic transmittance peaks observed for both the fruit extract and MP-AgNPs appeared at 3244, 2915, 2112, 1723, 1636, 1515, 1292, 1139, 1010, and 812 cm^{-1} . The broad and intense absorption band at 3244 cm^{-1} corresponds to hydroxyl (-OH) groups, indicating the presence of phenolic compounds [30]. Peaks at 1723 and 1636 cm^{-1} were attributed to carbonyl groups. High-performance liquid chromatography (HPLC) analysis of *Maclura pomifera* extracts revealed the presence of phenolic acids such as

protocatechuic acid, p-hydroxybenzoic acid, and caffeic acid [23], as well as isoflavones including osajin and pomiferin [18, 21] The absorption peak at 2915 cm^{-1} corresponds to C–H stretching vibrations of aliphatic $-\text{CH}_3$ and $-\text{CH}_2$ groups [31].

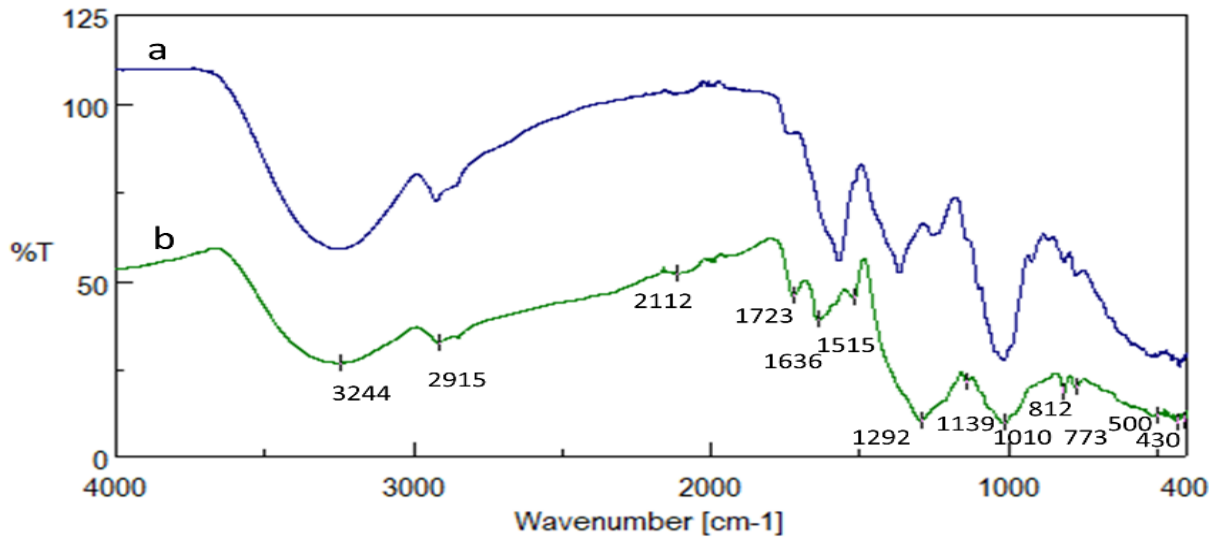
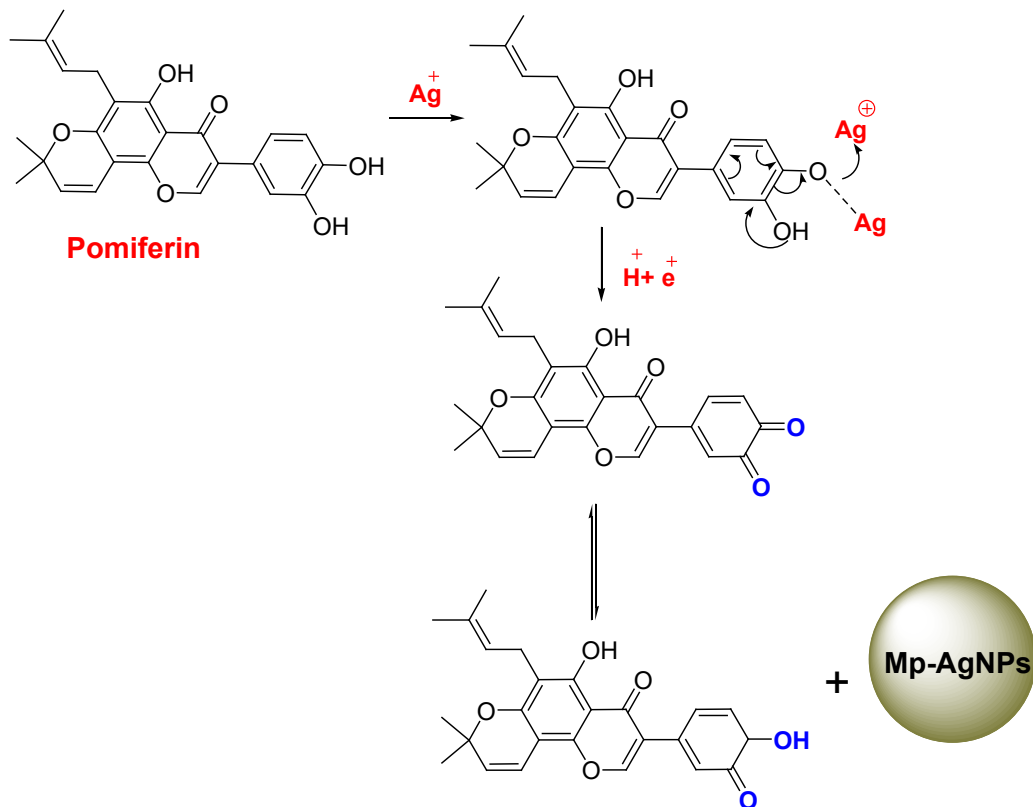


Fig. 6 FTIR spectrum of *Maclura pomifera* fruit extract (a) and *Maclura pomifera* fruit extract'silver nanoparticles (MP-AgNPs) (b)

Silver ions (Ag^+) are reduced by secondary metabolites present in *Maclura pomifera*, including isoflavones [17–18] and flavonoids [19]. The synthesis process involves sequential steps of reduction, aggregation, and growth of nanoparticles, ultimately leading to the formation of silver nanoparticles [32]. The proposed mechanism, adapted from Sadeghi-Kiakhani et al. [33] specifically for the pomiferin compound found in *Maclura pomifera* [21, 34], is illustrated in Scheme 1.



Scheme 1. Reasible reaction mechanism of synthesis of *Maclura pomifera* fruit extract'silver nanoparticles (MP-AgNPs)

The antioxidant activity results of the samples are presented in Fig. 7 and Table 2. In the DPPH• scavenging assay, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and Trolox were used as positive controls. The DPPH• scavenging activity of MP-AgNPs ($IC_{50} = 15.58 \pm 0.05 \mu\text{g/mL}$) was higher than that of the plant extract ($IC_{50} = 21.36 \pm 0.04 \mu\text{g/mL}$) and comparable to that of BHT ($IC_{50} = 10.96 \pm 0.02 \mu\text{g/mL}$). Similarly, in the ABTS assay using the same positive controls, the order of antioxidant activity was MP-AgNPs ($IC_{50} = 6.36 \pm 0.02 \mu\text{g/mL}$) > BHT ($IC_{50} = 7.27 \pm 0.00 \mu\text{g/mL}$) > plant extract ($IC_{50} = 10.58 \pm 0.01 \mu\text{g/mL}$).

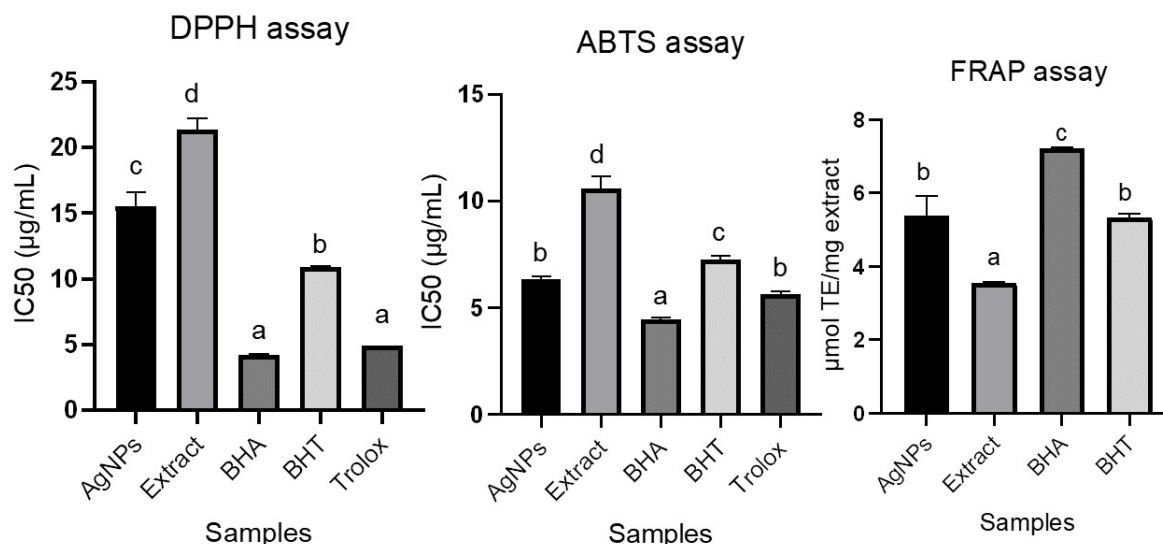


Fig. 7 Antioxidant activity of *Maclura pomifera* fruit silver nanoparticles (AgNPs) and *Maclura pomifera* fruit extract (Extract).

Table 2 The antioxidant activity results of *Maclura pomifera* fruit extract (Extract) and *Maclura pomifera* fruit silver nanoparticles (MP-AgNPs)

Samples name	DPPH ($\mu\text{g/mL}$)	ABTS ($\mu\text{g/mL}$)	FRAP ($\mu\text{mol TE/mg extract}$)
MP-AgNPs	15.58 ± 0.05^c	6.36 ± 0.02^b	5.39 ± 0.02^b
Extract	21.36 ± 0.04^d	10.58 ± 0.01^d	3.55 ± 0.00^a
BHA	4.26 ± 0.01^a	4.46 ± 0.00^a	7.21 ± 0.01^c
BHT	10.96 ± 0.02^b	7.27 ± 0.00^c	5.33 ± 0.01^b
Trolox	4.97 ± 0.03^a	5.65 ± 0.01^b	-

Note: The results were reported as mean values \pm SDs of three independent assays, means within columns followed by the same letter(s) are significantly different at $p < 0.05$ according to Duncan.

In the FRAP assay, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used as positive controls. The FRAP activities increased in the following order: plant extract ($3.55 \pm 0.00 \mu\text{mol TE/mg extract}$), BHT ($5.33 \pm 0.01 \mu\text{mol TE/mg extract}$), and MP-AgNPs ($5.39 \pm 0.02 \mu\text{mol TE/mg extract}$).

It is well established that the molecular structure plays a critical role in the antioxidant properties of compounds, with only certain phenolic structures containing hydroxyl groups capable of donating hydrogen atoms or electrons to neutralize free radicals [35]. Species of the genus *Maclura* are known to be rich in phenolic compounds, as reported for *Maclura cochinchinensis* [36–37], *Maclura tricuspidata* [38–39], *Maclura tinctoria* [40], and *Maclura pomifera* [21].

Several *Maclura* species have been employed in the green synthesis of metallic nanoparticles. For example, gold nanoparticles have been synthesized using the roots, stems, leaves, and fruits of

Maclura tricuspidata [41], while silver nanoparticles have been produced from the leaves of *Maclura pomifera* [42].

In the present study, silver nanoparticles (MP-AgNPs) were synthesized using the fruit extract of *Maclura pomifera*, and the antioxidant activities of both the extract and the synthesized nanoparticles were evaluated. To the best of our knowledge, this is the first report investigating the antioxidant potential of *Maclura pomifera* fruit extract and its corresponding AgNPs. Our findings suggest that both the extract and Mp-AgNPs have potential applications in biomedicine and nanotechnology.

The properties of different plant-based silver nanoparticles (e.g., particle size, zeta potential, IC₅₀ values) and those of the synthesized MP-AgNPs are presented comparatively in Table 3.

Table 3. Comparison of plant-based synthesized silver nanoparticles

Plant name	Nanoparticle Property				
	Particle size (nm)	Zeta (mV)	DPPH (IC ₅₀) (μmol)	ABTS (μmol)	FRAP (μmol TE/mg extract)
Mp-AgNPS	39.02	-34.6	15.58	6.36	5.39
Cakile maritima- seed	9.45 to 17.15	-37.7	-	-	-
Cassia occidentalis L.- seed	19.78	-	345	-	-
Ziziphora clinopodioides-leaves	below 100	-	22	-	-
Cucurbita spp- peel	81	-9.96	-	-	-
Thymus vulgaris- whole plant	44.6	-7.46	-	-	-
Tagetes erecta - flower	72.6	+0.719	-	-	-
Morus nigra L. - whole plant	170.17	-56.6	-	-	-
Moringa oleifera- leaves	26.19	-	-	-	-
Cichorium intybus- leaves	79	-38.2	-	-	-
Martynia annua L.- root	64	-21.6	66.71	-	84.64
Aerva lanata- flower	45.05	-	70.79	-	-
Hibiscus tiliaceus L.- leaves	25	-49	-	-	-

When Table 3 is examined, it can be observed that the synthesized MP-AgNPs fall within the average ranges reported in the literature in terms of particle size and zeta potential, and exhibit higher antioxidant activity compared to other plant-based nanoparticles in the literature [43–44].

Conclusion

The green synthesis of nanoparticles using plant extracts offers several advantages, including simplicity, accessibility, non-toxicity, environmental friendliness, and low production cost. In this study, silver nanoparticles (MP-AgNPs) were successfully synthesized using *M. pomifera* fruit extract. The formation of nanoparticles was confirmed by UV-Vis, SEM, EDX, FTIR, XRD, and zeta potential analyses. The MP-AgNPs exhibited a spherical morphology with an average particle size of 39.02 nm and a zeta potential of -34.6 mV, indicating good colloidal stability.

The antioxidant activities of both the *M. pomifera* extract and MP-AgNPs were evaluated using DPPH, ABTS, and FRAP assays. In all tests, MP-AgNPs demonstrated superior antioxidant capacity compared to the extract and showed comparable or better activity than synthetic standards such as BHT and BHA. These results suggest a synergistic enhancement of antioxidant potential due to the nanoparticle synthesis process.

To the best of our knowledge, this is the first study to report the antioxidant properties of silver nanoparticles synthesized from *Maclura pomifera* fruit extract. Given their effective antioxidant activity and eco-friendly synthesis method, MP-AgNPs hold significant promise for potential applications in biomedicine, pharmaceuticals, and nanotechnology.

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