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Eco-friendly biosynthesis of manganese oxide-silver bimetallic nanoparticles using *Cucumis melo* peel extract: characterization, antioxidant, antimicrobial, and antiviral activities

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Abstract

The employment of plant extracts for green production of bimetallic nanoparticles (BNPs) has gotten significant consideration because of its cheap, ecological, single-step, and easily scalable procedures. This methodology enables the manufacture of biocompatible nanoparticles (NPs) with improved activity. In this study, an environmentally friendly approach was utilized to biosynthesize manganese oxide-silver BNPs (MnO-Ag BNPs) using *Cucumis melo* (*C. melo*) peel extract (CPE), which served as the source of the required reducing and stabilizing materials. Several spectroscopic analytical methods, including ultraviolet-visible (UV-vis) spectroscopy, Fourier transform infrared (FTIR) spectroscopy, energy-dispersive X-ray (EDX) spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), and transmission electron microscopy (TEM), were applied for careful confirmation and characterization of successful MnO-Ag BNPs assembly. This work introduces a novel green route employing CPE for MnO-Ag BNPs synthesis, providing distinct phytochemical efficiency and multifunctional bioactivity compared with previously reported plant-based systems. The biosynthesized MnO-Ag BNPs bacterial inhibitory capability as well as free radical scavenging effect were evaluated. Also, human kidney normal epithelial-derived cells (Vero cell line CCL-81) was employed for assessment of the cytotoxic outcome of MnO-Ag BNPs at various concentrations. Regarding the elemental composition, the manganese (Mn) and Ag contents were detected by the UV-vis, XRD, and EDX studies with consequent validation of MnO-Ag BNPs biosynthesis. The range of the assessed BNPs size was 2 to 10 nm with average diameter of 5.8 ± 1.7 nm and an average area of 22.7 nm^2 . Analysis based on EDX technique revealed the presence of Mn and Ag metals with 23.7–46.6% of the atomic percentages and 32.2–28.0% of the weight percentages, respectively. The biosynthesized NPs showed strong free radical scavenging, achieving 85–90% inhibition at higher concentrations. The cytotoxic activity

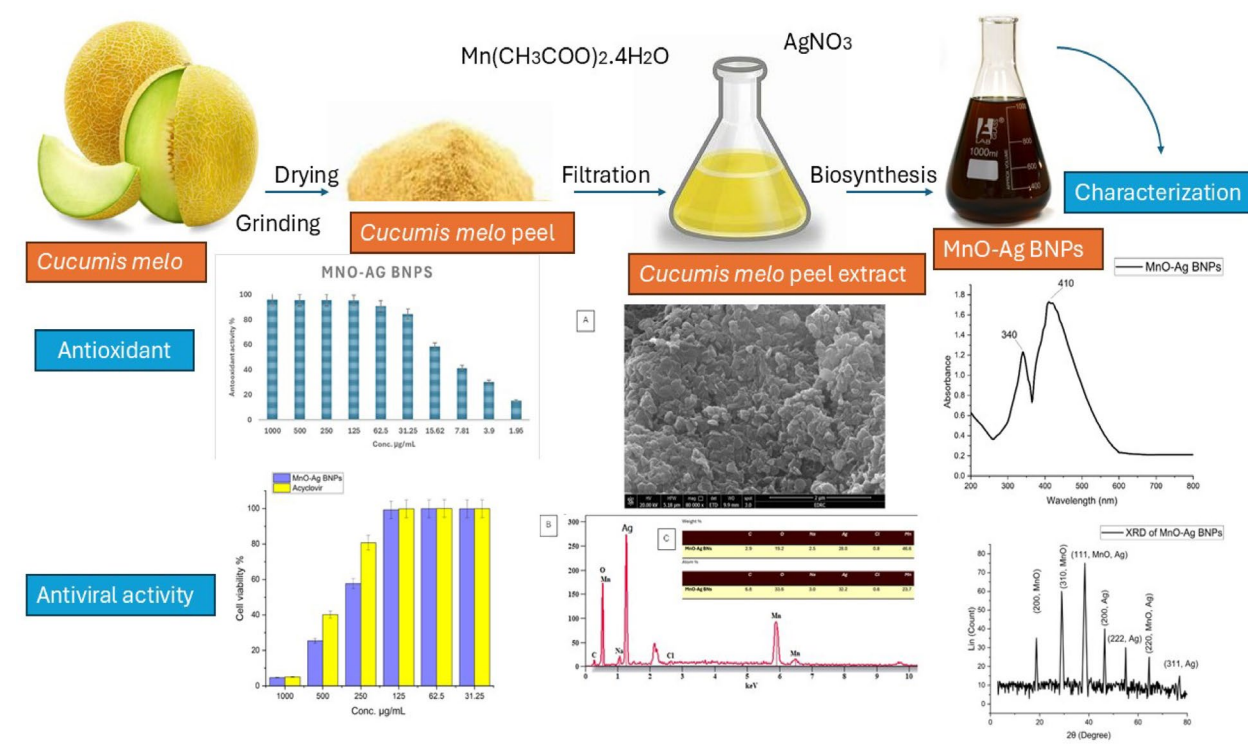
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findings indicated no significant harmful effects, at concentration range of 31.25–250 µg/mL, on Vero cell line. Additionally, the viability of the tested cell line infected with herpes simplex virus type-1 (HSV-1) significantly increased from 43% (untreated) to 78–99% when treated with 125 µg/mL MnO–Ag BNPs and acyclovir, respectively. Moreover, the inhibition rates achieved against the tested virus were 73% for MnO–Ag BNPs and 99% for acyclovir. These outcomes highlight the potential of MnO–Ag BNPs as promising candidates for biomedical and antiviral applications.

Keywords Bimetallic nanoparticles, Cost-effective, *Cucumis melo* peel extract, Antimicrobial and antioxidant capabilities, Cytotoxic activity, Antiviral Activities

Graphical abstract



Introduction

Nanotechnology has revolutionized modern science, providing innovative solutions in medicine, materials science, and environmental remediation (Tabrez et al. 2022a). Among various applications of nanomaterials (NMs), their biological potential particularly antioxidant and antiviral activities has received increasing attention (Bhattacharyya et al. 2023). However, monometallic NPs (MNPs) often suffer from drawbacks such as high surface energy, oxidation susceptibility, and limited reusability, which restrict their broad applicability (Crawley et al. 2022). Recent research aimed to overcome such drawbacks has focused on the development of BNPs which involve the integration of two metals within a single nanostructure to achieve enhanced physicochemical and biological properties. These BNPs exhibit superior catalytic, antimicrobial, and antioxidant characters compared with monometallic equivalents which resulted

from their synergistic interactions (Idris and Roy 2023). For instance, Ag–copper and gold (Ag)–palladium BNPs demonstrate improved stability and reactivity, leading to promising results in biomedical and environmental applications (Nanda and Yi 2024). The combination of Ag's strong antimicrobial properties with Mn's potent antioxidant and catalytic activity creates a multifunctional system suitable for combating oxidative stress (OS), infections, and environmental pollutants (Ahmad et al. 2025). Furthermore, MnO–Ag BNPs have shown potential in catalysis, sensing, and energy storage, benefiting from Ag's optical properties and Mn's redox characteristic (Ipadeola et al. 2024).

Integrating insights from materials science, chemistry, and biology, nanotechnology offers a unique platform to address biomedical and ecological challenges (Malik et al. 2023a). Nanoparticles are particularly efficient in reactive oxygen species (ROS) scavenging with

consequent reduction of OS, processes strictly associated with chronic diseases and cellular damage (Patel et al. 2024). Traditional antioxidants, though effective, often suffer from instability and poor bioavailability (Demirci-Çekiç et al. 2022). In contrast, NPs provide superior stability, controlled release, and enhanced bio-interaction, making them attractive alternatives for biomedical use (Liu et al. 2023). Moreover, the antiviral activity of NPs through disruption of viral envelopes, inhibition of replication, and modulation of immune responses has further broadened their therapeutic potential (Ahmed et al. 2025; Bhatti and DeLong 2023). Conventional physical and chemical methods for NPs synthesis, while effective, based on hazardous reagents, high cost, with environmental and health risks (Kirubakaran et al. 2026). Conversely, biosynthesis has emerged as an ecological and sustainable alternative that utilizes plant-derived materials with high phytochemical constituents, including phenolics, alkaloids, and flavonoids, that employed for natural reduction and stabilization of the produced NPs (Edo et al. 2025). This method not only minimizes toxicity but also provides NPs with improved stability and biocompatibility and consequently appropriate for various medical applications (Raudone and Savickiene 2024).

Plant-mediated synthesis has been extensively explored for fabrication of Ag NPs with extraordinary bioactivity and stability when combined with natural or polymeric stabilizers (Shahzadi et al. 2025). Recent examples include the use of *Equisetum diffusum* extract for mercury detection and cellulose-based compounds for Ag NPs stabilization (Jabbar et al. 2023). (Alserihi et al. 2023) inspected the therapeutic potential of epigallocatechin gallate (EGCG) and its nano-formulation for prostatic cancer management. Such study focus the increased prominence of green nanotechnology for development of cheap, biocompatible, as well as sustainable NMs. Different plant parts including leaves, seeds, peels, and stems have been utilized as bio-factories in NPs biosynthesis (Kirubakaran et al. 2026). *Cucumis melo* L. (muskmelon), a member of the *Cucurbitaceae* family, is well known for its phytochemical richness and pharmacological properties, including antimicrobial, antiviral, antidiabetic, and antioxidant activities (Yadav et al. 2025). Accordingly, CPE may represent a natural, renewable, and eco-friendly source for the biosynthesis of BNPs, offering a sustainable route for NPs production. Theoretical models such as classical nucleation theory and the synergistic effect model provide valuable insights into the mechanisms underlying BNPs formation, explaining their enhanced reactivity and stability. Furthermore, NPs morphology, size, and surface chemistry interpreted through quantum and ligand exchange theories are key determinants of their biological behavior (Tabrez et al. 2022b; Zhdanov 2025).

In this context, the current research aimed for development of a novel, environmentally benign, and economically viable synthetic procedures for MnO–Ag BNPs bio-fabrication using CPE. The obtained BNPs were comprehensively characterized based on several analytical techniques. Also, their biological activities including antioxidant, antibacterial, and antiviral potentials were systematically evaluated to explore their biomedical relevance.

Materials and methods

Materials

All of the compounds were analytical grade with no additional purification requirement. All subsequent experimental studies, including the production of MnO–Ag BNPs, employed deionized water (dH₂O). *Cucumis melo* was purchased at the Carrefour local market during the summer season in July, as the time of collection is a well-known factor influencing its constituents. Silver nitrate (AgNO₃, 98%), manganese acetate tetrahydrate (Mn(CH₃COO)₂·4H₂O, 99%), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were the product of Sigma Aldrich, Cairo, Egypt. Additionally, the tested normal cell line and viral strain were obtained from VACSERA, Cairo, Egypt.

Preparation of CPE

Fresh fruits were collected and peeled in order to make fresh CPE. After thoroughly cleaning the peels with tap water, they were then rinsed using dH₂O. The fresh peels weighing 500 gm were air-dried at room temperature under indirect sunlight. To obtain the fresh extract, the *C. melo* peels were extracted in dH₂O at 70 °C for 50 min. The extract was filtered using Whatman filters to remove any remaining peel pieces. The final powder obtained from the dried peels weighed 80 gm, resulting in a fresh plant-to-powder ratio of 6.25:1. The CPE was immediately placed in dark and cool until the beginning of MnO–Ag BNPs fabrication (Khan et al. 2025).

Synthesis of MnO–Ag BNPs

Thirty milliliters of Mn(CH₃COO)₂·4H₂O and 10 mL of AgNO₃ (3.0 mM), were mixed together and stirred for about 30 min at room temperature followed by addition of the prepared CPE (70 ml). When we checked the pH of the mixed solution after it had been prepared, we found that it was 10.0. For optimal synthesis of MnO–Ag BNPs, we fixed the reaction parameters at agitation (250 rpm) in a shaking incubator for about 6 h, incubation was performed at 35 °C. The weakly bound biomolecules were extracted from the peels by washing the resulting MnO–Ag BNPs 5 times with dH₂O. Centrifugation was then used for approximately 5 min at 10,000 rpm to further clean the BNPs. The resulting NPs were carefully

oven-dried at 50 °C after being cleaned with dH₂O (Chen et al. 2025).

Characterization of MnO–Ag BNPs

Different analytical procedures were employed for determination of the obtained BNPs' size and shape. A UV–vis Spectrophotometer (JENWAY 6305 Spectrophotometer, Staffordshire, UK) was utilized to record absorption spectra in the 200–800 nm range at room temperature. For FTIR analysis, the Cary 630 model was employed. The XRD (XRD–6000, Shimadzu Scientific Instruments, Japan) was employed for examination of phase structure of the BNPs using CuK α radiation (λ = 0.154 nm) with a 2 θ range of 10° to 90°.

Moreover, MnO–Ag BNPs surface morphology was studied based on SEM imaging (SEM, ZEISS, EVO–MA10, Germany). The composition, purity, and elemental distribution of the biogenic MnO–Ag BNPs were examined using EDX (Bruker, Germany). Likewise, the average and exact sizes of the produced MnO–Ag BNPs were determined using TEM (JEM–2100 Plus, JEOL, Japan). The DLS analysis (Malvern, UK) with the Zetasizer Nano Series (Nano ZS) was applied to decide the sizes and distribution of MnO–Ag BNPs in the examined colloidal suspension (Bekmezci et al. 2023).

Antibacterial activity

The biosynthesized MnO–Ag BNPs were screened against 5 microbial strains: *Bacillus subtilis* (*B. subtilis*) ATCC 6633, *Staphylococcus aureus* (*S. aureus*) ATCC 6538, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 9027, *Escherichia coli* (*E. coli*) ATCC 25922, and *Candida albicans* (*C. albicans*) ATCC 10231. With the help of an agar diffusion method, each investigated pure microbial strain grown in Mueller–Hinton broth was equally spread on sterile petri plates containing Mueller–Hinton agar. Sterilized cork borer used to make 4 circular holes of 7 mm diameter in each plate. The inhibitory effects of the MnO–Ag BNPs (200 μ g/mL), manganese acetate tetrahydrate (200 μ g/mL), silver nitrate (200 μ g/mL), and standard antimicrobial agents [ampicillin/sulbactam (200 μ g/mL) in case of bacterial species and nystatin (200 μ g/mL) in case of fungal species] were tested by adding 100 μ L of each tested solution into separate well. The Mn and Ag monometallic precursors were used as comparative controls to determine the synergistic antibacterial effect of the MnO–Ag BNPs. Consequently, all plates were incubated for 24 h at 37 °C for bacteria and for 72 h at 30 °C for fungi followed by measurement of each treatment's inhibitory zone diameter (IZD). Also, evaluation of the minimum inhibitory concentration (MIC) against microbial strains was carried out using different concentrations of MnO–Ag BNPs, ampicillin/sulbactam, and nystatin ranged from 200 to 3.12 μ g/mL (Hashem et al. 2023a). All

assays were performed in triplicate and the outcomes are expressed as the mean $\pm$ standard deviation (mean $\pm$ SD). Using specialized software (SPSS version 21), one-way ANOVA, Duncan's multiple series, and the least significant difference (LSD) were used to statistically analyze the inhibitory findings.

DPPH radical scavenging capacity

The DPPH radical scavenging assay was conducted to quantify MnO–Ag BNPs antiradical potential. Different concentrations of bio-synthesized MnO–Ag BNPs, Mn salt, Ag salt, CPE, and ascorbic acid standard (1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9 and 1.95 μ g/mL), were employed to assess DPPH radical scavenging capacity. The preparation of DPPH solution and experimental procedure were conducted following the protocol described by (Hasanin et al. 2022).

Using 95% ethanol, a DPPH radical solution (1 mM) was made. Each tested concentration (200 μ L) was mixed with 800 μ L of DPPH solution with well shaken followed by incubation in dark for 30 min at 25 °C. Centrifugation was then carried out for 5 min at 13,000 rpm. Each concentration's absorbance (A), at 517 nm, was measured in comparison to the ascorbic acid standard and untreated experiment as a blank. All assays were performed in triplicate and the experiment findings showed the DPPH inhibition percentage was calculated according to Eq. (1)

$$\text{Antioxidant activity (\%)} = \frac{\text{Absorbance for ascorbic acid} - \text{Absorbance for tested sample}}{\text{Absorbance for ascorbic acid}} \times 100 \quad (1)$$

Cytotoxic activity

The effectiveness of acyclovir and MnO–Ag BNPs on Vero cell line was determined following MTT assay. The ecologically synthesized MnO–Ag BNPs and acyclovir maximum non-toxic concentration (MNTC) were measured using normal Vero cells CCL–81 (as host cells) by implementing them into a 96–well plate. In this procedure, 100 μ L of growth medium containing 10⁴ Vero cells/well was incubated in a CO₂ incubator (5%) at 37 °C for 24 h until formation of confluent cell sheet. After discarding the excess medium, the attached Vero cells were exposed to serially diluted concentrations of MnO–Ag BNPs (1000 to 31.25 μ g/mL). Acyclovir was treated under the same conditions. All tests were performed in triplicate using dimethyl sulfoxide (DMSO) as the solvent. Untreated cells were employed as the negative control, while acyclovir-treated cells were used as the positive control. After 48 h of incubation at 37 °C, the plates were examined under an inverted microscope for cytopathic effects, including monolayer rupture, cell granulation, rounding, and shrinkage. Subsequently, 20 μ L of MTT solution (5 mg/mL) was added to each well

with constant shaking at 150 rpm for 5 min followed by incubation at 37 °C for 5 h. Following the disposal of the well contents, DMSO (200 µL) was used to dissolve the formed MTT metabolic product, namely formazan crystals (Abdel-Rahman et al. 2024). An ELISA reader was utilized to determine the absorbance at 570 nm. Cell viability was then calculated following Eq. (2). On the other hand, Eq. (3) was applied to calculate the cell toxicity percentage.

$$\text{Cell viability (\%)} = \frac{\text{Treated cells absorbance}}{\text{Untreated cells absorbance}} \times 100 \quad (2)$$

$$\text{Inhibition \%} = 100 - \text{Viability \%} \quad (3)$$

Furthermore, using regression analysis, CC_{50} (the concentration of the BNPs that inhibits 50% of Vero cell growth in comparison to untreated cells) was determined. Experiments were conducted in triplicate, and statistical significance was determined using one-way ANOVA ($P < 0.05$).

Antiviral assay

The antiviral activity of the MnO–Ag BNPs and acyclovir was assessed against HSV–1 using the MTT cell viability assay. Vero cells were seeded in 96–well plates at a density of 1×10^4 cells/well followed by overnight incubation at 37 °C in a humidified atmosphere containing 5% CO_2 for permission of the tested cell attachment. The tested concentrations of MnO–Ag BNPs and acyclovir were selected based on preliminary cytotoxicity assays, where non-lethal doses maintaining more than 80% Vero cell viability were considered safe. This concentration range was further used to establish the antiviral dose–response curve, consistent with previously reported NPs–based antiviral studies. A non-lethal concentration of the tested BNPs was mixed with the viral suspension at a ratio of 1:1 (v/v) and incubated at 35 °C for 1 h. Subsequently, 100 µL of the virus–sample mixture was inoculated to each well with gentle plates shaken for 5 min at 150 rpm to ensure even distribution followed by incubation at 37 °C in presence of 5% CO_2 for 24–48 h to allow viral infection. After that, 20 µL of MTT solution (5 mg/mL in phosphate buffered saline) was inoculated into each well followed by incubation for about 3 h which allow the formation of formazan crystals. Careful removal of the medium was then was performed followed by solubilization of the resulting crystals in 200 µL of DMSO with gentle shaking for 5 min at 150 rpm. The optical density (OD) of each well was measured at 560 nm using a microplate reader, with background correction at 620 nm (Alshallash et al. 2024).

Consequently, the viability of the tested cell was measured relative to the untreated controls to determine the

antiviral efficacy of the tested samples. Control wells containing only cells (non-infected) and virus-infected cells without treatment were included in each experiment. All treatments were performed in triplicate to ensure reproducibility. The assay procedure was adapted from (Alshallash et al. 2024), with slight modifications. The antiviral activity of MnO–Ag BNPs against HSV–1 was evaluated via determination of inhibition percentage using Eq. 4.

$$\begin{aligned} \text{Inhibition (\%)} \\ = [1 - (\text{OD (infected + treated)} / \text{OD (infected)})] \times 100 \end{aligned} \quad (4)$$

Different concentrations of MnO–Ag BNPs and acyclovir were used to generate dose–response curves. The concentration that achieved 50% viral inhibition (IC_{50} or EC_{50}) was determined using regression analysis performed in SPSS version 21 (IBM Corp., Armonk, NY, USA).

Statistical analysis

The mean $\pm$ SD was used for all triplicated findings expression. Statistical analyses were conducted using SPSS software, version 21 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed to determine significant differences among groups, with $P < 0.05$ considered statistically significant.

Results and discussion

Biosynthesis of MnO–Ag BNPs using CPE

The biosynthesis of BNPs utilizing plant extracts offers numerous advantages compared to microbial methods. These benefits include being cost-efficient, reducing the risk of contamination, enabling rapid synthesis, utilizing a straightforward single-step process, and ensuring safe handling. Furthermore, the process is environmentally friendly, suitable for large-scale production, requires minimal laboratory infrastructure, and results in NPs with enhanced stability resulted from the presence of abundance plant metabolites (Saleh and Hassan 2023). Plant-derived secondary metabolites, including alkaloids, phenols, flavonoids, proteins, carbohydrates, tannins, and terpenoids, not only aid in reducing and stabilizing NPs but also enhance their antimicrobial and antiviral properties (Dlamini et al. 2023). This eco-friendly and efficient method holds significant ability to synthesizing both single-metal and BNPs for various biomedical applications.

The present work investigates the biosynthesized MnO–Ag BNPs using CPE. The active metabolites in this extract demonstrated fundamental role in reduction of silver nitrate and manganese acetate. Throughout the synthetic process, these metabolites also contributed to BNPs capping and stabilization. The peel extract was

adopted a green method for BNPs biosynthesis, which reflects key advantages over conventional methods that rely on toxic chemicals or organic solvents (Edo et al. 2025). In recent years, melon peel extract has become a popular source in environmental and nanotechnology research, particularly for the sustainable synthesis of NPs (Ali et al. 2024). The use of CPE helps for reduction of environmental impact associated with traditional NPs synthesis processes, as the extract serves as both a reducing and stabilizing agent, thereby minimizing chemical waste and enhancing the sustainability of the manufacturing process (Shameem and Ahmad 2025). Similarly, (Al-Hamoud et al. 2025) successfully obtained Ag–Mn BNPs utilizing *Euphorbia cactus* extract, while (Alshallash et al. 2024) utilized *Zingiber officinale* to mediate the biosynthesis of Au/Ag nanoalloys. Malik et al. (Malik et al. 2023b) showed biogenic synthesis of Ag–Cu BNPs using *Salvia officinalis* plant extract. Furthermore, a green chemistry approach was utilized by (Khormi et al. 2025) to simultaneously synthesize Ag–Se BNs using an extract from *Salvia hispanica* seeds. (Ali and Abdul Kareem 2024) explored biosynthesis of MnO_2 NPs using leaf extracts of *Punica granatum* and in vitro evaluation of their biological applications. Additionally, Ag NPs have been synthesized from CPE by (Rani et al. 2023), further emphasizing the versatility of plant-based NPs synthesis.

Characterization

UV–vis spectroscopy

The biosynthesized BNPs were initially characterized using a UV–Visible spectrophotometer. A notable color change to pale brown in the aqueous CPE, upon mixing with $\text{Mn}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ and AgNO_3 , served as the first visual indicator of MnO–Ag BNPs synthesis. This color change confirmed the role of metabolites in metal ions reduction and promoting nanoscale structures formation. The solution's absorbance was analyzed within the 200 to 800 nm wavelength range for identification of maximum surface plasmon resonance (SPR). As shown in Fig. 1, the MnO–Ag BNPs exhibit two separate absorption peaks at about 340 nm and 410 nm, corresponding to MnO and Ag NPs, respectively. These peaks are indicative of the successful synthesis of phase-separated Mn and Ag NPs, with each metal contributing to the observed plasmon resonance at their characteristic wavelengths.

Similar observations have been documented in earlier studies. For example, (Alshallash et al. 2024) identified two prominent SPR peaks at 440 nm and 545 nm, attributed to Ag and Au NPs in the bimetallic Au/Ag nanoalloys. For instance, Ag-doped MnO_2 NPs exhibited an SPR peak at 410 nm, while undoped MnO_2 NPs showed a peak at 348.0 nm, as reported by (Ali and Abdul Kareem 2024). Similarly, (Malik et al. 2023b) noted

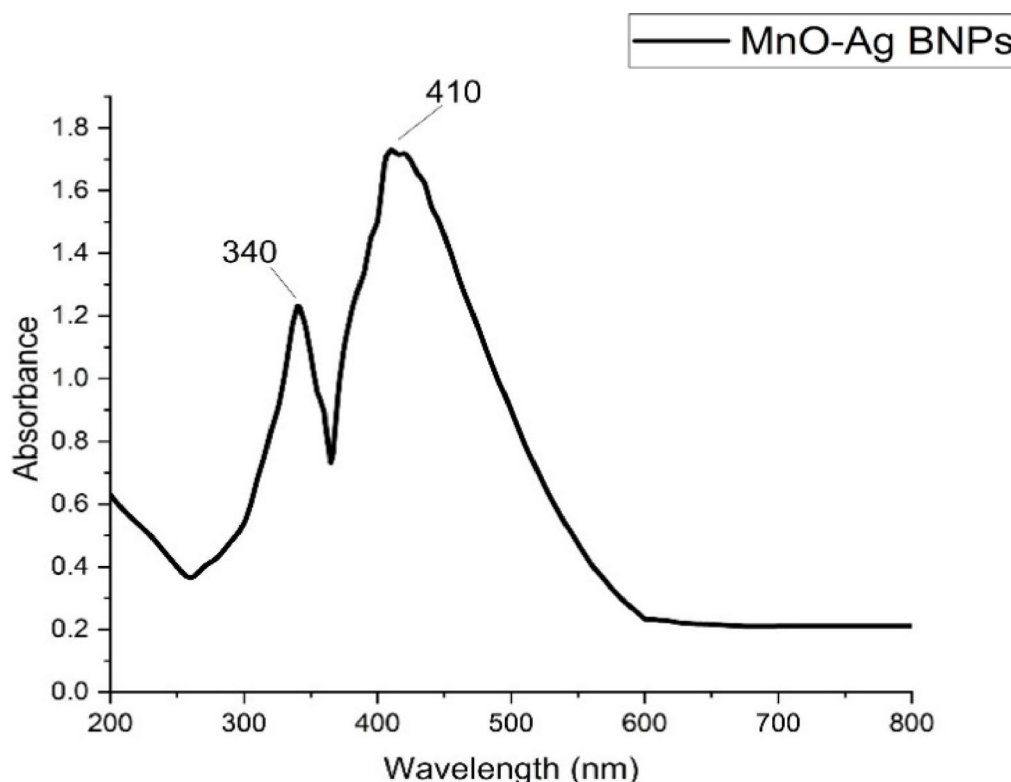


Fig. 1 UV–vis spectra indicated the successful MnO–Ag BNPs biosynthesis using CPE based on the detection of SPR characteristic peaks

an absorption peak at 409 nm for biosynthesized Ag–Cu NPs. In another study Aly Khalil et al. (Aly Khalil et al. 2024) identified an SPR band at 238 and 374 nm for Se–Au BNPs derived from *Pluchea indica* leaves. Moreover, (Hassanisaadi et al. 2022) observed a prominent SPR peak at 440 nm for Ag NPs synthesized using *Aloysia citrodora* leaf extract, while Losetty et al. (Losetty et al. 2024) reported SPR bands in the range of 350–500 nm for Ag NPs obtained from *Malachra alceifolia* (wild okra). In the case of NPs synthesized using *Ziziphus spina-Christi* aqueous leaves extract, (Zohra et al. 2023) recorded the absorption spectra of the Zn/Mn₂O₄ NC showed a lower intensity absorbance band around 282 nm, in contrast to the absorption edge of pure MnO₂ NPs at 268 nm.

For MnO NPs, an absorption maximum was detected around 410 nm in the visible region (Saod et al. 2022). In contrast, MnO₂ NPs showed broad absorption features spanning from 292 to 368 nm (Dileepan and Jeyaram 2025). The SPR characteristics observed in UV–vis spectroscopy offer valuable information regarding NPs morphology. Variations in peak intensity and wavelength position are often indicative of differences in particle size and geometry; smaller particles generally lead to red-shifted peaks, whereas larger ones may cause a blue-shift. Beyond confirming NPs formation, UV–vis spectral analysis plays a crucial role in predicting their functional properties (Ahmad 2024). Notably, the SPR behavior is strongly associated with the NPs' performance in areas such as catalysis, antimicrobial action, and optical

applications across environmental and biomedical fields (Arif et al. 2024). According to (Selim et al. 2025a), UV–vis spectroscopy analysis of the biosynthesized MgO–ZnO nanocomposite revealed a characteristic absorbance peak at 300 nm, indicating successful formation of NMs by using *Pluchea indica* leaf extract. Elkady et al. (Elkady 2025) confirmed the successful synthesis of CuO–Se BNPs using *Lagenaria siceraria* leaf extract through the appearance of distinct SPR bands in the UV–vis spectra. The SPR peak was observed at a maximum wavelength of 330 nm, indicating NPs formation.

TEM, FTIR, and DLS analysis

The TEM serves as a powerful tool for NPs morphological characterization, offering high-resolution insights into their size, shape, and degree of aggregation. In this study, the TEM images demonstrated the capability of CPE to synthesize spherical MnO–Ag BNPs with diameter range of 2–10 nm (Fig. 2A). Analysis of particle size distribution revealed a mean diameter of 5.8 ± 1.7 nm and an estimated average surface area of 22.7 nm², reflecting the consistency and precision of the synthesis method (Fig. 2B,C). The TEM images clearly show that the MnO–Ag BNPs exhibit excellent dispersion, with no observable aggregation, a crucial parameter for NPs stability and performance in different applications. The well-dispersed nature of the BNPs is indicative of the effectiveness of the CPE in stabilizing the BNPs during the biosynthesis process. This absence of aggregation enhances the potential

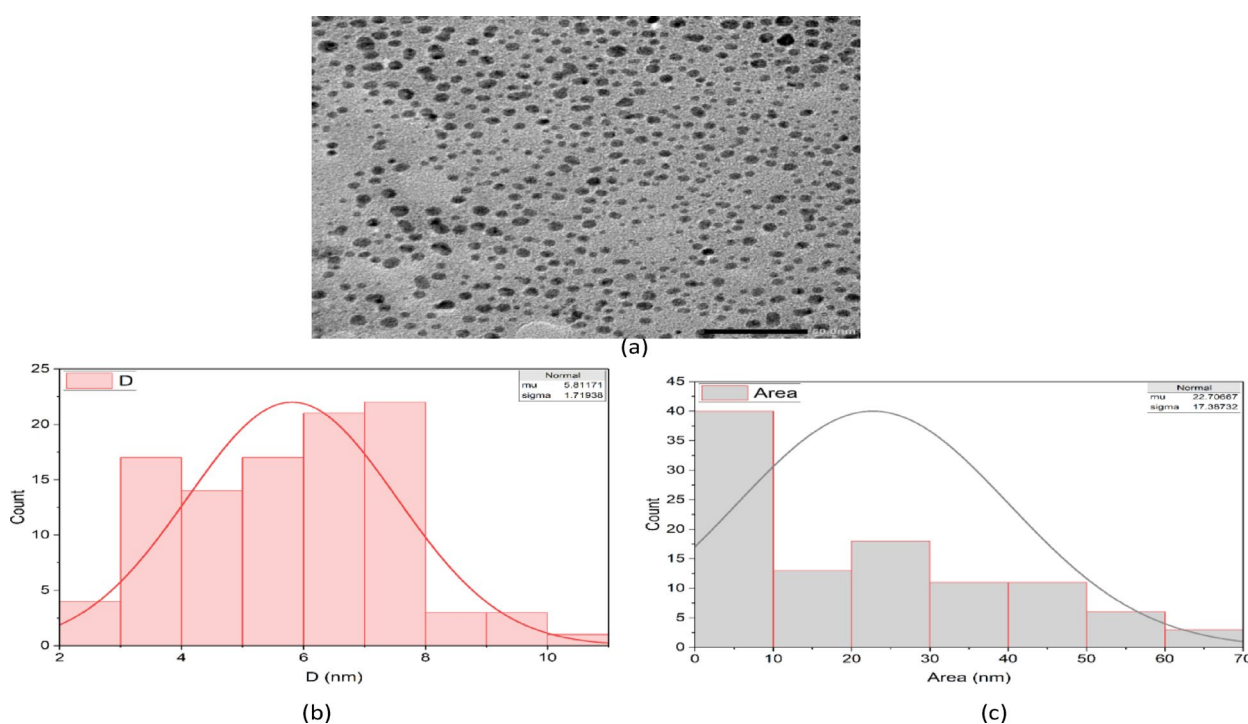


Fig. 2 TEM image (a), size distribution (b), and surface area distribution (c) of the biosynthesized MnO–Ag BNPs

for these BNPs to be used in systems where uniformity and stability are key (Lashari et al. 2022). The combination of MnO and Ag in the BNPs leads to unique properties, such as improved catalytic activity, enhanced stability, and synergistic effects, which are often superior to those observed in MNPs (Liu and Corma 2023). These characteristics are attributed to the interaction between the two metals at the nanoscale, which can significantly alter the properties of the individual components. Comparative studies have reported similar findings in terms of NPs morphology and size distribution. (El-Amier et al. 2023) reported TEM images of silver, selenium, and zinc nanoparticles, all showing particle sizes below 100 nm. Specifically, selenium nanoparticles exhibited sizes of approximately 78.16 nm and 89.64 nm. (Selim et al. 2025b) achieved green synthesis of Zn–MnO nanocomposites using *Penicillium rubens*. TEM analysis revealed irregular, nearly spherical particles with sizes ranging from 25.13 to 36.21 nm. (Malik et al. 2023b) reported similar results for Ag–Cu BNPs synthesized from *Salvia officinalis*, with particle sizes range of 3.3–57.5 nm, as determined by TEM analysis. (Selim et al. 2025a) reported that TEM confirmed the structural stability of the biosynthesized MgO–ZnO nanocomposite, with consistent particle sizes range of 5–35 nm. (Elkady et al. 2025) recorded greenly synthesized spherical CuO–Se

BNPs with approximately 30 nm average diameter and size distribution range of 5–50 nm. (Jabir et al. 2024) reported that the TEM analysis revealed Cu_4O_3 NPs with a spherical morphology and a particle size below 100 nm. (Tabrez et al. 2022c) synthesized spherical ZnO NPs (50–60 nm) using pumpkin seed extract, as confirmed by SEM and TEM analysis, and demonstrated their potent anticancer activity.

The FTIR spectra of the CPE as well as the biosynthesized MnO–Ag BNPs revealed distinct absorption peaks indicating the functional groups involved in BNPs formation (Fig. 3A). The extract displayed characteristic bands at 3382, 2927, 2850, 2003, 1581, 1368, 1105, 667, and 492 cm^{-1} , corresponding to hydroxyl, aliphatic, carbonyl, aromatic, and ether groups, mainly derived from phenolics, flavonoids, and sugars acting as reducing agent and capping stabilizer (Yadav et al. 2024). After green synthesis BNPs, the FTIR spectrum showed peaks at 3330, 2229, 2148, 2003, 1581, 1368, 1105, 725, and 492 cm^{-1} . The shift of the O–H band ($3382 \rightarrow 3330 \text{ cm}^{-1}$) confirmed its role in metal ion reduction (Wu et al. 2023), while new peaks at 2229 and 2148 cm^{-1} suggested the formation of $\text{C}\equiv\text{N}$ or $\text{C}\equiv\text{C}$ bonds due to interactions between biomolecules and the metallic surface (Azeez and Shenbagaraman 2025). The persistence of peaks at 1581, 1368, and 1105 cm^{-1} indicates that some functional groups

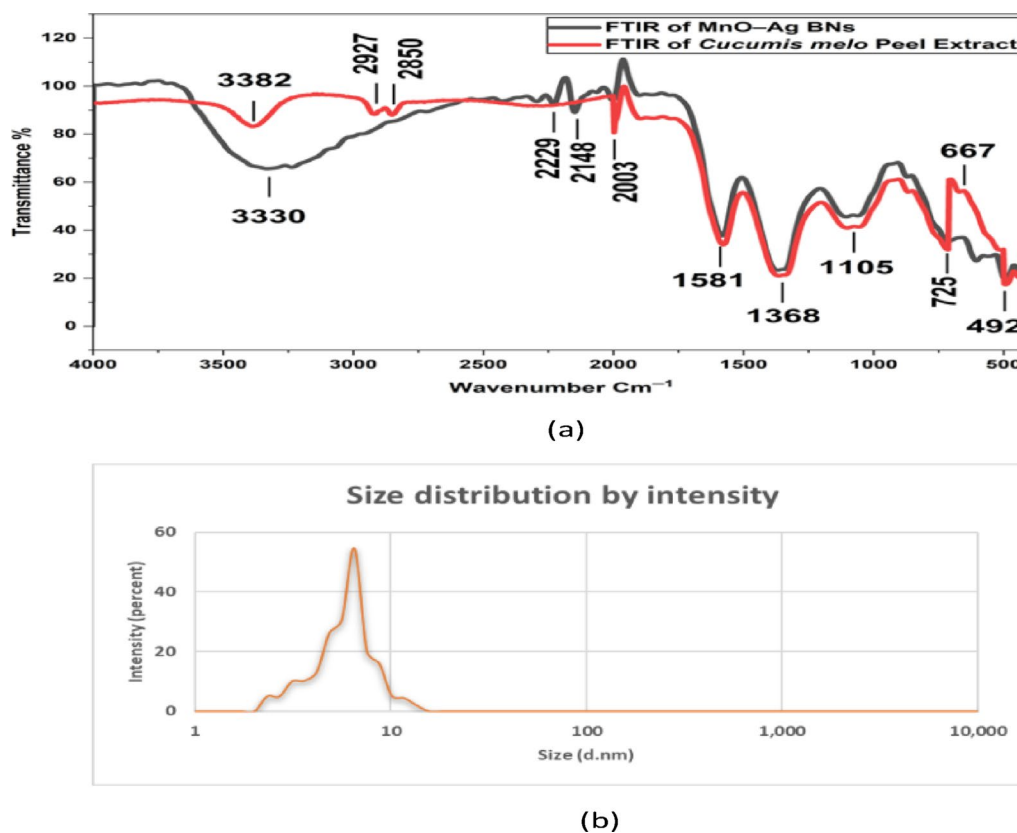


Fig. 3 FTIR spectrum of CPE and biosynthesized MnO–Ag BNPs (a) and DLS analysis of MnO–Ag BNPs (b)

remained adsorbed on the NPs surface, providing stabilization (Wu et al. 2023). The strong band at 492 cm^{-1} corresponds to metal–oxygen (M–O) stretching, confirming BNPs formation (Li et al. 2022).

Overall, the observed spectral shifts and new bands demonstrate strong interactions between the extract's biomolecules and metal ions, supporting their role in reduction and stabilization. These results align with previous studies using plant extracts such as *Camellia sinensis*, and *Zingiber officinale*, where hydroxyl and carbonyl groups facilitated NPs synthesis (Ongtanasup et al. 2024; Tran et al. 2025). This confirms that CPE acts as an efficient and ecological reducing agent in sustainable nanotechnology.

Additionally, the size and distribution of the biosynthesized MnO–Ag BNPs were examined using DLS based on their hydrodynamic diameters. The histogram (Fig. 3B) illustrated BNPs with 7 nm average hydrodynamic diameter and volume intensity of 54%. The larger BNPs size observed in the DLS measurements of colloidal solution may result from uneven BNPs distribution. As well, DLS method offers insight into the BNPs homogeneity via calculation polydispersity index (PDI). Homogeneity is considered higher when the PDI is below 0.4, while values above 0.4 suggest more variability in the particle sizes, as noted in earlier studies (Jusril et al. 2022). However, a solution with PDI above 1.0 classified as highly heterogeneous. In this study, a PDI value of 0.187 was reported for the biogenic MnO–Ag BNPs, indicating a well-distributed colloidal solution. For comparison, Eid et al. (Saied et al. 2022) reported an average particle size of 51.4 nm for biosynthesized CuO–NPs. According to

Elattar et al. (Elattar et al. 2025), both Ag/CeO₂ and CuO/CeO₂ nanocomposites exhibit comparable hydrodynamic sizes, measuring 622.9 d.nm and 602.9 d.nm, respectively. However, CuO/CeO₂ displays a markedly more uniform atomic-scale distribution, as reflected by its lower PDI of 0.027, compared to 0.073 observed for Ag/CeO₂. (Elkady et al. 2025) recorded biosynthesized NPs with an average particle size of approximately 79 nm. (Selim et al. 2025a) reported also MgO–ZnO BNPs with 51 nm average particle size and a PDI of 0.31, indicating moderate size distribution uniformity.

XRD and SEM–EDX analysis

Characterization of Mn–Ag BNPs was revealed by XRD (Fig. 4). Confirmed biogenic MnO–Ag BNPs crystalline nature was accomplished by the presence of XRD discrete patterns with characteristic diffraction peaks at various 2θ angles. The distinctive diffraction peaks detected at 2θ angles observed at 38.4° , 46.4° , 55.0° , 64.5° , and 76.9° corresponded to (111), (200), (222), (220), and (311) crystallographic planes, respectively. The detected peaks proven the metallic silver face-centered cubic (fcc) structure. (Baran et al. 2023) in the green synthesis of silver NPs from *Allium cepa* L. peel extract reported diffraction peaks at 2θ values of 37.89° , 44.02° , 64.25° , and 77.04° , corresponding to the (111), (200), (220), and (311) planes of cubic silver (Ag, JCPDS No. 65–2871). In addition to Ag, MnO was also identified in the XRD patterns. Diffraction angles (2θ) at 18.7° , 29.0° , 38.4° , and 64.5° corresponded to crystallographic planes (200), (310), (111), and (220), indicative of MnO NPs. These results were consistent with (El-Moslami et al. 2024) study, which

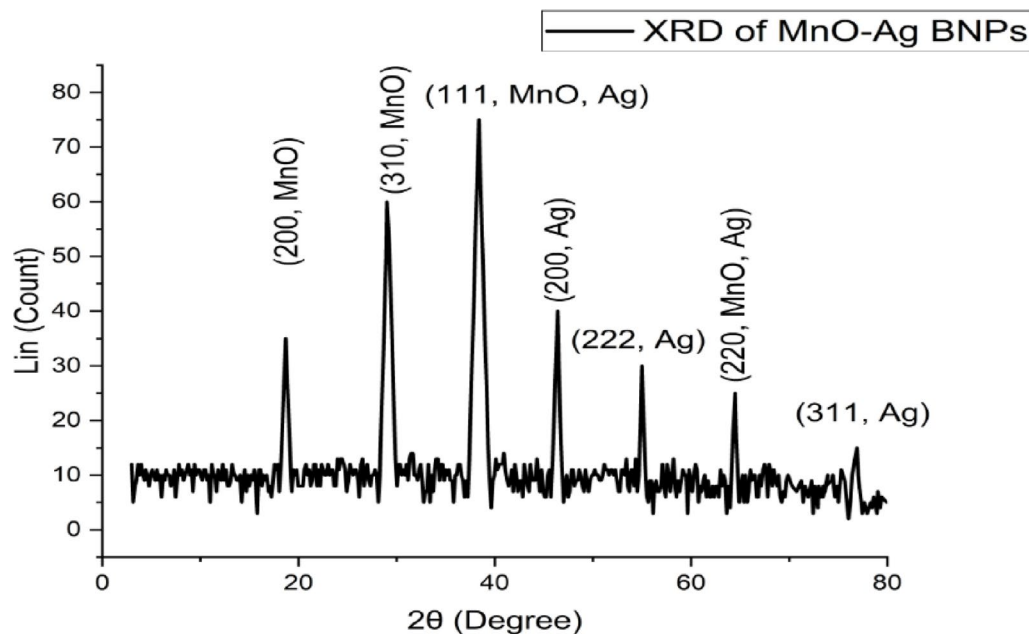


Fig. 4 XRD spectrum of biosynthesized MnO–Ag BNPs using CPE

recorded sharp peaks at 18.56°, 28.90°, 37.34°, and 63.74°, indexed to the (200), (310), (211), and (512) facets of MnO NPs. Furthermore, MnO–Ag BNPs average crystallite size of approximately 6 nm was obtained from Scherrer equation $d = K\lambda/\beta\cos\theta$ (Hassan et al. 2021). Similarly, (Malik et al. 2023b) reported a crystalline nature for Ag–Cu NPs with distinct fcc peaks, with average crystallite size of 17 nm calculated using the same method. These XRD analyses collectively demonstrate the crystalline nature of MnO–Ag BNPs, confirming the presence of both Ag and MnO components in the synthesized material.

SEM imaging at 80,000× magnification (Fig. 5A) reveals the morphology of the MnO–Ag BNPs, showing an irregularly aggregated structure with a rough, sheet-like topology. This morphology suggests that the BNPs have a higher surface area, which could be beneficial for catalytic and adsorption-based applications. The scale bar indicates a resolution of 2 μm, providing a closer look at the particle-level topology. Also, EDX spectrum (Fig. 5B) clarified MnO–Ag BNPs elemental composition. The detected peaks correspond to elements Mn, Ag, sodium (Na), carbon (C), oxygen (O), and chlorine (Cl). The weight and atomic percentages of each element are presented in Fig. 5C, with Mn and Ag accounting for 46.6–23.7% by weight, respectively, and 28.0–32.2% by

atomic percentage. Oxygen's significant presence suggests oxidation states, likely from MnO. While, presence of minor constituent including Na, C, and Cl could be impurities or remnants from the synthesis process (Roy et al. 2024). These results validate the successful synthesis of MnO–Ag BNPs with significant contributions from Mn and Ag as the primary components, alongside oxygen, which is consistent with oxides formation.

Moreover, SEM imaging highlights BNPs agglomeration, which may be attributed Mn and Ag phases interactions or van der Waals forces (Abbas et al. 2024). The rough morphology and aggregated clusters are advantageous for many applications, where higher surface areas improve light absorption and reactive site availability (Mahajan et al. 2025). (Malik et al. 2023b) used SEM analysis to reveal the spherical shape and uniform dispersion of Ag–Cu NPs and the EDX confirmed 47.83 wt% Ag, 28.95 wt% Cu, and traces of carbon and oxygen from phytochemicals, highlighting the NPs' stabilization and crystalline nature. (Elkady et al. 2025) showed that SEM revealed aggregated CuO–Se NPs with columnar and needle-like structures, while EDX confirmed the presence of 32.7 wt% Cu and 14.8 wt% Se. Elattar et al. (Elkady et al. 2025) reported that SEM analysis revealed spherical to slightly elongated morphologies for both Ag/CeO₂ and CuO/CeO₂ nanocomposites, with uniform

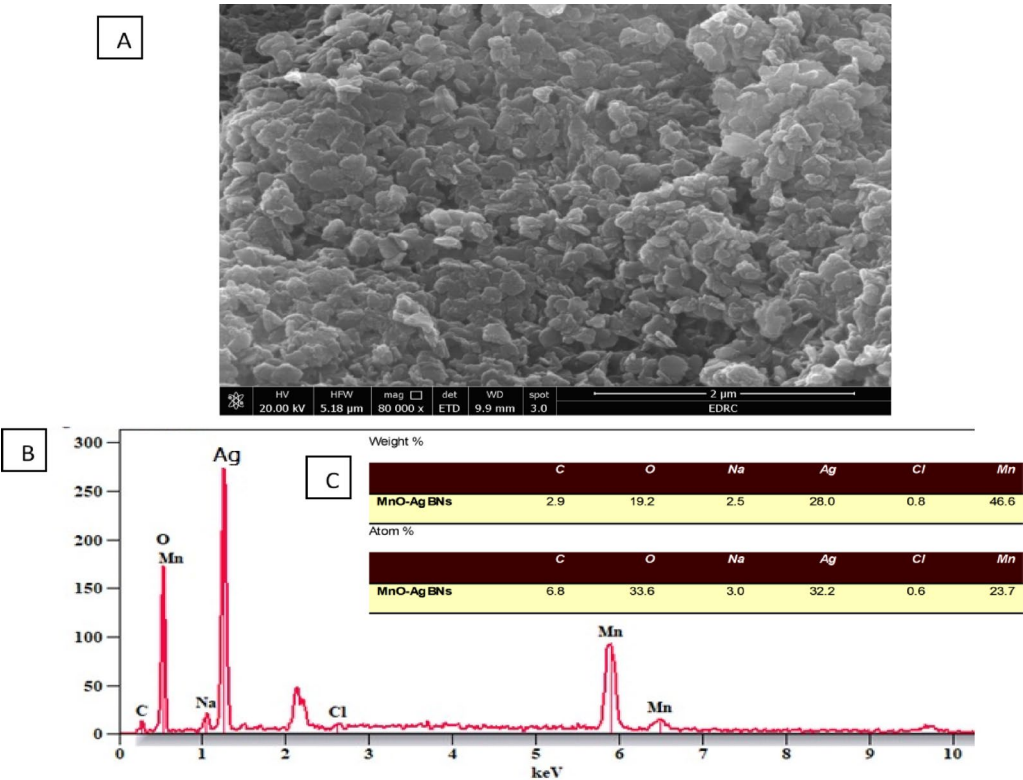


Fig.5 SEM image of MnO–Ag BNPs synthesized using CPE (a), EDX spectrum shows elemental composition (b), and Table of elemental weight and atomic percentages (c)

size distribution. EDX confirmed elemental composition, showing dominant presence of cerium, oxygen, and Ag/CeO₂, while CuO/CeO₂ contained copper, cerium, oxygen, and minor elements like potassium and zinc.

Antimicrobial activity

Bimetallic NPs have attracted significant interest due to their superior efficacy compared to MNPs, particularly against multidrug-resistant (MDR) pathogens. In the present study, MnO–Ag BNPs exhibited broad-spectrum microbial inhibitory activity against both bacterial and fungal strains (Table 1). Amongst microbial strains under investigation, *B. subtilis* exhibited the highest sensitivity, with an IZD measuring 20.00 ± 1.00 mm when exposed to Mn–Ag BNPs at 100 µg/mL. The agar–well diffusion assay revealed that *B. subtilis* was the most sensitive, showing an inhibition zone diameter (IZD) of 20.00 ± 1.00 mm at 200 µg/mL. Notably, *S. aureus*, *E. coli*, and *P. aeruginosa* recorded IZDs of 18.71 ± 0.75 , 17.22 ± 1.28 , and 16.15 ± 0.66 mm, respectively, while *C. albicans* exhibited a moderate inhibition zone of 11.33 ± 1.39 mm, indicating limited antifungal activity. The minimum inhibitory concentration (MIC) results further confirmed strong antibacterial efficacy, with *C. albicans* and *P. aeruginosa* showing low MIC values of 12.5 and 25 µg/mL, respectively, and *S. aureus*, *B. subtilis*, and *E. coli* displaying MICs of 50 µg/mL. Comparable studies support these findings. (Malik et al. 2023b) reported that Ag–Cu NPs effectively inhibited *S. epidermidis*, *S. aureus*, *E. coli*, and *K. pneumoniae*. Similarly (Hasanin et al. 2023) studied TiO₂@Cr₂O₃ BNPs. Antimicrobial testing showed the highest MIC against *A. niger* (50 µg/mL) and the lowest MIC against Gram-positive bacteria (3.12 µg/mL). (Sher et al. 2022) reported that Ag/Au bimetallic nanoparticles exhibited strong antimicrobial activity against multiple Gram-positive bacteria (*S. aureus*, *Actinomyces meriye*, *B. cereus*, *S. pyogenes*, MRSA, *Micrococcus luteus*, *S. pneumoniae*), Gram-negative bacteria (*Klebsiella pneumoniae*, *E. coli*, *Serratia marcescens*), and fungi (*Aspergillus niger*, *A. fumigatus*, *A. flavus*), outperforming HH extract, HH-AgNPs, and HH-AuNPs. (Hashem et al. 2023a) also documented strong

antimicrobial activity of Se–Ag BNPs against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *K. oxytoca*, and *C. albicans*.

Further study by (Krishnan et al. 2023) showed that BiPO₄/ZnO nanocomposites (B1Z4-75:300; NCs-4) produced a maximum inhibition zone of 20 mm against *A. baumannii* and a minimum of 12 mm against *B. filamentosus* at 80 µg/mL, suggesting NCs-4 as a potential antibacterial agent. Other reports similarly described synergistic microbial inhibitory effects of bimetallic oxides including MgO–ZnO (Selim et al. 2025a), CuO–CeO₂ and Ag–CeO₂ (Mohamed et al. 2025; Nagajyothi et al. 2025), and CuO–Se NPs (Elkady et al. 2025), with the latter also exhibiting quorum–quenching activity against MDR *P. aeruginosa*. Moreover, (Vasiliev et al. 2023) noted that a mixture of Cu NPs and Ag NPs produced antibacterial effects up to six times greater than either metal alone. The superior antimicrobial performance of MnO–Ag BNPs can be also related to synergistic interactions between the two metals (Elhawary et al. 2025). These interactions promote the generation of ROS, release of metal ion, and direct disruption of microbial membranes (Yan et al. 2023). The NPs' small size as well as their high surface area facilitate strong electrostatic attraction between their positively charged surfaces and the negatively charged microbial cell membranes, causing leakage of intracellular components and cell lysis (Mondal et al. 2024). Additionally, metal ions bind to thiol (–SH) groups in peptidoglycan layers, destabilizing bacterial cell walls, while ROS generation further damages DNA, proteins, and lipids (Rana et al. 2023). The release of Ag⁺ ions amplifies this effect through interaction with microbial enzymes and nucleic acids, leading to broad-spectrum antimicrobial action. Amin et al. (Amin et al. 2022) further demonstrated that functionalization of Ag NPs with sulfonamide groups improved stability, cellular penetration, and antibacterial efficiency supporting a similar synergistic mechanism proposed for MnO–Ag BNPs.

Antioxidant activity

Generation of ROS by molecular oxygen can damage biomolecules including DNA, lipids, and proteins, leading to OS with consequent cellular dysfunction. The MnO–Ag BNPs exhibited pronounced antioxidant properties due

Table 1 Biosynthesized MnO–Ag BNPs observed IZD and MICs for assessed pathogens

Tested materials*	Mn	Ag	MnO–Ag BNPs		Ampicillin/sulbactam		Nystatin	
Microbial strain	IZD (mm)	IZD (mm)	IZD (mm)	MIC (µg/mL)	IZD (mm)	MIC (µg/mL)	IZD (mm)	MIC (µg/mL)
<i>B. subtilis</i>	ND	ND	20.00 ± 1.05^a	50	11.44 ± 0.87^d	100	ND	ND
<i>S. aureus</i>	ND	ND	18.71 ± 0.75^b	50	ND	ND	ND	ND
<i>P. aeruginosa</i>	ND	ND	16.15 ± 0.66^c	25	9.88 ± 1.15^e	50	ND	ND
<i>E. coli</i>	ND	ND	17.22 ± 1.28^{bc}	50	ND	ND	ND	ND
<i>C. albicans</i>	ND	ND	11.33 ± 1.39^e	12.5	ND	ND	ND	ND

Mn; manganese acetate tetrahydrate, Ag; silver nitrate, IZD; inhibition zone diameter, MIC; minimum inhibitory concentration, ND; not detected. All experiments were conducted in triplicate, and results were expressed as mean $\pm$ SD. Different superscript letters (a–e) within the same column indicate significant differences ($P < 0.05$) according to one-way ANOVA followed by Tukey's post hoc test

to their increased surface area and reactive metal sites, enabling efficient neutralization of ROS. The synergistic effect between MnO and Ag enhanced the scavenging capacity, as confirmed by the DPPH assay. This observation in accordance with (Ullah et al. 2023), study which recorded phyto-mediated Ag NPs with enhanced antioxidant and antimicrobial activities owing to the synergistic interaction between Ag ions and phytochemicals in olive extract. Such NPs effectively interact with cellular biomolecules, mitigating free radical-induced damage.

The accumulation of free radicals is implicated in several diseases, including cardiovascular, neurodegenerative, and metabolic disorders (Chaudhary et al. 2023). Therefore, antioxidants are essential in reducing OS. In our research, MnO–Ag BNPs antioxidant activity was evaluated using the DPPH assay at concentrations ranging from 1.95 to 1000 $\mu\text{g/mL}$ (Fig. 6). Among the tested samples ascorbic acid (standard), MnO–Ag BNPs, Mn^{2+} ions, Ag^+ ions, and CPE, ascorbic acid showed nearly 100% inhibition at high concentrations. The biogenic MnO–Ag BNPs exhibited strong scavenging activity (85–90% inhibition at 500–1000 $\mu\text{g/mL}$) with a low IC_{50} value of 27.8 $\mu\text{g/mL}$, outperforming the other samples. Also, Mn^{2+} ions showed moderate antioxidant activity at higher concentrations (~70% inhibition), whereas Ag^+ ions achieved 70–80% inhibition with decreasing efficacy at lower doses. The CPE exhibited comparatively weak antioxidant capacity. Comparable findings have been reported for other bimetallic and oxide NPs. For example, Cu–Ag BNPs achieved up to 85% DPPH scavenging within 16 min (Ali et al. 2023), while Ag–ZnO BNPs displayed an IC_{50} of 340 $\mu\text{g/mL}$, superior to that of ascorbic acid (289.74 $\mu\text{g/mL}$) (Suresh et al. 2024). (El-Khateeb

2025) synthesized Ag/ Fe_2O_3 nanoparticles using *Syzygium aromaticum* (clove) extract. The nanoparticles exhibited strong DPPH radical-scavenging activity with an IC_{50} of 0.0098 mg/mL, comparable to the clove extract (0.0097 mg/mL) and superior to ascorbic acid (0.0222 mg/mL). Biosynthesized Vr–Mn–Cu NPs exhibited concentration-dependent DPPH scavenging with 51.44% inhibition at 1000 $\mu\text{g/mL}$ (Ahmad et al. 2022), while Se NPs demonstrated >90% inhibition between 250–4000 $\mu\text{g/mL}$ with an IC_{50} of 27.8 $\mu\text{g/mL}$ (Hashem et al. 2023b).

The antioxidant mechanism of the biosynthesized MnO–Ag BNPs primarily involves the neutralization of ROS and free radicals through multiple pathways (Chaudhary et al. 2023). These NPs act as electron donors, stabilizing free radicals by converting them into less reactive or harmless molecules. The presence of Mn and Ag enhances this process, as both metals can cycle between different oxidation states, allowing them to scavenge radicals efficiently (Dai et al. 2024). Additionally, the NPs can enhance the cellular antioxidant defence by stimulating the activity of natural enzymes including catalase, superoxide dismutase, and glutathione peroxidase (Jomova et al. 2024). The mixed activities of direct radical scavenging with indirect specific enzymes activation helps to reduce OS within biological systems. The SPR of Ag further contributes to electron transfer reactions, increasing the overall redox potential of the NPs (Zhang et al. 2023). Consequently, these properties collectively protect biomolecules like DNA, proteins, and lipids from oxidative damage and maintain cellular integrity. The enhanced antioxidant capacity of MnO–Ag BNPs likely derives from the combined redox properties

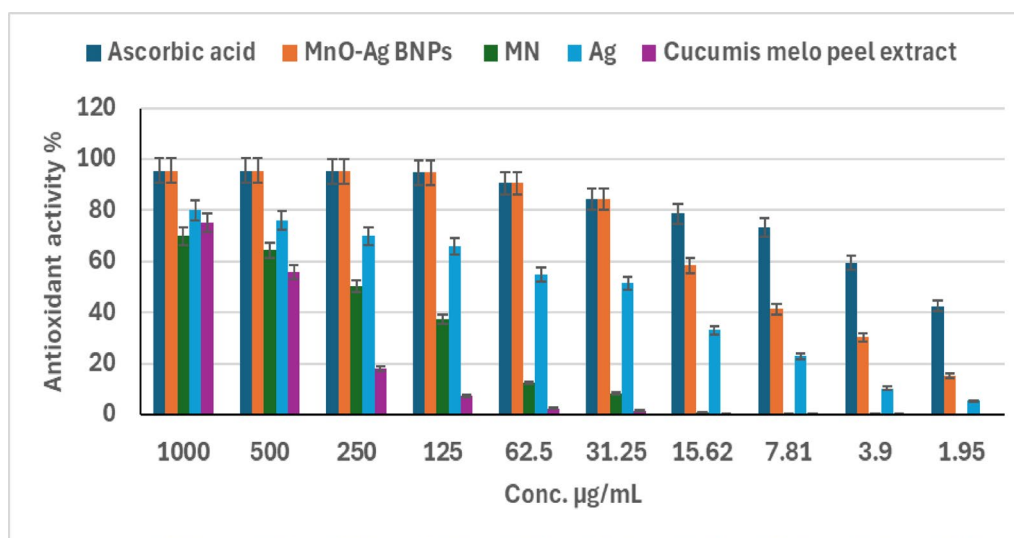


Fig. 6 Antioxidant activity (% DPPH inhibition) of ascorbic acid (positive control), Mn (manganese acetate tetrahydrate), Ag (silver nitrate), MnO–Ag BNPs, and CPE at different concentrations (1.95–1000 $\mu\text{g/mL}$). Data are presented as mean $\pm$ SD ($n=3$). Statistical differences between treatments at each concentration were evaluated by one-way ANOVA followed by Tukey's post-hoc test ($P<0.05$)

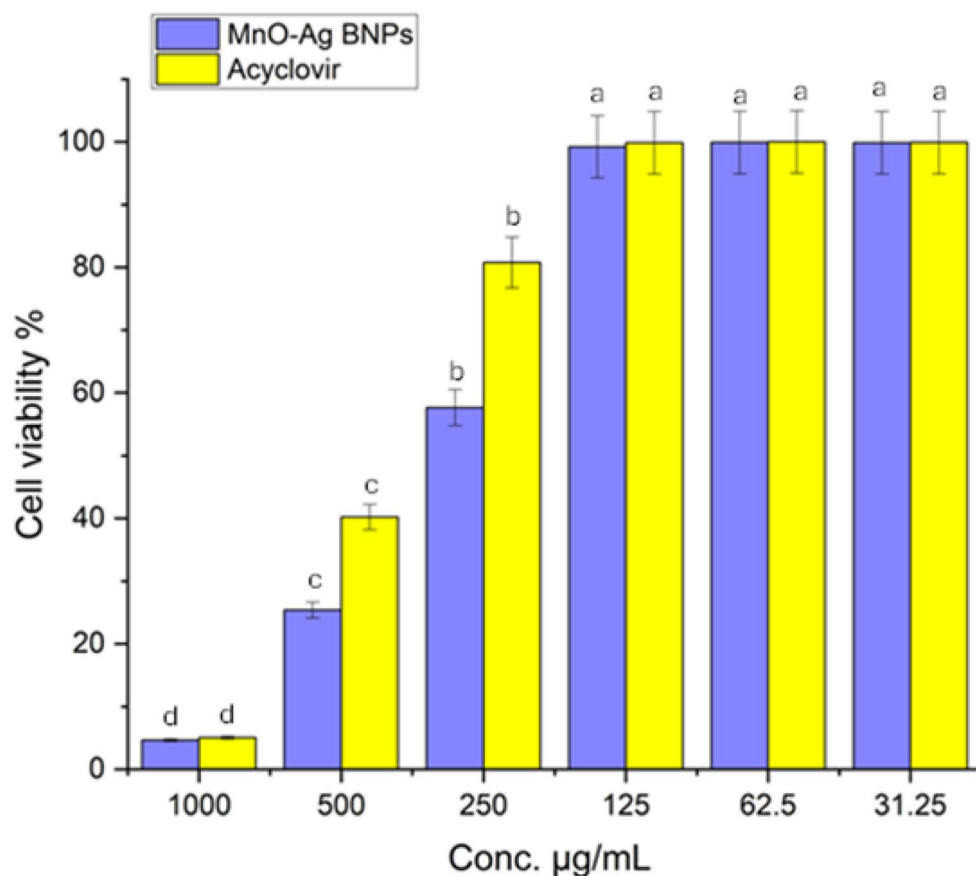


Fig. 7 Mtt assay showing cell viability (%) after exposure to varying concentrations of mno-ag bnps and acyclovir. Results are presented as mean $\pm$ sd from three independent experiments. statistical significance was set at $p < 0.05$. Superscript letters (a–d) indicate significant differences across the tested groups

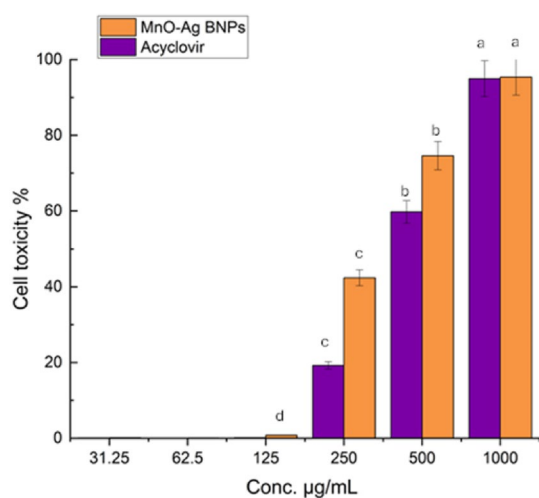


Fig. 8 MTT assay showing cytotoxicity (%) of cells treated with varying concentrations of MnO–Ag BNPs and acyclovir. Data are expressed as mean $\pm$ SD from three independent replicates. Statistical significance was considered at $P < 0.05$. Variation in superscript letters (a–d) reflects statistically significant differences among the groups

of MnO and Ag and the stabilizing/capping phytochemicals from CPE, which together increase surface reactivity and radical interaction compared with single-metal species or crude extract.

Antiviral activity of MnO–Ag BNPs

Cytotoxicity against vero cells

The biosafety of newly synthesized materials, assessed via their cytotoxic effect using regular human cell lines, is a crucial measure in ensuring their suitability for potential human applications in the future. The cytotoxic effects of MnO–Ag BNPs on Vero cells were examined across a wide concentration range (Fig. 7). The results indicated that low to moderate concentrations (31.25–250 $\mu\text{g/mL}$) exhibited no significant cytotoxicity, maintaining cell viability comparable to untreated controls. However, the elevated MnO–Ag BNPs concentrations (500 and 1000 $\mu\text{g/mL}$) markedly reduced cell viability by 74–95%, respectively (Fig. 8). The calculated half-maximal cytotoxic concentration (CC_{50}) was $345.26 \pm 4.66 \mu\text{g/mL}$, indicating that MnO–Ag BNPs can be considered non-toxic,

as materials with $IC_{50} \geq 90 \mu\text{g/mL}$ are generally regarded as biocompatible. For comparison, the cytotoxicity of acyclovir was also evaluated under the same conditions. Similar to MnO–Ag BNPs, acyclovir exhibited no cytotoxicity at concentrations up to 250 $\mu\text{g/mL}$, while higher concentrations (500 and 1000 $\mu\text{g/mL}$) caused 59.7–94.9% growth inhibition, respectively (Fig. 8). The CC_{50} value of acyclovir was $439.6 \pm 1.52 \mu\text{g/mL}$, with a maximum non-toxic concentration (MNTC) of 125 $\mu\text{g/mL}$ for both acyclovir and MnO–Ag BNPs. These findings confirm that biosynthesized MnO–Ag BNPs, produced using CPE, exhibit minimal cytotoxicity toward normal human cell lines at suitable concentrations (Fig. 9A,B).

The cytotoxic mechanisms of MnO–Ag BNPs may involve NPs diffusion through ion channels, leading to interactions with intracellular proteins and nucleic acids. This can result in mitochondrial dysfunction, disruption of the cell cycle, and apoptosis (Misra et al. 2023). Similarly, (Aly Khalil et al. 2024) reported that Se–Au bimetallic nanoparticles showed an IC_{50} value of 116.8 $\mu\text{g/mL}$ against the normal Wi-38 cell line, indicating that

all previously reported MIC values were within a safe range. (Sayed et al. 2024) synthesized *Staphylococcus aureus*-mediated Ag/Cu bimetallic nanocomposites. The nanocomposites showed anticancer activity against the HepG-2 cell line, while exhibiting low cytotoxicity toward the normal BHK cell line. Additionally, (Zadeh et al. 2022) reported that Cu–Zn BNPs exhibited strong cytotoxic activity towards MCF–7 breast cancer cells with an IC_{50} of 54 $\mu\text{g/mL}$, leading to complete cell death above 600 $\mu\text{g/mL}$. On another study, (Rashid Khan et al. 2024) bio-fabricate MgO NPs with enhanced ROS generation and apoptosis while inhibiting cell migration in MDA–MB–231 cells, indicating strong dose-dependent cytotoxic and therapeutic effects.

Antiviral activity toward HSV-1

This study aimed to evaluate the antiviral efficacy of MnO–Ag BNPs against HSV–1 while minimizing host cell damage. Cytotoxic effects were confirmed through decreased cell proliferation and morphological alterations, such as rounding, detachment, and aggregation

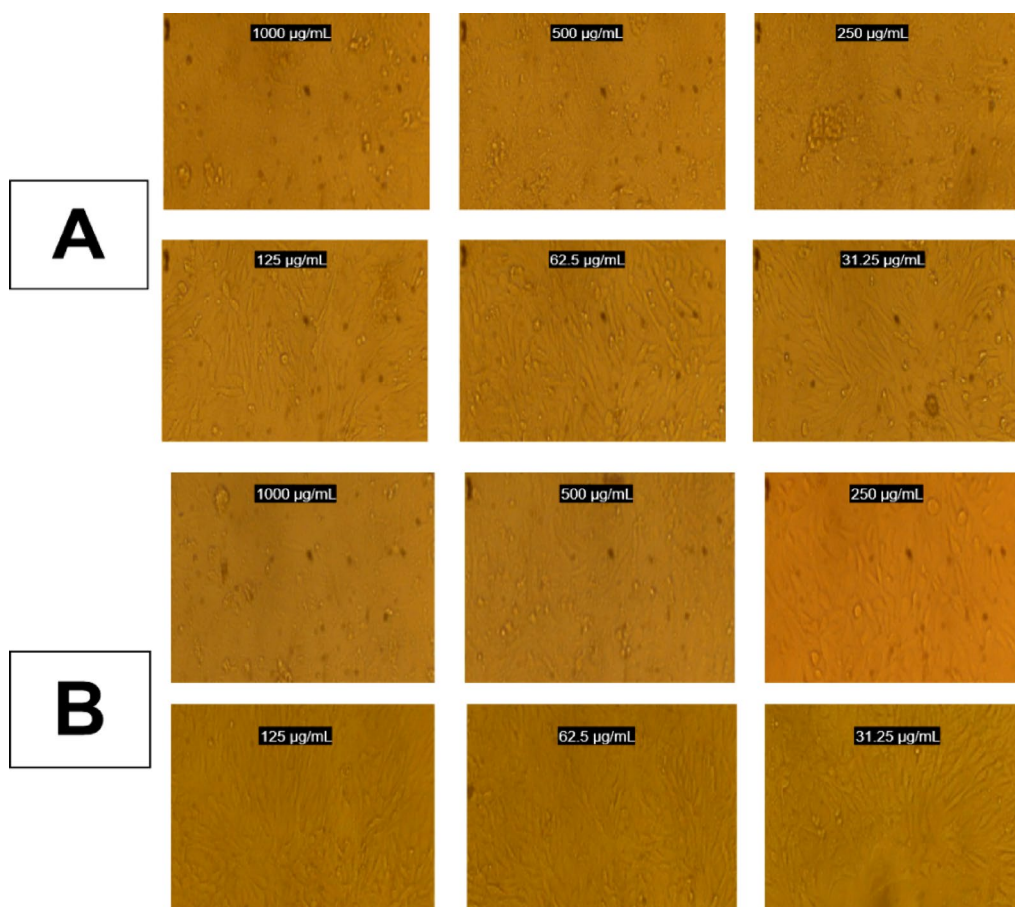


Fig. 9 Microscopic examination of MnO–Ag BNPs (a) and acyclovir (b) following cells exposure to serial concentrations (31.25–1000 $\mu\text{g/mL}$) for 48 h and observation under an inverted light microscope at 200 $\times$ magnification. Control cells (untreated) displayed a normal, confluent epithelial morphology. Increasing concentrations of both MnO–Ag BNPs and acyclovir induced concentration-dependent cytopathic effects, including cell rounding, detachment, and loss of confluence, particularly at $\geq 500 \mu\text{g/mL}$

of cells. The concentration of 125 µg/mL, determined as the MNTC from the cytotoxicity assay, was selected for antiviral testing. Acyclovir, a well-established antiviral drug used to treat HSV-1 and varicella-zoster virus (VZV) infections, was employed as a positive control. Its mechanism of action involves inhibiting viral replication by targeting viral DNA polymerase (Kleiner et al. 2023). MnO-Ag BNPs synthesized using CPE were tested alongside acyclovir. The extract is known to contain bioactive phytochemicals such as flavonoids, phenolics, and antioxidants that contribute to antimicrobial and antiviral activity. The results demonstrated a substantial increase in the viability of HSV-1-infected Vero cells following treatment, from $43.6 \pm 0.5\%$ in untreated controls to 78.4 ± 0.9 – $99.2 \pm 0.5\%$ after exposure to MnO-Ag BNPs and acyclovir, respectively (Table 2). Both agents exhibited strong antiviral activity at 125 µg/mL, with inhibition rates of $73 \pm 0.7\%$ for MnO-Ag BNPs and $98.6 \pm 0.4\%$ for acyclovir (Table 2).

The antiviral effect of MnO-Ag BNPs likely involves multiple mechanisms, including interference with viral attachment and entry, inhibition of replication, induction of OS, modulation of host immune responses, prevention of virus-induced apoptosis, and reduction of viral load (Elbas et al. 2025). The combined presence of Mn and Ag may further enhance antiviral potency through synergistic interactions. MnO-Ag BNPs may bind to viral surface proteins or lipid envelopes, disrupting their ability to attach to host cell receptors an essential early step in infection. This inhibition prevents viral entry and subsequent replication. Bimetallic NPs can also damage viral nucleic acids or inhibit key enzymes required for genome replication and protein synthesis (Bharti 2024).

Comparable findings were reported by (Alshallash et al. 2024), which reported strong inhibitory effect (78.2–69.6%) of Au/Ag nanoalloys against hepatitis A and Cox-B4 viruses. Similarly, (Zubair et al. 2023) reported that Au-Ag bimetallic nanostructures exhibited

enhanced antiviral activity against H9N2 influenza virus compared to monometallic AuNPs and AgNPs. (Alrabayah et al. 2023) found that ZnO-NPs ($IC_{50}=7.01$, $CC_{50}=145.77$ µg/mL) and *C. dirnum* L. extract ($IC_{50}=61.15$, $CC_{50}=145.87$ µg/mL) exhibited antiviral activity against HCoV-229E, while their combination ($IC_{50}=2.41$, $CC_{50}=179.23$ µg/mL) showed superior efficacy. (El-Sheekh et al. 2022) reported that Ag₂O|AgO-NPs and Au-NPs reduced HSV-1 CPE by 90% at 31.25 µL, with Ag₂O|AgO-NPs showing a higher reduction rate (49.23%) than Au-NPs (42.75%), demonstrating their effectiveness as green antiviral agents against HSV-1 replication. Additionally, Zubair et al. (2023) showed that Ag-Ag BNPs possess enhanced viral inhibitory effect towards the H9N2 influenza virus due to synergistic effects. Similarly, the combined MnO-Ag BNPs may produce synergistic antiviral action, contributing to their potent antiviral activity towards HSV-1.

Proposed mechanism of action of MnO-Ag BNPs

The schematic diagram (Fig. 10) illustrates the integrated mechanism of MnO-Ag BNPs. Their antioxidant activity arises from the scavenging of ROS, while the antimicrobial potential is attributed to cell membrane disruption and protein denaturation. Additionally, the antiviral effect involves interference with viral attachment and replication within host cells.

Conclusion

Our study presents, for the first time, the green biosynthesis of MnO-Ag BNPs using CPE as a sustainable and eco-friendly approach. Comprehensive characterization using UV-vis, XRD, and EDX confirmed successful biosynthesis and elemental integration, with Mn and Ag accounting for 23.7–46.6% atomic percentages, respectively. The synthesized BNPs exhibited nanoscale size distribution (2–10 nm), with an average diameter of 5.8 ± 1.7 nm and mean area of 22.7 nm². The biosynthesized MnO-Ag BNPs exhibited potent antimicrobial activity, particularly against *B. subtilis* with an IZD of 20.00 ± 1.00 mm at 200 µg/mL. Moderate to strong effects were also observed against *S. aureus*, *E. coli*, and *P. aeruginosa*, while limited antifungal activity was noted against *C. albicans*. The MIC values further confirmed the efficacy, with low MICs of 12.5 µg/mL for *C. albicans* and 25 µg/mL for *P. aeruginosa*. Functionally, the biosynthesized NPs showed strong free radical scavenging, achieving 85–90% inhibition at higher concentrations. Cytotoxicity assays showed that concentrations up to 250 µg/mL were non-toxic to Vero cells, indicating good biocompatibility. Importantly, antiviral assays revealed that treatment with MnO-Ag BNPs enhanced Vero cell viability from 43% (HSV-1 infected control) to 78%, approaching the 99% viability observed with acyclovir.

Table 2 The MNTC of MnO-Ag BNPs and acyclovir against HSV-1

Treatments* Effect (%)	Vero cell line			
	Normal uninfected cells (Control)	HSV-1 infected cells		
		Untreated cells	MnO-Ag BNPs treated cells (125 µg/mL)	Acyclovir treated cells
Cell viability	100 ± 0.0^a	43.6 ± 0.5^d	78.4 ± 0.9^b	99.2 ± 0.5^a
Cell toxicity	0.0 ± 0.0^d	56.3 ± 0.7^b	21.6 ± 0.5^c	0.78 ± 1.7^d
Viral activity	0.0 ± 0.0^d	100 ± 0.0^a	22 ± 1.3^c	1.38 ± 2.3^d
Antiviral effect	0.0 ± 0.0^d	0.0 ± 0.0^d	73 ± 0.7^b	98.6 ± 0.4^a

The results were presented as the mean $\pm$ SD from three separate triplicated experiments ($P < 0.05$). Groups sharing different superscript letters (a–d) in the same row are significantly different ($P < 0.05$), based on one-way ANOVA followed by Tukey's post hoc analysis

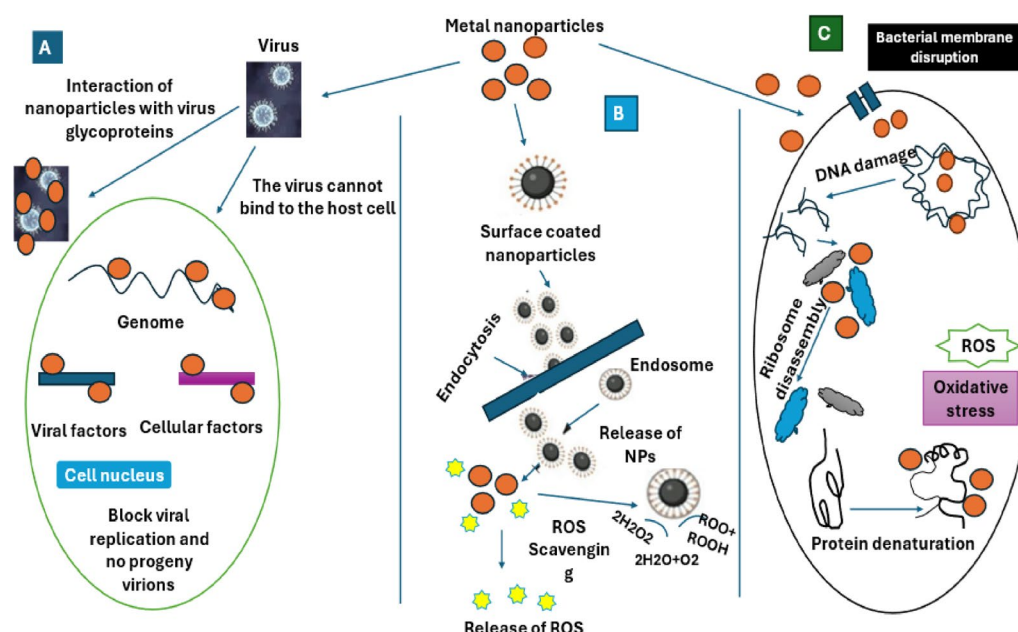


Fig. 10 Proposed mechanism of action of MnO–Ag BNPs showing their (a) antiviral (inhibition of viral entry and replication); (b) antioxidant activity (ROS scavenging), and (c) antimicrobial activity (cell membrane disruption)

At 125 µg/mL, the BNPs achieved 73% viral inhibition, underscoring their potential as effective antiviral agents. The novelty of this work lies in utilizing agricultural waste for the green fabrication of biologically active BNPs with dual antioxidant and antiviral properties. However, limitations include the absence of mechanistic insights into NPs–virus interactions and the need for in vivo validation. Further studies should focus on elucidating the molecular mechanisms and evaluating long-term biosafety and therapeutic efficacy in animal models.

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Author contributions

Conceptualization and research design: E.S. Methodology: all authors contributed to the research required material preparation and data collection; all authors participated formal analysis and investigation of the obtained data; original draft preparation, review and editing: All authors reviewed the final version; All authors read and approved the submitted and final manuscript.

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Data availability

Data for this article, including FTIR spectra, particle size distribution, antioxidant activity, cytotoxicity assays, and SEM imaging, are available at Figshare at <https://doi.org/10.6084/m9.figshare.29954423>.

Declarations

Ethical approval and consent to participate

Not applicable.

Human participation and animals traits

Not applicable.

Informed consent

Not applicable.

Conflict of interests

The authors declare that they have no conflict of interest.

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