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**Sustained-release Ag₂O/UiO-66@*Heracleum persicum* nanocomposite exhibits potent
antibacterial and antibiofilm activities against vancomycin-resistant *Staphylococcus aureus***

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Abstract

Background: Vancomycin-resistant *Staphylococcus aureus* (VRSA)-associated urinary tract infections (UTIs) represent a major therapeutic challenge due to multidrug resistance and robust biofilm formation. This study aimed to develop an Ag₂O/UiO-66@*H. persicum* nanoformulation and evaluate its antibacterial, antibiofilm, and cytocompatibility properties against clinical VRSA isolates.

Methods: The synthesized nanoformulation was characterized by physicochemical and structural analyses, including particle size, polydispersity index (PDI), zeta potential, encapsulation efficiency (EE%), and release behavior. Antibacterial activity was assessed using minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), disk diffusion, and time-kill assays. Antibiofilm activity was evaluated by crystal violet (CV), minimum biofilm eradication concentration (MBEC), and extracellular polymeric substance (EPS) assays. Expression levels of biofilm- and resistance-associated genes (*icaA*, *icaD*, *vanA*, and *vanB*) were quantified by qRT-PCR, and cytocompatibility was determined using the MTT assay.

Results: The nanoformulation exhibited favorable physicochemical properties, including a hydrodynamic size of 154.6 nm, PDI of 0.324, zeta potential of −17.5 mV, and high EE% (81.20 ± 2.9%), together with a sustained release profile compared with free extract. Among 200 UTI isolates, three biofilm-forming VRSA isolates carrying *vanA* and/or *vanB* genes were identified. Ag₂O/UiO-66@*H. persicum* demonstrated markedly enhanced antibacterial activity (MIC: 12.5–25 µg/mL; MBC: 25–50 µg/mL), superior growth inhibition in disk diffusion and time-kill

assays, and potent antibiofilm effects, evidenced by significant reductions in biofilm biomass, viable biofilm cells, and EPS production. Moreover, the nanoformulation significantly downregulated *icaA*, *icaD*, *vanA*, and *vanB* expression and exhibited improved cytocompatibility compared with free Ag₂O nanoparticles.

Conclusions: Ag₂O/UiO-66@*H. persicum* provides sustained release, potent antibacterial and antibiofilm activities, and improved biocompatibility, highlighting its potential as a multifunctional nanoplatform for combating biofilm-associated VRSA infections.

Keywords: Vancomycin-resistant *Staphylococcus aureus* (VRSA), UiO-66, silver oxide nanoparticles, *Heracleum persicum*, Biofilm.

1. Introduction

Urinary tract infections (UTIs) are among the most prevalent bacterial infections worldwide, affecting millions of individuals annually and imposing a substantial burden on healthcare systems. Complicated UTIs, particularly those associated with urinary catheterization, diabetes mellitus, immunosuppression, and prolonged hospitalization, are frequently caused by multidrug-resistant (MDR) pathogens. The increasing prevalence of antimicrobial resistance among uropathogens has significantly reduced treatment options and is associated with higher recurrence rates, prolonged hospital stays, increased healthcare costs, and elevated mortality rates [1,2].

Among Gram-positive uropathogens, *Staphylococcus aureus* (*S. aureus*) is an important opportunistic pathogen capable of causing a wide range of infections, from superficial skin and soft tissue infections to severe invasive diseases such as bacteremia, endocarditis, osteomyelitis, septic arthritis, and pneumonia. In the urinary tract, *S. aureus* infections are particularly

problematic in hospitalized and catheterized patients due to its ability to colonize medical devices and establish persistent infections [3].

The extensive use and misuse of antibiotics have accelerated the emergence and dissemination of resistant strains worldwide. Methicillin-resistant *S. aureus* (MRSA) remains one of the most clinically significant antimicrobial-resistant pathogens because of its resistance to nearly all β -lactam antibiotics. Furthermore, the emergence of vancomycin-resistant *S. aureus* (VRSA) has raised serious therapeutic concerns, as vancomycin has long been considered a last-resort treatment for severe MRSA infections [4,5]. Resistance is primarily associated with the acquisition of resistance determinants such as *mecA* and, in VRSA isolates, the *vanA* gene cluster, which collectively compromise the efficacy of conventional antimicrobial therapy [6].

Biofilm formation represents a critical virulence mechanism that contributes to bacterial persistence, immune evasion, and reduced susceptibility to antimicrobial agents. In *S. aureus*, biofilm development is mediated by microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and regulated by several genetic determinants [7,8].

Among these, the intercellular adhesion (*ica*) operon plays a central role. The *icaA* gene encodes N-acetylglucosaminyltransferase involved in the synthesis of poly-N-acetylglucosamine (PNAG), a major component of the biofilm extracellular matrix, while *icaD* enhances enzymatic activity and promotes biofilm maturation. The expression of *icaA* and *icaD* is therefore closely associated with biofilm establishment and persistence. Biofilm-associated infections are notoriously difficult to eradicate because biofilm-embedded cells exhibit substantially greater tolerance to antimicrobial agents than their planktonic counterparts [9,10].

The growing limitations of conventional antibiotics have stimulated interest in alternative antimicrobial strategies based on natural products and nanotechnology. *Heracleum persicum* (*H. persicum*), commonly known as Golpar, has been widely used in traditional medicine for the treatment of various disorders, including respiratory, gastrointestinal, and inflammatory conditions [11].

Phytochemical investigations have demonstrated that *H. persicum* contains diverse bioactive constituents, including monoterpenes, sesquiterpenes, saponins, glycosides, alkaloids, vitamins, and mucilaginous compounds, which contribute to its antimicrobial, antioxidant, anti-inflammatory, and analgesic activities [12,13].

Nevertheless, the practical application of plant-derived bioactive compounds is often limited by poor stability, volatility, hydrophobicity, and susceptibility to environmental degradation [14]. Encapsulation technologies have therefore been developed to enhance stability, improve bioavailability, and increase therapeutic efficacy [15,16].

Metal–organic frameworks (MOFs) have emerged as attractive nanocarriers for biomedical applications due to their highly porous structures, large surface areas, and tunable physicochemical properties [17]. Among them, UiO-66, a zirconium-based MOF, has gained considerable attention because of its exceptional thermal stability, chemical resistance, biocompatibility, and high loading capacity for therapeutic agents [18,19]. These characteristics enable the efficient encapsulation and controlled release of bioactive compounds, thereby improving their biological performance.

Silver oxide (Ag₂O) nanoparticles are also recognized for their broad-spectrum antimicrobial activity against bacteria, fungi, and viruses [20,21].

Their antimicrobial effects are attributed to multiple mechanisms, including disruption of cell membrane integrity, induction of reactive oxygen species (ROS), interaction with cellular proteins, and interference with nucleic acid synthesis [22,23]. Owing to these multifunctional properties, Ag₂O nanoparticles have been extensively investigated for applications in antimicrobial coatings, drug delivery systems, biosensors, and biomedical devices [23].

The integration of phytochemicals with advanced nanostructured delivery systems may provide synergistic antimicrobial and antibiofilm effects. Despite growing interest in MOF-based antimicrobial platforms, limited information is available regarding the efficacy of Ag₂O/UiO-66 nanocomposites loaded with *H. persicum* against urinary VRSA isolates. Therefore, the present study aimed to evaluate the antibacterial and antibiofilm activities of an Ag₂O/UiO-66 nanocomposite loaded with *H. persicum* extract against VRSA strains isolated from patients with UTIs. In addition, the effects of the nanocomposite on the expression of key resistance- and biofilm-associated genes, including *vanA*, *icaA*, and *icaD*, were investigated. By simultaneously targeting antimicrobial resistance and biofilm formation, this study proposes a promising nanotechnology-based strategy for combating persistent MDR urinary tract infections.

2. Materials and Methods

2.1. Materials

Zirconium(IV) chloride (ZrCl₄, ≥99%), terephthalic acid (H₂BDC, 98%), N,N-dimethylformamide (DMF, analytical grade), silver nitrate (AgNO₃, ≥99%), sodium hydroxide (NaOH, ≥98%), chloroform (≥99%), anhydrous sodium sulfate (Na₂SO₄), and all other analytical-grade reagents were purchased from Merck (Darmstadt, Germany), unless otherwise stated. Deionized (DI) water was used throughout the study.

Dried fruits of *H. persicum* were obtained from the Iranian Biological Resource Center (IBRC, Tehran, Iran) and authenticated prior to use. All chemicals were used as received without further purification.

2.2. Synthesis of UiO-66 nanoparticles via solvothermal method

UiO-66 nanoparticles were synthesized using a solvothermal method with slight modifications to a previously reported procedure [24]. Briefly, 0.480 g of ZrCl_4 and 3.66 g of H_2BDC were dissolved in 45 mL of DMF under magnetic stirring at room temperature for 1 h until a homogeneous solution was obtained. The resulting solution was transferred to a sealed glass reaction vial and heated at 140 °C for 36 h.

After completion of the reaction, the mixture was allowed to cool naturally to room temperature. The resulting white precipitate was separated from the reaction medium, and the supernatant was discarded. The collected solid was washed with fresh DMF (45 mL) and aged for 24 h to facilitate the removal of residual reactants and entrapped solvent molecules. Subsequently, the washing procedure was repeated twice using chloroform to promote solvent exchange within the porous framework. Finally, the purified product was dried under vacuum at 60 °C until a constant weight was achieved, yielding UiO-66 nanoparticles as a fine white powder.

2.3. Hydrothermal synthesis of Ag_2O nanoparticles

Ag_2O nanoparticles were synthesized via a hydrothermal method adapted from Mohan et al.[25]. Initially, 0.5 g of AgNO_3 was dissolved in 50 mL of deionized water under continuous magnetic stirring for 30 min to obtain a clear solution. In a separate vessel, a 5 M NaOH solution was prepared by dissolving the appropriate amount of NaOH pellets in 50 mL of deionized water under similar stirring conditions.

The NaOH solution was then added dropwise to the AgNO₃ solution under constant stirring until the pH reached 12, resulting in the formation of a dark brown suspension. The obtained mixture was transferred into a Teflon-lined stainless-steel autoclave and heated at 125 °C for 2 h under hydrothermal conditions.

After natural cooling to room temperature, the resulting precipitate was collected by filtration and washed several times with deionized water to remove residual ions. The final product was dried under ambient conditions to obtain Ag₂O nanoparticles.

2.4. Fabrication of Ag₂O/UiO-66 nanocomposite via hydrothermal assembly

The Ag₂O/UiO-66 nanocomposite was prepared via a facile wet chemical assembly method with slight modification of a previously reported procedure [26]. Initially, the as-synthesized UiO-66 nanocrystals were dispersed in deionized water and ultrasonicated to obtain a stable and homogeneous suspension. Subsequently, the prepared Ag₂O nanoparticles were introduced into the UiO-66 suspension and magnetically stirred for 10 min to ensure uniform dispersion and initial interaction between the components. The resulting mixture was then transferred into a Teflon-lined stainless-steel autoclave and heated at 90 °C for 6 h to facilitate the deposition and immobilization of Ag₂O nanoparticles onto the UiO-66 framework.

After natural cooling to room temperature, the obtained composite was collected by filtration and washed thoroughly with deionized water to remove unbound particles. Finally, the product was dried in a vacuum oven at 80 °C overnight until a constant weight was achieved, yielding the Ag₂O/UiO-66 nanocomposite powder.

2.5. Extraction and GC analysis of *H. persicum* EO

The EO of *H. persicum* was extracted from 100 g of dried fruits using hydrodistillation with a Clevenger-type apparatus. The plant material was immersed in 600 mL of distilled water in a 2-L round-bottom flask and subjected to hydrodistillation for 3 h. The extraction procedure was performed in triplicate to ensure reproducibility.

The obtained EO was separated and dried over anhydrous sodium sulfate (Na_2SO_4) to remove residual moisture and stored at $-20\text{ }^\circ\text{C}$ until further analysis. The extraction yield (% v/w) was recorded accordingly. Gas chromatographic (GC) analysis was performed using an HP-5 MS capillary column ($30\text{ m} \times 0.25\text{ mm i.d.}$, $0.25\text{ }\mu\text{m}$ film thickness). The oven temperature was programmed from $50\text{ }^\circ\text{C}$ (held for 3 min) to $260\text{ }^\circ\text{C}$ at a rate of $3\text{ }^\circ\text{C/min}$, and then held at $260\text{ }^\circ\text{C}$ for 5 min. The injector and detector temperatures were maintained at $240\text{ }^\circ\text{C}$ and $270\text{ }^\circ\text{C}$, respectively. Nitrogen was used as the carrier gas at a constant flow rate of 1 mL/min [27].

2.6. Loading of *H. persicum* EO onto $\text{Ag}_2\text{O}/\text{UiO-66}$ nanocomposite

The loading of *H. persicum* EO onto the $\text{Ag}_2\text{O}/\text{UiO-66}$ nanocomposite was carried out via a physical adsorption method. Briefly, the synthesized $\text{Ag}_2\text{O}/\text{UiO-66}$ nanocomposite was dispersed in ethanol containing a predetermined amount of EO and subjected to continuous stirring at room temperature to facilitate the diffusion of EO molecules into the porous structure of UiO-66 and their adsorption onto the surface of Ag_2O nanoparticles.

After the loading process, the mixture was centrifuged to collect the EO-loaded nanocomposite, and the obtained solid was washed lightly with ethanol to remove unadsorbed EO. The final product was dried under controlled conditions to obtain the $\text{Ag}_2\text{O}/\text{UiO-66}/\text{EO}$ nanocomposite.

This hybrid system integrates the high surface area and porosity of UiO-66, the intrinsic antimicrobial activity of Ag_2O nanoparticles, and the bioactive phytochemical constituents of *H.*

persicum EO, resulting in a multifunctional nanoplatform for antimicrobial and anti-biofilm applications.

2.7. Physicochemical characterization of Ag₂O/UiO-66/EO nanocomposite

The morphology and microstructural characteristics of the Ag₂O/UiO-66/EO nanocomposite were investigated using scanning electron microscopy (SEM; TESCAN VEGA 3SB, Czech Republic) and transmission electron microscopy (TEM; Philips CM30, Netherlands). SEM was employed to evaluate particle morphology, surface topology, and aggregation behavior, whereas TEM was used to examine particle size, structural features, and the distribution of Ag₂O nanoparticles relative to the UiO-66 matrix.

The hydrodynamic diameter, zeta potential, and polydispersity index (PDI) of the nanocomposite were determined by dynamic light scattering (DLS) using a Zetasizer Nano instrument (Malvern Instruments Ltd., Worcestershire, UK) at 25 °C. Prior to analysis, samples were dispersed in DI water by ultrasonication to ensure uniform suspension.

The crystalline structure and phase composition of the samples were analyzed by X-ray diffraction (XRD) using a Bruker D8 Advance diffractometer (Bruker, Germany) equipped with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) and operated at 40 kV and 40 mA. Diffraction patterns were recorded over a 2θ range of 10–80° with a step size of 0.02° and a scanning rate of 2° min⁻¹.

FTIR spectroscopy was performed using a Tensor 27 spectrophotometer (Bruker, Germany) in the spectral range of 4000–400 cm⁻¹ to investigate the functional groups present in the samples and assess the formation of the nanocomposite as well as EO loading. The obtained spectra were evaluated based on the characteristic absorption bands associated with the UiO-66 framework, Ag₂O, and EO constituents.

TGA was conducted using a TGA 4000 instrument (PerkinElmer, USA) under a nitrogen atmosphere. Samples were heated from room temperature to 800 °C at a heating rate of 10 °C min⁻¹. The resulting thermograms were used to evaluate thermal stability and estimate the EO loading content within the nanocomposite.

The specific surface area, pore volume, and pore size distribution were determined by BET analysis using a Micromeritics ASAP 2020 surface area analyzer (Micromeritics, USA). Nitrogen adsorption–desorption isotherms were recorded at 77 K after vacuum degassing of the samples. BET measurements were performed to evaluate the porous characteristics of UiO-66 and the changes induced by Ag₂O incorporation and EO loading.

2.8. Determination of EO entrapment efficiency in Ag₂O/UiO-66/EO nanocomposite

The loading efficiency of EO within the Ag₂O/UiO-66 nanocomposite was determined by measuring the amount of non-loaded EO remaining after the loading process [16]. Following preparation of the Ag₂O/UiO-66/EO nanocomposite, the suspension was transferred into Ultracel-30K centrifugal filter units (Millipore, USA) with a molecular weight cut-off (MWCO) of 30,000 Da. Briefly, 500 µL of the formulation was placed in the upper chamber of the filtration device and centrifuged at 4000 × g for 20 min at 4 °C using a refrigerated centrifuge (Eppendorf® 580R, Germany). The non-loaded EO passed through the membrane into the filtrate compartment, whereas the nanocomposite was retained in the upper chamber.

The concentration of free EO in the filtrate was determined using a UV–visible spectrophotometer (JASCO V-530, Japan) at $\lambda_{\text{max}} = 257$ nm, based on a previously established calibration curve. The loading efficiency percentage (LE%) was calculated according to the following equation:

$$EE(\%) = \frac{(C_{total} - C_{free})}{C_{total}} \times 100$$

where C_{total} is the initial amount of essential oil used for loading and C_{free} represents the concentration of unencapsulated essential oil in the filtrate.

2.9. *In vitro* release profile of EO from Ag₂O/UiO-66/EO nanocomposite

The *in vitro* release profile of EO from the Ag₂O/UiO-66/EO nanocomposite was evaluated using the dialysis bag diffusion method [28]. Prior to the experiment, a dialysis membrane (MWCO 12 kDa; Merck, Germany) was soaked in distilled water according to the manufacturer's instructions. Approximately 2 mL of the Ag₂O/UiO-66/EO suspension was transferred into the dialysis bag, which was subsequently sealed and immersed in 50 mL of phosphate-buffered saline containing sodium dodecyl sulfate (PBS–SDS) to maintain sink conditions. The release study was conducted at pH 7.4 and 37 ± 0.5 °C under continuous magnetic stirring at 300 rpm.

At predetermined time intervals, aliquots were withdrawn from the release medium and replaced with an equal volume of fresh pre-warmed PBS–SDS to maintain a constant volume throughout the experiment. The concentration of released EO was quantified using UV–visible spectrophotometry at $\lambda_{max} = 257$ nm. The cumulative percentage of EO release was calculated using a previously established calibration curve, and the release profile was expressed as cumulative EO release (%) versus time.

2.10. Isolation, identification, and characterization of VRSA isolates

2.5 Bacterial isolation and characterization

S. aureus isolates were recovered from 200 clinical specimens collected from hospitalized patients at Baqiyatallah Hospital, Tehran, Iran, between January and April 2025. The clinical

specimens included blood, sputum, and urine samples. Samples were inoculated onto appropriate selective and differential culture media and incubated under standard conditions. Presumptive identification of *S. aureus* was initially performed based on colony morphology, including colony size, pigmentation, and hemolytic characteristics, followed by conventional microbiological and biochemical tests. Gram staining was performed to confirm the presence of Gram-positive cocci arranged predominantly in clusters. The isolates were further characterized by catalase testing to differentiate staphylococci from catalase-negative Gram-positive cocci, and coagulase testing was used to differentiate *S. aureus* from coagulase-negative staphylococci. Additional conventional identification procedures, including mannitol fermentation and other standard biochemical characteristics, were performed to support the phenotypic identification of the isolates [29]. To confirm the species-level identification of the presumptive *S. aureus* isolates, molecular confirmation was subsequently performed by PCR amplification of the *femA* gene, a species-associated genetic marker used for the identification of *S. aureus*. Genomic DNA was extracted from the confirmed bacterial isolates, and PCR amplification of the *femA* gene was performed using the specific primers and PCR conditions described in a previous study [30]. The resulting PCR products were evaluated by agarose gel electrophoresis. Isolates showing the expected *femA*-specific amplification product (132 bp) were considered molecularly confirmed *S. aureus* and were included in the subsequent analyses.

Biofilm-forming ability was initially screened using Congo red agar (CRA) and subsequently quantified by the microtiter plate crystal violet (CV) assay [31].

The presence of resistance- and biofilm-associated genes, including *vanA*, *vanB*, *icaA*, and *icaD*, was determined by PCR using gene-specific primers. Antimicrobial susceptibility testing was initially performed using the Kirby–Bauer disk diffusion method according to the Clinical and

Laboratory Standards Institute guidelines. Because disk diffusion alone is not sufficient for the definitive classification of VRSA, the minimum inhibitory concentration (MIC) of vancomycin was additionally determined using the broth microdilution method in accordance with the CLSI M100, 35th edition (2025). According to the CLSI interpretive criteria for *Staphylococcus* spp., vancomycin MIC values of ≤ 2 $\mu\text{g/mL}$, 4–8 $\mu\text{g/mL}$, and ≥ 16 $\mu\text{g/mL}$ were interpreted as susceptible, intermediate, and resistant, respectively. Accordingly, isolates with a vancomycin MIC of ≥ 16 $\mu\text{g/mL}$ were classified as VRSA. The presence of vancomycin resistance-associated genes, including *vanA* and *vanB*, was evaluated as a molecular characterization of vancomycin resistance. Isolates meeting the phenotypic criteria for vancomycin resistance were selected for subsequent antimicrobial and antibiofilm analysis.

For quality control and assay validation, *S. aureus* ATCC 70069 was included as a positive control strain in the antimicrobial and antibiofilm assays. The use of the reference strain was intended to ensure the reliability and reproducibility of the experimental procedures and to provide a standardized comparator for the tested clinical isolates.

2.11. Evaluation of the antibacterial activity of Ag₂O/UiO-66/EO against VRSA isolates

The antibacterial activity of Ag₂O NPs, EO, and Ag₂O/UiO-66/EO was evaluated by determining MIC and minimum bactericidal concentration (MBC) using the broth microdilution method according to CLSI guidelines. Serial dilutions ranging from 12.5 to 400 $\mu\text{g/mL}$ for Ag₂O NPs and 400 to 1600 $\mu\text{g/mL}$ for EO were prepared in Mueller–Hinton broth (MHB) using sterile 96-well microplates. Bacterial suspensions adjusted to 0.5 McFarland standard were further diluted to achieve a final inoculum density of 5×10^5 CFU/mL in each well. Following incubation at 37 °C for 24 h, the MIC was defined as the lowest concentration showing no visible bacterial growth.

For MBC determination, 10 μ L aliquots were collected from wells exhibiting no visible growth and spread onto Mueller–Hinton agar (MHA) plates. After incubation, the MBC was defined as the lowest concentration resulting in $\geq 99.9\%$ bacterial killing [31].

The antibacterial effects of the tested formulations were further evaluated through growth inhibition kinetics. Equal volumes (100 μ L) of bacterial suspension (10^5 CFU/mL) and test compound at the MIC concentration were mixed in 96-well plates and incubated at 37 °C. Optical density was measured at 600 nm every 8 h for up to 72 h using a microplate reader (Epoch, BioTek Instruments, USA). Untreated bacterial cultures served as growth controls [32].

The antibacterial activity against VRSA isolates was additionally assessed using the disk diffusion method. Sterile 6-mm filter paper disks (Oxoid, UK) were impregnated with 10 μ L of each formulation at $0.5 \times \text{MIC}$, air-dried, and placed on MHA plates inoculated with the test strain. All experiments were performed in triplicate [33].

2.12. Comparative anti-biofilm activity of Ag₂O NPs, EO, and Ag₂O/UiO-66/EO against VRSA isolates

The antibiofilm activity of Ag₂O NPs, EO, and Ag₂O/UiO-66/EO against VRSA isolates was evaluated using the 96-well microtiter plate crystal violet assay. Briefly, bacterial biofilms were allowed to develop for 24 h at 37 °C. Subsequently, preformed biofilms were exposed to the MIC of each test formulation and incubated for an additional 24 h.

Following treatment, the wells were gently washed with phosphate-buffered saline (PBS) to remove non-adherent cells and stained with 1% (w/v) crystal violet. After staining, the bound dye was solubilized with 95% ethanol, and biofilm biomass was quantified by measuring absorbance

at 570 nm using a microplate reader (Stat Fax 2100, USA). Results were expressed relative to untreated controls [34].

The minimum biofilm eradication concentration (MBEC) was determined to evaluate the ability of the formulations to disrupt preformed biofilms. After biofilm establishment, wells were washed with PBS and treated with different concentrations of the test compounds for 24 h at 37 °C. Residual biofilm biomass was quantified by measuring absorbance at 570 nm.

Uninoculated Tryptic Soy Broth (TSB) served as the negative control, whereas untreated bacterial cultures were used as the positive control. The MBEC was defined as the lowest concentration producing an absorbance value equal to or lower than that of the negative control. All experiments were performed in duplicate [35].

2.13. Quantification of extracellular polymeric substances (EPS) production

The effect of Ag₂O NPs, EO, and Ag₂O/UiO-66/EO on EPS production was evaluated using a modified ethanol precipitation method [36]. Briefly, bacterial suspensions adjusted to 0.5 McFarland standard were inoculated into Luria–Bertani (LB) broth supplemented with sub-MIC concentrations of each test compound and incubated at 37 °C for 24 h, while untreated cultures served as controls. Cultures were centrifuged at 10,000 × g for 30 min at 4 °C, and EPS present in the supernatant were precipitated by adding cold 95% ethanol (1:3, v/v) followed by incubation at 4 °C for 18 h. The precipitated EPS were collected by centrifugation, resuspended in double-distilled water, filtered through a 0.45 µm cellulose acetate membrane, and freeze-dried. The dry weight of EPS was then measured and expressed as mg per 100 mL of culture. All experiments were performed in triplicate.

2.14. Analysis of gene expression in VRSA after treatment with Ag₂O NPs, EO, and Ag₂O/UiO-66/EO

The effects of Ag₂O NPs, EO, and Ag₂O/UiO-66/EO on the expression levels of *vanA*, *vanB*, *icaA*, and *icaD* genes in VRSA isolates were evaluated using quantitative real-time PCR (qRT-PCR) following exposure to sub-MIC concentrations of each formulation.

Total RNA was extracted from treated bacterial cultures, and complementary DNA (cDNA) was synthesized using standard reverse transcription procedures. The relative expression levels of target genes were analyzed by qRT-PCR using gene-specific primers listed in Table 1[37,38].

Total RNA was extracted using the RNX Plus kit (CinnaGen, Iran) according to the manufacturer's instructions. First-strand complementary DNA (cDNA) was synthesized using the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, USA). The quality and concentration of cDNA were assessed using a NanoDrop spectrophotometer.

Quantitative real-time PCR (qRT-PCR) was performed using SYBR Green Master Mix (Ampliqon, Denmark) in 25 µL reaction volumes containing diluted cDNA and gene-specific primers. Amplification was carried out under the following cycling conditions: initial denaturation at 95 °C for 60 s, followed by 30 cycles of 95 °C for 5 s, 60 °C for 1 min, and 72 °C for 45 s, with a final extension step at 72 °C for 10 min.

The 16S rRNA gene served as the internal reference gene, and relative gene expression levels were calculated using the comparative $2^{-\Delta\Delta Ct}$ method, with untreated samples used as calibrators [39].

Table 1. Oligonucleotide primers used for this study.

Gene	Primer sequence (5'-3')	Product (bp)
<i>vanA</i>	F: TCTGCAATAGAGATAGCCGC R: GGAGTAGCTATCCCAGCATT	400
<i>vanB</i>	F: GTGACAAACCGGAGGCGAGGA R: CCGCCATCCTCCTGCAAAAAA	430
<i>icaA</i>	F: ATGGTCAAGCCCAGACAGAG R: CGTGTTTTCAACATTTAATGCAA	188
<i>icaD</i>	F: ATGGTCAAGCCCAGACAGAG R: CGTGTTTTCAACATTTAATGCAA	198
<i>16S rRNA</i>	F: ATCAGAGCGCGGATCTTTGCCG R: ATCAGAGCGCGGATCTTTGCCG	155

368

369 2.15. MTT cytotoxicity assay

370 The cytotoxicity of Ag₂O NPs, EO, and Ag₂O/UiO-66/EO was evaluated using the MTT assay
371 according to ISO 10993-5 and previously reported methods [40]. L929 fibroblast cells were
372 cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher
373 Scientific, USA) supplemented with 10% fetal bovine serum (FBS) under standard conditions
374 (37 °C, 5% CO₂). Cells were trypsinized, counted using a Neubauer hemocytometer, and seeded
375 at a density of 5 × 10⁴ cells per well in 24-well plates. After 24 h of incubation, cells were treated
376 with different concentrations (5.31–85 µg/mL) of Ag₂O NPs, EO, and Ag₂O/UiO-66/EO and
377 incubated for an additional 24 h under standard culture conditions. MTT solution (1 mg/mL) was
378 then added to each well, and cells were incubated for 3–5 h at 37 °C. The resulting formazan

crystals were dissolved in isopropanol, and absorbance was measured at 560 nm. Cell viability was calculated relative to untreated control cells using the following equation:

Viable cell percentage (%) = treated cells mean absorbance/control cells mean absorbance × 100

2.16. Statistical analysis

Statistical analysis were performed using SPSS software (version 20) and GraphPad Prism (version 8). Data were analyzed using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. All results are presented as mean ± standard deviation (SD), and statistical significance was defined as $P < 0.05$.

3. Results

3.1. Morphology of Ag₂O/UiO-66/EO nanocomposite

SEM micrographs (Figure 1A) of the Ag₂O/UiO-66/EO nanocomposite show the formation of predominantly quasi-spherical to irregular particles with an agglomerated morphology. The particles appear as clustered nanostructures distributed across the sample. At higher magnification (×50k), nanoscale primary particles are observed decorating the UiO-66 framework, indicating successful incorporation of the inorganic phase without visible collapse of the MOF structure. The surface of the nanocomposite appears rough and porous.

TEM images (Figure 1B) confirm the formation of nanoscale particles with quasi-spherical morphology and partial aggregation. Dark contrast regions corresponding to Ag₂O nanoparticles are observed distributed on and within the UiO-66 framework. The particles exhibit nanoscale dimensions and are dispersed along the MOF structure.

3.2. Dynamic size, PDI, zeta potential and EE%

DLS analysis of the Ag₂O/UiO-66/EO nanocomposite showed a particle size distribution with an average hydrodynamic diameter of 154.6 nm (Figure 1C). The PDI was 0.324, indicating a moderately uniform size distribution.

The zeta potential of the nanocomposite was measured to be −17.5 mV (Figure 1D), indicating a negatively charged surface.

Encapsulation efficiency (EE%) analysis showed that Ag₂O/UiO-66 exhibited negligible loading capacity, whereas Ag₂O/UiO-66/EO achieved an EE% of $81.20 \pm 2.9\%$ (Figure 1E).

3.3. XRD analysis

The X-ray diffraction (XRD) pattern of the Ag₂O/UiO-66/EO nanocomposite recorded in the range of $2\theta = 10\text{--}80^\circ$ is shown in Figure 1F. Characteristic diffraction peaks at approximately $2\theta \approx 12.02^\circ$, 17.09° , 25.725° , and 30.765° correspond to the crystalline structure of the UiO-66 framework. In addition, diffraction peaks observed at around $2\theta \approx 32.115^\circ$, 37.525° , 50.44° , and 64° are attributed to the crystalline phases of Ag₂O, confirming its successful incorporation into the composite. No additional impurity peaks were detected, indicating the high phase purity of the synthesized nanocomposite.

3.4. FTIR, thermal (TGA), surface (BET), and release analyses of the nanocomposite

The FTIR spectrum of Ag₂O/UiO-66/EO (Figure 2A) shows characteristic absorption bands confirming successful formation of the hybrid nanocomposite. A broad band at 3432 cm^{-1} corresponds to O–H stretching vibrations from surface hydroxyl groups, adsorbed water, and EO-derived phenolic compounds. Peaks at 2924 and 2855 cm^{-1} are assigned to aliphatic C–H stretching vibrations. Bands at 1741 and 1656 cm^{-1} correspond to C=O stretching vibrations from phytochemical constituents and carboxylate groups in UiO-66.

The bands at 1584 and 1394 cm^{-1} are attributed to asymmetric and symmetric stretching of carboxylate groups of the UiO-66 linker, indicating preservation of framework integrity. Peaks in the region 1154–1018 cm^{-1} correspond to C–O and C–O–C vibrations of EO constituents. Low wavenumber bands at 547 and 477 cm^{-1} confirm Zr–O and Ag–O bonding, respectively.

TGA results (Figure 2B) show multi-stage weight loss behavior. The first stage ($<150\text{ }^{\circ}\text{C}$) corresponds to removal of adsorbed water and solvents. The second stage (150–300 $^{\circ}\text{C}$) is attributed to decomposition of EO phytochemicals and partial degradation of organic groups. Above 300 $^{\circ}\text{C}$, gradual weight loss is associated with decomposition of the UiO-66 framework. A stable residue at high temperature corresponds to inorganic Ag_2O and Zr-based species, indicating good thermal stability.

Nitrogen adsorption–desorption isotherms (Figure 2C) reveal a predominantly microporous structure with mesoporous contribution. The BET surface area is 554.56 $\text{m}^2\text{ g}^{-1}$, with a pore volume of 0.373 $\text{cm}^3\text{ g}^{-1}$. The average pore diameter is 2.876 nm. BJH analysis confirms pore formation in the nanoscale range, suitable for guest molecule diffusion (Table 2).

The cumulative release profiles of free EO and $\text{Ag}_2\text{O}/\text{UiO-66}/\text{EO}$ are presented in Figure 2D. Free EO exhibited a pronounced burst release, reaching $83.6 \pm 2.1\%$ within the first 8 h and gradually increasing to $99.9 \pm 1.5\%$ after 72 h. In contrast, $\text{Ag}_2\text{O}/\text{UiO-66}/\text{EO}$ showed a significantly slower and sustained release behavior, with cumulative release values of $22.0 \pm 1.3\%$, $27.0 \pm 1.1\%$, and $36.0 \pm 1.0\%$ at 8, 24, and 72 h, respectively. At all sampling points, the cumulative release from $\text{Ag}_2\text{O}/\text{UiO-66}/\text{EO}$ was significantly lower than that of free EO ($P < 0.001$).

444

445 Table 2. Textural properties of the Ag₂O/UiO-66/EO nanocomposite determined by BET and
 446 BJH analyses.

Parameter	Value	Unit
BET Analysis		
Monolayer adsorption capacity (V _m)	124.79	cm ³ (STP) g ⁻¹
Specific surface area (SBET)	554.65	m ² .g ⁻¹
BET constant (C)	22510	—
Total pore volume (P/P ₀ = 0.99)	0.373	cm ³ .g ⁻¹
Mean pore diameter	2.876	nm
BJH Analysis (Adsorption Branch)		
Pore volume (V _p)	0.1784	cm ³ .g ⁻¹
Peak pore radius (r _p , peak area)	1.21	nm
Pore surface area (a _p)	69.688	m ² .g ⁻¹

447

448 3.5. Identification of VRSA isolates and their biofilm-forming ability

449 Among the 200 clinical specimens collected from patients with UTIs, 34 isolates were identified
 450 as *S. aureus* based on colony morphology, Gram staining, and conventional biochemical tests,
 451 including catalase, oxidase, and coagulase reactions. Of these, three isolates were classified as

VRSA based on their MIC and the molecular detection of vancomycin resistance-associated genes (*vanA* and/or *vanB*) by PCR analysis. All three VRSA isolates exhibited strong biofilm-forming ability in the CV assay.

3.6. Antibacterial activity of Ag₂O, EO, and Ag₂O/UiO-66/EO against VRSA isolates

3.6.1. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the formulations

The antibacterial activities of Ag₂O, EO, and Ag₂O/UiO-66/EO against clinical VRSA isolates and the reference strain are summarized in Table 3.

Ag₂O exhibited moderate antibacterial activity, with MIC values ranging from 50 to 100 µg.mL⁻¹ and MBC values between 100 and 200 µg.mL⁻¹. EO showed comparatively lower antibacterial efficacy, with MIC and MBC values of 400–800 µg.mL⁻¹ and 800–1600 µg.mL⁻¹, respectively.

In contrast, Ag₂O/UiO-66/EO demonstrated the highest antibacterial activity, with MIC values ranging from 12.5 to 25 µg.mL⁻¹ and MBC values of 25–50 µg.mL⁻¹ against VRSA isolates. The nanocomposite exhibited significantly lower inhibitory and bactericidal concentrations than either Ag₂O or EO alone ($P < 0.05$).

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Ag₂O, EO, and Ag₂O/UiO-66/EO against VRSA isolates recovered from urinary tract infections.

Isolate	MIC (Ag ₂ O)*	MIC (EO)	MIC (Ag ₂ O/UiO- 66/EO)†	MBC (Ag ₂ O)	MBC (EO)	MBC (Ag ₂ O/UiO- 66/EO)†
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VRSA-1	100	400	12.5/100	200	800	25/100
VRSA-2	50	400	12.5/50	100	800	25/100
VRSA-3	100	800	25/100	200	1600	50/100
ATCC 700699	50	400	12.5/25	100	800	25/50

* All values are expressed as $\mu\text{g.mL}^{-1}$.

† For the $\text{Ag}_2\text{O}/\text{UiO-66}/\text{EO}$ formulation, values are expressed as Ag_2O concentration/ EO concentration ($\mu\text{g.mL}^{-1}$).

3.6.1. Disk diffusion findings

The antibacterial activities of Ag_2O , EO , and $\text{Ag}_2\text{O}/\text{UiO-66}/\text{EO}$ against clinical VRSA isolates and the reference strain were further assessed using the disk diffusion assay (Figure 3A). The $\text{Ag}_2\text{O}/\text{UiO-66}/\text{EO}$ nanocomposite exhibited the highest antibacterial activity against all tested strains, producing inhibition zone diameters ranging from approximately 31 to 33 mm. These values were significantly greater than those observed for Ag_2O and EO alone ($P < 0.001$).

Ag_2O demonstrated moderate antibacterial activity, with inhibition zones of approximately 24–26 mm, whereas EO exhibited comparatively lower activity, producing inhibition zones of approximately 18–20 mm. Similar inhibition zone diameters were observed among all clinical isolates and the reference strain, indicating a relatively strain-independent antibacterial effect of the tested formulations.

3.6.3. Time-kill kinetic assay

The bactericidal kinetics of Ag_2O , EO , and $\text{Ag}_2\text{O}/\text{UiO-66}/\text{EO}$ against VRSA were evaluated over a 72-h incubation period by monitoring optical density at 600 nm (OD_{600}) (Figure 3B).

The untreated control exhibited a continuous increase in bacterial growth throughout the experiment, reaching an OD₆₀₀ of approximately 3.8 after 72 h. Treatment with EO partially inhibited bacterial proliferation; however, bacterial growth gradually increased over time, resulting in a final OD₆₀₀ of approximately 2.1 at 72 h. Ag₂O demonstrated a more pronounced inhibitory effect, limiting bacterial growth to an OD₆₀₀ of approximately 1.3 at the end of the incubation period.

Among all formulations, Ag₂O/UiO-66/EO exhibited the greatest antibacterial activity. The OD₆₀₀ values remained consistently low throughout the experiment and reached only approximately 0.5 after 72 h. No appreciable rebound in bacterial growth was observed during the incubation period.

3.7. Antibiofilm activity of Ag₂O/UiO-66@EO against VRSA isolates

3.11.1. Inhibition of biofilm formation assessed by crystal violet assay

The effects of Ag₂O NPs, EO, and Ag₂O/UiO-66@EO on biofilm formation by VRSA isolates were evaluated using the crystal violet (CV) microtiter plate assay, and biofilm biomass was quantified by measuring OD₅₇₀ values (Figure 4A). Prior to treatment, all VRSA isolates exhibited strong biofilm-forming capacity, with OD₅₇₀ values ranging from approximately 0.48 to 0.53. Treatment with EO significantly reduced biofilm biomass, resulting in OD₅₇₀ values of approximately 0.38–0.42 (***P < 0.001 versus untreated control). Similarly, Ag₂O NPs significantly decreased biofilm formation, with OD₅₇₀ values ranging from approximately 0.30 to 0.35 (***P < 0.001 versus untreated control). Among the tested formulations, Ag₂O/UiO-66@EO exhibited the greatest inhibitory effect on biofilm formation, reducing OD₅₇₀ values to approximately 0.12–0.16 in all clinical isolates and the reference strain ATCC 700699. The

reductions observed for the nanocomposite were highly significant compared with both the untreated control ($***P < 0.001$) and the individual treatments ($####P < 0.001$).

3.7.2. Eradication of preformed biofilms evaluated by the MBEC

The ability of Ag₂O NPs, EO, and Ag₂O/UiO-66@EO to eradicate mature VRSA biofilms was determined using the MBEC assay, and the surviving bacterial populations were expressed as log₁₀(CFU/mL) (Figure 4B). Untreated mature biofilms contained approximately 7.0–7.5 log₁₀(CFU/mL) viable bacterial cells. Treatment with EO significantly reduced viable cell counts to approximately 5.0–5.6 log₁₀(CFU/mL) ($***P < 0.001$ versus untreated biofilms). Exposure to Ag₂O NPs further reduced bacterial viability, with counts ranging from approximately 4.0 to 4.5 log₁₀(CFU/mL) ($***P < 0.001$ versus untreated biofilms). In contrast, treatment with Ag₂O/UiO-66@EO resulted in the greatest reduction in viable biofilm-associated bacteria, decreasing cell counts to approximately 1.1–1.7 log₁₀(CFU/mL) in all tested strains, including ATCC 700699. These reductions were highly significant compared with both untreated biofilms ($***P < 0.001$) and single-component treatments ($####P < 0.001$). Relative to untreated controls, the nanocomposite achieved reductions exceeding 5 log₁₀(CFU/mL).

3.7.3. Inhibition of extracellular polymeric substance production

The effects of Ag₂O NPs, EO, and Ag₂O/UiO-66@EO on extracellular polymeric substance (EPS) production by VRSA isolates were determined by measuring EPS dry weight (mg/100 mL) (Figure 4C). Untreated VRSA isolates produced substantial amounts of EPS, with values ranging from approximately 13.0 to 13.8 mg/100 mL. Treatment with EO significantly reduced EPS production to approximately 10.3–10.8 mg/100 mL ($***P < 0.001$ versus untreated control). Ag₂O NPs caused a greater reduction, decreasing EPS levels to approximately 8.5–9.1 mg/100

mL (**P < 0.001 versus untreated control). The most pronounced inhibitory effect was observed in the Ag₂O/UiO-66@EO-treated group, in which EPS production decreased to approximately 4.0–4.5 mg/100 mL across all tested isolates and the reference strain ATCC 700699. The reductions achieved by the nanocomposite were highly significant compared with both the untreated control (**P < 0.001) and the individual treatments (###P < 0.001).

3.8. Real-time PCR analysis of biofilm- and vancomycin resistance-associated genes (*icaA*, *icaD*, *vanA* and *vanB*)

The expression levels of biofilm-associated genes (*icaA* and *icaD*) and vancomycin resistance genes (*vanA* and *vanB*) were evaluated in VRSA isolates (strains 1–3 and ATCC 700699) following treatment with Ag₂O NPs, *H. persicum* extract, and Ag₂O/UiO-66@*H. persicum* (Figure 5).

For biofilm-related genes, untreated control samples showed baseline expression levels of 1.0-fold. Treatment with *H. persicum* extract resulted in a reduction in *icaA* and *icaD* expression across all isolates, with fold changes approximately ranging from 0.60 to 0.75 compared with control. Ag₂O NPs further decreased the expression of both genes, with relative expression levels ranging from approximately 0.40 to 0.55. The most significant reduction was observed in the Ag₂O/UiO-66@*H. persicum* group, where *icaA* and *icaD* expression levels decreased to approximately 0.22–0.30-fold in all tested strains (Figure 5A and 5B).

Regarding vancomycin resistance genes, untreated VRSA isolates exhibited baseline expression levels of 1.0-fold for both *vanA* and *vanB*. Treatment with *H. persicum* extract produced a slight reduction in gene expression, with fold changes ranging from approximately 0.85 to 0.95. Ag₂O NPs led to a more pronounced decrease, with expression levels ranging from approximately 0.50

to 0.65 for both genes. The lowest expression levels were observed in the Ag₂O/UiO-66@*H. persicum* treated group, where *vanA* and *vanB* expression decreased to approximately 0.28–0.40-fold relative to control across all isolates (Figure 5C and 5D). All gene expression changes were consistent across clinical isolates (strains 1–3) and the reference strain ATCC 700699.

3.9. Cytotoxicity evaluation against normal cells by MTT assay

The cytocompatibility of Ag₂O NPs, *H. persicum* extract, and Ag₂O/UiO-66@*H. persicum* was assessed in HFF cells using the MTT assay across a range of concentrations (Figure 6). Untreated cells showed ~100% viability, confirming normal metabolic activity. At low concentrations (≤ 10.63 $\mu\text{g/mL}$), all tested formulations maintained high cell viability (>85 – 90%), with no significant difference compared to the control group ($p > 0.05$). At 21.5 $\mu\text{g/mL}$, a significant reduction in viability was observed only for Ag₂O NPs ($p < 0.001$), whereas *H. persicum* extract and Ag₂O/UiO-66@*H. persicum* did not show comparable toxicity at this level.

A clear concentration-dependent cytotoxic effect was observed at higher doses. Ag₂O NPs induced the strongest reduction in cell viability, decreasing to approximately ~60% at 85 $\mu\text{g/mL}$ ($***p < 0.001$). *H. persicum* extract exhibited relatively low cytotoxicity, maintaining ~85% viability even at the highest concentration ($p < 0.01$). Importantly, Ag₂O/UiO-66@*H. persicum* showed improved biocompatibility compared to Ag₂O NPs, with cell viability remaining above ~77.8% across most tested concentrations ($####p < 0.001$ vs. Ag₂O NPs).

Overall, the results demonstrate that incorporation of *H. persicum* and Ag₂O into the UiO-66 framework significantly reduces the cytotoxic effects associated with free Ag₂O nanoparticles while maintaining acceptable cell viability at antibacterial concentrations.

4. Discussion

The development of multifunctional nanoplateforms that integrate metallic nanoparticles, metal-organic frameworks (MOFs), and plant-derived bioactive compounds has emerged as a promising strategy to overcome the limitations of conventional antimicrobial therapies, particularly against multidrug-resistant (MDR) pathogens [41]. In this context, the successful construction of an Ag₂O/UiO-66/EO nanocomposite represents a significant advancement due to the simultaneous contribution of inorganic antimicrobial agents, high-surface-area porous carriers, and natural phytochemical components. Such hybrid systems are expected to enhance bacterial targeting efficiency, improve stability of active compounds, and promote synergistic antibacterial effects [42].

In the present study, morphological analyses demonstrated the formation of quasi-spherical to irregular nanoparticles with pronounced aggregation. SEM results revealed clustered nanostructures with a rough and porous surface, while TEM images confirmed the presence of well-dispersed Ag₂O domains anchored on the UiO-66 framework without structural collapse. The observed particle distribution in the nanoscale range suggests successful integration of all components into a unified hybrid architecture. Notably, Ag₂O nanoparticles appeared as darker contrast regions uniformly distributed along the MOF matrix, indicating effective loading and stabilization within the porous framework.

Although partial agglomeration was observed, this phenomenon is commonly reported in Ag-based nanocomposites and can be attributed to the inherently high surface energy of Ag₂O nanoparticles, which drives interparticle attraction during synthesis and drying processes. Additionally, the presence of EO-derived phytochemicals may have influenced nucleation and growth behavior by acting as mild reducing and capping agents, thereby modulating particle aggregation without compromising structural integrity [43].

598 Importantly, the preservation of the UiO-66 framework after incorporation of Ag₂O and EO is a
599 critical finding, as Zr-based MOFs such as UiO-66 are known for their high chemical and
600 thermal. This structural robustness ensures that the porous architecture remains available for
601 interaction with bacterial cells and facilitates diffusion of bioactive species. The rough and
602 porous morphology observed in SEM analysis is particularly relevant, as increased surface
603 heterogeneity has been shown to enhance bacterial adhesion and improve antimicrobial contact
604 efficiency, which may contribute to the enhanced bioactivity observed in subsequent assays
605 [44,45]. Overall, the morphological characteristics of the Ag₂O/UiO-66/EO nanocomposite
606 confirm the successful synthesis of a structurally stable hybrid system with nanoscale
607 architecture, which is expected to play a key role in its antibacterial and anti-biofilm
608 performance against VRSA isolates.

609 The physicochemical properties of nanocarriers play a crucial role in determining their biological
610 performance, particularly in antimicrobial and antibiofilm applications where particle stability,
611 cellular interaction, and biofilm penetration are key determinants. In this study, the Ag₂O/UiO-
612 66/EO nanocomposite exhibited a hydrodynamic size of 154.6 nm, which is within the optimal
613 nanoscale range previously associated with efficient bacterial interaction and enhanced
614 bioavailability [44].

615 The observed discrepancy between DLS and electron microscopy results is consistent with
616 earlier findings reported for UiO-66-based nanostructures, where hydrodynamic diameter
617 includes solvation layers and surface-associated organic molecules. Similar size variations were
618 previously observed in Ag-based MOF composites reported in this study's comparative literature
619 section, including UiO-66-NH₂@AgNP systems (131–166 nm range) , confirming that the
620 present system follows expected colloidal behavior [44].

621 The PDI value of 0.324 indicates a moderately homogeneous dispersion. As previously discussed
622 in the literature cited in this work, PDI values around 0.3 are generally considered acceptable for
623 hybrid nanomaterials, particularly those containing organic–inorganic interfaces where slight
624 heterogeneity is inevitable due to surface functionalization and phytochemical adsorption [46-
625 48].

626 The zeta potential value of -17.5 mV reflects a moderately stable colloidal system. This behavior
627 is consistent with earlier observations for UiO-66- and UiO-66-NH₂-based nanostructures
628 reported in this study's referenced literature, which typically exhibit mildly negative surface
629 charges depending on surface chemistry and medium conditions. In the present system, the
630 negative surface charge is primarily influenced by EO-derived phytochemicals and MOF
631 functional groups, while partial neutralization occurs due to interaction with Ag₂O species,
632 leading to a balanced electrostatic profile [49,50].

633 As previously reported for plant-derived functional coatings and nanocarriers in this manuscript,
634 phenolic, hydroxyl, and carboxyl functional groups contribute significantly to surface charge
635 modulation. This balanced charge is particularly important for antimicrobial applications, as it
636 may enhance interaction with negatively charged bacterial membranes and facilitate penetration
637 into biofilm matrices [49,50].

638 EE% analysis demonstrated a high EO loading capacity of $81.20 \pm 2.9\%$, which is significantly
639 higher than that of the bare Ag₂O/UiO-66 system. This improvement confirms the strong affinity
640 of EO phytochemicals for the porous UiO-66 framework, consistent with previously reported
641 UiO-66-based drug delivery systems cited in this work, including cisplatin ($\sim 70\%$) and curcumin
642 ($\sim 65\%$) loaded nanocarriers [51]. The high EE% highlights the role of MOF porosity and surface

643 interactions in stabilizing bioactive compounds through hydrogen bonding and van der Waals
644 interactions.

645 Overall, the combination of nanoscale size (154.6 nm), moderate negative surface charge (-17.5
646 mV), and high EE% (81.20%) confirms the successful fabrication of a stable and biologically
647 functional Ag₂O/UiO-66/EO nanoplatform suitable for antimicrobial applications against MDR
648 pathogens.

649 The XRD analysis confirms the successful formation of the Ag₂O/UiO-66/EO nanocomposite
650 with preserved crystallinity of the UiO-66 framework. The characteristic UiO-66 peaks ($\approx 12^\circ$ –
651 31°) indicate structural stability after incorporation of Ag₂O and EO-derived components,
652 consistent with the robust nature of Zr-based MOFs reported previously in this study [52,53].

653 The appearance of distinct Ag₂O reflections ($\approx 32^\circ$ – 64°) confirms successful loading of the
654 crystalline Ag₂O phase within the composite. The coexistence of both phases without additional
655 impurity peaks suggests high purity and successful composite formation.

656 The observed peak broadening and reduced intensity of Ag₂O reflections indicate nanoscale
657 crystallite size and restricted crystal growth, a behavior commonly reported in Ag-based MOF
658 hybrid systems. This may also reflect interfacial interactions between Ag₂O, UiO-66, and EO-
659 derived phytochemicals, which contribute to nanoparticle stabilization during synthesis [52,53].

660 Overall, the XRD results verify the structural integrity and successful hybridization of the
661 nanocomposite, which is essential for its subsequent antimicrobial performance.

662 The FTIR results confirm the successful integration of EO-derived phytochemicals, Ag₂O, and
663 UiO-66 within a single hybrid framework. The preservation of characteristic UiO-66 carboxylate
664 vibrations (1584 and 1394 cm^{-1}) indicates structural stability of the MOF after modification,

665 consistent with the robustness of Zr-based frameworks previously reported in this study [54].
666 The appearance of Zr–O and Ag–O vibrations further confirms successful incorporation of
667 inorganic phases, supporting the XRD and morphological results.

668 The thermal behavior observed in TGA reflects the multi-component nature of the
669 nanocomposite. The degradation profile of EO components at lower temperatures and the
670 stability of the inorganic residue at higher temperatures align with previously reported UiO-66-
671 based systems loaded with organic molecules [28]. The gradual decomposition above 300 °C
672 further supports the thermal stability of the hybrid structure, while the remaining residue
673 confirms the presence of stable inorganic phases.

674 BET analysis demonstrates that the nanocomposite retains a high surface area ($554.56 \text{ m}^2 \text{ g}^{-1}$),
675 although reduced compared to pristine UiO-66 reported in earlier studie in this work [28],
676 indicating successful pore occupation by Ag_2O and EO molecules. The mesoporous contribution
677 (2.876 nm) is particularly favorable for diffusion-controlled antimicrobial delivery, as previously
678 reported for MOF-based drug carriers.

679 The release profile clearly demonstrates the functional advantage of encapsulation. The burst
680 release observed for free EO (~99.9% at 72 h) is effectively controlled in the nanocomposite,
681 where only 36% release occurs over the same period. This sustained release behavior is
682 attributed to diffusion barriers within the UiO-66 framework and interactions with Ag_2O , which
683 collectively regulate payload release. Similar controlled release behavior has been reported in
684 MOF-based delivery systems, confirming the suitability of UiO-66 as a sustained-release carrier
685 [55].

Overall, the combined FTIR, TGA, BET, and release results confirm the successful construction of a structurally stable, porous, and controlled-release nanopatform suitable for antimicrobial applications.

The emergence of vancomycin-resistant and biofilm-forming *S. aureus* strains has severely limited the efficacy of conventional antibiotics and necessitated the development of alternative antimicrobial strategies capable of simultaneously targeting bacterial growth, persistence, and resistance mechanisms [56]. In the present study, the antibacterial performance of Ag₂O/UiO-66/EO was comprehensively evaluated using MIC/MBC determination, disk diffusion, and growth kinetic analyses. Collectively, these assays consistently demonstrated that the nanocomposite exhibited markedly superior antibacterial activity compared with Ag₂O and EO alone.

Ag₂O alone displayed moderate antibacterial activity, with MIC and MBC values of 50–100 and 100–200 µg.mL⁻¹, respectively, which is consistent with previous studies reporting bactericidal activity of silver-based nanoparticles against *S. aureus* and other pathogenic bacteria within a similar concentration range [57,58]. The antimicrobial properties of Ag₂O are primarily mediated through bacterial membrane disruption, intracellular reactive oxygen species (ROS) generation, protein denaturation, and interference with nucleic acid synthesis [58]. In contrast, free EO exhibited comparatively weaker antibacterial activity, with MIC and MBC values ranging from 400–800 and 800–1600 µg.mL⁻¹, respectively. Although *H. persicum* contains numerous bioactive terpenoids, phenolics, and flavonoids with documented antimicrobial properties, its efficacy is often limited by poor aqueous solubility, volatility, rapid degradation, and restricted penetration into bacterial biofilms [59,60].

Remarkably, incorporation of Ag₂O and EO into the UiO-66 framework significantly potentiated antibacterial activity, reducing MIC values to 12.5–25 µg mL⁻¹ and MBC values to 25–50 µg.mL⁻¹. Compared with free EO, the nanocomposite achieved approximately a 16–32-fold reduction in MIC values. These findings were further corroborated by the disk diffusion assay, in which Ag₂O/UiO-66/EO generated substantially larger inhibition zones (31–33 mm) than Ag₂O (24–26 mm) and EO (18–20 mm). Moreover, growth kinetic analysis demonstrated prolonged antibacterial activity, with the nanocomposite maintaining an OD₆₀₀ of approximately 0.5 after 72 h, whereas the control, EO, and Ag₂O groups reached OD₆₀₀ values of approximately 3.8, 2.1, and 1.3, respectively. The absence of bacterial regrowth in the Ag₂O/UiO-66/EO-treated group indicates durable bactericidal activity and sustained suppression of bacterial proliferation.

The enhanced antibacterial efficacy of Ag₂O/UiO-66/EO is likely multifactorial. Characterization studies demonstrated that the nanocomposite possessed a high BET surface area (554.65 m² g⁻¹), high EE% (81.20 ± 2.9%), nanoscale particle size (154.6 nm), and a moderately negative zeta potential (−17.5 mV). These physicochemical properties collectively provide an ideal platform for efficient loading, stabilization, and controlled release of EO constituents [28,61]. Indeed, the release study demonstrated a sustained release pattern, with only approximately 36% of the encapsulated EO released after 72 h, indicating prolonged retention of bioactive compounds within the MOF matrix. Such controlled release behavior likely maintains effective antibacterial concentrations at the bacterial interface over extended periods and prevents the rapid depletion commonly observed with free EO formulations [62].

Furthermore, the porous architecture of UiO-66 may improve the dispersion of Ag₂O nanoparticles and minimize nanoparticle aggregation, thereby maximizing the available reactive surface area and promoting continuous release of Ag⁺ ions [20]. This interpretation is supported

by SEM and TEM analyses, which demonstrated successful anchoring of Ag₂O nanoparticles onto the preserved UiO-66 framework without structural collapse. Simultaneously, the nanoscale particle size and moderate surface charge may facilitate intimate interactions with bacterial cell surfaces and enhance diffusion through EPS within biofilms [43,63].

The antibacterial activity of the nanocomposite is therefore likely mediated through several complementary mechanisms acting simultaneously. Ag₂O can induce ROS generation, membrane destabilization, and intracellular macromolecular damage, whereas EO-derived phytochemicals may further disrupt membrane integrity and interfere with quorum sensing, virulence signaling pathways, and biofilm-associated adaptive responses [20,21]. The convergence of these mechanisms may explain not only the substantially lower MIC and MBC values but also the larger inhibition zones and prolonged suppression of bacterial growth observed during the 72-h kinetic assay.

Interestingly, similar inhibition zone diameters were observed among all clinical isolates and the reference strain, suggesting that the antibacterial activity of Ag₂O/UiO-66/EO is largely independent of strain-specific phenotypic variations. This finding is particularly important because it indicates robust antibacterial activity against highly resistant and strongly biofilm-forming VRSA isolates. Comparable observations have been reported for other UiO-66-based antimicrobial systems, including ciprofloxacin-loaded UiO-66 nanocomposites and vancomycin/amikacin-loaded UiO-66-NH₂/PEG formulations, which exhibited enhanced antibacterial efficacy through sustained drug release and improved delivery efficiency [38].

Collectively, the present findings demonstrate that Ag₂O/UiO-66/EO functions not merely as a passive carrier system but as a multifunctional antimicrobial nanoplatform capable of significantly potentiating antibacterial activity against VRSA. The substantial reduction in

effective bactericidal concentrations, combined with sustained antibacterial activity and prevention of bacterial regrowth, suggests that this nanocomposite may represent a promising therapeutic strategy for the management of persistent and biofilm-associated VRSA infections while potentially reducing cytotoxicity and minimizing the selective pressure that drives the emergence of additional resistance mechanisms [20,59].

Biofilm formation is one of the major virulence determinants of *S. aureus* and represents a critical challenge in the treatment of VRSA-associated urinary tract infections. The EPS matrix acts as a physical and biochemical barrier that limits antimicrobial penetration, facilitates intercellular communication, and protects bacterial cells from host immune responses and antibiotic-mediated killing. Consequently, successful management of biofilm-associated infections requires not only inhibition of biofilm formation but also eradication of mature biofilms and disruption of the EPS matrix itself [59,64].

In the present study, Ag₂O/UiO-66@EO exhibited remarkably superior antibiofilm activity compared with Ag₂O NPs and free EO alone. Treatment with the nanocomposite reduced biofilm biomass from approximately 0.48–0.53 to 0.12–0.16 OD₅₇₀ units, corresponding to nearly a 70% reduction in biofilm formation. Moreover, viable bacterial populations within mature biofilms decreased from approximately 7.0–7.5 log₁₀ CFU/mL to 1.1–1.7 log₁₀ CFU/mL, representing a reduction exceeding 5 log₁₀ units and meeting the accepted criterion for effective biofilm eradication. Likewise, EPS production was markedly suppressed from approximately 13–13.8 mg/100 mL in untreated controls to 4.0–4.5 mg/100 mL following nanocomposite treatment. Collectively, these findings demonstrate that Ag₂O/UiO-66@EO not only inhibits biofilm establishment but also efficiently eradicates mature biofilms and substantially disrupts extracellular matrix production.

777 Several complementary mechanisms may account for this pronounced antibiofilm activity. The
778 high specific surface area of the UiO-66 framework ($554.65 \text{ m}^2\text{g}^{-1}$) and the high EE% of EO
779 ($81.20 \pm 2.9\%$) provide efficient loading and sustained delivery of bioactive constituents, thereby
780 maintaining prolonged antibiofilm pressure at the bacterial interface [20,38]. Consistent with this
781 interpretation, the release study demonstrated a controlled release pattern, with only
782 approximately 36% of the encapsulated EO released after 72 h, suggesting prolonged availability
783 of active compounds capable of continuously interfering with biofilm maturation and
784 maintenance [38].

785 Furthermore, the nanoscale hydrodynamic diameter (154.6 nm) and moderate negative surface
786 charge (-17.5 mV) of the nanocomposite may facilitate close interactions with bacterial cells and
787 improve penetration through the dense EPS matrix [65]. Immobilization of Ag_2O nanoparticles
788 within the UiO-66 framework likely prevents nanoparticle aggregation and increases the
789 available reactive surface area, as supported by SEM and TEM analyses showing uniform
790 anchoring of Ag_2O within the preserved MOF architecture. Continuous release of Ag^+ ions may
791 subsequently induce oxidative stress, membrane disruption, and intracellular macromolecular
792 damage through ROS generation [63]. Simultaneously, EO-derived phytochemicals may
793 interfere with quorum-sensing pathways, suppress polysaccharide biosynthesis, and impair cell–
794 cell adhesion processes that are essential for biofilm maturation and structural stability [66].

795 The marked reduction in EPS production observed in the present study is particularly important
796 because EPS constitutes the structural backbone of bacterial biofilms and is directly responsible
797 for antimicrobial tolerance and persistence. Depletion of EPS weakens biofilm architecture,
798 increases matrix permeability, and exposes embedded bacterial cells to antimicrobial agents and
799 host defense mechanisms [67]. Therefore, the substantial decrease in EPS production achieved

800 by Ag₂O/UiO-66@EO likely contributed to the pronounced reduction in viable biofilm-
801 associated bacterial cells observed in the MBEC assay.

802 Our findings are consistent with previous investigations demonstrating the enhanced antibiofilm
803 efficacy of UiO-66-based nanoplateforms. Eskandari et al. reported that Thymol@UiO-66
804 exhibited approximately 25% greater antibiofilm activity than free thymol and ZnO
805 nanoparticles, whereas Thymol/ZnONPs@UiO-66 further increased antibiofilm efficacy by
806 nearly 46% against *S. aureus* isolates [68]. Similarly, other UiO-66-based antimicrobial systems
807 have demonstrated improved biofilm penetration, sustained release behavior, and enhanced
808 disruption of bacterial virulence mechanisms [69].

809 As a result, the simultaneous reductions in biofilm biomass (~70%), viable biofilm-associated
810 bacterial populations (>5 log₁₀ CFU/mL), and EPS production (~68–70%) indicate that
811 Ag₂O/UiO-66@EO functions as a multifunctional antibiofilm nanoplateform rather than a simple
812 antimicrobial carrier. The ability to inhibit biofilm formation, eradicate mature biofilms, and
813 disrupt matrix integrity highlights the considerable potential of this nanoformulation for the
814 management of persistent and biofilm-associated VRSA urinary tract infections.

815 Biofilm formation has been described as a successful survival strategy for many bacterial species
816 in most natural environments. Biofilm formation in *Staphylococcus* spp. is largely driven by the
817 *ica* operon, which regulates production of PIA/PNAG, a key extracellular matrix polysaccharide
818 that protects bacteria and enhances antimicrobial resistance. *icaA* encodes the enzyme
819 responsible for synthesizing PNAG, while *icaD* is essential for its optimal activity; together, they
820 synergistically enable efficient PIA production and robust biofilm accumulation [70].

821 The expression levels of the biofilm-related genes *icaA* and *icaD* were evaluated in VRSA
822 isolates (strains 1–3 and ATCC 70069) following treatment with Ag₂O NPs, *H. persicum* extract,
823 and Ag₂O/UiO-66@*H. persicum* using quantitative real-time PCR (Figure 5A and 5B).
824 Compared with the control gene, *H. persicum* moderately but significantly downregulated the
825 expression of both genes ($***p < 0.001$), whereas treatment with Ag₂O NPs resulted in a more
826 pronounced reduction in transcript levels. Notably, Ag₂O/UiO-66@*H. persicum* exhibited the
827 strongest inhibitory effect, leading to a marked suppression of *icaA* and *icaD* expression across
828 all tested strains, with fold changes reduced to approximately 0.22–0.3 relative to the control
829 ($***p < 0.001$; $### p < 0.001$ compared with Ag₂O NPs and extract). The consistent
830 downregulation pattern observed for both genes indicates effective disruption of the *ica* operon,
831 which is essential for PIA synthesis and biofilm maturation [70]. Various studies indicated the
832 antibiofilm activity of silver-based nanoparticles by downregulation of *icaA* and *icaD* genes
833 expression [71-73]. Furthermore, PCR results of different investigations indicated that in the
834 presence of plant-derived extract-loaded nanocarriers or metal-doped nanocarriers can reduce the
835 expression of the biofilm-associated genes (*icaA* and *icaD*) compared to the free extract and free
836 metal NPs [74-77]. These molecular findings are in strong agreement with the crystal violet,
837 MBEC, and EPS results, confirming that Ag₂O/UiO-66@*H. persicum* suppresses VRSA biofilm
838 formation at the transcriptional level.

839 VRSA represents a growing global public health concern, particularly in clinical settings where
840 resistance is most prevalent. The resistance is mainly mediated by the *vanA* and *vanB* genes,
841 which alter peptidoglycan biosynthesis by replacing the vancomycin target D-Ala-D-Ala termini,
842 thereby markedly reducing the drug's binding affinity and rendering vancomycin—once a
843 last-line therapy for MRSA—increasingly ineffective [78]. In current study, the expression levels

of the vancomycin resistance genes *vanA* and *vanB* were quantified in VRSA isolates following treatment with Ag₂O NPs, *H. persicum* extract, and Ag₂O/UiO-66@*H. persicum* using quantitative real-time PCR (Figure 5C and 5D). Relative to the control, *H. persicum* induced a light but not significant reduction in *vanA/vanB* expression, whereas treatment with Ag₂O NPs resulted in a more pronounced downregulation across all tested strains ($P < 0.001$). Notably, the Ag₂O/UiO-66@*H. persicum* nanocomposite exhibited the strongest inhibitory effect, reducing transcript levels to approximately 0.28–0.4-fold of the control ($***p < 0.001$; $### p < 0.001$ compared with other groups). This consistent suppression pattern observed for both *vanA* and *vanB* suggests effective interference with the molecular machinery responsible for vancomycin resistance. In line with our findings, Rahmanian et al., study found that the expression level of the *mecA*, *vanA*, *icaA*, and *icaD* genes in vancomycin/amikacin-UiO-66@NH₂/PEG treated VRSA isolates was substantially decreased compared to other groups [38]. Importantly, these transcriptional changes are in strong agreement with the disc diffusion, time-kill, MBEC, and biofilm assays, collectively confirming that Ag₂O/UiO-66@*H. persicum* enhances VRSA susceptibility by downregulating key vancomycin resistance determinants at the gene expression level.

Biofilm formation in *S. aureus* is primarily regulated by the *ica* operon, which governs the synthesis of polysaccharide intercellular adhesin (PIA/PNAG), a key component of the extracellular matrix that enhances bacterial adhesion, biofilm maturation, and antimicrobial tolerance. Within this operon, *icaA* encodes the N-acetylglucosaminyltransferase responsible for initiating PIA synthesis, while *icaD* functions as an essential cofactor that enhances enzymatic activity and stabilizes polymer formation, together contributing to robust biofilm development [79,80].

867 The expression levels of *icaA* and *icaD* in VRSA isolates (strains 1–3 and ATCC 700699) were
868 significantly altered following treatment with Ag₂O NPs, *H. persicum* extract, and Ag₂O/UiO-
869 66@*H. persicum* (Figures 5A and 5B). Compared with untreated controls, *H. persicum* extract
870 induced a moderate reduction in gene expression, whereas Ag₂O NPs resulted in a more
871 pronounced downregulation. The strongest inhibitory effect was observed in the Ag₂O/UiO-
872 66@*H. persicum* group, where expression levels of both genes decreased to approximately 0.22–
873 0.30-fold relative to control (***P < 0.001; ####P < 0.001 versus single-component treatments).
874 This coordinated suppression of *icaA* and *icaD* indicates effective disruption of the *ica* operon,
875 thereby inhibiting PIA synthesis and biofilm development at the transcriptional level [79]. These
876 molecular findings are consistent with the observed reductions in biofilm biomass, MBEC, and
877 EPS production, confirming the antibiofilm efficacy of the nanocomposite at both phenotypic
878 and genetic levels.

879 In addition to biofilm-related genes, the expression of vancomycin resistance determinants *vanA*
880 and *vanB* was also evaluated in the same VRSA isolates following treatment (Figures 5C and
881 5D). These genes are responsible for altering the D-Ala-D-Ala target site in peptidoglycan
882 precursors, leading to reduced binding affinity of vancomycin and consequent clinical resistance
883 [81]. Compared with the control group, *H. persicum* extract produced a slight but non-significant
884 reduction in *vanA* and *vanB* expression, whereas Ag₂O NPs significantly downregulated both
885 genes (P < 0.001). Notably, Ag₂O/UiO-66@*H. persicum* exerted the most substantial inhibitory
886 effect, reducing transcript levels to approximately 0.28–0.40-fold of control (***P < 0.001; ####P
887 < 0.001 versus Ag₂O NPs and extract).

888 The observed downregulation of both biofilm-associated and resistance-associated genes
889 suggests that the nanocomposite interferes with key regulatory pathways involved in virulence

890 and antimicrobial resistance. Similar findings have been reported in previous studies, where
891 UiO-66-based and nanoparticle-loaded systems significantly suppressed the expression of *icaA*,
892 *icaD*, and *vanA*-related genes in resistant *S. aureus* strains [38]. Importantly, these transcriptional
893 changes align well with the results of disc diffusion, time-kill kinetics, MBEC, and EPS assays,
894 collectively demonstrating that Ag₂O/UiO-66@*H. persicum* enhances VRSA susceptibility
895 through multi-level interference with both biofilm formation and vancomycin resistance
896 mechanisms.

897 The cytotoxicity findings indicate a clear concentration-dependent response, with Ag₂O NPs
898 exhibiting the highest toxicity toward HFF cells. This effect is primarily attributed to excessive
899 Ag⁺ ion release, which promotes intracellular ROS generation, mitochondrial dysfunction, and
900 oxidative stress-mediated apoptosis, as widely reported for silver-based nanomaterials [82,83].

901 In contrast, *H. persicum* extract showed comparatively low cytotoxicity, likely due to its phenolic
902 and flavonoid constituents, which possess antioxidant properties capable of mitigating ROS-
903 mediated cellular damage. Such phytochemicals are known to enhance cellular resilience against
904 oxidative stress and nanoparticle-induced inflammation [84].

905 Importantly, the Ag₂O/UiO-66@*H. persicum* nanocomposite exhibited significantly improved
906 biocompatibility compared with Ag₂O NPs. This reduction in cytotoxicity can be attributed to the
907 UiO-66 framework, which enables controlled release of Ag⁺ ions and phytochemicals while
908 limiting direct nanoparticle–cell interactions. Zirconium-based MOFs such as UiO-66 have been
909 widely reported to improve the safety profile of loaded antimicrobial agents by regulating release
910 kinetics and reducing acute cellular exposure [85,86].

Notably, the nanocomposite maintained high cell viability at concentrations that were effective against VRSA isolates, suggesting an improved therapeutic window. This balance between antimicrobial potency and reduced cytotoxicity is a key advantage of MOF-based hybrid systems. Similar findings have been reported for other MOF–silver composites, which demonstrate reduced mammalian toxicity while preserving strong antibacterial activity, supporting their potential for biomedical applications [87,88].

4. Conclusion

The present study successfully developed an Ag₂O/UiO-66@*H. persicum* nanoformulation with favorable physicochemical characteristics, high loading efficiency, and sustained release behavior. The nanoformulation exhibited markedly enhanced antibacterial and antibiofilm activities against clinical VRSA isolates compared with free Ag₂O NPs and *H. persicum* extract, accompanied by significant downregulation of biofilm- (*icaA* and *icaD*) and vancomycin resistance-related (*vanA* and *vanB*) genes. Moreover, the formulation demonstrated improved cytocompatibility relative to free Ag₂O nanoparticles, indicating a favorable therapeutic profile. Although the findings are limited to in vitro investigations, they suggest that Ag₂O/UiO-66@*H. persicum* represents a promising multifunctional nanoplatform for combating biofilm-associated VRSA infections. Further studies involving in vivo efficacy, comprehensive safety evaluation, and mechanistic investigations are warranted to support its translational potential.

Declarations

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board (IRB) of the Islamic Azad University, Tehran, Iran, under the ethical code IR.IAU.TNB.REC.1404.213. All procedures

933 involving human participants were conducted in accordance with the ethical standards of the
934 institutional and/or national research committee and with the 1964 Helsinki Declaration and its
935 later amendments or comparable ethical standards. Written informed consent was obtained from
936 all participants or their legal guardians, as applicable.

937 **Clinical trial number**

938 Not applicable

939 **Consent for publication**

940 Not applicable

941 **Availability of data and materials**

942 The data that support the findings of this study are available from the corresponding authors as
943 the corresponding author, upon reasonable request.

944 **Conflict of Interests**

945 The authors declare that there are no conflicts of interest.

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948 **Author's contribution**

949 S.K. performed the laboratory experiments and collected the data; F.A. designed the study; E.S.
950 wrote the manuscript; P.H. drew the graphs and performed the analysis; All authors reviewed and
951 approved the manuscript.

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Figure 1. (A) Scanning electron microscopy (SEM) images showing the surface morphology and aggregation state of the Ag₂O/UiO-66/EO nanocomposite; (B) transmission electron microscopy (TEM) micrographs illustrating nanoscale structure and distribution of Ag₂O nanoparticles on the UiO-66 framework; (C) dynamic light scattering (DLS) analysis showing hydrodynamic particle size distribution of the nanocomposite; and (D) X-ray diffraction (XRD) pattern confirming the crystalline phases of UiO-66 and Ag₂O and successful formation of the Ag₂O/UiO-66/EO nanocomposite.

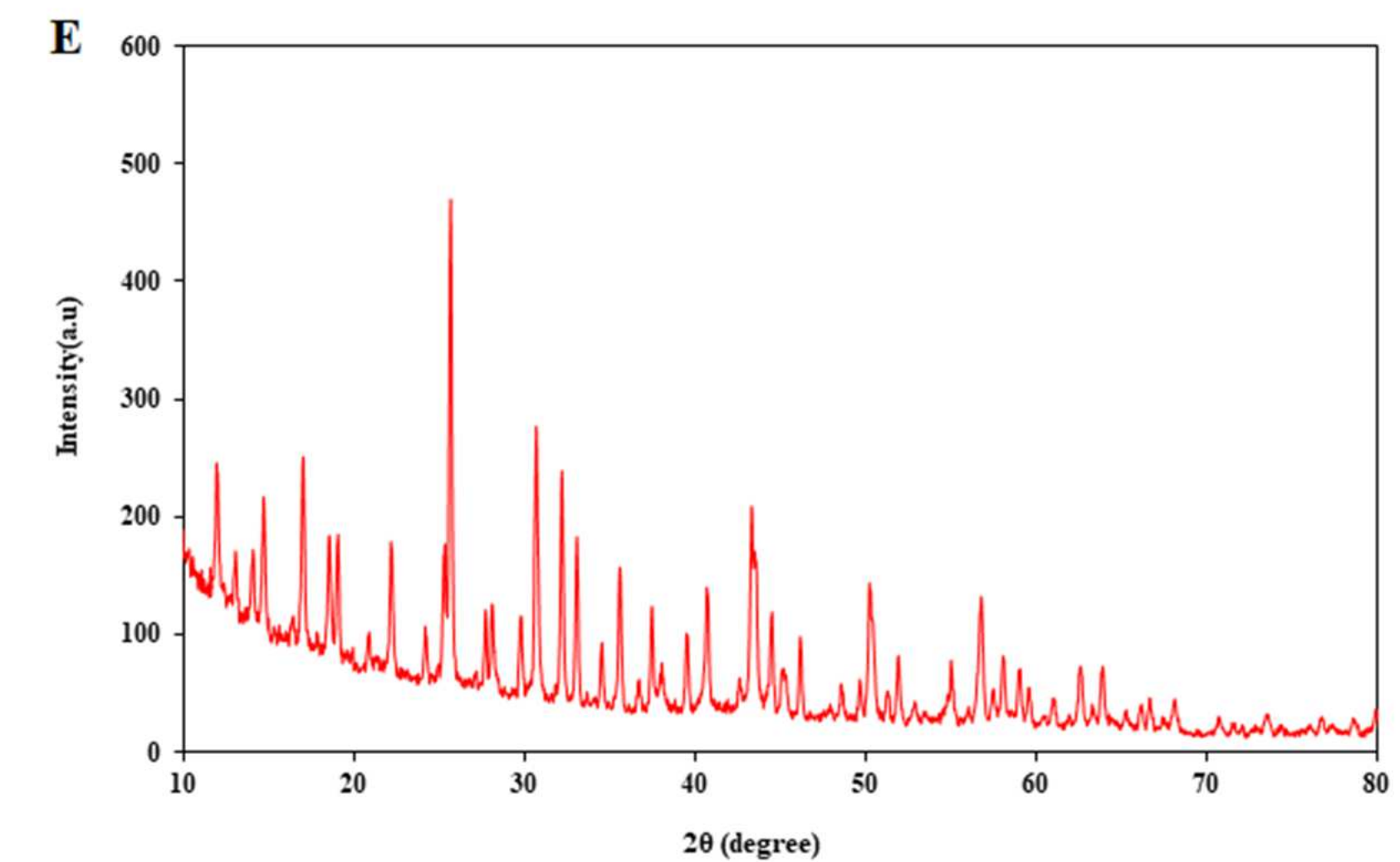
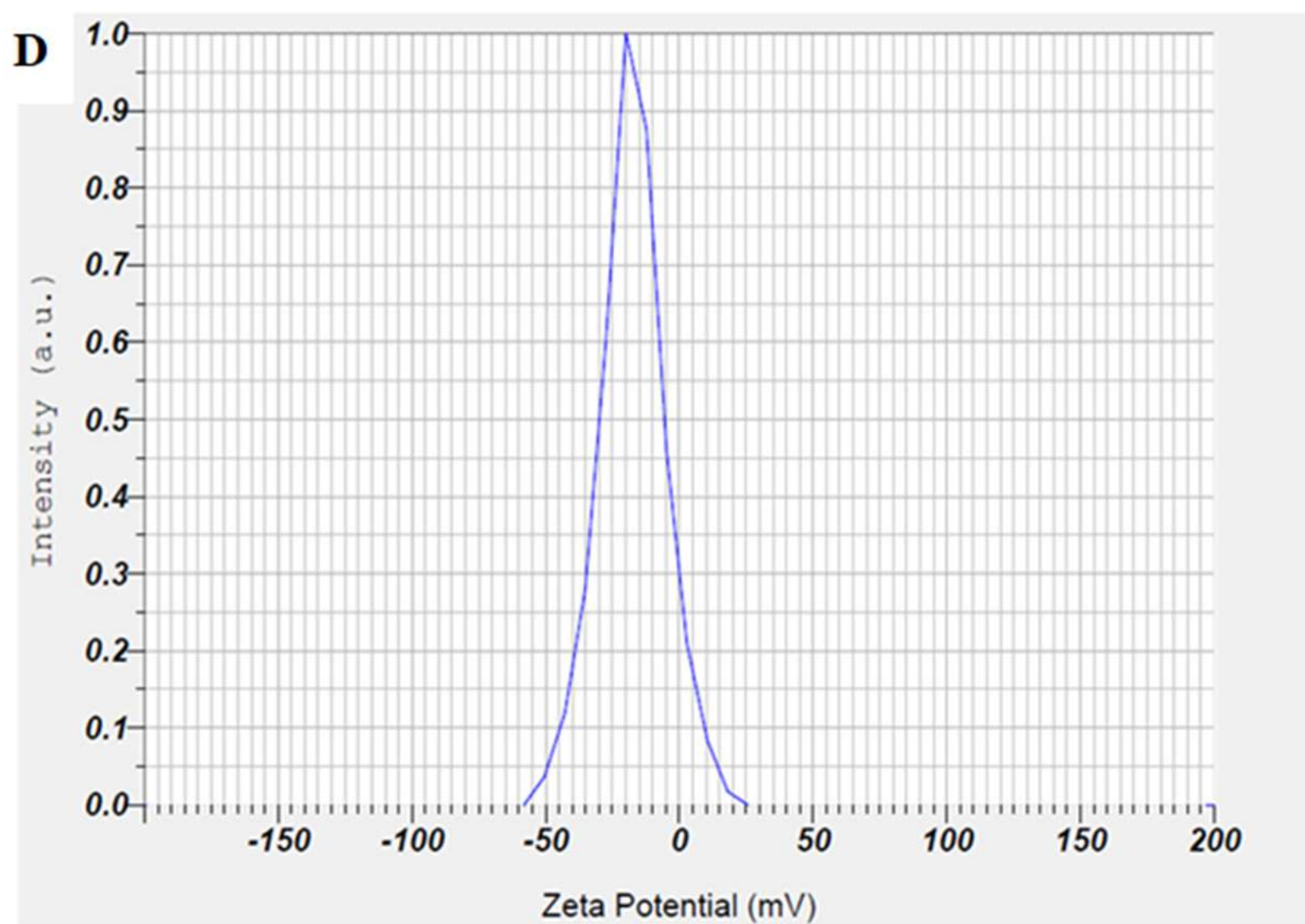
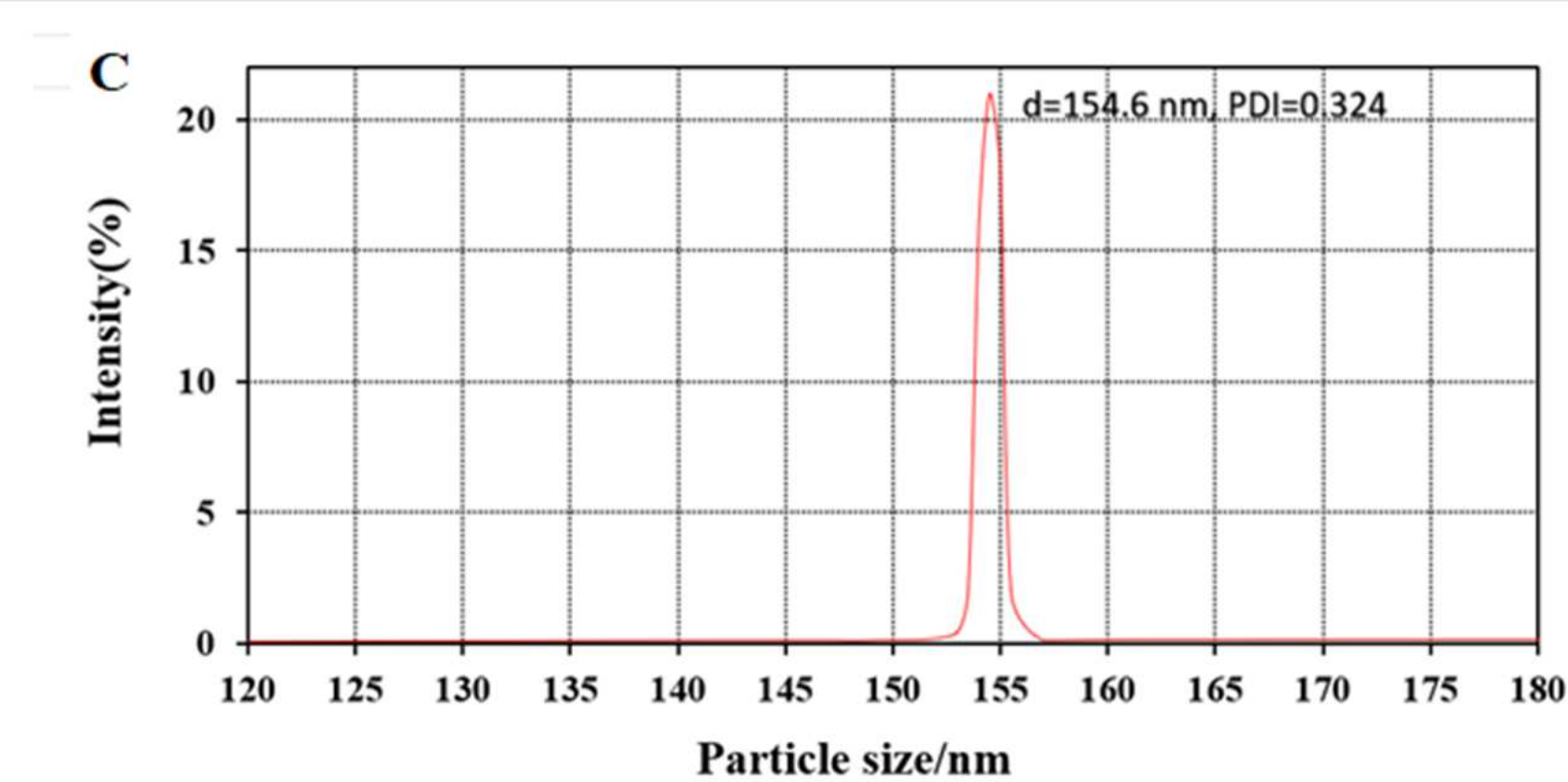
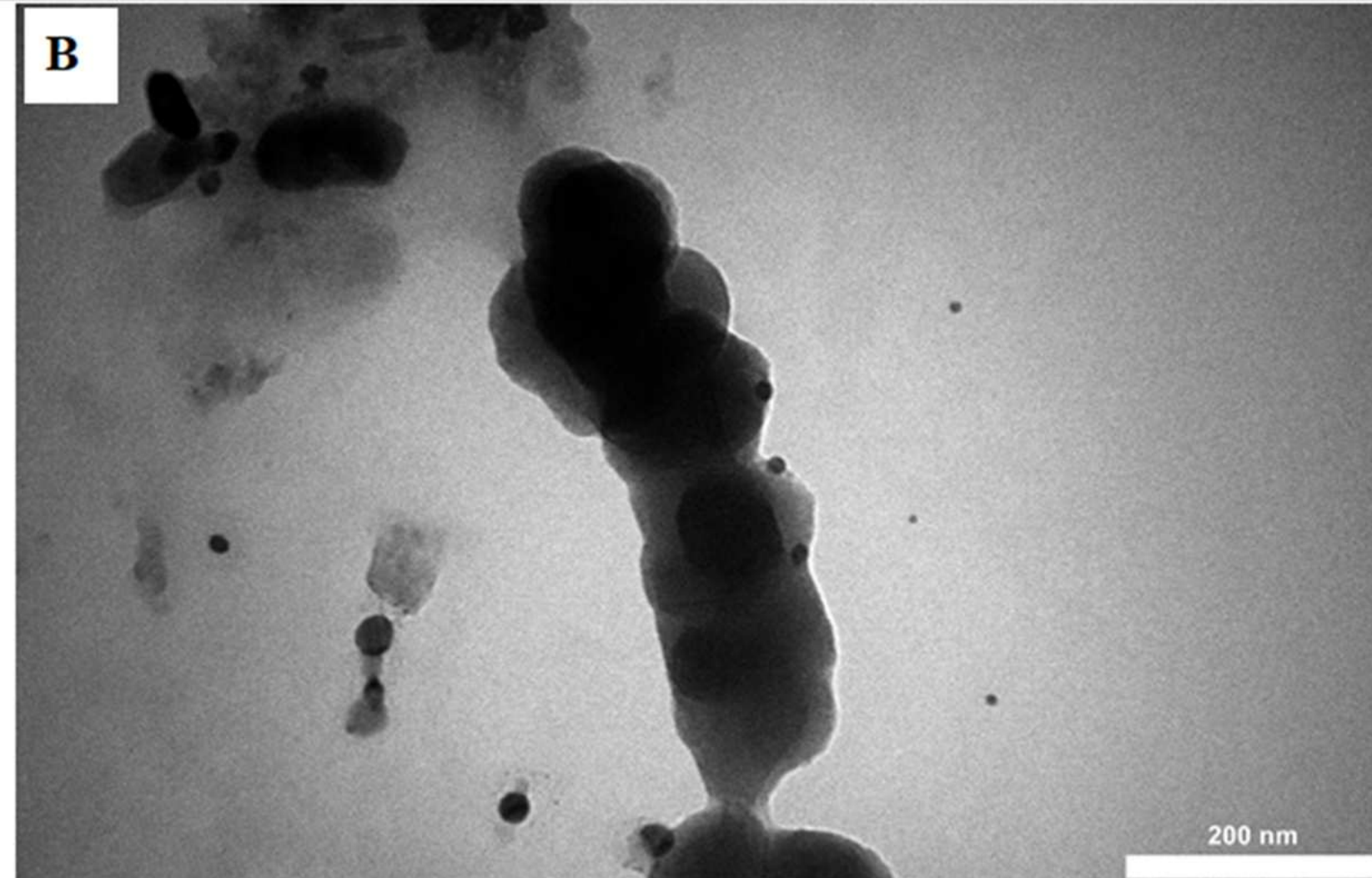
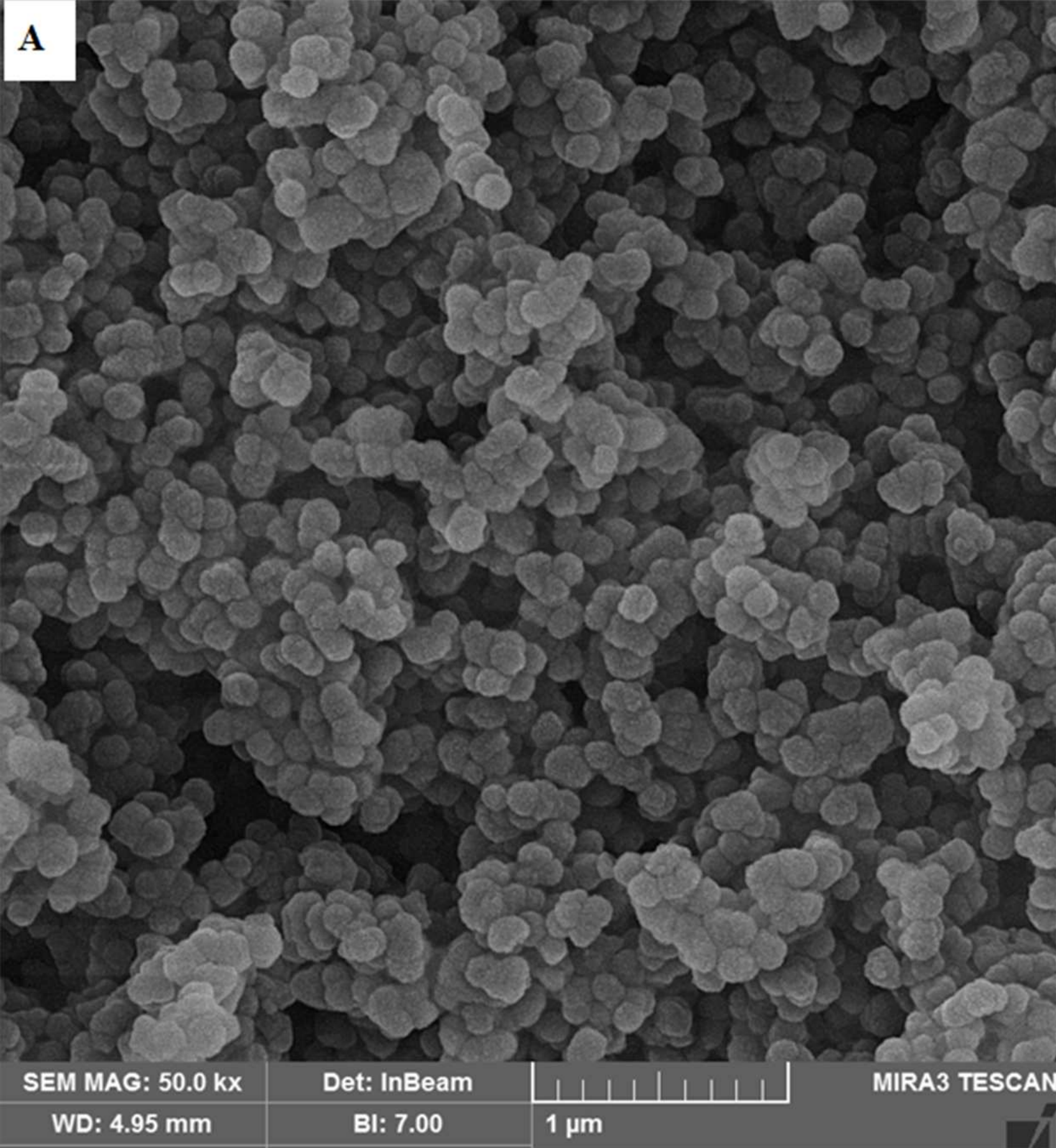
Figure 2. (A) Fourier transform infrared (FTIR) spectrum confirming the presence of functional groups from UiO-66, Ag₂O, and EO-derived phytochemicals in the Ag₂O/UiO-66/EO nanocomposite; (B) thermogravimetric analysis (TGA) showing the thermal stability and multi-stage decomposition behavior of the nanocomposite; (C) nitrogen adsorption–desorption isotherm and Brunauer–Emmett–Teller (BET) analysis indicating surface area, pore structure, and porosity of the material; and (D) cumulative *in vitro* release profiles of EO from the Ag₂O/UiO-66/EO nanocomposite compared to free EO, demonstrating sustained release behavior.

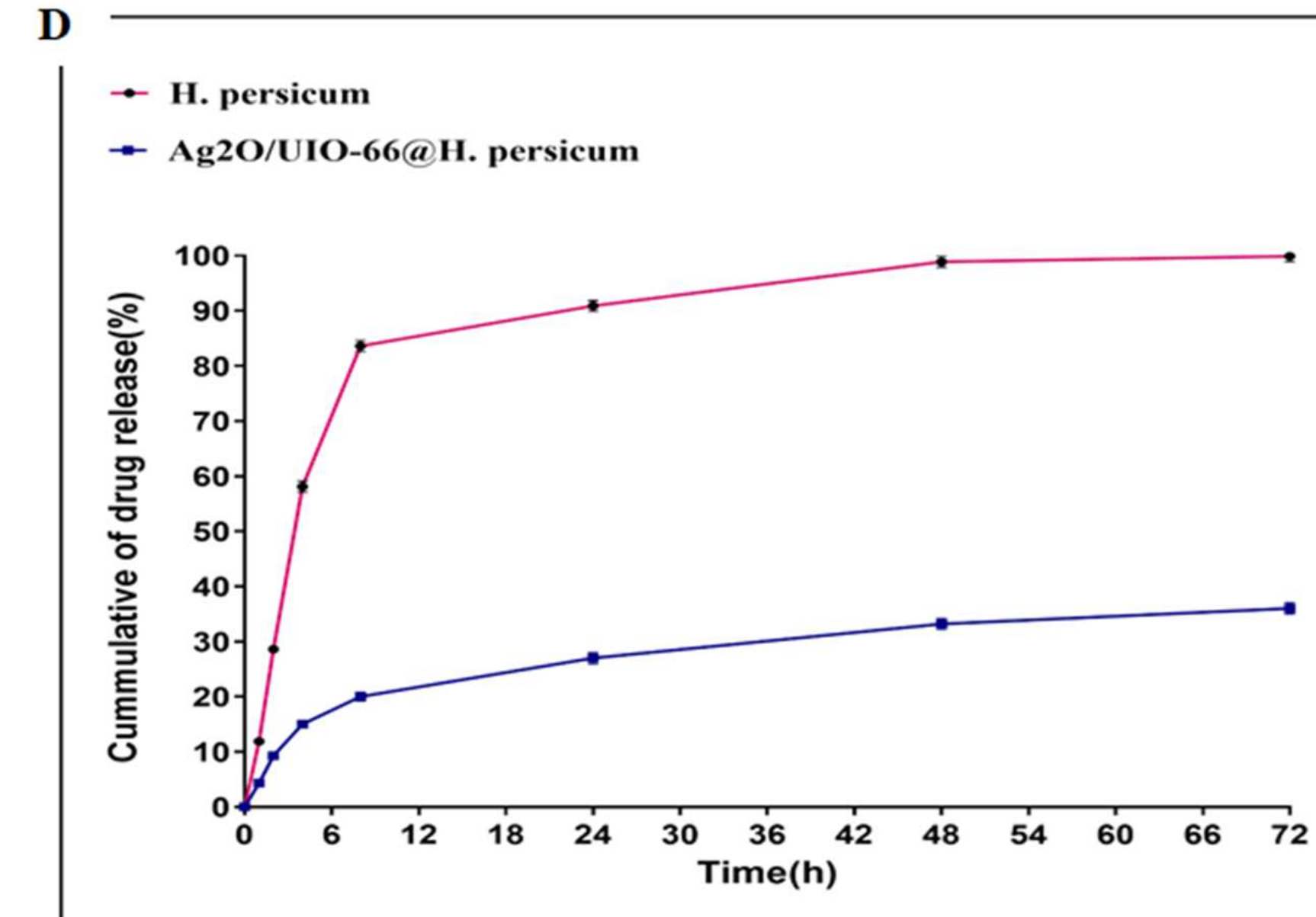
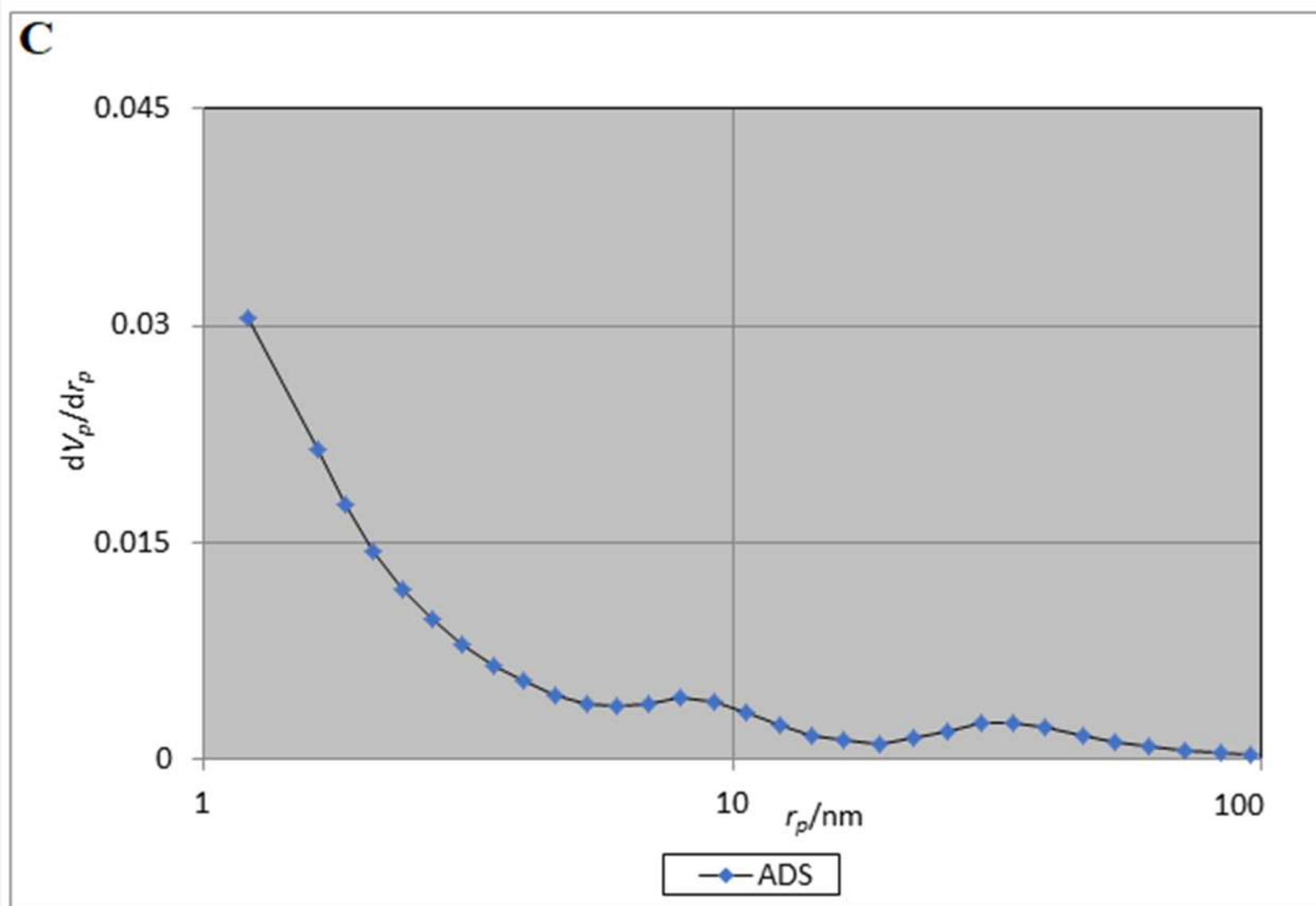
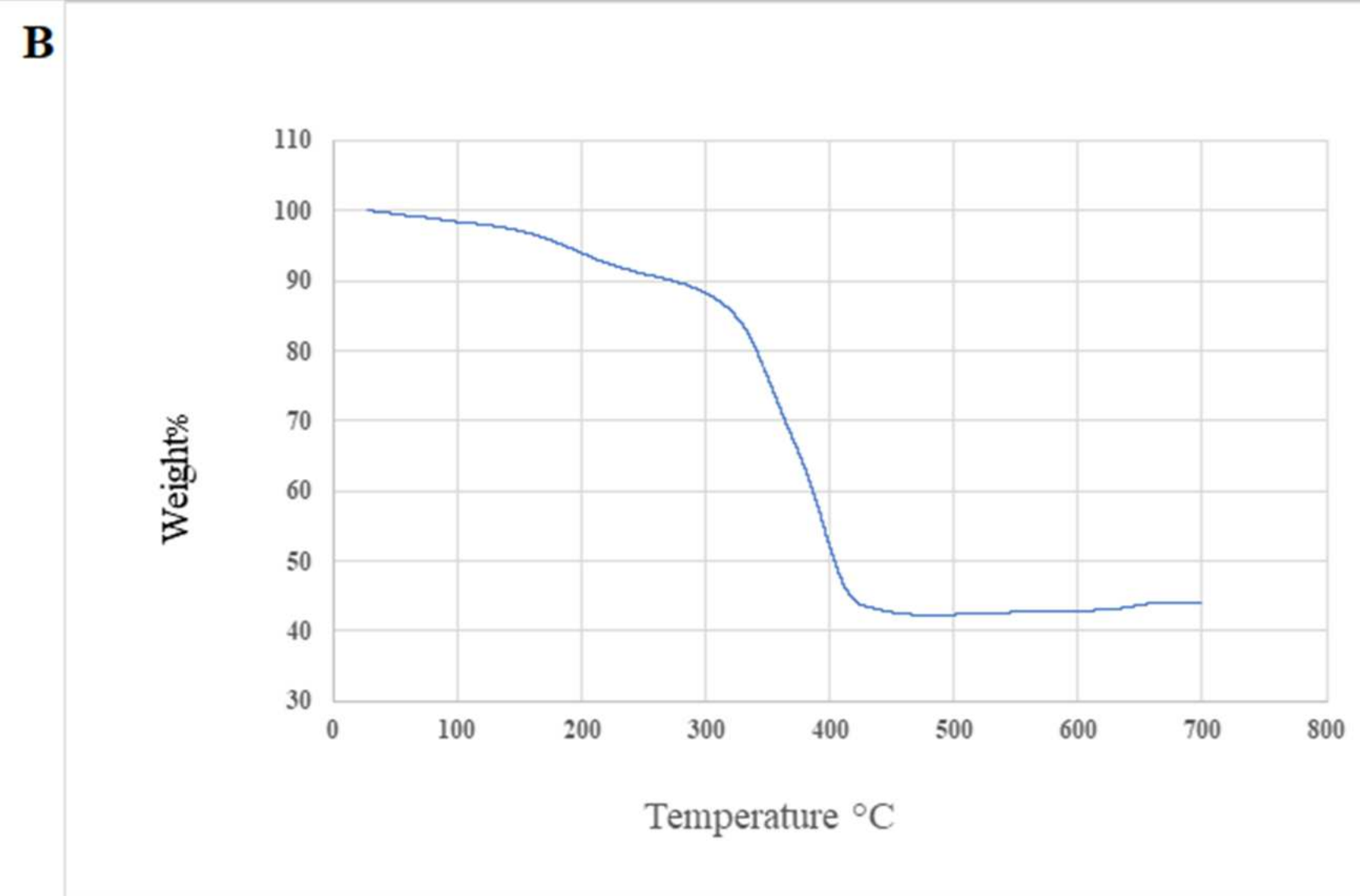
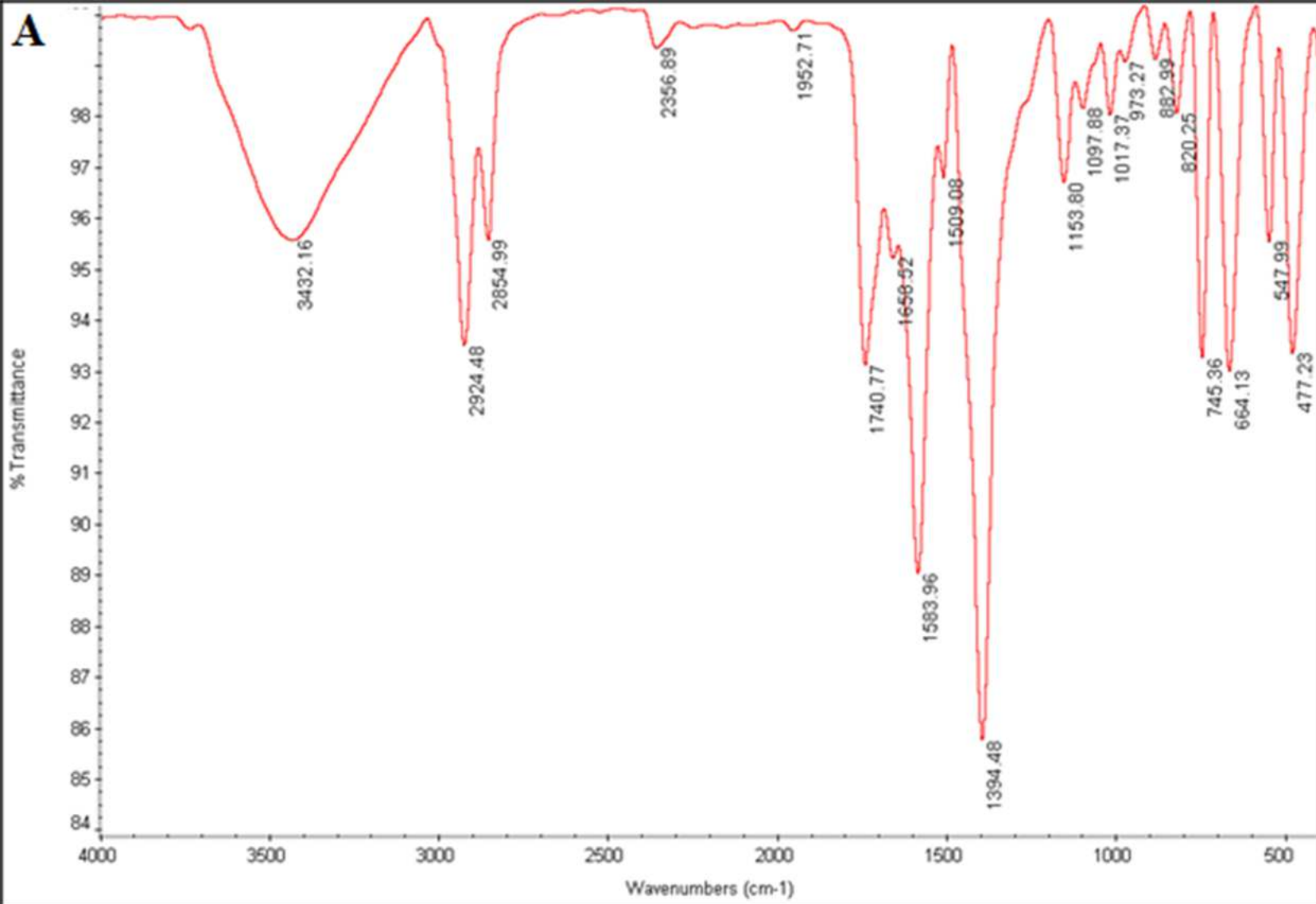
Figure 3. Antibacterial activity of Ag₂O, EO, and the Ag₂O/UiO-66/EO nanocomposite against VRSA isolates, as determined by (A) disk diffusion assay (inhibition zone diameters, mm) and (B) bacterial growth kinetic (time–kill) assay based on OD₆₀₀ measurements over a 72-h incubation period. Data are presented as mean ± SD of three independent experiments. ***P < 0.001 compared with the corresponding Ag₂O- and EO-treated groups.

Figure 4. Antibiofilm activity of Ag₂O NPs, *H. persicum* extract, and Ag₂O/UiO-66@*H. persicum* nanocomposite against VRSA isolates evaluated using (A) crystal violet (CV) biofilm formation assay, (B) MBEC assay for eradication of preformed biofilms, and (C) EPS production assay. Data are presented as mean ± SD (n = 3). Statistical significance was determined as follows: ***P < 0.001 versus control group; ###P < 0.001 versus Ag₂O NPs and *H. persicum* extract treated groups.

Figure 5. Relative expression levels of biofilm-associated genes (A) *icaA* and (B) *icaD*, and vancomycin resistance-related genes (C) *vanA* and (D) *vanB* in VRSA isolates treated with Ag₂O NPs, *H. persicum* extract, and Ag₂O/UiO-66@*H. persicum* nanocomposite, analyzed by real-time PCR. Data are presented as mean ± SD (n = 3). *P < 0.05, **P < 0.01, ***P < 0.001 versus control group; #P < 0.05, ##P < 0.01, ###P < 0.001 for Ag₂O/UiO-66@*H. persicum* compared with Ag₂O NPs and *H. persicum* extract treated groups.

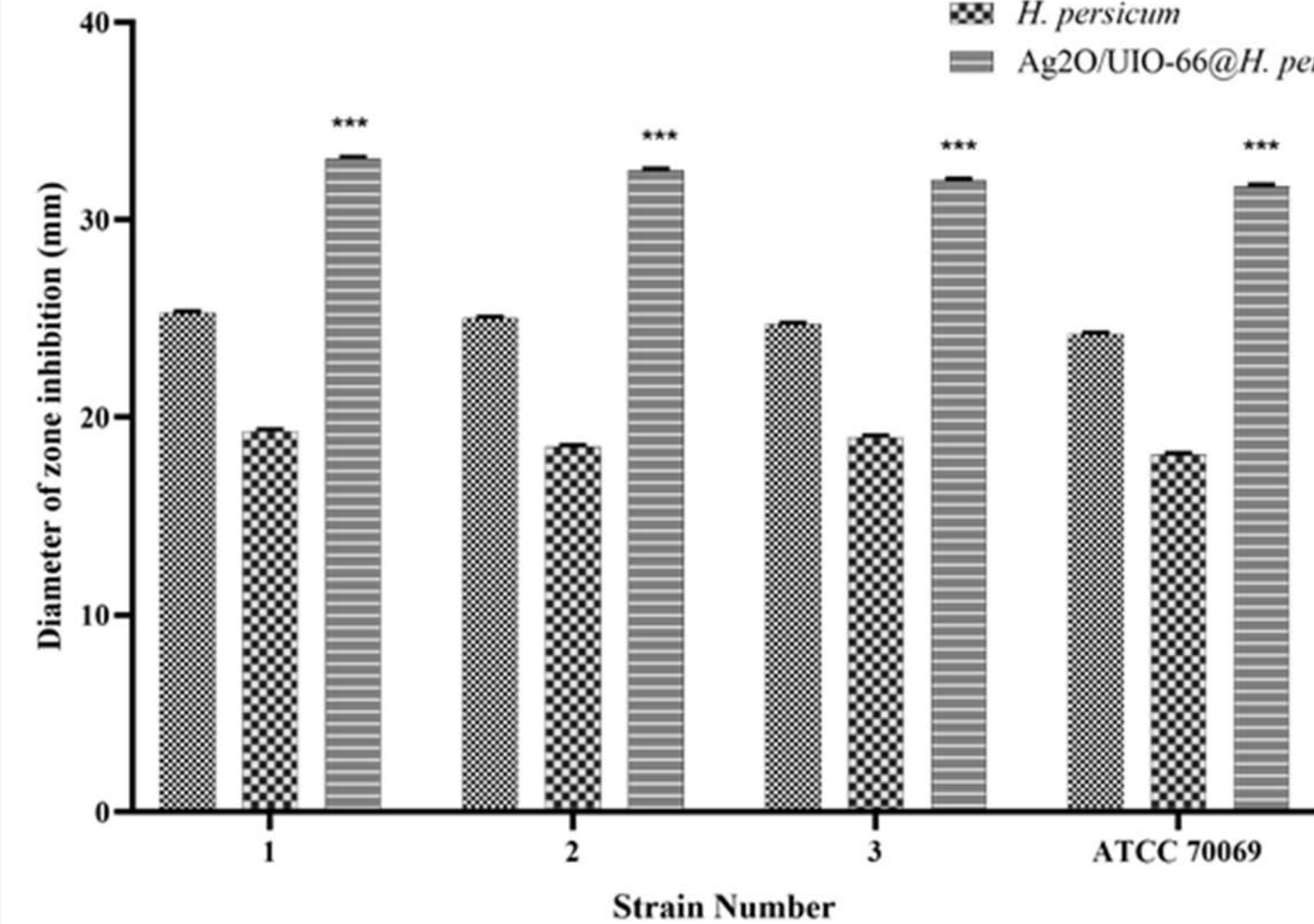
Figure 6. Cytocompatibility of Ag₂O NPs, *H. persicum* extract, and Ag₂O/UiO-66@*H. persicum* nanocomposite in HFF cells following exposure to increasing concentrations (5.31–85 µg/mL), as determined by the MTT assay. Data are presented as mean ± SD (n = 3). **P < 0.01 and ***P < 0.001 versus the untreated control group; ###P < 0.001 for Ag₂O/UiO-66@*H. persicum* compared with Ag₂O NPs and *H. persicum* extract-treated groups.





A*VRSA*

Ag2O NPs
H. persicum
Ag2O/UIO-66@*H. persicum*

**B**

Time-Kill Assay

Medium
Ag2O NPs
H. persicum
Ag2O/UIO-66@*H. persicum*

