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Berberine-functionalized Biogenic Silver Nanoparticles Inhibit Quorum-Regulated Curli mediated Biofilms in Emerging MDR Uropathogenic *Enterobacter hormaechei*

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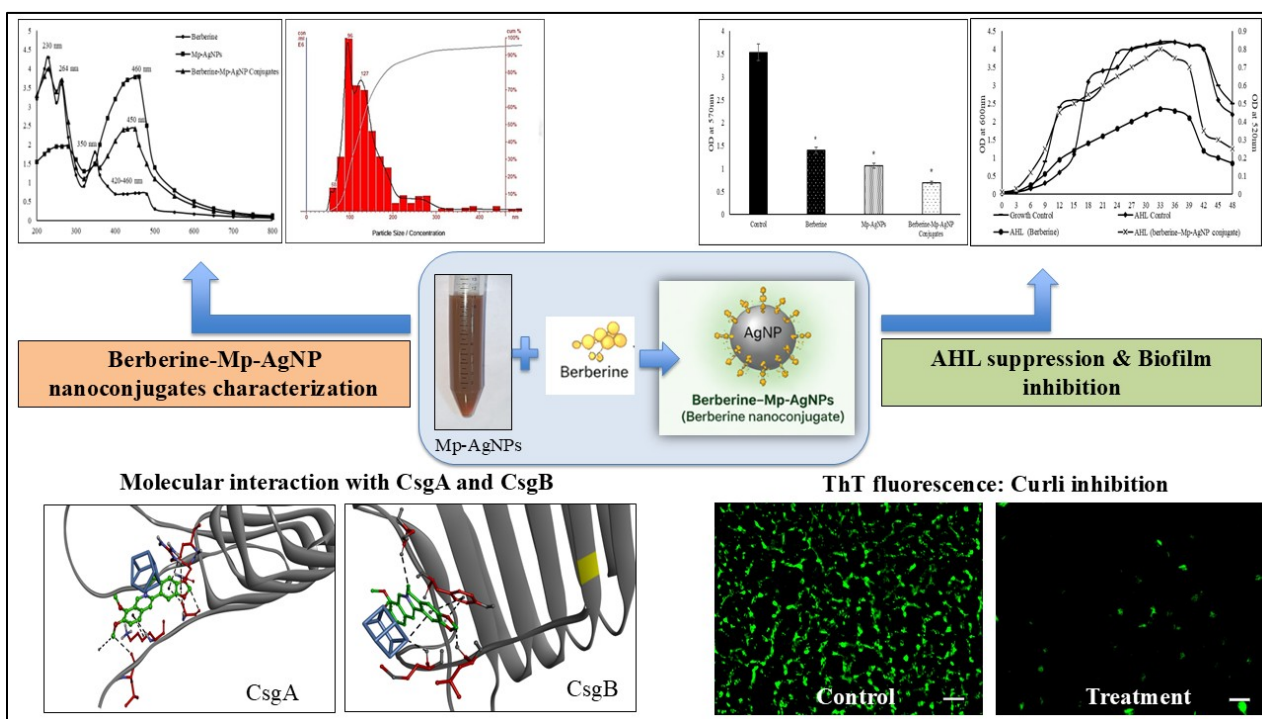
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Graphical Abstract



Highlights:

- First experimental evidence for the curli-rich amyloid biofilms in *E. hormaechei* and its suppression by berberine.
- Mp-AgNP@berberine nanocomplex achieved superior inhibition of *E. hormaechei* biofilms (80.57%) versus individual components.
- Suppression of curli-rich amyloid assembly, reveals a previously underexplored antibiofilm target.
- Simultaneous attenuated virulence (QS, EPS, ECM, curli) indicates multifaceted action.
- In silico analysis supports stable binding to CsgA/CsgB, supports mechanism-driven antibiofilm activity.

Abstract

Multidrug-resistant *Enterobacter hormaechei* is being increasingly reported in urinary tract infections (UTIs), where its ability to form quorum-regulated, curli-rich biofilms contributes to antibiotic tolerance and persistence. Targeting both quorum sensing (QS) and amyloid-mediated biofilm stability may provide an alternative strategy to conventional antibiotics. In this study, we developed a Berberine-functionalized biogenic silver nanoparticle (AgNPs synthesized using *Mussaenda philippica* leaf extract) to enhance phytochemical delivery and antibiofilm efficacy.

Physicochemical characterization confirmed successful association between Berberine (BBR) and *M. philippica* mediated AgNPs (Mp-AgNP@BBR), with a hydrodynamic size of 217 nm, polydispersity index of 0.230, and a stable zeta potential of -33.2 mV. At sub-MIC Mp-AgNP@BBR nanocomplex inhibited biofilm formation by 80.57%, compared to 60.37% for BBR and 67.78% for Mp-AgNPs alone. Treatment also reduced total extracellular polymeric substances, decreasing protein content from 549 to 159 $\mu\text{g/mL}$ and carbohydrate content from 659 to 196 $\mu\text{g/mL}$, indicating substantial matrix disruption. QS activity, assessed via AHL quantification, showed a reduction in OD_{520} from 0.83 (control) to 0.33 following nanocomplex treatment, without affecting bacterial growth. Cell surface hydrophobicity decreased from 60% to 23.34%, suggesting impaired adhesion. Curli amyloid production was strongly suppressed, as confirmed by Congo Red binding and Thioflavin T fluorescence assays. Molecular docking analysis further supported stable interactions between BBR-Ag8 complex and curli protein subunits CsgA and CsgB, indicating potential structural interference.

Overall, this study demonstrates that nano-enabled BBR delivery effectively disrupts quorum-regulated curli biofilms in UTI-associated, MDR *E. hormaechei*, providing a promising strategy to combat biofilm-mediated persistence.

Keywords: Antibiofilm, Amyloid, Berberine (BBR), Curli, *Enterobacter hormaechei*, MDR, Urinary Tract Infections (UTIs).

1. Introduction

Biofilm formation is a critical virulence strategy that enables bacteria to survive hostile conditions, including antibiotic exposure and host immune responses. In urinary tract infections (UTIs), this biofilm mode of growth plays a decisive role in disease persistence and recurrence, particularly in catheter-associated cases. The burden of UTIs is particularly high in women, elderly populations, and patients requiring long-term catheterization (He et al. 2025). The increasing prevalence of multidrug-resistant (MDR) uropathogens has intensified global concern, as treatment options continue to narrow and recurrence rates rise. Organisms such as uropathogenic *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are commonly implicated in complicated and catheter-associated UTIs, often exhibiting resistance to multiple frontline antibiotics. In addition, *Enterobacter hormaechei*, a member of the *Enterobacter cloacae* complex, has been increasingly reported in clinical infections, including UTIs, and is frequently associated with multidrug resistance and biofilm formation, contributing to persistence and therapeutic challenge (Scharf et al. 2020).

A major challenge in treating UTIs is the ability of bacterial pathogens to form biofilms-multicellular communities encased in an extracellular matrix that adhere strongly to uroepithelial surfaces and indwelling catheters (Lila et al. 2023). Biofilm-embedded cells exhibit dramatically increased tolerance to antibiotics and host immune defenses, contributing to chronic and recurrent infections. Within these communities, bacteria coordinate group behaviors through QS, a cell-density-dependent communication system that regulates biofilm formation, virulence factor production, and stress responses (De et al. 2026). Disrupting QS, known as quorum quenching, has therefore emerged as a promising strategy for weakening biofilms and restoring antimicrobial susceptibility.

While QS orchestrates the regulatory framework of biofilm development, structural stabilization of mature biofilms in Enterobacteriaceae is critically mediated by curli amyloid fibers. Curli

assembly (consists of total 2 subunits), governed by the *csg* operon, involves CsgA as the principal β -sheet-forming structural subunit and CsgB as the surface-associated nucleator that initiates CsgA polymerization (Perov et al. 2019). This CsgB-mediated nucleation enables extracellular CsgA monomers to assemble into highly stable networks that promote adhesion to uroepithelial tissues and catheter materials, enhance intercellular aggregation, and confer mechanical resilience and antibiotic tolerance to mature biofilms (Tursi and Tükel 2018). These extracellular amyloid networks reinforce adhesion, mechanical resilience, and antibiotic tolerance.

Many MDR UTI pathogens, including *E. hormaechei*, utilize QS-regulated pathways to strengthen biofilm integrity and enhance survival during antibiotic exposure (Cieřlik et al. 2025). The formidable persistence of these pathogens is further exacerbated by the presence of plasmid-encoded carbapenemases, extended-spectrum β -lactamases (ESBLs), and target-site mutations that collectively reduce susceptibility to multiple drug classes (Liu et al. 2022). Despite its increasing clinical significance and its strong association with catheter-related infections, *E. hormaechei* remains comparatively under-explored in UTIs research; many clinical reports historically group isolates under the *Enterobacter cloacae* complex or focus on more studied uropathogens such as *E. coli* and *K. pneumoniae*, leaving important gaps in understanding the biology, biofilm behavior, and resistance mechanisms of *E. hormaechei*.

Natural compounds capable of interfering with QS and biofilm formation are gaining attention as adjunct or alternative approaches to combat MDR uropathogens. BBR, an isoquinoline alkaloid found in species such as *Berberis* and *Coptis*, has been reported in several studies to exhibit antibacterial and anti-biofilm activity against common pathogenic bacteria. Evidence from in-vitro and in-silico work indicates that BBR can modulate QS signaling, inhibit biofilm development, and reduce virulence in organisms such as *Pseudomonas aeruginosa* and *Salmonella enterica* by

interacting with QS receptors (e.g., LasR, RhlR) and suppressing QS-regulated phenotypes (Matijašević et al. 2025). Despite these promising properties, its clinical application is limited by poor solubility, low permeability across Gram-negative outer membranes, and insufficient intracellular accumulation. Adjuvating such compounds with the nanoparticles could solve these issues to a much greater extent [10].

Green-synthesized silver nanoparticles (AgNPs) have emerged as potent antimicrobial agents with multiple mechanisms of action, including membrane disruption, oxidative stress induction, and interference with biofilm architecture (Alharbi et al. 2022). AgNPs produced using plant extracts often display enhanced stability and bioactivity because phytochemicals act as natural reducing and capping agents. Notably, AgNPs synthesized from *M. philippica* (Mp-AgNPs), a plant rich in diverse secondary metabolites, exhibit strong antimicrobial and antibiofilm potential and have been shown to suppress QS-mediated virulence, extracellular polymeric substance production, and biofilm formation in uropathogenic *Klebsiella pneumoniae* (Mhavarkar and Jadhav 2025). Combining BBR with biogenic Mp-AgNPs could offer a synergistic strategy to overcome the limitations of each component. Therefore, the present study investigates BBR-functionalized Mp-AgNPs as a dual-action nanotherapy designed to enhance and inhibit QS-associated biofilm formation and mitigate drug tolerance in the under-explored MDR pathogen *E. hormaechei* associated with UTIs.

2. Material and Methods

Our previous work documented the preparation and physicochemical attributes of Mp-AgNPs and their activity against *Klebsiella pneumoniae* (Mhavarkar and Jadhav 2025). In the present investigation, these data were used for comparison, and new analyses were carried out to evaluate the antibiofilm effects of BBR and Mp-AgNP@BBR nanocomplex against *Enterobacter hormaechei*.

2.1 Isolation and Identification of Uropathogenic Bacteria

A previously isolated uropathogen was characterized and identified as *E. hormaechei* (Mhavarkar et al. 2025) and has been utilized in this study. The organism was maintained on nutrient agar slants at 4 °C to retain viability for subsequent experimental work.

2.2 Spectroscopic and Structural Characterization of AgNPs@BBR

In perusal to explore how BBR interacts with Mp-AgNPs, UV-Visible spectra were first recorded using a Shimadzu UV-1900i spectrophotometer. Samples of pure BBR, Mp-AgNPs, and the Mp-AgNP@BBR were scanned from 200-800 nm at room temperature. The optical fingerprints were examined for changes in the nanoparticle SPR band and additional absorption features characteristic of BBR. For FTIR (Fourier Transform Infrared) analysis, powdered samples were placed on an Everest ATR diamond crystal and analyzed with a Nicolet™ Summit X FTIR spectrometer (Thermo Fisher Scientific, USA). Spectra were collected from 4000–500 cm⁻¹ at 2 cm⁻¹ resolution. Structural information was gathered using X-ray diffraction (XRD) using MiniFlex 600 (Rigaku, Japan) with CuK α radiation (λ = 1.5405 Å). Scans were run between 10° and 80° (2 θ).

2.3 Evaluation of Physicochemical Attributes of Mp-AgNP@BBR

Dynamic Light Scattering (DLS) was performed to determine the size distribution profile and polydispersity index (PDI) of the functionalized NPs. The samples were dispersed in distilled water and analyzed using a Zetasizer Nano ZS90 (Malvern Panalytical Ltd., UK) equipped with Zetasizer Software Version 7.11. Zeta potential and electrophoretic mobility measurements were also conducted to evaluate the surface charge and colloidal stability of the functionalized nanoparticles.

For Nanoparticle Tracking Analysis (NTA), samples were first diluted with distilled water, and 0.5 mL of the prepared suspension was loaded into the LM20 Nanosight Nanoparticle Analysis System (Nanosight, UK). The particles were visualized using the system's CCD camera, with both images and videos captured. Data analysis was conducted using Nanosight NTA 2.3 software to assess particle size distribution and concentration.

2.4 Checkerboard-based combinatorial antibiofilm assay

Two-fold serial dilutions of Mp-AgNPs and BBR were prepared in a sterile 96-well microtiter plate to obtain final concentrations ranging from 128 to 2 µg/mL. Aliquots (50 µL) of each compound were dispensed along the horizontal and vertical axes of the plate to generate different treatment combinations. Subsequently, 100 µL of bacterial suspension (10⁶ CFU/mL) prepared in fresh Luria-Bertani medium was added to each well. The plates were incubated statically at 37 °C for 48 h to allow biofilm formation. After incubation, planktonic cells were removed, and the wells were gently washed with sterile phosphate-buffered saline to remove non-adherent bacteria. Biofilm biomass was quantified using the crystal violet staining method, and absorbance was measured at 570 nm using a microplate reader (Wypij et al. 2018). The calculation of the fractional inhibitory concentration index (FICI) was done using the following equations:

$$FIC_a = (MIC \text{ of } Mp - AgNPs \text{ in combination}) / (MIC \text{ of } Mp - AgNPs) \\ FIC_b = (MIC \text{ of } Mp - AgNP@BBR) / (MIC \text{ of } BBR)$$

$$FICI = FIC_a + FIC_b$$

The interaction was interpreted as – synergistic (≤ 0.5), additive ($>0.5-1$), indifferent ($>1-4$), or antagonistic (>4). All experiments were performed in triplicates.

2.5 Quantitative Biofilm Inhibition by individual treatments

Initially, the MIC of the three treatments was established by standard broth dilution method and were used to guide the concentration ranges applied in subsequent antibiofilm and checkerboard assays. The anti-biofilm potential of BBR and Mp-AgNP@BBR complex was determined by 96-well microtiter crystal violet assay. Briefly, overnight cultures of *E. hormaechei* were exposed to sub-inhibitory concentrations of BBR (16 $\mu\text{g/mL}$) or the Mp-AgNP@BBR (8 $\mu\text{g/mL}$) nanocomplex, whereas untreated cultures served as controls. The prepared microtiter plates were incubated at 37 °C for 48 h to allow structured biofilm formation. Following incubation, planktonic cells were gently removed by washing the wells with phosphate-buffered saline (PBS). The adherent biofilms were then stained with 0.4% crystal violet for 10 min at RT and rinsed with distilled water to eliminate unbound dye. The retained crystal violet was solubilized using 20% glacial acetic acid, and the resulting biofilm biomass was quantified by measuring absorbance at 570 nm with a UV-visible microplate reader (Synergy H1, Agilent, USA) (Moreno-Chamba et al. 2023).

2.6 Analysis of total EPS

The production of extracellular polymeric substances (EPS) by *E. hormaechei* was analyzed to assess the effect of BBR and Mp-AgNP@BBR. Bacterial cultures were incubated in nutrient broth containing sub-inhibitory concentrations of the test compounds, while untreated cultures served as control. After 48 h of incubation, the cells were harvested and washed with 0.9% NaCl to remove planktonic cells. Equal volumes of 5% phenol were added to the washed cells, followed by the slow addition of concentrated H_2SO_4 . The reaction mixtures were incubated in the dark for 1

h for color development. Samples were then centrifuged at $10,000 \times g$ for 20 min, and the absorbance of the supernatant was measured at 490 nm using a spectrophotometer (Shimadzu UV-1900i).

2.6.1 Analysis of Extracellular Matrix Components: Proteins and Carbohydrates.

Extracellular matrix (ECM) components were isolated from biofilms exposed to three treatments. Following incubation, biofilms were collected and resuspended in 1 mL of 1.5 M NaCl solution. The suspensions were centrifuged at $5000 \times g$ for 10 min at 4 °C, and the resulting supernatants were passed through a 0.22 μm membrane filter. The filtered supernatants were designated as ECM fractions. Untreated biofilms processed under the same conditions served as controls.

2.6.2 Determination of Protein Content

Protein concentration in the ECM fractions from treated and untreated biofilms was quantified using the Lowry assay using bovine serum albumin standard curve. Briefly, 0.5 mL of diluted sample was combined with 0.5 mL of Lowry reagent and incubated at room temperature for 20 min. This was followed by the addition of 0.5 mL of Folin-Ciocalteu's phenol reagent and further incubation for 30 min. Absorbance was measured at 620 nm, and protein content was calculated from the standard calibration curve and expressed as $\mu\text{g/mL}$.

2.6.3 Determination of Carbohydrate Content

Carbohydrate levels in the ECM of treated and control biofilms were estimated using anthrone. A volume of 100 μL of tenfold-diluted ECM sample was reacted with 200 μL of anthrone reagent (0.125% w/v prepared in 94.5% v/v conc. sulfuric acid). The reaction mixture was heated in a boiling water bath for 15 min and allowed to cool to room temperature. Absorbance was

recorded at 595 nm using a microplate reader (Synergy H1, Agilent, USA), and glucose was used as the reference standard.

2.7 Evaluation of Curli Amyloid Fibers Using CRCBB assay and ThT Fluorescence Assay

2.7.1 Congo Red-Coomassie Brilliant Blue (CRCBB) agar test for curli detection

Curli fiber production was evaluated using Congo Red (CR) and Coomassie Brilliant Blue (CBB) dye-supplemented agar, as previously described by (Shrestha et al. 2025). LB agar was prepared, autoclaved, and cooled to approximately 55 °C before supplementation with sterile-filtered CR (40 µg/mL) and CBB G-250 (20 µg/mL). The medium was poured into sterile Petri plates and allowed to solidify under aseptic conditions. Overnight bacterial cultures were standardized to an optical density (OD₆₀₀) of approximately 0.5 and subjected to four experimental conditions at sub-MICs. Aliquots (5 µL) from each condition were spot-inoculated onto CRCBB agar plates and incubated statically at 28 °C for 48 h to allow curli expression.

2.7.2 Thioflavin T fluorescence assay for Curli distribution

Curli amyloid fiber production in biofilms was monitored using a Thioflavin T (ThT) fluorescence staining assay. Biofilm formation was carried out on sterile glass slides under static conditions. An overnight culture of *E. hormaechei* was inoculated into fresh LB broth and equally distributed into four experimental groups. One group served as untreated control, while the remaining groups were supplemented with sub-MIC concentrations of three compounds. Sterile glass slides were immersed in each experimental setup and incubated at 37 °C for 48 h to allow surface-associated biofilm formation. Following incubation, the glass slides were gently removed and washed three times with sterile PBS to eliminate non-adherent cells. The

attached biofilms were then stained with 20 μ M ThT solution for 30 min. at RT in the dark for staining the curli fibers. Excess dye was removed by washing three times with PBS (Kyle et al. 2023).

ThT stained biofilms were imaged using a Leica DMI3000 fluorescence microscope (excitation at 450 nm and emission at 482 nm using a 100 $\times$ objective) (Siri et al. 2024). Fluorescence images were acquired under identical imaging conditions across control and treated groups to assess curli amyloid fiber disruption. Fluorescence was quantitatively analyzed in Fiji ImageJ, where %Area measured relative curli abundance within fixed regions and the Integrated Density ($\times 10^6$ a. u.) quantified total ThT fluorescence for all the variables. Three biological replicates (≥ 200 fields/biofilm) were analyzed by one-way ANOVA with Tukey HSD post-hoc test (mean $\pm$ SD).

2.8 Assessment of Bacterial Cell Surface Hydrophobicity Index (HI)

The effect of BBR and its nanocomplex on the cell surface hydrophobicity of *E. hormaechei* was assessed using a modified hydrocarbon adherence assay based on the method described by (Srinivasan et al. 2018). Briefly, bacterial cultures were standardized to an optical density (OD) of 1.0 at 530 nm, and 1 mL of each suspension was transferred into sterile glass tubes. The bacterial cells were then treated with sub-inhibitory concentrations of Mp-AgNPs, BBR, or the Mp-AgNP@BBR, while untreated suspensions were maintained as controls. Following treatment, 1 mL of analytical-grade toluene (Sigma-Aldrich) was added to each tube, and the mixtures were vortexed vigorously for 2 min to promote interaction between the bacterial cell surface and the hydrocarbon phase. The tubes were subsequently left undisturbed at room temperature for 10 min to allow complete separation of the aqueous and organic phases. Aqueous phase was carefully collected, and its absorbance was recorded at 530 nm using a UV-Vis spectrophotometer.

The HI was calculated according to the formula:

$$\text{HI (\%)} = [1 - (\text{OD}_{530} \text{ after vortexing} / \text{OD}_{530} \text{ before vortexing})] \times 100$$

2.9 Determination of Acyl-Homoserine Lactones (AHLs) Levels

The production of acyl-homoserine lactones (AHLs) by *E. hormaechei* was assessed using a colorimetric protocol adapted from Taghadoshi et al. [24]. Bacterial cultures were incubated in sterile Mueller-Hinton broth at 37 °C, either in the presence of BBR or functionalized-Mp-AgNPs. At three-hour intervals, 3 mL aliquots were collected under aseptic conditions and centrifuged at 10,000 rpm for 15 min to separate the cells. The supernatant was extracted with an equal volume of ethyl acetate, and the organic layer was evaporated to dryness. The dried residue was redissolved in a small volume of HPLC-grade acetonitrile and stored at -20 °C until analysis. For detection of AHLs, 40 µL of the extract was added to each well of a 96-well microplate, followed by 50 µL of a 1:1 (v/v) mixture of hydroxylamine (2 M) and NaOH (3.5 M), and then 50 µL of a 1:1 (v/v) mixture of ferric chloride (10% w/v in 4 N HCl) and 95% ethanol. Formation of the ferric-hydroxamate complex was measured at 520 nm using a microplate reader (Synergy H1, Agilent, USA), and AHL concentrations were calculated based on the measured absorbance values.

2.10 Statistical Analysis

All experiments were performed in triplicate, and data were analyzed using Jamovi version 2.7.23. Results are expressed as mean $\pm$ standard deviation (SD). Statistical significance was found by One-way ANOVA with Tukey's post-hoc multiple comparison test ($p < 0.05$). were used.

2.11 Literature search and data compilation

Data compilation for the antibiofilm activity of BBR and for the antibiofilm studies on *E. hormaechei*, a systematic literature search was

performed in Google Scholar and PubMed databases. Combinations of the following keywords: ('berberine' and 'antibiofilm'), and ('berberine' and ('biofilm inhibition')) were used for summarizing BBR's antibiofilm activity data. Studies reporting only the quantitative % inhibition or % eradication (disruption) of biofilm by free-berberine only were selected for inclusion in the respective summary table, while formulations based on berberine-loaded nanoparticles, liposomes, or other derivatives were excluded from the main table.

Combinations of the following keywords: ('*Enterobacter hormaechei*' and 'antibiofilm'), and ('*Enterobacter hormaechei*' and 'biofilm inhibition') were used for antibiofilm studies on *E. hormaechei*. Only the studies reporting antibiofilm activity against *E. hormaechei* were included in the respective summary table while antibacterial/antimicrobial reports/data were excluded.

2.12 Molecular Docking Analysis with Curli subunits

Curli proteins involved in biofilm formation were selected as receptor targets for molecular docking studies. The three-dimensional structure of the *Enterobacter hormaechei* curli proteins CsgA and CsgB was retrieved from the UniProt database (AlphaFold ID: AF-A0A155BS15-F1; CsgB: AF-A0A431SJI7-F1). Receptor preparation was carried out using AutoDock Tools 1.5.6 by adding polar hydrogens and assigning Kollman united atom charges, and the processed structures were saved in PDBQT format (Morris et al. 2009). BBR (CID: 2353) structure was obtained from the PubChem database in SDF format and converted into PDB format, while the Ag8 octahedral nanoparticle was constructed using BIOVIA Discovery Studio Visualizer. Energy minimization of the nanoparticle was performed in PyRx using the Universal Force Field with the conjugate gradient optimization algorithm, after which the minimized structures were converted to PDBQT format.

Initial docking of BBR with the Ag8 nanoparticle was conducted using AutoDock Tools, employing a docking grid of $44 \times 18 \times 40$ with a grid spacing of $0.375 \text{ \AA}$. The Lamarckian Genetic Algorithm was used with 100 runs and a maximum of 27,000 GA operations, and conformational clustering was performed at an RMSD tolerance of $1 \text{ \AA}$. The most favorable BBR-Ag8 complex was selected based on the lowest binding energy and the highest cluster population.

The optimized nanocomplex and BBR alone were subsequently docked against curli proteins CsgA and CsgB. Docking of the BBR-Ag8 complex with *E. hormaechei* CsgA was performed using a grid size of $100 \times 124 \times 126$ with a grid spacing of $0.397 \text{ \AA}$, while docking with CsgB employed a grid size of $126 \times 126 \times 126$ with a grid spacing of $0.375 \text{ \AA}$. Blind docking of BBR with CsgA was carried out in PyRx using AutoDock Vina at an exhaustiveness of 8, with the grid box centered at $x = -1.5371$, $y = 3.4088$, and $z = 4.1657$ and grid dimensions of $37.7416 \text{ \AA} \times 42.2367 \text{ \AA} \times 53.8688 \text{ \AA}$, while BBR docking with CsgB was performed using a grid center at $x = -0.1651$, $y = 2.085$, and $z = 1.8073$ and grid dimensions of $51.5584 \text{ \AA} \times 45.6585 \text{ \AA} \times 48.9349 \text{ \AA}$.

For all docking runs, AutoDock Tools evaluated binding affinities and clustered poses based on conformational overlap, with the best docked conformations selected according to the lowest binding free energy (ΔG , kcal/mol) and highest cluster population. The resulting docked complexes were analyzed for hydrogen bonding, hydrophobic interactions, and metal coordination using BIOVIA Discovery Studio Visualizer to elucidate potential mechanisms of biofilm inhibition (Barage et al. 2017); (Trott and Olson 2010).

3. Results

3.1 Structural characterization of Mp-AgNP@BBR

The UV-visible absorption spectra of the three tested components were recorded over the wavelength range of 200-800 nm (Fig. 1). BBR displayed distinct absorption maxima at approximately 230 nm and 264 nm, attributable to $\pi \rightarrow \pi^*$ electronic transitions of its aromatic isoquinoline framework. An additional, comparatively weak absorption band was observed near 350 nm, consistent with extended conjugation within the BBR molecule (Bhanumathi et al. 2017). The Mp-AgNPs exhibited a characteristically pronounced and broad surface plasmon resonance (SPR) band centered at approximately 460 nm for AgNPs. In the Mp-AgNP@BBR, absorption features were observed at 230 nm and 264 nm, together with a plasmon resonance band appearing at approximately 450 nm. This band showed slight spectral broadening, reflecting modifications in the optical profile of the adjuvanted system (Nguyen et al. 2022).

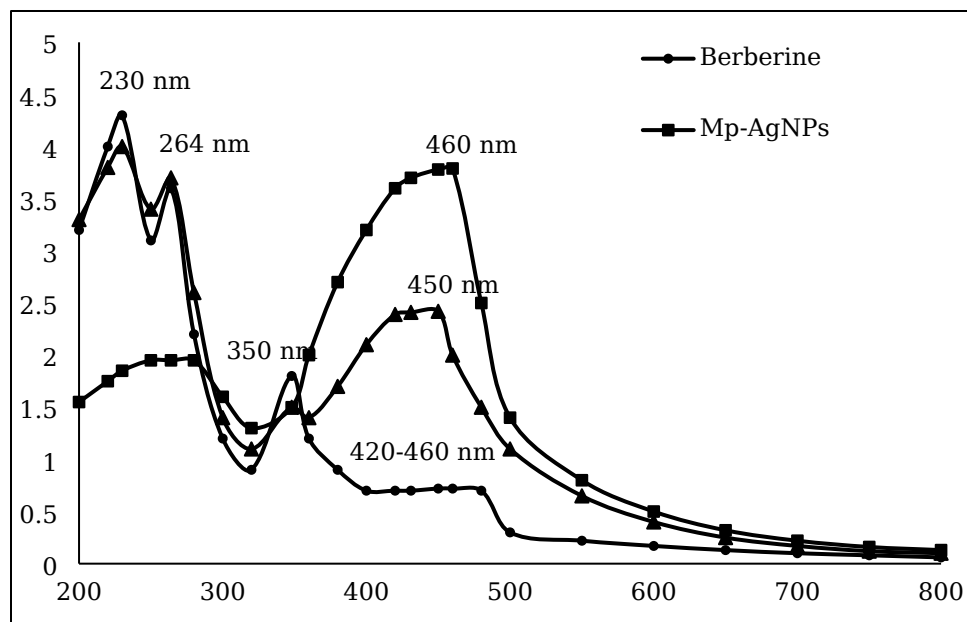
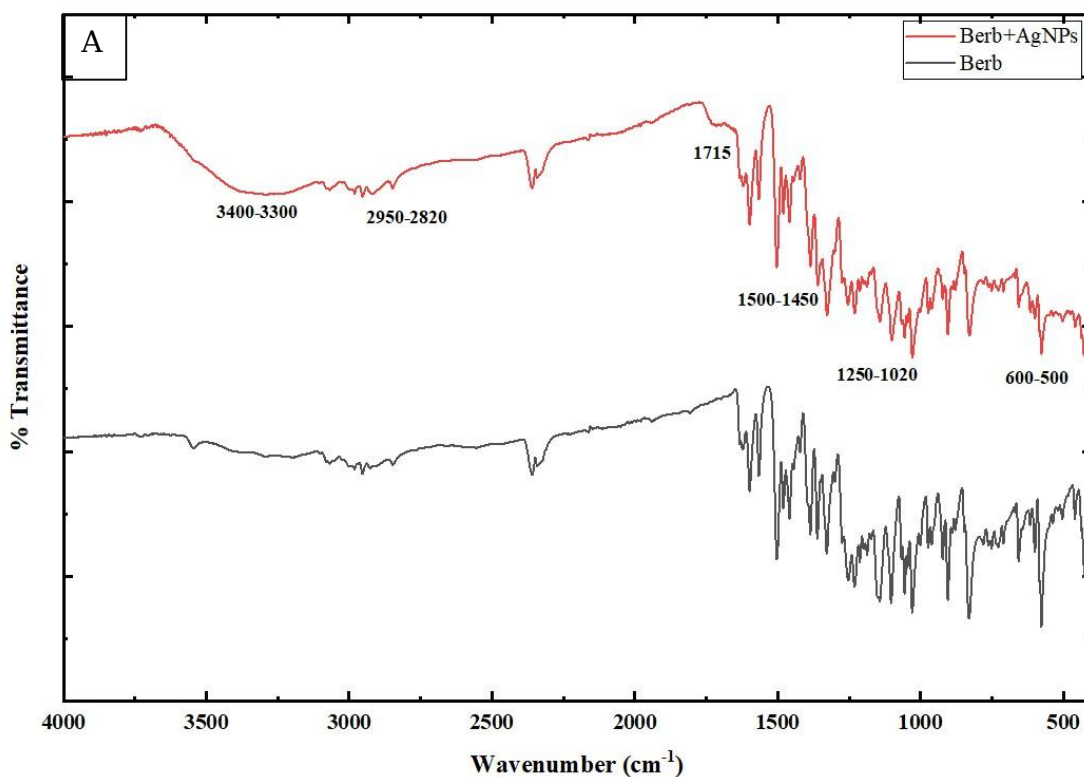


Fig. 1

The FTIR spectra of free BBR exhibited a broad absorption band in the range of $3400\text{--}3300\text{ cm}^{-1}$, corresponding to O-H and N-H stretching vibrations (Fig. 2 A). The bands observed at $2950\text{--}2820\text{ cm}^{-1}$ were assigned to aliphatic C-H stretching. A distinct peak at 1715 cm^{-1} indicated C=O stretching of conjugated carbonyl groups. Characteristic aromatic skeletal vibrations appeared in the region $1500\text{--}1450\text{ cm}^{-1}$, while peaks between 1250 and 1020 cm^{-1} were attributed to C-O and C-N stretching vibrations. The low-frequency bands around $600\text{--}500\text{ cm}^{-1}$ correspond to out-of-plane bending of aromatic C-H bonds (Bhanumathi et al. 2018). In the functionalized-Mp-AgNPs spectrum, all major functional group peaks were retained; however, noticeable shifts in peak positions and changes in intensities were observed, particularly in the -OH/-NH ($3400\text{--}3300\text{ cm}^{-1}$), C=O (1715 cm^{-1}), and aromatic C-N/C=C ($1500\text{--}1450\text{ cm}^{-1}$) regions, indicating interaction of BBR functional groups with the Mp-AgNP surface (Yeh et al. 2022; Tahan et al. 2024a)

The structural nature, crystallinity, and phase purity of the coated-NPs were investigated by XRD. The diffraction peaks observed at 2θ values of

38.11°, 44.31°, 64.51°, and 77.36° were indexed to the (111), (200), (220), and (311) planes of the face-centered cubic (fcc) crystal structure of metallic silver, respectively. These values were identical to those obtained for the biogenically synthesized Mp-AgNPs reported in our earlier study, indicating that the crystalline phase and lattice structure of silver were preserved upon BBR association. In addition, low-intensity diffraction peaks in the lower 2θ region (18-32°) (Supplementary Fig. S1) were assigned to the characteristic crystalline likenesses of BBR, confirming its successful association with the Mp-AgNP surface. The coexistence of the characteristic fcc Ag peaks together with BBR-related notations demonstrate the formation of a crystalline Mp-AgNP@BBR without any phase transformation or structural distortion of the silver core (Fig. 2 B).



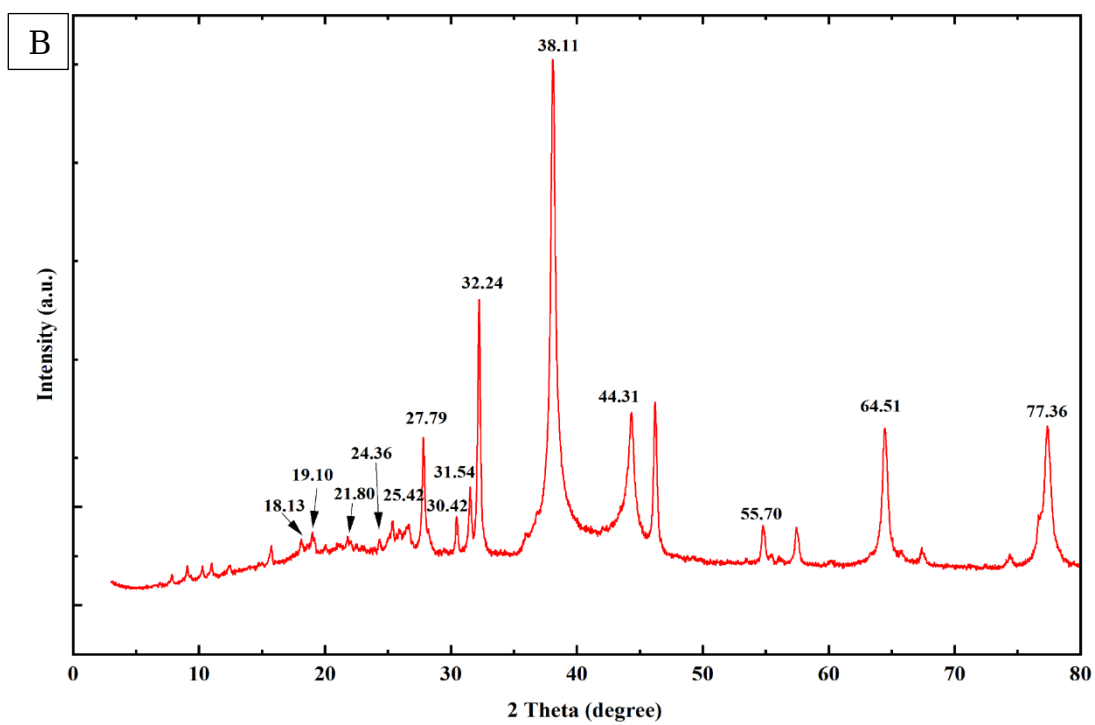
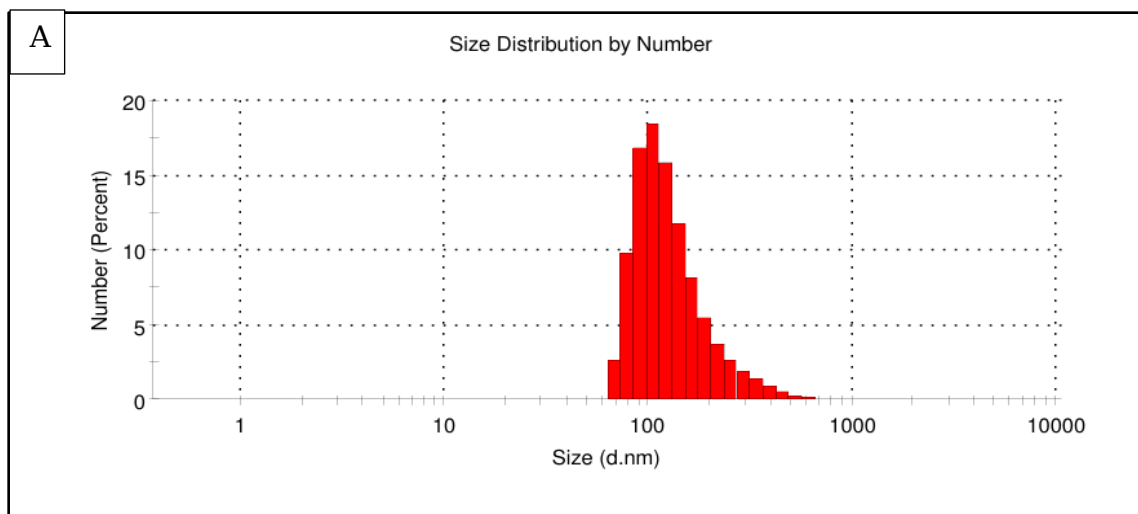


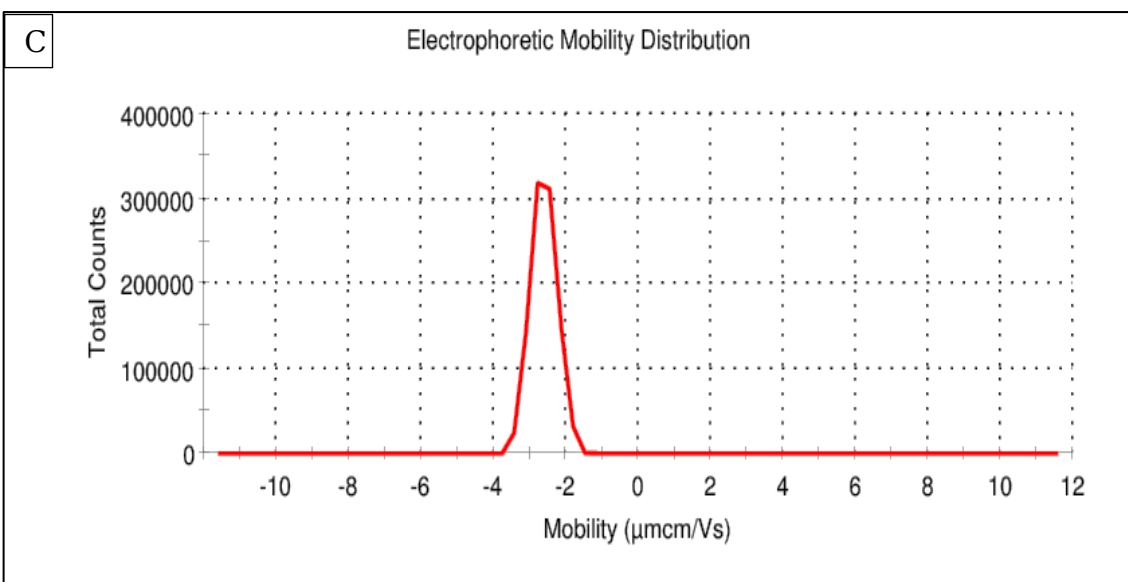
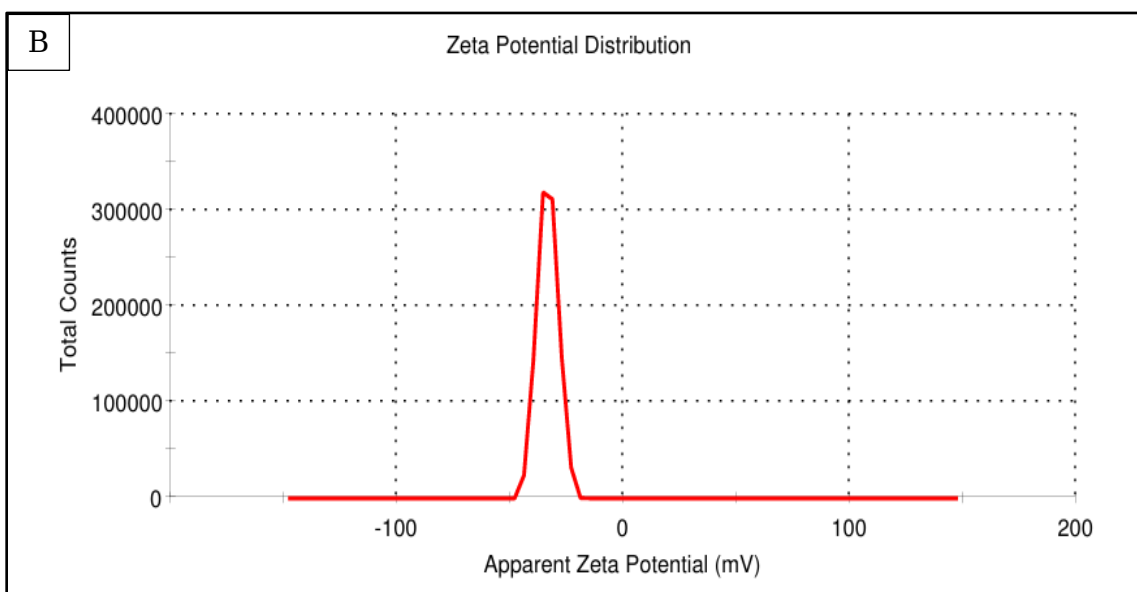
Fig. 2. (A, B)

3.2 Physicochemical Attributes of nanocomplex

DLS analysis of the Mp-AgNP@BBR revealed a single, well-defined particle population with a Z-average hydrodynamic diameter of 217.0 d.nm. The number-based size distribution showed a single peak at 137.3 nm (100%), indicating a uniform size distribution following nanocomplex formation. The PDI 0.230 (as compared to PDI 0.434 of Mp-AgNPs) suggested good size homogeneity for this biological system, while the absence of secondary peaks confirmed stable complexion without aggregation (Fig. 3A). Zeta potential analysis of the Mp-AgNP@BBR showed a single, monomodal charge distribution with a mean zeta potential of -33.2 mV (Fig. 3 B). The distribution accounted for 100% of the particles, with a zeta deviation of 4.54 mV, indicating uniform surface charge. The electrophoretic mobility of the functionalized NPs was -2.603 , indicating a negatively charged surface and confirming its applicability for assessing nanoparticle surface charge and colloidal stability (Fig. 3 C). NTA further demonstrated a marked increase in hydrodynamic diameter following surface functionalization with BBR, with this nanocomplex exhibiting a mode particle size of 96 nm (Fig.4) compared to 17 nm mode for bare Mp-AgNPs. This confirms successful coating of BBR onto the nanoparticles surface, resulting in stable nanocomplex with a well-defined size distribution and absence of severe aggregation.

Fig.3. (A, B, C)





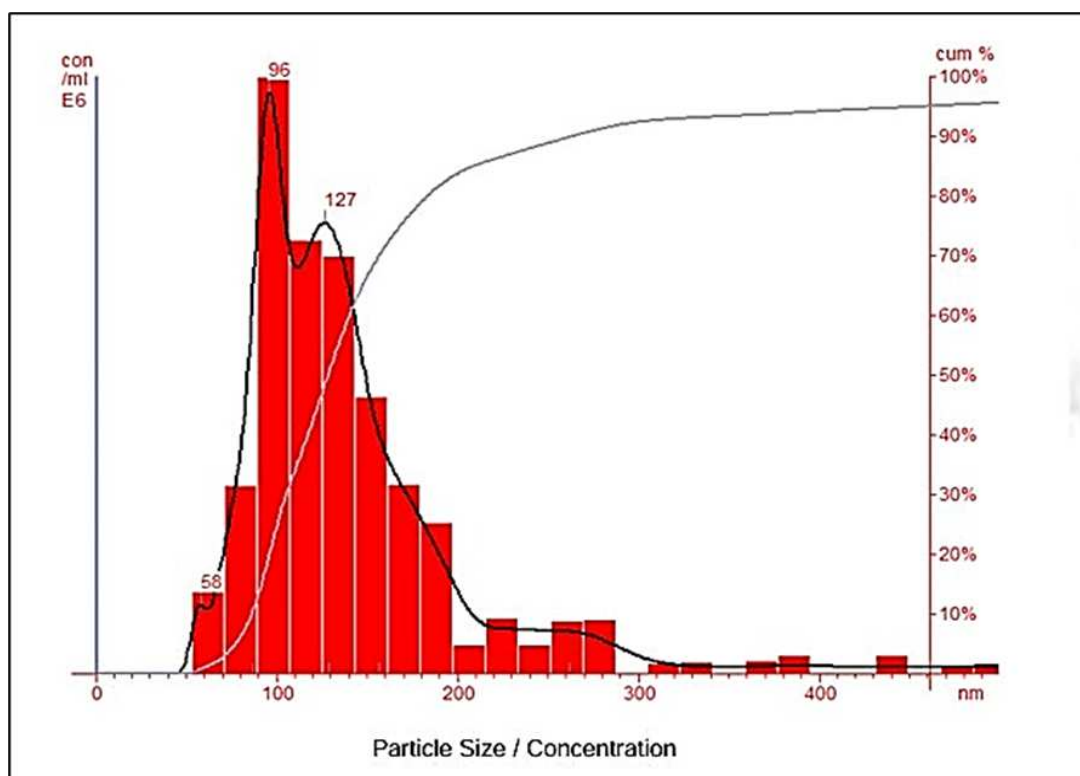


Fig.4.

3.3 Synergistic Interaction of BBR and Mp-AgNPs

The potential interaction between BBR and Mp-AgNPs was assessed using a checkerboard-based combinatorial assay (Supplementary S1 Table 1). In this experiment, strong inhibition of biofilm formation was observed at sub-MIC combinations, particularly at the 8 $\mu\text{g/mL}$ combination of BBR and Mp-AgNPs, resulting in a FICI value of 0.5, which indicates synergistic interaction. The results demonstrate that the combination treatment effectively inhibited biofilm formation at lower concentrations compared to the individual treatments. This synergism provided a strong rationale for the development of Mp-AgNP@BBR nanocomplex, which was subsequently tested for its antibiofilm properties against *E. hormaechei*.

Table 1. Molecular docking analysis of BBR and BBR-Ag8 complex with curli proteins.

Protein	Ligand	Binding energy (kcal/mol)	Key interacting residues	Major interactions observed	Interpretation
CsgA	BBR	- 6.20	Phe4, Ala7, Pro41, Arg64	C-H bond; alkyl; π -alkyl	Predominantly hydrophobic interactions with limited H-bonding
CsgA	BBR-Ag8 nanocomplex	- 5.81	Lys2, Leu3, Lys5, Ala7, Gln25, Arg64	H-bond; C-H bond; π -donor; π -sigma; alkyl; π -alkyl	Increased interaction types despite slightly lower affinity
CsgB	BBR	- 6.90	Phe7, Met5, Ser29, Tyr31	C-H bond; π - π stacking; alkyl; π -alkyl	Strong π - π and hydrophobic stabilization
CsgB	BBR-Ag8 nanocomplex	- 6.19	Leu6, Met8, Ser29, Tyr31	C-H bond; metal-acceptor (Ag); π -cation; π - π ; π -alkyl	Metal-mediated interactions alter binding mode

3.4 *E. hormaechei* Biofilm Inhibition by BBR and Mp-AgNP@BBR

Initial investigations determined 16 µg/mL as a sub-MIC value for Mp-AgNPs and BBR, and for the nanocomplex as 8 µg/mL. Hence, these sub-MIC values were considered for the antibiofilm assays to avoid the growth inhibition-driven effects. The antibiofilm activity of bare and complexed molecules against *E. hormaechei* was evaluated using the microtiter plate crystal violet assay. At sub-inhibitory concentrations, all treatments significantly reduced biofilm formation compared to the untreated control (Fig. 5). BBR alone inhibited biofilm formation by $60.3 \pm 1.2\%$ ($p = 0.05$), whereas Mp-AgNPs exhibited greater antibiofilm activity, marked with $67.78 \pm 1.0\%$ ($p = 0.04$) inhibition. Notably, the Mp-AgNP@BBR demonstrated the highest efficacy, achieving $80.5 \pm 0.9\%$ ($p = 0.04$) inhibition of biofilm formation. Not only inhibition, but treatments were also evaluated for their biofilm disruption abilities with qualitative Tube assay and microscopic observation. As depicted in Supplementary Fig. S2 and S3, these assays respectively revealed the biofilm disruption, where the nanocomplex exhibited highest activity.

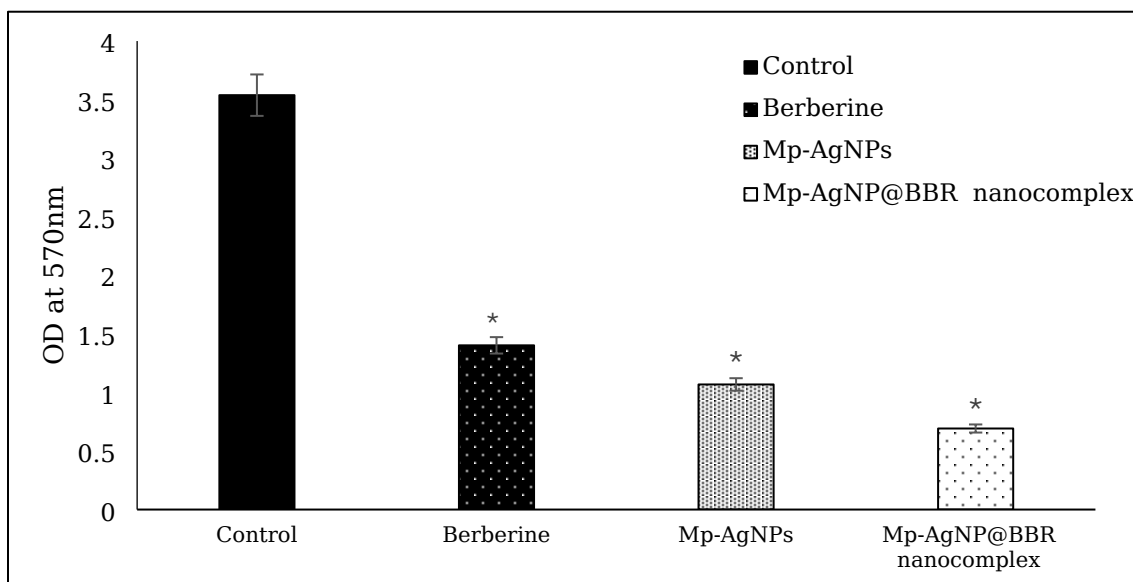


Fig. 5.

3.5 Assessment of Biofilm Matrix Integrity and Biochemical Composition

Spectrophotometric quantification demonstrated a marked reduction in total EPS production in *E. hormaechei* following treatment with Mp-AgNPs, BBR (16 µg/mL), and Mp-AgNP@BBR at a sub-MIC concentration (8 µg/mL). Mp-AgNPs reduced EPS synthesis by $49.02 \pm 1.1\%$ ($p = 0.04$), while BBR alone resulted in $40.5 \pm 1\%$ ($p = 0.03$) relative to untreated controls. The Mp-AgNP@BBR exhibited the strongest inhibitory effect, suppressing EPS production by $63.3 \pm 1.0\%$ ($p = 0.03$) (Fig. 6). EPS constitutes the primary structural scaffold of bacterial biofilms, playing a critical role in surface adhesion, biofilm maturation, and tolerance to antimicrobial stress.

The pronounced reduction observed with the nanocomplex formulation indicates enhanced impairment of the biofilm matrix compared to individual treatments, highlighting the superior antibiofilm potential of the BBR-nanoparticle hybrid system.

ECM content analysis of (untreated) biofilms of *E. hormaechei* exhibited a high, 549 µg/mL proteins. Treatment with a phytochemical resulted in a moderate reduction (486 µg/mL), whereas Mp-AgNPs exposure caused a pronounced decrease in protein levels (269 µg/mL). Notably, the Mp-AgNP@BBR exhibited maximum inhibitory effect, reducing the EPS protein content to 159 µg/mL, indicating a strong suppression of proteinaceous matrix components. Similarly, carbohydrate estimation revealed that the control biofilms contained 659 µg/mL of total polysaccharides. BBR treatment reduced this level to 539 µg/mL, while Mp-AgNPs further lowered the carbohydrate content to 398 µg/mL. The nanocomplex treatment led to a marked depletion of EPS carbohydrates, with a residual concentration of only 196 µg/mL, demonstrating a substantial suppression of the polysaccharide backbone of the biofilm matrix.

Overall, both protein and carbohydrate analyses consistently showed that the Mp-AgNP@BBR exerted significantly stronger inhibitory effects on EPS biosynthesis than either BBR or Mp-AgNPs alone, highlighting its superior potential to destabilize the structural integrity of the *E. hormaechei* biofilm matrix.

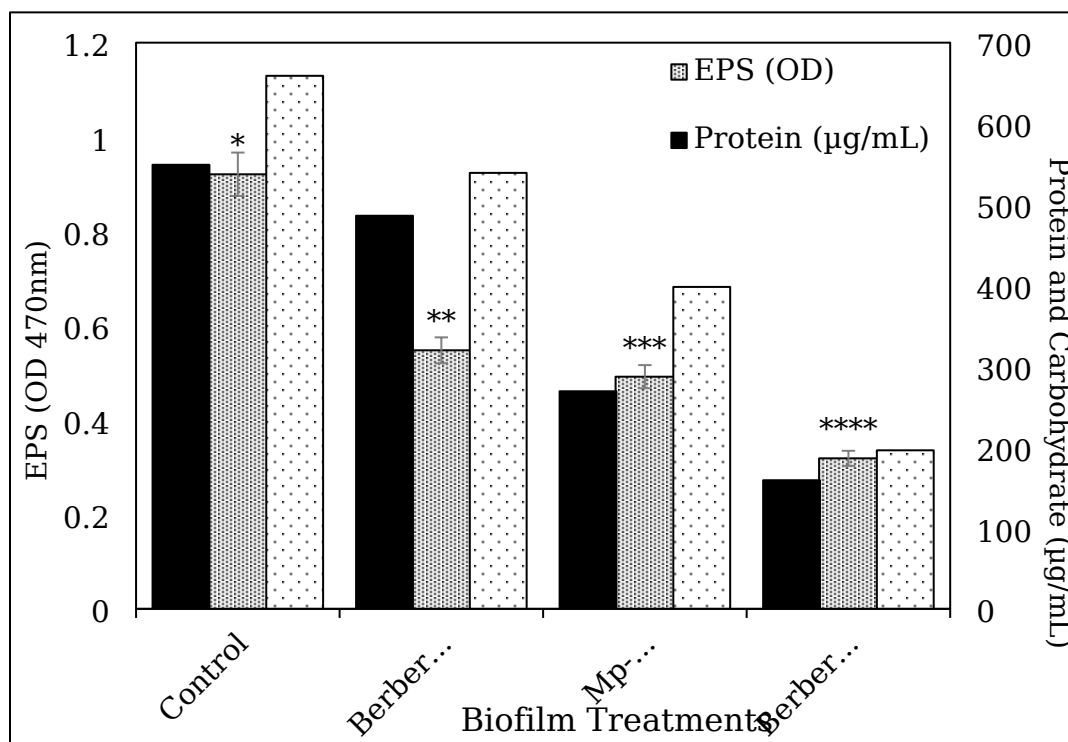


Fig. 6.

3.6 Assessment of Curli Amyloid Fibers distribution

Qualitative evaluation of curli fiber production on CRCBB agar revealed distinct treatment-dependent differences in colony morphology and dye-binding behavior (Fig. 7). The untreated control colonies (Fig. 7A) exhibited intense dark red coloration with a rough, wrinkled surface, indicative of strong Congo Red binding and robust curli fiber expression. In contrast, colonies exposed to BBR alone (Fig. 7B) displayed a noticeable reduction in dye uptake, appearing light red to pink with a comparatively smoother and less wrinkled morphology, suggesting partial inhibition of

curli production. Similarly, colonies treated with Mp-AgNPs (Fig. 7C) also showed reduced Congo Red binding, exhibiting a lighter red to orange hue with a smoother colony morphology, indicating moderate suppression of curli fiber expression. Notably, colonies treated with Mp-AgNP@BBR (Fig. 7D) showed a pronounced loss of Congo Red binding, presenting pale pink to nearly white coloration with smooth and moist colony architecture. This marked phenotypic shift indicates a substantial suppression of curli fiber synthesis in the nanocomplex-treated group.

ThT fluorescence staining (Fig. 8 A-D) revealed distinct differences in curli amyloid fiber production among treated and untreated *E. hormaechei* biofilms formed on glass slides. ThT specifically binds to β -sheet-rich amyloid structures, such as curli fibers, resulting in enhanced green fluorescence that directly reflects curli abundance within the biofilm matrix. The untreated biofilms exhibited intense green fluorescence under 100 $\times$ magnification, indicating robust curli expression and a dense amyloid-rich architecture. In comparison, BBR-treated biofilms showed a moderate reduction in fluorescence intensity, suggesting partial inhibition of curli fiber assembly. A more pronounced decrease in ThT fluorescence was observed following Mp-AgNP treatment, indicating stronger impairment of amyloid fiber formation. Notably, biofilms exposed to the Mp-AgNP@BBR displayed minimal and discontinuous fluorescence signals, visually reflecting near-complete suppression of curli amyloid production. When this visual fluorescence was studied in further depths by quantitative fluorescence image analyses, this too revealed dramatic reductions in curli amyloid content across all treatments. The Nanocomplex treatment resulted in a pronounced reduction in curli coverage (Fig. 8 E), decreasing it by 94.20% ($0.6 \pm 0.1\%$ coverage area compared to $10.8 \pm 0.7\%$ area in Control biofilms ($p < 0.001$). BBR and Mp-AgNPs treatments also showed substantial reduction in residual curli coverage, achieving decreases of 70.22% and 74.01% corresponding to respective values of $3.2 \pm 0.4\%$ and $2.8 \pm 0.3\%$

(both $p < 0.001$). Nanocomplex exhibited significantly superior inhibitory efficacy than AgNPs ($p < 0.01$). Further quantification using integrated fluorescence intensity (IntDen) provided a more sensitive measure of the curli abundance. A consistent decrease in curli-associated signals was observed in all the treatments, displaying values as $66.82\% \pm 0.3$, $74.56\% \pm 0.3$, and $94.26\% \pm 0.7$, respectively, for BBR, Mp-AgNPs, and nanocomplex. This pattern closely mirrored the reductions observed in surface coverage. Notably, unlike %Area output, IntDen measurements revealed that there were statistically significant differences between all the treatment groups, indicating that intensity-based quantification may provide enhanced discriminatory sensitivity in capturing subtle variations in curli-associated fluorescence in the biofilm inhibition study. These findings confirm that the progressive loss of ThT fluorescence corresponds to impaired curli biogenesis, highlighting the superior anti-amyloid and anti-biofilm efficacy of the nanocomplex.

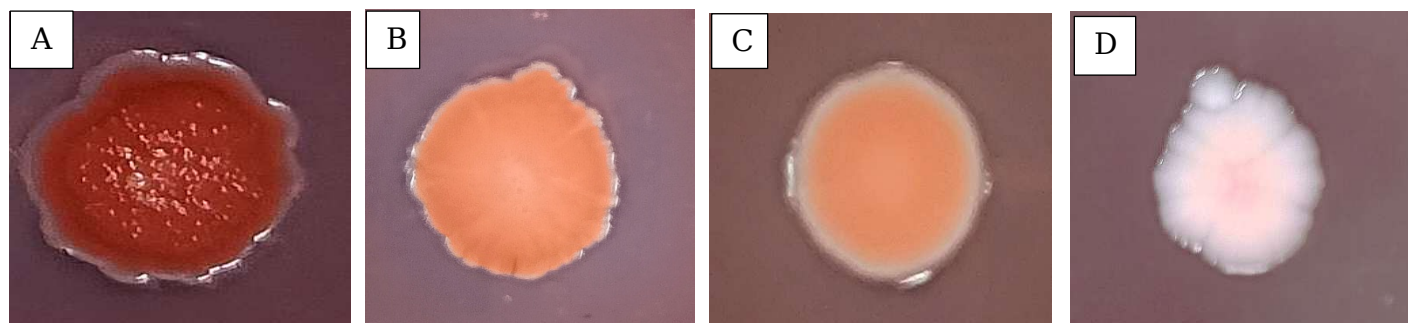


Fig.7.

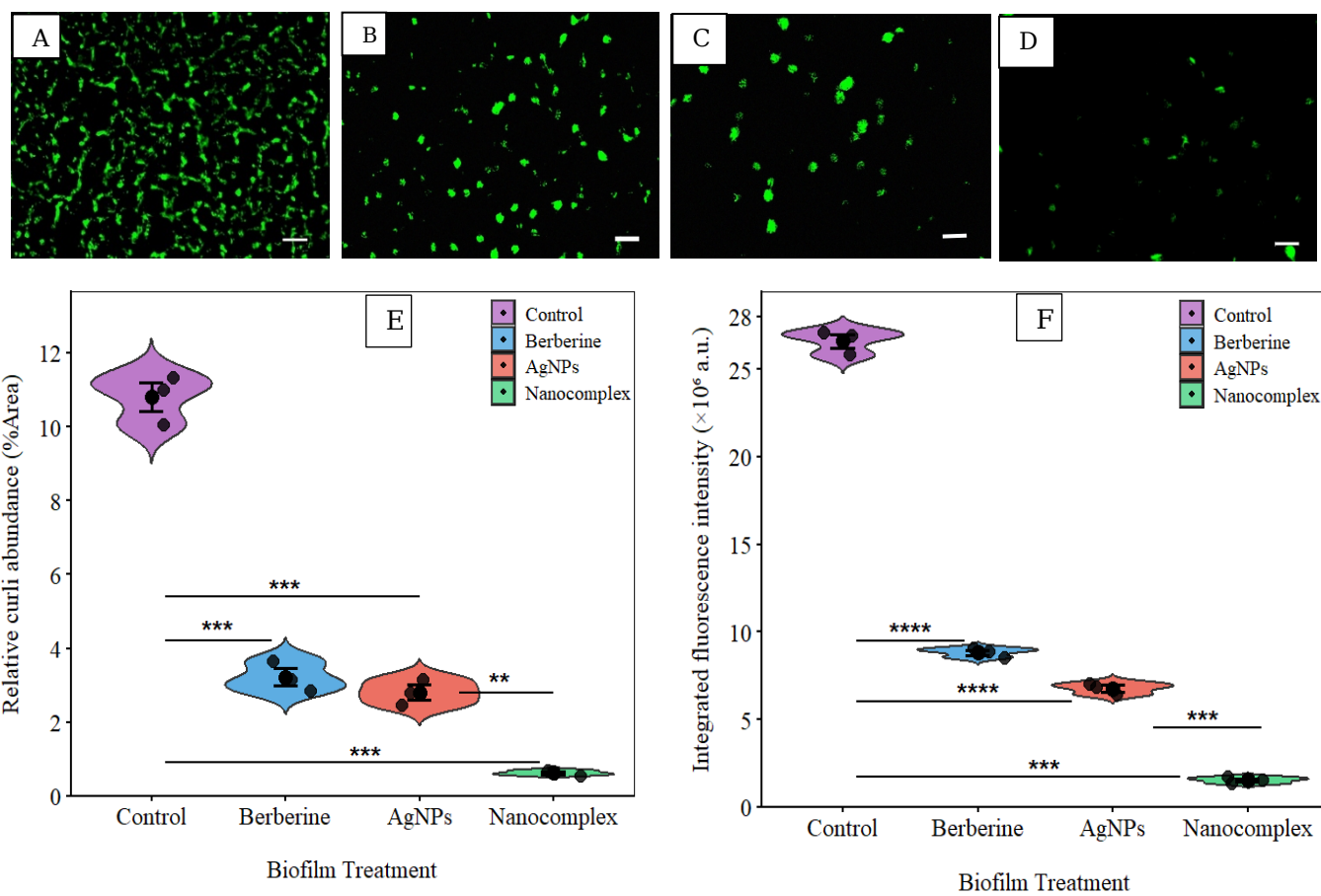


Fig.8 (A, B, C, D, E, F).

3.7 Assessment of Bacterial Cell Surface Hydrophobicity (HI Index)

The microbial adhesion to hydrocarbon assay using toluene was conducted to examine the effect of nanoparticles, individually and in complex, on the hydrophobicity index (HI) of *E. hormaechei* biofilms. The untreated control exhibited high cell surface hydrophobicity, with an HI of $60 \pm 1.0\%$. Exposure to sub-inhibitory concentrations of Mp-AgNPs significantly reduced the HI to $35.5 \pm 0.9\%$. ($p = 0.05$). Treatment with BBR alone caused a moderate decrease in hydrophobicity, yielding an HI of $47 \pm 0.8\%$ ($p = 0.05$), while the Mp-AgNP@BBR nanocomplex produced the greatest reduction, lowering the HI to $23.34 \pm 0.8\%$ ($p = 0.05$) (Fig.9).

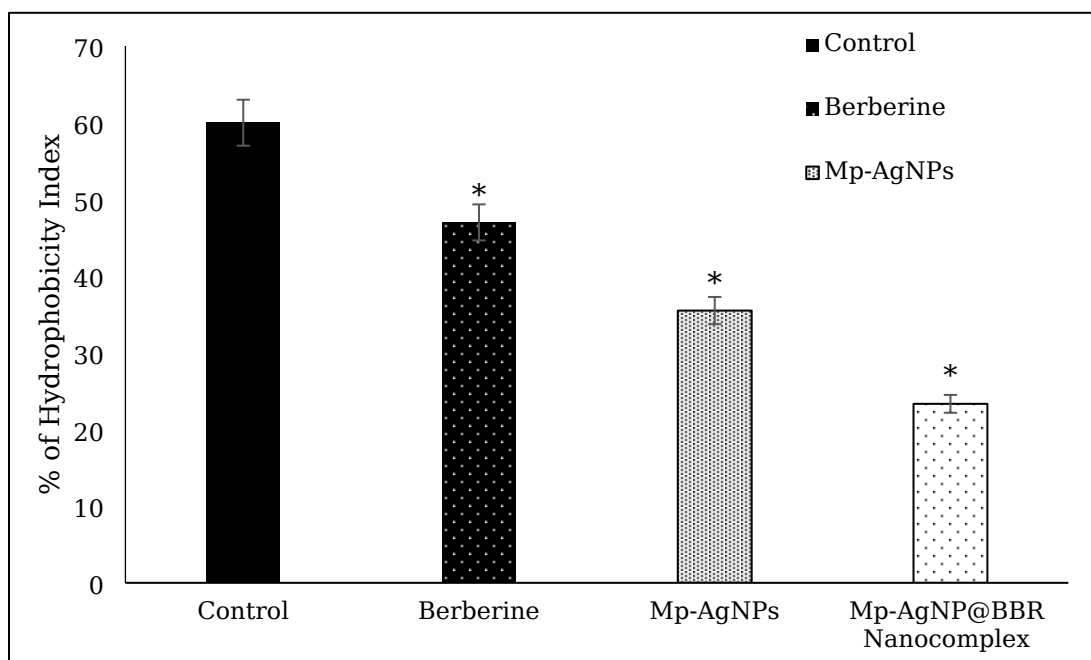


Fig. 9.

3.8 Disruption of quorum sensing signals

AHLs production was monitored over a 48 h incubation period by measuring absorbance at 520 nm (Fig. 10). In the untreated AHLs control, signal intensity was minimal during the early time points (6–12 h), followed

by a gradual accumulation phase. Peak AHL levels were recorded at 33 h with an OD₅₂₀ of approximately 0.83. Thereafter, a progressive decline in AHLs signal was evident, with values decreasing to 0.50 at 42 h and further to 0.44 at 48 h. Cultures exposed to free BBR exhibited weakened AHLs accumulation throughout the time course, reaching a reduced maximum OD₅₂₀ of 0.45 at 33 h, followed by a decrease to 0.22 at 48 h. Mp-AgNPs-treated cultures exhibited consistently low AHL levels throughout the incubation period, with only a slight increase between 12–15 h, reaching a markedly reduced peak at 33 h, followed by a sharp decline to 0.18 at 42 h and 0.15 at 48 h. Growth curve analysis of *E. hormaechei* confirmed that the observed reduction in AHLs production was not attributable to growth inhibition.

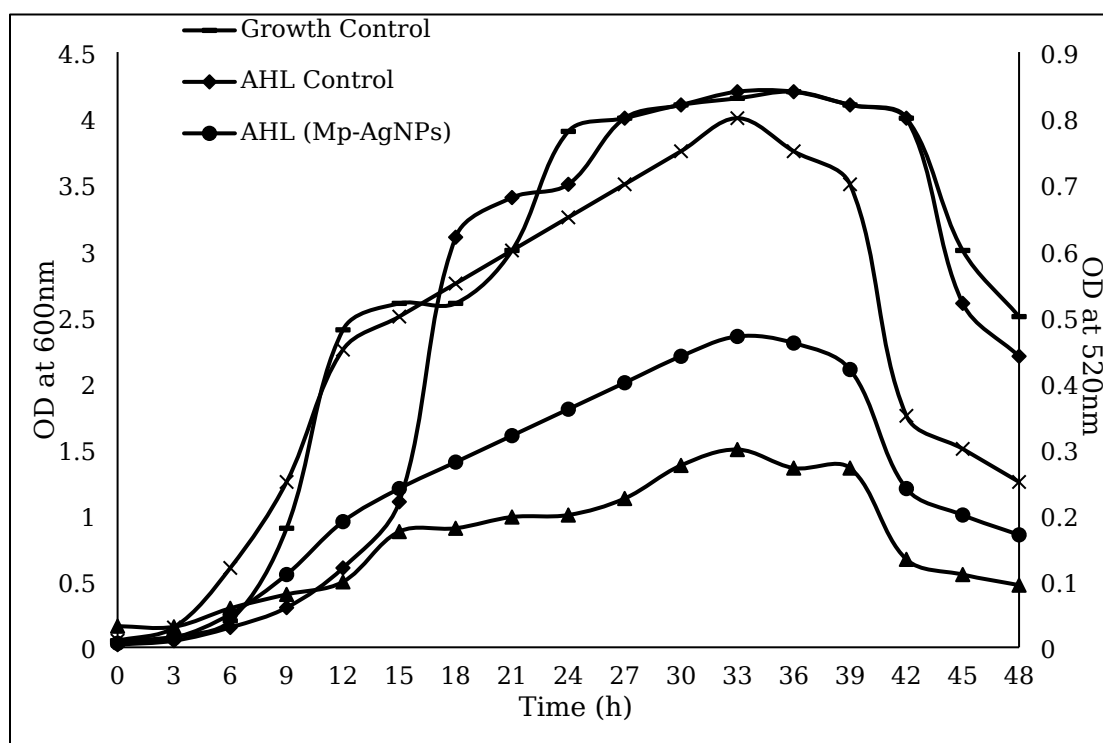


Fig. 10.

3.9 Molecular Docking Analysis of BBR and Mp-AgNP@BBR

Molecular docking analysis was performed to investigate the binding behavior of BBR and its Ag8- complex with the curli proteins – CsgA and CsgB, which play critical roles in amyloid-mediated biofilm architecture. Binding energies and interaction profiles are summarized in Table 1.

BBR interacted with CsgA with a binding energy of -6.2 kcal/mol, stabilized mainly through carbon-hydrogen bonds and hydrophobic interactions (alkyl and π -alkyl) involving Ala7, Pro41, Arg64, and Phe4. The interaction pattern was limited to weak hydrogen bonding and nonpolar contacts (Fig. 11 A).

The BBR-Ag8 complex exhibited slightly reduced binding energy toward CsgA (-5.81 kcal/mol); however, the interaction profile was substantially more complex. It formed conventional hydrogen bonds, carbon-hydrogen bonds, π -donor hydrogen bonds, π -sigma, alkyl, and π -alkyl interactions, engaging residues Ala7, Gln25, Leu3, Lys2, Lys5, and Arg64 (Fig.11 B).

BBR showed stronger binding affinity toward CsgB (-6.9 kcal/mol) compared to CsgA. The interaction was dominated by π - π stacking, carbon-hydrogen bonds, and hydrophobic contacts, particularly involving Phe7, Tyr31, Ser29, and Met5. Recurrent π - π stacking with Tyr31 suggests strong aromatic stabilization within the CsgB binding region (Fig.12 A).

The BBR-Ag8 nanocomplex bound CsgB with a binding energy of -6.19 kcal/mol. Notably, Ag8 incorporation introduced metal-acceptor interactions involving the silver atom (IAG327), along with π -cation and π - π stacked interactions, in addition to hydrophobic contacts. Residues such as Ser29, Leu6, Met8, and Tyr31 participated in these interactions, indicating a distinct binding mode compared to BBR alone (Fig.12 B).

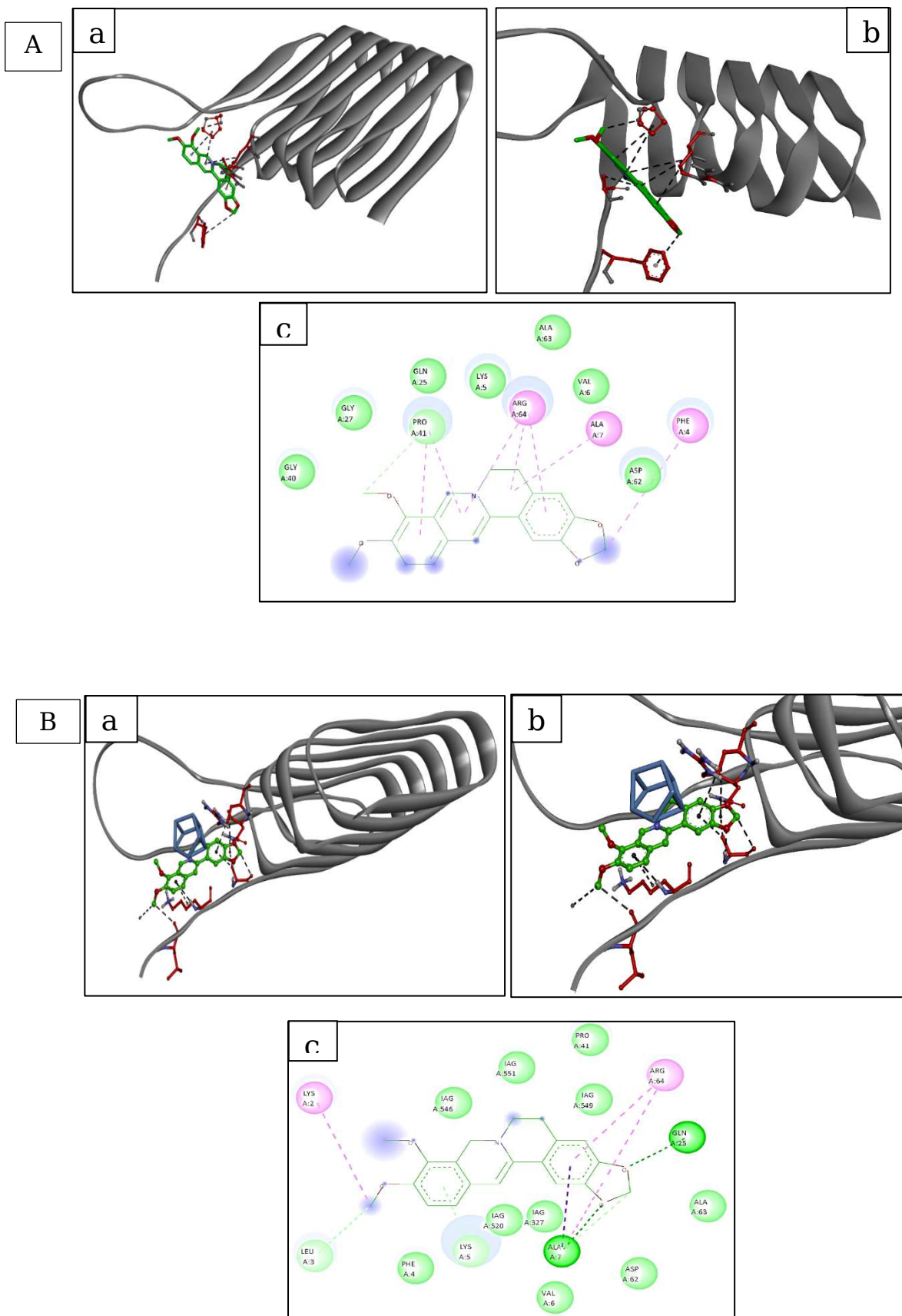


Fig.11. (A, B)

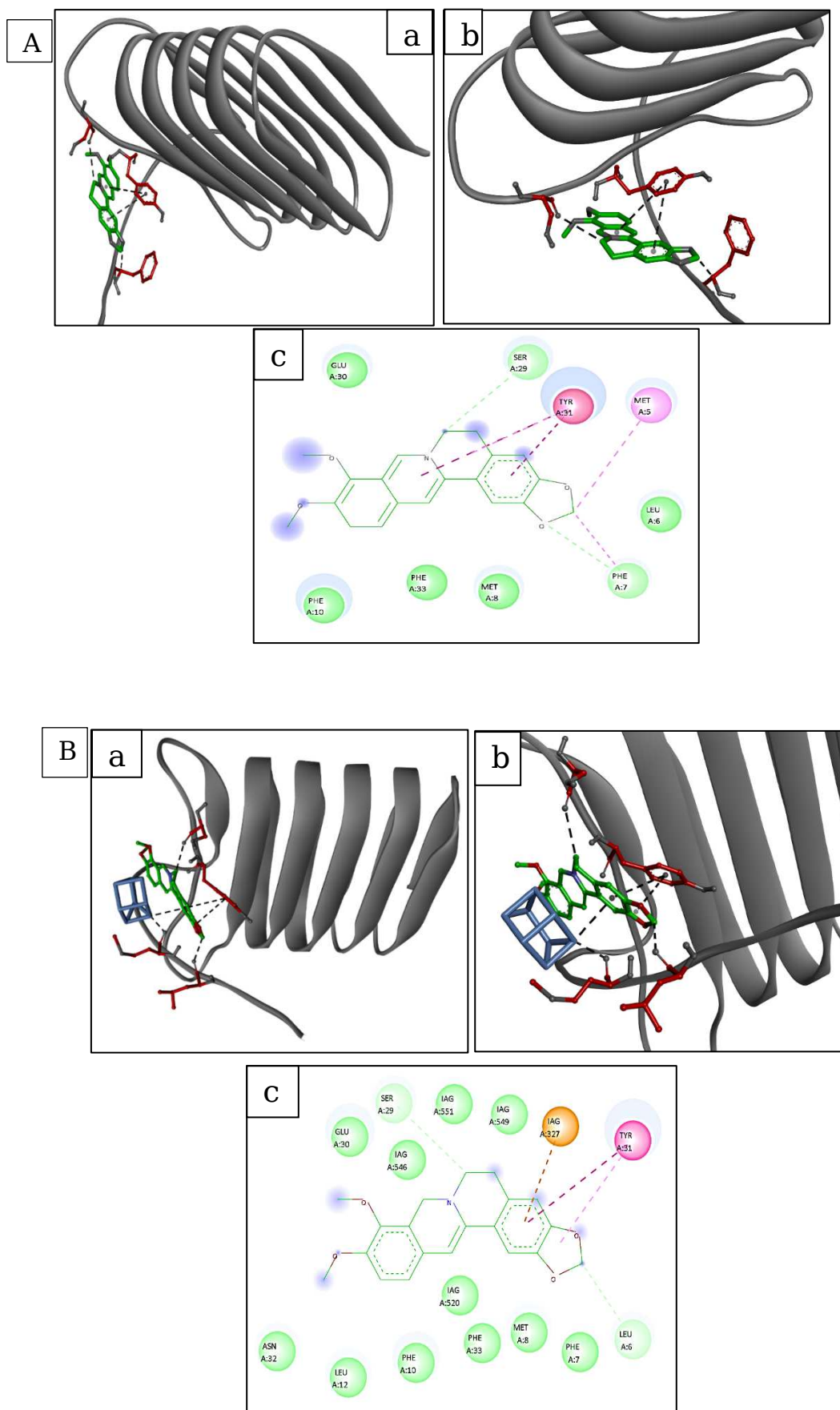


Fig.12. (A, B)

4. Discussion:

UTIs represent one of the global health burdens, further exacerbated by emergence of MDR-pathogens and their ability to form resilient biofilms. Among these, *Enterobacter hormaechei* has recently gained attention as an emerging and hence underexplored uropathogen, characterized by pronounced genomic plasticity and a high propensity to acquire resistance determinants through plasmids and mobile genetic elements (Cieřlik et al. 2025), (Yeh et al. 2022), (Singh et al. 2022; Mayer et al. 2025). Its ability to form curli mediated, quorum regulated biofilms further contributes to its persistence and therapeutic recalcitrance.

In such serious context, the development of potent antibiofilm strategies remains a critical unmet need (Table 3). Therefore, the present study explores the nano-enabled therapeutic approach by evaluating the antibiofilm efficacy of BBR-functionalized silver nanoparticle nanocomplex against curli-associated and quorum-regulated biofilm formation in *E. hormaechei*.

BBR, a plant isoquinoline alkaloid, has gained a specific attention in recent times because of its broad-spectrum biological activities viz. – antimicrobial, antibiofilm, anti-inflammatory, antioxidant, and anticancer. Although it has proven its pharmacological potential and adjuvant properties, the literature is still limited in its standalone efficacy, particularly in robust biofilm-forming MDR-pathogens and uropathogens in general (Imenshahidi & Hosseinzadeh, 2018); (Neag et al., 2018); (Yang et al., 2025); (Duda-Madej et al., 2026, 2025).

Table 3. Quantitative antibiofilm activities of BBR.

Organism		Sub-MIC	% Inhibition/Disruption	Reference*
<i>Salmonella enterica</i> <i>serovar Typhimurium</i>		625 µg/mL (1/2 MIC)	30.21% Inhibition	(Aswathanarayan and Vittal 2018)
<i>Pseudomonas aeruginosa</i> <i>PA01</i>		19 µg/mL (1/4 MIC) 1000 µg/mL (1/2 MIC)	71.70% Inhibition 49.34% Inhibition	
<i>Staphylococcus aureus</i> (ATCC 6538P)		0.125 µmol/mL (1/4 MIC)	34.56% Disruption	(Li et al. 2019)
<i>Multidrug-Resistant</i> <i>Staphylococcus aureus</i>		0.1 µmol/ mL (NR)	32.98% Disruption	(Huang et al. 2020)
<i>Candida krusei</i> ATCC 6258		10 µg/mL	96.93% Inhibition	(Xie et al. 2020)
<i>Candida glabrata</i> ATCC 90030		20 µg/mL	92.36% Inhibition	
<i>Candida albicans</i> ATCC 10231		80 µg/mL	43.54% Inhibition	
<i>Candida dubliniensis</i> MYA 646		40 µg/mL	21.62% Inhibition	
<i>Candida albicans</i> ATCC 90028		160 µg/mL	19.89% Inhibition	
<i>Candida albicans</i> strain SC531 + <i>Staphylococcus aureus</i> strain HNS0029 (Dual biofilms)		128 µg/mL (NR)	~37% Disruption	(Gao et al. 2021)
<i>Candida albicans</i> strain SC531 + <i>Staphylococcus aureus</i> strain ATCC25923 (Dual Biofilms)			~21% Disruption	
<i>Salmonella Typhimurium</i> CMCC50115 (LT2 derivative)	Wild type	56.25 µg/mL (1/16 MIC)	66.29% Disruption	(Yang et al. 2025)
	<i>ΔfimH</i>	56.25 µg/mL (1/16 MIC)	26.59% Disruption	
	<i>ΔfimA</i>	62.5 µg/mL (1/16 MIC)	7.58% Disruption	
MRSA		50 µg/mL (NR)	41.20% Inhibition	(Ning et al. 2022)
MRSA Multi-locus Sequence Type (MLST) strain STA239		514.56 µg/mL (×4 MIC)	95.65% Inhibition	(Xia et al. 2022)

<i>Staphylococcus aureus</i>	50 µg mL ⁻¹ (NR)	> 60% Disruption	(Xie et al. 2022)
<i>Candida albicans</i>	64 µg/mL (1/8 MIC)	40% Inhibition 42% Disruption	(Lin et al. 2023)
MRSA	> 75 µM (NR)	39.16% Inhibition	(Lu et al. 2023)
VRSA <i>Staphylococcus aureus</i> (I - IV) strains	0.5 - 1 µg/mL (1/2 MIC)	99.76-99.97%	(Abd El-Hamid et al. 2024)
<i>Pseudomonas aeruginosa</i>	640 µg/mL (1/2 MIC)	50.50%	(Yi et al. 2024)
MRSA	200 µg/mL (MIC NR)	60%	(Kandaswamy et al. 2024)
<i>Staphylococcus aureus</i> ATCC 6538	82.5 µg/mL (×4 MIC)	50.50% Disruption	(Sadeghi et al. 2024)
MRSA strain 1 - 5	65 µg/mL (×4 MIC)	19.88% - 41.58% Disruption	
Uropathogenic <i>Enterobacter hormaechei</i>	16 µg/mL (1/2 MIC)	60.37% Inhibition (berberine alone)	Present study
	8 µg/mL (1/4 MIC)	80.57% Inhibition (by nanocomplex)	

*Related literature shows several studies reporting significant antibiofilm action of BBR, but quantitative data are very limited. This table includes only studies that report the percentage inhibition of biofilm formation by BBR alone.

Pursuing this scientific niche, the present study provides mechanistic insights into the enhanced antibiofilm activity of the BBR-Mp-AgNP nanocomplex, particularly in the context of curli-mediated robust biofilm inhibition, thereby highlighting its potential as a promising strategy to combat emerging MDR uropathogens.

Initial physicochemical characterization of the BBR-Mp-AgNPs nanocomplex established a clear progression from nanoparticles formation to controlled surface functionalization and finally to biologically enhanced performance. UV-visible spectroscopy first confirmed distinct optical identities of each component. While Mp-AgNPs were already characterized in our previous study, the spectral properties of BBR too were consistent with the literature. However, the nanocomplex developed in this study exhibited a new absorption profile, indicating potential interaction between the two agents. Following interaction, retention of BBR's characteristic bands demonstrates preservation of its aromatic electronic structure without any structural degradation. The modification around 230-270 nm and importantly, the SPR band shift to 450 nm from 460 nm with slight broadening indicates modification of the nanoparticle surface dielectric environment rather than metallic core alteration. These spectral changes initially marked the association of BBR with Mp-AgNPs leading to partial surface coverage and modification of the local refractive index surrounding the nanoparticles, resulting in damping and slight broadening of the plasmon band. Such spectral changes are commonly associated with ligand adsorption and formation of nanoparticle-drug surface nanocomplexes. Similar plasmonic modulation during ligand adsorption has been reported by Kopal et al. (Kopal and Smeliková 2025) where surface-bound organic molecules altered electron oscillation behavior without disrupting nanoparticle integrity. In summary, UV-Vis data in this study indicated a stable surface coordination between BBR and Mp-AgNPs rather than simple

physical blending owing to a simple conjugation strategy i.e. mixing and shaking, used in synthesis of this nanocomplex.

FTIR analysis provided the molecular-level confirmation of this interaction. Largely, the functional groups characteristic of BBR was seen maintained. The broadening and displacement of -OH/-NH stretching vibrations indicate involvement of hydrogen bonding and possible coordination with surface silver atoms. The shift of the carbonyl band ($\sim 1715\text{ cm}^{-1}$) suggests partial electron donation to Ag⁰, implying formation of a surface complex. Changes in aromatic C=C and C-N vibrational regions further indicate participation of the isoquinoline nitrogen in metal-ligand interaction. These collective shifts confirm that BBR anchors to Mp-AgNPs through multi-point attachment rather than single-site adsorption, creating a stabilized organic corona. The appearance of BBR-associated functional group vibrations, along with shifts due to interaction with the nanoparticle surface, validated the formation of the nanocomplex. Such cooperative binding is critical because it enhances dispersion stability while maintaining accessibility of functional groups necessary for biological interaction. This is an initial indication of adsorption-mediated binding in which organic ligands interact with nanoparticle surfaces through weak electrostatic interactions, hydrogen bonding, or π -metal interactions. The preservation of the main functional groups signs the structural integrity and stability of nanocomplex.

XRD analysis too demonstrated that this AgNPs-surface engineering by BBR does not perturb the metallic core. The preservation of fcc silver indices (consistent with the standard JCPDS file for metallic Ag (No. 04-0783)), after organic association confirms that functionalization occurs exclusively at the nanoparticle interface. Maintenance of crystalline phase purity is biologically significant because surface atom availability and antimicrobial reactivity are influenced by lattice integrity. Comparable retention of Ag crystalline nature after phytochemical immobilization is a known phenomenon, where surface-bound bioactives enhanced the

biological function without altering the intrinsic metallic architecture (Baidoo et al. 2025). In the present system, this structural preservation ensures that the intrinsic redox and surface reactivity of Mp-AgNPs remain intact while surface chemistry is selectively modified.

Colloidal characterization strengthens these structural interpretations by providing further insights. The unimodal DLS distribution with low PDI (0.230) indicated uniform coating rather than aggregation. The increase in hydrodynamic diameter observed in both DLS and NTA reflects formation of an organic conjugation layer and not a nanoparticle clustering. Zeta potential shifted from -22.1 mV to -33.2 mV, pointing increased electrostatic stabilization due to surface reorganization and exposure of negatively charged functional groups. Such $\geq \pm 30$ mV ZP value is indicative of high colloidal stability as per the well documented literature (Bhattacharjee 2016), (Wu et al. 2018). The increased negative electrophoretic mobility (-2.603) further supports enhanced dispersion predictability, consistent with the stabilization mechanisms discussed by (Piri et al. 2024). Therefore, physicochemical evidence converges to demonstrate that BBR coating yielded a structurally intact, electrostatically stabilized nanopatform optimized for biological interface interaction.

Collectively, these structural and physicochemical analyses consistently point out towards surface-association mediated functionalization of Mp-AgNPs by BBR resulting into stable nanoparticle system while preserving the crystalline integrity of the metallic core and the phytochemical characteristics.

This phytochemical engineered stability directly translated into enhanced antibiofilm activity against *E. hormaechei*. Biofilm inhibition at sub-MIC concentrations confirms that suppression is not a secondary effect of bactericidal toxicity but reflects targeted interference with biofilm-associated regulatory pathways. While BBR alone exhibited significant inhibition (60.37%) and Mp-AgNPs had already demonstrated somewhat stronger suppression (67.78% inhibition), the AgNP@BBR nanocomplex

remarkably produced the highest biofilm formation inhibition (80.57%). This enhanced efficacy reflects complementary mechanisms – AgNPs mediate physicochemical alteration through redox-mediated stress, direct membrane damage, silver-ion release, oxidative stress, biofilm penetration, while BBR may exert more regulatory, reported mechanisms - QS inhibition, biofilm-associated gene regulation, modulation of dispersal pathways actions, curli/amyloid interference.

Literature survey stamps the measurable antibiofilm activity of BBR, although comparatively few studies report the detailed percentage inhibition values. We listed such studies in Table 2, which compares the % inhibition outcome of the BBR in previous reports with present study. Although BBR action towards *E. hormaechei* biofilms (present study) is moderately placed in that table, Mp-AgNP@BBR nanocomplex holds a higher position in terms of biofilm inhibition potency. Also, this comparison reveals that BBR suppresses the biofilms to a varying degree, depending on the organisms and the conditions. The key significance of the present study is that we extend our observation with BBR to a biochemical analysis that constitutes monitoring of the architecture of the uropathogenic biofilms, and it better reflects the disruption of the biofilm rather than only transcriptional response.

Table 2. A consolidated analysis of emerging antibiofilm evidence against *E. hormaechei*.

Source of Strain	Antibiofilm Agent	MIC	Biofilm Inhibition / Outcome	Reference
Clinical isolate (nosocomial pathogen)	Gallic acid (phytochemical)	> 4 mg/mL	Significant dose-dependent inhibition at 0.5 – 4 mg/mL; QS inhibition (violacein reduction)	(Rajan et al. 2023)
Soil invertebrate-derived isolates	<i>Xanthium strumarium</i> extract	Doses 0.012 to 50 mg/ml (range tested)	Strain ETU-7 to ETU-17 85.2 – 97.8% Inhibition	(Hassan et al. 2024)
MDR clinical isolate	Essential oils (<i>Thymus leptobotrys</i> , <i>Lavandula mairei</i>)	MIC: 1.56–6.25 mg/mL	>80% Inhibition <50% Disruption	(Laktib et al. 2024)
Clinical isolate (GC26)	Ferulic acid (phytochemical)	> 4096 µg/mL	Strong reduction in biofilm biomass and metabolic activity (dose-dependent)	(Vaikkathillam et al. 2025)
Clinical isolate	Naringin (flavonoid)	Not specified	Significant antibiofilm activity with dose-dependent inhibition	(Babu et al. 2025)
Clinical MDR isolates (ECC)	Bacteriophage + colistin	Colistin MIC 32.71 µg/mL	Effective biofilm eradication using sequential phage-antibiotic treatment	(Singh et al. 2025)
Clinical isolate	SeNPs (<i>Lysinibacillus odyseey</i> cell free extract derived - Selenium nanoparticles)	Not specified (25 - 100 µg/mL)	73.59 % Inhibition (at maximum conc.)	(Mangudi and Pappu 2025)
Uropathogenic clinical isolate	AgNPs (<i>Mussaenda philippica</i> - plant extract derived)	16 µg/mL (1/2 MIC)	67.78% Inhibition	(Mhavarkar et al. 2025)
Not specified	CuNPs (<i>Ononis natrix</i> - plant extract derived)	0.078 mg/mL	72.11% Inhibition	(El-Ouahmani et al. 2026)

	copper nanoparticles)			
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Conversely, the interaction of BBR with bacterial biofilms is not universally inhibitory, rather it is context dependent. BBR can exert conditional effects on biofilm dynamics, where sub-inhibitory concentrations may promote biofilm persistence especially, in Methicillin-resistant *Staphylococcus aureus* (MRSA) strains. It inhibits biofilm dispersal and enhances matrix production while higher concentrations are inhibitory (Zhang et al. 2022). Biofilm enhancement has been reported in *S. aureus* as evidenced by the increased biofilm biomass and upregulation key matrix associated genes *icaA/B* and adhesin related factors, underlining the strong stress exerting nature of BBR and survival stress response (biofilm enhancement) in *S. aureus* (Tan et al. 2019).

The potent antibiofilm activity observed for Mp-AgNP@BBR was confirmed as synergistic by the checkerboard analysis, yielding a FICI value of 0.5, as per the standard interpretative criteria ($FICI \leq 0.5$ = synergy). This method is generally used to evaluate interactions between antimicrobial agents based on changes in MIC values (Ribeiro et al. 2023), employed here in the antibiofilm context. The finding suggests that the combination reduced the effective inhibitory concentrations required to suppress bacterial growth compared with individual treatments. Synergistic interactions have been reported in studies where silver nanoparticles enhanced the antimicrobial efficacy of conventional compounds, leading to reduced MIC values and improved antibacterial activity (Tahan et al. 2024a).

EPS production is a fundamental requirement for the establishment of mature biofilm architecture and hence it was analyzed. There was a significant decrease in total EPS content of the uropathogenic biofilms treated with individual substances and their nanocomplex. Further, biochemical dissection through ECM quantification revealed substantial reduction in both carbohydrate and protein fractions of *E. hormaechei* biofilms, particularly with the surface functionalized Mp-AgNPs. Carbohydrates form the primary structural scaffold of biofilms, whereas matrix-associated proteins mediate biofilm cohesion, surface attachment

and enzymatic activity. Silver nanoparticles are known to bind thiol and amine residues, disrupting enzyme systems involved in polymer secretion, while oxidative stress impairs biosynthetic pathways (Estevez et al. 2020). BBR disrupts biofilm matrix biosynthesis, particularly polysaccharide and protein components (Duda-Madej et al. 2026). The combined formulation therefore likely exerts multi-level suppression inhibiting glycosyltransferases, impairing secretion systems, and destabilizing protein scaffolding leading to collapse of matrix architecture rather than superficial detachment (Rainey et al. 2018; Yang et al. 2025a). Reduced cell surface hydrophobicity further supported the early-stage adhesion impairment. Hydrophobic interactions facilitate initial attachment and stabilization of biofilm microcolonies. The pronounced decrease observed with the nanocomplex suggests remodeling of outer membrane composition and altered surface charge dynamics. Silver-induced membrane destabilization combined with BBR-mediated metabolic modulation likely restructured lipopolysaccharide exposure and surface protein arrangement, weakening adhesion strength and subsequent matrix development.

To distinguish between transcriptional (gene-level) and structural (protein-level) antibiofilm effects due to interference by individual and nanocomplexed BBR, we examined the amyloid architecture in *E. hormaechei* biofilms. Direct structural validation of curli suppression was obtained through CRCBB phenotyping and ThT fluorescence staining. Curli forms structural backbone of Enterobacteriaceae biofilms (Barnhart and Chapman 2006). Diminished congo red binding and loss of wrinkled colony morphology confirmed phenotypic suppression of amyloid assembly. (Nguyen et al. 2015) linked reduced CR affinity to interference with curli-associated pathways.

Reduced ThT fluorescence in treated biofilms indicated impaired β -sheet fibrillogenesis. Quantitative measurements of ThT-amyloid fluorescence were performed by using %Area and IntDen. Differential trends observed in these analyses offered an important mechanistic insight.

While %Area gives the extent of the spatial distribution of the curli, IntDen accounts for the cumulative fluorescence intensity of the total amyloid content and hence may offer a sensitivity of revealing subtle biological changes. In this case, the later resolved statistical differences between all treatment groups, which the %Area could not reveal. Absence of statistical significance between the AgNPs and BBR group in %Area meant that both of these treatments reduced the surface coverage of curli structures. However, significant separation in IntDen indicated that Mp-AgNPs exert stronger effect on overall curli density and packing in this uropathogenic biofilm, as compared to BBR. This distinction marks the importance of inclusion of both spatial and intensity-based metrics in assessing the biofilm inhibition comprehensively. Nevertheless, both metrics observed the similar trend of amyloid inhibition activity i.e. nanocomplex > Mp-AgNPs > BBR, highlighting the enhanced efficacy by BBR functionalization of AgNPs. In the present study, the magnitude of curli reduction with the nanocomplex surpassed that of BBR and AgNPs alone, demonstrating enhanced amyloid-targeted activity of the Mp-AgNP@BBR. This enhanced efficacy likely arises from Mp-AgNP-mediated improvements in BBR solubility and bioavailability, coupling the nanoparticle biofilm penetration and BBR's targeted modulation of amyloid pathways. Although, curli-dependent biofilm formation in *Enterobacter* spp. has been reported previously, this study provides the first ThT fluorescence-based experimental evidence of curli rich amyloid matrix in uropathogenic *E. hormaechei* and its modulation by BBR and its Mp-AgNP@BBR nanocomplex.

QS analysis corroborated the targeted biofilm architectural findings of this study. The significant reduction in AHL levels without affecting the growth of *E. hormaechei* indicates communication (signaling) interference rather than cytotoxicity, aligning with the QS regulatory mechanisms described by (Aswathanarayan and Vittal 2018). AHL-mediated QS coordinates biofilm development in *Enterobacter* species (Rodríguez-Urretavizcaya et al. 2024), so signal attenuation suppresses collective EPS

production and maturation. The antivirulence pattern observed in present investigation paralleled the findings of (Tahan et al. 2024b), where nanomaterial systems disrupted QS without exerting lethal pressure, thereby reducing resistance development risk.

Curli gene cluster (*csgA*, *csgB*, *csgD*) and curli like functional amyloids have been reported in the *Enterobacter cloacae* complex, including *E. hormaechei* isolated, where they have been shown to form biofilms on abiotic surfaces and clinical devices. Such genomic and phenotypic evidence supports the plausibility of curli-rich amyloid networks in *E. hormaechei* biofilms and provides the mechanistic rationale for docking BBR and its nanocomplex with AgNP against CsgA like subunits in this study. Molecular docking investigations revealed complementary inhibition strategies rather than simple affinity competition of antibiofilm agents studied here with the Curli subunits. That is, binding energy alone does not fully explain the enhanced antibiofilm performance observed experimentally for BBR-Ag8 surface functionalized nanosystem. BBR docking demonstrated superior favorable binding energies toward both CsgA (-6.20 kcal/mol) and CsgB (6.90 kcal/mol) with interaction profile primarily dominated by hydrophobic contacts and π - π stacking, which favor aromatic surface accommodation within amyloidogenic domains. This reflects capacity of BBR to securely obstruct fibril elongation ends at amyloid steric zipper interfaces formed by cross- β spine segments that govern monomer addition to growing fibrils. This elongation blockade mechanism can be directly correlated with the 70.22% curli inhibition (%Area reduction observed in ThT analyses).

Conversely, the BBR-Ag8 nanocomplex, despite marginally lower negative docking energies, exhibited superior functional performance (94.20% reduced curli coverage), owing to expanded interaction diversity. The nanocomplex formed 6 distinct interaction types versus BBR's 3 interaction types for CsgA, and introduced a unique Ag metal-acceptor coordination and π -cation interactions with key residues (CsgA: Arg64; CsgB: Tyr31). The introduction of directional hydrogen bonding and metal

coordination suggests a shift toward structurally disruptive engagement rather than passive surface stabilization. Metal-mediated interactions may alter the local electronic environment of key residues, potentially destabilizing intermolecular stacking interfaces essential for nucleation of amyloid fibers. This multivalent surface engagement mirrors transthyretin inhibition studies where position of amino acid residue A119, rather than binding affinity, determined anti-amyloid efficacy, demonstrating that binding position and interaction diversity supersede binding affinities (Jain et al. 2017)

This observed molecular docking discordance disclose a key principle of curli inhibition: preventing template formation proves more effective than slowing fibril growth. Tight binding at elongation surfaces restricts fibre extension, while broad nucleation interface coverage eliminates the initial oligomeric seeds entirely – representing an 'upstream blockade' that achieves near-complete curli suppression as observed in this study. The expanded epitope engagement seen in case of BBR-Ag8 likely stabilizes these transient nucleation sites in non-productive conformations, preventing the β -arcade transitions essential for amyloid propagation.

Importantly, amyloid inhibition is a kinetic interference process rather than a simple thermodynamic binding event; therefore, interaction multiplicity and binding persistence at aggregation-prone regions may be more critical than absolute docking scores. This mechanistic distinction aligns with the stronger inhibition observed in CRCBB and ThT assays for the Mp-AgNP@BBR nanosystem. Furthermore, the intrinsic limitations of BBR, particularly its poor aqueous solubility and low bioavailability, can restrict effective local concentration and sustained interaction within the biofilm matrix. A8g association likely enhances dispersibility, surface retention, and microenvironmental stability, thereby improving functional interference with curli assembly. Collectively, these findings support a mechanistic shift from affinity-dominated binding to multi-modal structural

perturbation, explaining the superior anti-curli and antibiofilm efficacy of the Ag-surface functionalized formulation.

Collectively, the data establishes a coherent mechanistic continuum in which controlled surface functionalization preserves nanoparticle crystallinity while enhancing colloidal stability; improved physicochemical properties enable effective biological interface engagement; synergistic nanoparticle phytochemical integration disrupts quorum sensing, EPS biosynthesis, cell surface hydrophobicity, and curli amyloid assembly; and docking analysis mechanistically validates targeted interference with fibrillogenic proteins. The Mp-AgNP@BBR nanocomplex thus operates as a rationally engineered anti-amyloid antibiofilm system, achieving superior efficacy through coordinated physicochemical stabilization and multi-target biological interference.

5. Conclusion

Biofilm persistence driven by QS and functional amyloids represents a fundamental barrier to treating multidrug-resistant infections. UTIs being one of the global diseases presents a great health concerns with emerging bacteria like *E. hormaechei*, has been shown to be effectively inhibited by Mp-AgNP@BBR in this study.

Through integrated biofilm matrices, and ThT fluorescence quantification of curli inhibition, and molecular docking of CsgA/CsgB curli segment interactions, this study establishes Mp-AgNP@BBR nanocomplexes as a rationally engineered platform that possess therapeutic antibiofilm potential. By preserving nanoparticle crystallinity while enabling superior QS suppression, EPS matrix disruption, cell surface adhesion impairment, and amyloid blockade, the nanosystem achieves synergistic multi-target interference against MDR *E. hormaechei* UTI biofilms.

This work provides following novel outcomes: first experimental validation of BBR-curli interactions via direct ThT fluorescence quantification, strongly corroborated by docking analysis; mechanistic

resolution of the docking outcome paradox that demonstrated ‘nucleation’ blockade is superior than ‘elongation’ inhibition of curli β -sheets in curli-mediated biofilms; and proof-of-principle for nanoparticle-mediated repurposing of phytochemical that shifts BBR from moderate QS inhibitor to potent anti-amyloid agent. These findings reveal that therapeutic efficacy depends directly to, and critically on intervention hierarchy within biofilm developmental cascades, establishing a new paradigm for combating persistent, quorum-regulated infections.

CRedit authorship contribution statement

S.R.M.: Experimental and computational work, Writing original draft, Methodology, Investigation, Formal analysis, Data curation. S.H.B.: Molecules preparation, In-Silico studies Supervision, Manuscript editing. S.P.: Analysis Manuscript writing, Methodology. S.B.J.: Manuscript – Writing, review & editing, Data analysis, Validation, Supervision, Project administration, Conceptualization.

Declaration of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration about generative AI & AI tools

Authors declare that they have NOT used any Generative AI & AI tools to create or alter images of this manuscript.

Ethical declaration

Uropathogenic strains used in this study were obtained as anonymized clinical isolates from urine samples processed in a diagnostic laboratory. No identifiable patient information was received and no direct human

participation was occurred. The Institutional ethical approval is not applicable in this case.

Funding Declaration

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Data Availability Declaration

All the data generated during this study is are included in this article and its supplementary information files. Further queries can be addressed to the Corresponding author.

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References

1. Alharbi NS, Alsubhi NS, Felimban AI (2022) Green synthesis of silver nanoparticles using medicinal plants: Characterization and application. *J Radiat Res Appl Sci* 15:109-124. <https://doi.org/10.1016/j.jrras.2022.06.012>
2. Aswathanarayan JB, Vittal RR (2018) Inhibition of biofilm formation and quorum sensing mediated phenotypes by berberine in: *Pseudomonas aeruginosa* and *Salmonella typhimurium*. *RSC Adv* 8:36133-36141. <https://doi.org/10.1039/c8ra06413j>
3. Baidoo I, Sarbadhikary P, Abrahamse H, George BP (2025) Metal-based nanoplatforms for enhancing the biomedical applications of berberine: current progress and future directions. *Nanomedicine* 20:851-868
4. Barage S, Kulkarni A, Pal JK, Joshi M (2017) Unravelling the structural interactions between PKR kinase domain and its small molecule inhibitors using computational approaches. *J Mol Graph Model* 75:322-329. <https://doi.org/10.1016/j.jmgm.2017.06.009>
5. Barnhart MM, Chapman MR (2006) Curli biogenesis and function. *Annu. Rev. Microbiol.* 60:131-147
6. Bhanumathi R, Manivannan M, Thangaraj R, Kannan S (2018) Drug-Carrying Capacity and Anticancer Effect of the Folic Acid- and Berberine-Loaded Silver Nanomaterial to Regulate the AKT-ERK Pathway in Breast Cancer. *ACS Omega* 3:8317-8328. <https://doi.org/10.1021/acsomega.7b01347>
7. Bhanumathi R, Vimala K, Shanthi K, et al (2017) Bioformulation of silver nanoparticles as berberine carrier cum anticancer agent against breast cancer. *New Journal of Chemistry* 41:14466-14477. <https://doi.org/10.1039/c7nj02531a>
8. Bhattacharjee S (2016) DLS and zeta potential - What they are and what they are not? *Journal of Controlled Release* 235:337-351
9. Cieřlik M, Wójcicki M, Migdał P, et al (2025) Fighting biofilm: bacteriophages eliminate biofilm formed by multidrug-resistant *Enterobacter hormaechei* on urological catheters. *Med Microbiol Immunol* 214:. <https://doi.org/10.1007/s00430-025-00844-0>
10. De R, Barik S, Hossain ST, Mukherjee SK (2026) Combinatorial antibiofilm and anti-virulence effects of silibinin-BSA nanoparticles and gentamicin against *Pseudomonas aeruginosa*: Insights into quorum sensing-mediated molecular mechanisms. *Int J Biol Macromol* 354:151398. <https://doi.org/10.1016/j.ijbiomac.2026.151398>

11. Duda-Madej A, Bazan H, Łabaz J, Viscardi S (2026) Berberine Interferes with the Molecular Landscape of Biofilm-Driven Pathogenicity. *Pathogens* 15
12. Duda-Madej A, Viscardi S, Bazan H, Sobieraj J (2025) Exploring the Role of Berberine as a Molecular Disruptor in Antimicrobial Strategies. *Pharmaceuticals* 18
13. El-Ouahmani N, Amjlef A, Yacoubi B, et al (2026) Copper nanoparticles derived from the extract of *Ononis natrix* (L.): Characterization, antibacterial, and antibiofilm activities. *Medicine* 105:e47746. <https://doi.org/10.1097/MD.00000000000047746>
14. Esnaashari F, Alidoust FA, Jafari N, et al (2026) Berberine-ZnO loaded chitosan nanoparticles inhibits biofilm formation in *Pseudomonas aeruginosa* PAO1 through targeting extracellular polymeric substances. *BMC Biotechnol* 26:. <https://doi.org/10.1186/s12896-026-01103-7>
15. Estevez MB, Raffaelli S, Mitchell SG, et al (2020) Biofilm eradication using biogenic silver nanoparticles. *Molecules* 25:. <https://doi.org/10.3390/molecules25092023>
16. Gao S, Zhang S, Zhang S (2021) Enhanced in vitro antimicrobial activity of amphotericin B with berberine against dual-species biofilms of *Candida albicans* and *Staphylococcus aureus*. *J Appl Microbiol* 130:1154–1172. <https://doi.org/10.1111/jam.14872>
17. Hassan MM, Albogami B, Mwabvu T, et al (2024) Molecular characterization of virulence genes and influence of *Xanthium strumarium* extract against two *Enterobacter* species isolated from some soil invertebrates. *Cell Mol Biol* 70:166–175. <https://doi.org/10.14715/cmb/2024.70.11.24>
18. He Y, Zhao J, Wang L, et al (2025) Epidemiological trends and predictions of urinary tract infections in the global burden of disease study 2021. *Sci Rep* 15:. <https://doi.org/10.1038/s41598-025-89240-5>
19. Huang X, Wang P, Li T, et al (2020) Self-Assemblies Based on Traditional Medicine Berberine and Cinnamic Acid for Adhesion-Induced Inhibition Multidrug-Resistant *Staphylococcus aureus*. *ACS Appl Mater Interfaces* 12:227–237. <https://doi.org/10.1021/acsami.9b17722>
20. Jain N, Ådén J, Nagamatsu K, et al (2017) Inhibition of curli assembly and *Escherichia coli* biofilm formation by the human systemic amyloid precursor transthyretin. *Proc Natl Acad Sci U S A* 114:12184–12189. <https://doi.org/10.1073/pnas.1708805114>
21. Kandaswamy K, Prasad Panda S, Subramanian R, et al (2024) Synergistic berberine chloride and Curcumin-Loaded nanofiber

- therapies against Methicillin-Resistant *Staphylococcus aureus* Infection: Augmented immune and inflammatory responses in zebrafish wound healing. *Int Immunopharmacol* 140:.. <https://doi.org/10.1016/j.intimp.2024.112856>
22. Kopal I, Smeliková V (2025) Silver-Surface-Enhanced Raman Spectra of Berberine: Analyte-Induced Surface Changes, Variable Concentration Correlation, and Excitation Wavelength Dependence. *Langmuir* 41:16725–16735. <https://doi.org/10.1021/acs.langmuir.4c05174>
 23. Kyle JJ, Sharma S, Tiarks G, et al (2023) Fast Detection and Quantification of Interictal Spikes and Seizures in a Rodent Model of Epilepsy Using an Automated Algorithm. *Bio Protoc* 13:.. <https://doi.org/10.21769/BioProtoc.4632>
 24. Laktib A, Nayme K, Fayzi L, et al (2024) Antibacterial and antibiofilm activities of *Thymus leptobotrys* and *Lavandula mairei* essential oils against extended spectrum β -lactamase producing Enterobacteriaceae. *Journal of Essential Oil-Bearing Plants* 27:356–373. <https://doi.org/10.1080/0972060X.2024.2325101>
 25. Li T, Wang P, Guo W, et al (2019) Natural Berberine-Based Chinese Herb Medicine Assembled Nanostructures with Modified Antibacterial Application. *ACS Nano* 13:6770–6781. <https://doi.org/10.1021/acsnano.9b01346>
 26. Lila ASA, Rajab AAH, Abdallah MH, et al (2023) Biofilm Lifestyle in Recurrent Urinary Tract Infections. . 13
 27. Lin Q, Li Y, Sheng M, et al (2023) Antibiofilm effects of berberine-loaded chitosan nanoparticles against *Candida albicans* biofilm. *LWT* 173:.. <https://doi.org/10.1016/j.lwt.2022.114237>
 28. Liu S, Chen L, Wang L, et al (2022) Cluster Differences in Antibiotic Resistance, Biofilm Formation, Mobility, and Virulence of Clinical *Enterobacter cloacae* Complex. *Front Microbiol* 13:.. <https://doi.org/10.3389/fmicb.2022.814831>
 29. Lu J, Wang Z, Cai D, et al (2023) Carrier-Free Binary Self-Assembled Nanomedicines Originated from Traditional Herb Medicine with Multifunction to Accelerate MRSA-Infected Wound Healing by Antibacterial, Anti-Inflammation and Promoting Angiogenesis. *Int J Nanomedicine* 18:4885–4906. <https://doi.org/10.2147/IJN.S422944>
 30. Mangudi S, Pappu S (2025) Biologically synthesized selenium nanoparticles from cell-free supernatant of *Lysinibacillus odysseyi* with therapeutic potential. *Microb Pathog* 209:.. <https://doi.org/10.1016/j.micpath.2025.108109>
 31. Matijašević D, Malešević M, Ćurčić J, et al (2025) Inhibition of *Pseudomonas aeruginosa* quorum sensing-regulated behaviors by

- mushroom extracts. J Ethnopharmacol 353:.. <https://doi.org/10.1016/j.jep.2025.120466>
32. Mayer C, Soukarieh F, Simões M, et al (2025) AHL-based QS signalling promotes uropathogenic *Escherichia coli* settlement through the de-repression of biofilm formation by SdiA. PLoS One 20:.. <https://doi.org/10.1371/journal.pone.0328837>
 33. Mhavarkar SR, Barage SH, Jadhav K, Jadhav SB (2025) Swift-kinetics driven RSM optimized, biocompatible AgNPs inhibit uropathogenic biofilms via AHL interference: mechanistic insights from Ag8-SdiA in-silico docking
 34. Mhavarkar SR, Jadhav SB (2025) Antibiofilm potential of silver nanoparticles from *M. Philippica* mediated via reduction in AHLs activity against uropathogenic *K. pneumoniae*. South African Journal of Botany 181:537–548. <https://doi.org/10.1016/j.sajb.2025.04.040>
 35. Moreno-Chamba B, Salazar-Bermeo J, Navarro-Simarro P, et al (2023) Autoinducers modulation as a potential anti-virulence target of bacteria by phenolic compounds. Int J Antimicrob Agents 62:.. <https://doi.org/10.1016/j.ijantimicag.2023.106937>
 36. Morris GM, Ruth H, Lindstrom W, et al (2009) Software news and updates AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem 30:2785–2791. <https://doi.org/10.1002/jcc.21256>
 37. Nguyen HT, Pham TN, Le AT, et al (2022) Antibacterial activity of a berberine nanoformulation. Beilstein Journal of Nanotechnology 13:641–652. <https://doi.org/10.3762/bjnano.13.56>
 38. Nguyen Y, Nguyen NX, Rogers JL, et al (2015) Structural and mechanistic roles of novel chemical ligands on the SdiA quorum-sensing transcription regulator. mBio 6:.. <https://doi.org/10.1128/mBio.02429-14>
 39. Ning Y, Wang X, Chen P, et al (2022) Targeted inhibition of methicillin-resistant *Staphylococcus aureus* biofilm formation by a graphene oxide-loaded aptamer/berberine bifunctional complex. Drug Deliv 29:1675–1683. <https://doi.org/10.1080/10717544.2022.2079768>
 40. Perov S, Lidor O, Salinas N, et al (2019) Structural insights into curli CsgA cross- β fibril architecture inspire repurposing of anti-amyloid compounds as anti-biofilm agents. PLoS Pathog 15:.. <https://doi.org/10.1371/journal.ppat.1007978>
 41. Piri M, Mahdavi AH, Hajian M, et al (2024) Effects of nano-berberine and berberine loaded on green synthesized selenium

- nanoparticles on cryopreservation and in vitro fertilization of goat sperm. *Sci Rep* 14:.. <https://doi.org/10.1038/s41598-024-75792-5>
42. Rainey K, Michalek SM, Wen ZT, Wu H (2018) Glycosyltransferase-mediated biofilm matrix dynamics and virulence of *Streptococcus mutans*. *Appl Environ Microbiol* 85:.. <https://doi.org/10.1128/AEM.02247-18>
 43. Rajan PP, Kumar P, Mini M, et al (2023) Antibiofilm potential of gallic acid against *Klebsiella pneumoniae* and *Enterobacter hormaechei*: in-vitro and in-silico analysis. *Biofouling* 39:948–961. <https://doi.org/10.1080/08927014.2023.2279996>
 44. Ribeiro AI, Vieira B, Dantas D, et al (2023) Synergistic Antimicrobial Activity of Silver Nanoparticles with an Emergent Class of Azoimidazoles. *Pharmaceutics* 15:.. <https://doi.org/10.3390/pharmaceutics15030926>
 45. Rodríguez-Urretavizcaya B, Vilaplana L, Marco MP (2024) Strategies for Quorum Sensing inhibition as a tool for controlling *Pseudomonas aeruginosa* infections. *Int. J. Antimicrob. Agents* 64
 46. Sadeghi S, Agharazi F, Hosseinzadeh SA, et al (2024) Gold nanoparticle conjugation enhances berberine's antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA). *Talanta* 268:.. <https://doi.org/10.1016/j.talanta.2023.125358>
 47. Scharf B, Schmidt TJ, Rabbani S, et al (2020) Antiadhesive natural products against uropathogenic *E. coli*: What can we learn from cranberry extract? *J Ethnopharmacol* 257:.. <https://doi.org/10.1016/j.jep.2020.112889>
 48. Shakibaie MR, Taghadosi R, Masoumi S (2015) Biochemical detection of N-Acyl homoserine lactone from biofilm-forming uropathogenic *Escherichia coli* isolated from urinary tract infection samples
 49. Shrestha A, Shringi S, Shah DH (2025) Rapid serotype-independent differential detection of biofilm-positive and biofilm-negative *Salmonella* using Fourier transform infrared biotyping. *One Health* 20:.. <https://doi.org/10.1016/j.onehlt.2025.101004>
 50. Singh G, Dizaji HB, Puttuswamy H, Sharma S (2022) Biogenic Nanosilica Synthesis Employing Agro-Waste Rice Straw and Its Application Study in Photocatalytic Degradation of Cationic Dye. *Sustainability* (Switzerland) 14:.. <https://doi.org/10.3390/su14010539>
 51. Singh S, Singh AK, Yadav VB, et al (2025) In vitro efficacy of bacteriophage and colistin combinations in eradicating biofilm formed by colistin-resistant *Enterobacter cloacae* complex.

- Microbe (Netherlands) 8:.
<https://doi.org/10.1016/j.microb.2025.100533>
52. Siri M, Mangiarotti A, Vázquez-Dávila M, Bidan CM (2024) Curli Amyloid Fibers in *Escherichia coli* Biofilms: The Influence of Water Availability on their Structure and Functional Properties. *Macromol Biosci* 24:.
<https://doi.org/10.1002/mabi.202300234>
 53. Srinivasan R, Vigneshwari L, Rajavel T, et al (2018) Biogenic synthesis of silver nanoparticles using *Piper betle* aqueous extract and evaluation of its anti-quorum sensing and antibiofilm potential against uropathogens with cytotoxic effects: an in vitro and in vivo approach. *Environmental Science and Pollution Research* 25:10538–10554. <https://doi.org/10.1007/s11356-017-1049-0>
 54. Tahan M, Zeraatkar S, Neshani A, et al (2024a) Antibacterial Potential of Biosynthesized Silver Nanoparticles Using Berberine Extract Against Multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. *Indian J Microbiol* 64:125–132. <https://doi.org/10.1007/s12088-023-01136-y>
 55. Tahan M, Zeraatkar S, Neshani A, et al (2024b) Antibacterial Potential of Biosynthesized Silver Nanoparticles Using Berberine Extract Against Multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. *Indian J Microbiol* 64:125–132. <https://doi.org/10.1007/s12088-023-01136-y>
 56. Tan J, Wang J, Yang C, et al (2019) Antimicrobial characteristics of Berberine against prosthetic joint infection-related *Staphylococcus aureus* of different multi-locus sequence types. *BMC Complement Altern Med* 19:.
<https://doi.org/10.1186/s12906-019-2558-9>
 57. Babu G, Vaikkathillam P, Rajan PP, Jayakumar D, Prabhu RR, Kumar P. Exploring the Antibiofilm Potential of Naringin Against *Enterobacter hormaechei*: Experimental and Computational Insights. *APMIS*. 2025 Dec;133(12):e70124.
 58. Trott O, Olson AJ (2010) AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* 31:455–461. <https://doi.org/10.1002/jcc.21334>
 59. Tursi SA, Tükel Ç (2018) Curli-Containing Enteric Biofilms Inside and Out: Matrix Composition, Immune Recognition, and Disease Implications. <https://doi.org/10.1128/MMBR>
 60. Vaikkathillam P, Mini M, Mohan A, et al (2025) Anti-biofilm effect of ferulic acid against *Enterobacter hormaechei* and *Klebsiella pneumoniae*: in vitro and in silico investigation. *Biofouling* 41:157–170.
<https://doi.org/10.1080/08927014.2024.2446927>

61. Wu Y, Huang X, Yang M, et al (2018) Ameliorative effect of berberine coated bio-active nanoparticles in acetaminophen induced hepato-renal damage in diabetic rats. *J Photochem Photobiol B* 189:250-257. <https://doi.org/10.1016/j.jphotobiol.2018.10.015>
62. Wypij M, Czarnecka J, Świecimska M, et al (2018) Synthesis, characterization and evaluation of antimicrobial and cytotoxic activities of biogenic silver nanoparticles synthesized from *Streptomyces xinghaiensis* OF1 strain. *World J Microbiol Biotechnol* 34:. <https://doi.org/10.1007/s11274-017-2406-3>
63. Xia S, Ma L, Wang G, et al (2022) In vitro Antimicrobial Activity and the Mechanism of Berberine Against Methicillin-Resistant *Staphylococcus aureus* Isolated from Bloodstream Infection Patients. *Infect Drug Resist* 15:1933-1944. <https://doi.org/10.2147/IDR.S357077>
64. Xie J, Meng Z, Han X, et al (2022) Cholesterol Microdomain Enhances the Biofilm Eradication of Antibiotic Liposomes. *Adv Healthc Mater* 11:. <https://doi.org/10.1002/adhm.202101745>
65. Xie Y, Liu X, Zhou P (2020) In vitro antifungal effects of berberine against candida spp. In planktonic and biofilm conditions. *Drug Des Devel Ther* 14:87-101. <https://doi.org/10.2147/DDDT.S230857>
66. Yang X, Wang Y, Li L, et al (2025a) Berberine and its nanoformulations and extracts: potential strategies and future perspectives against multi-drug resistant bacterial infections. *Front Microbiol* 16:. <https://doi.org/10.3389/fmicb.2025.1643409>
67. Yang Y, Wang H, Xue Q, et al (2025b) New advances of natural products in non-small cell lung cancer: From mechanisms to therapies. *J. Ethnopharmacol.* 346
68. Yeh TK, Lin HJ, Liu PY, et al (2022) Antibiotic resistance in *Enterobacter hormaechei*. *Int. J. Antimicrob. Agents* 60
69. Zhang C, Li Z, Pan Q, et al (2022) Berberine at sub-inhibitory concentration inhibits biofilm dispersal in *Staphylococcus aureus*. *Microbiology (United Kingdom)* 168:. <https://doi.org/10.1099/mic.0.001243>
70. Imenshahidi M, Hosseinzadeh H. Berberine and barberry (*Berberis vulgaris*): a clinical review. *Phytotherapy Research.* 2019 Mar;33(3):504-23.
71. Neag et_2018_Berberine- Botanical Occurrence, Traditional uses, Extraction Methods and Relevance in Neag MA, Mocan A, Echeverría J, Pop RM, Bocsan CI, Crişan G, Buzoianu AD. Berberine: Botanical occurrence, traditional uses, extraction

- methods, and relevance in cardiovascular, metabolic, hepatic, and renal disorders. *Frontiers in pharmacology*. 2018 Aug 21;9:557.
72. Abd El-Hamid MI, Ibrahim D, Elazab ST, Gad WM, Shalaby M, El-Neshwy WM, Alshahrani MA, Saif A, Algendy RM, AlHarbi M, Saleh FM. Tackling strong biofilm and multi-virulent vancomycin-resistant *Staphylococcus aureus* via natural alkaloid-based porous nanoparticles: perspective towards near future eradication. *Frontiers in Cellular and Infection Microbiology*. 2024 Jan 12;13:1287426.
73. Yi Y, Zhou Y, Lin S, Shi K, Mei J, Ying G, Wu S. Screening and isolation of quorum sensing inhibitors of *Pseudomonas aeruginosa* from *Phellodendron amurense* extracts using bio-affinity chromatography. *Journal of Separation Science*. 2024 Aug;47(15):2400222.
74. Yang X, Wang Y, Li L, Tang D, Yan Z, Li M, Jiang J, Bi D. Berberine and its nanoformulations and extracts: potential strategies and future perspectives against multi-drug resistant bacterial infections. *Frontiers in Microbiology*. 2025 Sep 2;16:1643409.

Figure caption

Fig. 1. UV-Vis spectra of BBR, Mp-AgNPs, and Mp-AgNP@BBR nanocomplex recorded from 200-800 nm.

Fig. 2. (A) Comparative FTIR spectra of BBR and Mp-AgNPs@BBR showing characteristic functional group regions (4000–500 cm^{-1}). (B) Structural characterization of Mp-AgNPs@BBR by powder X-ray diffraction (XRD).

Fig. 3. (A) Dynamic light scattering (DLS) analysis showing the number-based hydrodynamic size distribution of the Mp-AgNP@BBR (B) Surface charge distribution of the Mp-AgNP@BBR obtained by zeta potential analysis. (C) Electrophoretic mobility distribution profile of the Mp-AgNP@BBR.

Fig. 4. Particle size and concentration distribution of the Mp-AgNP@BBR determined by NTA.

Fig. 5. Effect of BBR, Mp-AgNPs, and Mp-AgNP@BBR at sub-MIC concentrations on biofilm biomass quantified by crystal violet staining. Values represent mean $\pm$ SD (n=3); (*) indicates statistically significant at ($p \leq 0.05$).

Fig. 6. Effect of BBR, Mp-AgNPs, and Mp-AgNP@BBR on EPS (OD_{470}), protein, and carbohydrate content of biofilms. EPS content was quantified at 470 nm and is represented on the primary Y-axis, while protein and carbohydrate concentrations ($\mu\text{g/mL}$) are shown on the secondary Y-axis. Data are expressed as mean $\pm$ SD, and * indicates statistical significance ($p \leq 0.05$) compared to control.

Fig.7. CR-CBB agar assay showing curli-associated colony morphology: (A) untreated control, (B) BBR-treated, and (C) Mp-AgNPs treated, (D) Mp-AgNP@BBR-treated groups.

Fig.8. ThT fluorescence reveals superior anti-biofilm efficacy of Mp-AgNP@BBR nanocomplex. (A-D) Representative fluorescence micrographs (ThT; green fluorescence). Treatments: (A) Control, (B) BBR, (C) Mp-AgNPs, (D) Mp-AgNP@BBR. Scale bar, 20 μ m.

(E) Violin plot of relative curli coverage (%Area, n=3 biological replicates, ≥ 200 fields/biofilm). Tukey HSD post-hoc: ***p<0.001 vs Control; **p<0.01 AgNPs vs Nanocomplex. (F) Integrated ThT fluorescence intensity ($\times 10^6$ a. u.). Tukey HSD: ***p<0.001 all pairwise comparisons (n=3 biological replicates, ≥ 200 fields/biofilm).

Fig. 9. Bacterial cell surface hydrophobicity determined by the microbial adhesion to hydrocarbons (MATH) assay and expressed as percentage hydrophobicity index following treatment with BBR, Mp-AgNPs, and Mp-AgNP@BBR. Value represent mean $\pm$ SD (n=3); (*) indicates statistically significant at (p $\leq$ 0.05).

Fig. 10. Time-dependent quantification of acyl-homoserine lactone (AHL) production in control and treated cultures, monitored spectrophotometrically at 520 nm alongside bacterial growth (OD₆₀₀).

Fig.11. (A)Molecular docking analysis of BBR with CsgA. (a) Three-dimensional binding conformation of BBR within the CsgA structure. (b) Enlarged view of the binding pocket highlighting ligand orientation and intermolecular interactions. (c) Two-dimensional interaction map depicting key amino acid residues involved in ligand stabilization. (B)Structural docking model of the BBR-Mp-AgNP in complex with CsgA. (a) Three-dimensional ribbon model showing conjugate positioning along the β -sheet

architecture of CsgA. (b) Close-up of the interaction region illustrating spatial complementarity within the binding groove. (c) Residue-level interaction diagram highlighting non-covalent contacts stabilizing the complex.

Fig.12. (A) In silico interaction profile of BBR with CsgB. (a) Overall docked complex showing BBR accommodated within the CsgB structural framework. (b) Close-up representation of the ligand-protein interface indicating spatial orientation and contact regions. (c) Schematic 2D interaction diagram depicting hydrogen bonding and hydrophobic interactions with surrounding residues. (B) Docked assembly of CsgB with the BBR-Mp-AgNP nanocomplex obtained from molecular modeling. (a) Surface representation highlighting the ligand-occupied region. (b) Sectional view of the contact zone illustrating depth of insertion. (c) Contact distribution diagram mapping interacting residues surrounding the conjugate.

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