

Biosynthesis of Chromium Oxide Nanoparticles Using *Klebsiella variicola* and Antioxidant Activities Against Multidrug-Resistant *Burkholderia cepacia* Isolated from Burn Infections

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Abstract: Antibiotic resistant bacteria are increasingly associated with burn wound infections and represent a critical threat to patient survival and treatment outcomes. This study investigated the biomedical activity of green synthesized chromic oxide (Cr_2O_3) nanoparticles against multidrug-resistant (MDR) *Burkholderia cepacia* isolates recovered from burn patients. *Burkholderia cepacia* is a Gram-negative bacterium usually found in various environments, particularly in hospitals. It can lead to severe infections in immunocompromised patients, such as those with severe burns. This bacterium thrives in harsh conditions and is resistant to many antibiotics, making treatment challenging. **Methods:** Fifty burn wound specimens were collected from the Al-Najaf Health Directorate, , and the Burn Center at Al-Sadr Teaching Hospital, yielding forty bacterial isolates (80% isolation rate), among which *B. cepacia* accounted for 10%. Biogenic Cr_2O_3 nanoparticles were produced using cell free supernatants of *klebsella variicola* (R9), a Gram-positive, spore-forming bacterium, efficiently biosynthesized Cr_2O_3 nanoparticles from burn isolates, demonstrating its biotechnological potential for antimicrobial applications and extensively characterized by UV–Vis, FESEM, XRD, and AFM. **Results :** The nanoparticles exhibited concentration against *B. cepacia*, significant DPPH radical scavenging capacity, and no detectable hemolytic effect on whole blood. **Conclusion:** These findings support the potential of green Cr_2O_3 nanoparticles as a supplementary or alternative strategy to conventional antibiotics for the control of MDR *B. cepacia* in burn infections. *k.variicola* are officially registered on the NCBI (National centre for Biotechnology Information) website with the following sequences: *K. variicola* (PX963638.1)

Keywords: Green chromic oxide nanoparticles (Cr_2O_3 NPs), synthesis of nanoparticles, Burn wound infections, DPPH inhibition.

1. Introduction

Burn injuries are a major global health problem caused by exposure to heat, chemicals, radiation, or electricity. These injuries damage the skin barrier and weaken immune responses, increasing susceptibility to microbial infections. Such infections are among the most serious

complications of burns, raising morbidity and mortality rates and increasing the risk of sepsis [1].

The burn wound environment, particularly the presence of exudates, promotes microbial growth and colonization. Among these pathogens, *Burkholderia cepacia* is an important opportunistic bacterium associated with severe infections and sepsis. Although specific mortality rates are not well defined, it is

considered a significant contributor to infection-related deaths, especially in the context of antimicrobial resistance [2-4].

Bloodstream infections caused by *B. cepacia* have a fatality rate of around 25% in high-income countries. Overall, antimicrobial resistance remains a major global health threat, causing over 700,000 deaths annually, with projections reaching up to 10 million deaths per year by 2050 [5,6].

In this context, chromium oxide nanoparticles (Cr_2O_3 NPs) have emerged as a promising approach for combating microbial infections. They exhibit strong antibacterial activity due to their high surface area and their ability to generate reactive oxygen species (ROS), which damage bacterial cell walls and disrupt vital cellular functions. Additionally, they may serve as an alternative or adjunct to conventional antibiotics, particularly against resistant strains, highlighting their potential role in the treatment of burn infections [7].

2. Methodology

Clinical sampling and bacterial isolation

Fifty specimens were collected from patients with Different degrees burn infections during September 2025 from Al-Sadr Teaching Hospital, and the Burn Center, and Al-Najaf Health Directorate. Swabs were obtained under aseptic conditions from clinically infected burn sites and transported immediately to the microbiology laboratory. Specimens were cultured on appropriate selective media such as MacConkey agar and nonselective media such as brain heart infusion broth (BHI) medium and incubated under standard conditions. Bacterial isolates were identified by colony morphology, Gram staining, and routine biochemical tests including catalase test, oxidase test, indole test, citrate utilization test, urease test, triple sugar iron (TSI) test, and motility test.

Advanced identification was performed using an automated system (VITEK2) and confirmed by molecular methods.

Screening of bacterial isolates for biogenic Cr_2O_3 synthesis

Ten bacterial isolates (R1–R10), obtained from various sources, were screened for their ability to biosynthesize Cr_2O_3 nanoparticles. Each isolate was grown in brain heart infusion (BHI) broth medium, and the cell free supernatant was collected by centrifugation and filtration. A chromium precursor solution (0.2 M) was added to the supernatant and incubated at 37 °C for 24 h in a shaking incubator. Formation of a greenish precipitate was used as the initial indicator of nanoparticle synthesis. Isolates were further evaluated for antibacterial activity of the resulting reaction mixtures against *B. cepacia*.

Based on the intensity of precipitate formation and inhibitory effect against *B. cepacia*, one isolate, labelled R9, were selected as the most efficient producers. Detailed phenotypic characterization, microscopic examination, VITEK2 identification, and molecular analysis identified R9 as *klabsella varicella*.

Biogenic synthesis of Cr_2O_3 nanoparticles

For preparative synthesis, *klebsiella variicola* (R9) was cultured in HBI broth medium under optimized conditions. Cell free supernatants were obtained by centrifugation and filtration to remove bacterial cells. A 0.2 M chromium oxide precursor solution was added to each supernatant and incubated at 37 °C for 24 h with shaking. Visible color change and precipitate formation indicated the biosynthesis of Cr_2O_3 nanoparticles. The precipitates were collected by centrifugation, washed several times with distilled water and ethanol to remove unbound biomolecules, and dried [8].

Physicochemical characterization of Cr_2O_3 nanoparticles

Biogenic Cr_2O_3 nanoparticles were characterized using several analyses, including UV–visible spectrophotometry, FESEM, XRD, and AFM. to determine optical properties, morphology, elemental composition, crystallinity, and surface topology [9, 10].

Antioxidant activity (DPPH assay)

The antioxidant potential of biogenic Cr_2O_3 nanoparticles was determined using the DPPH free radical scavenging assay. Different concentrations (125–

1000 µg/mL) of nanoparticle suspensions were mixed with DPPH solution, incubated in the dark, and the decrease in absorbance was measured using a microplate ELISA reader at 514 nm. Inhibition percentages were calculated relative to a control without nanoparticles [11].

Hemolysis assay

Hemolysis is a condition in which red blood cells (RBCs) rupture, leading to the release of intracellular components, which may consequently result in anemia, jaundice, and renal dysfunction. Since any substance entering the bloodstream inevitably interacts with RBCs, evaluating hemolytic potential is essential for assessing biological safety [12].

In the present study, hemolytic activity of chromium oxide nanoparticles (Cr_2O_3 NPs) biosynthesized using *K. variicola* and *B. siamensis* was investigated. Hemolysis was determined using Triton X-100 as a positive control, while phosphate-buffered saline (PBS) served as a negative control.

Statistical analysis

All experiments were performed in triplicate, and data were expressed as mean $\pm$ standard deviation. Statistical comparisons between treated and control groups. One-way ANOVA at a fixed significance level with $p < 0.05$ considered statistically significant.

3. Results and discussion

Bacterial Isolation of burn infections

Out of fifty burn wound specimens, forty yielded positive bacterial cultures, resulting in an 80% isolation rate. *Staphylococcus aureus* represented 14% of isolates, whereas *K. pneumoniae* and *A. baumannii* each accounted for 20%. *B. cepacia* and *E. coli* represented 10% and 6% of isolates, respectively Figure 1. These findings are consistent with the recognized predominance of MDR Gram-negative organisms in burn settings and highlight *B. cepacia* as a clinically relevant pathogen.

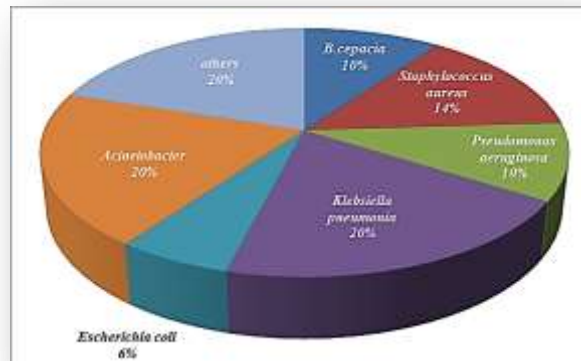


Fig 1: Bacterial percentage among clinical Specimen.

Molecular typing of selected *B. cepacia* isolates using universal PCR revealed polymorphic patterns and distinct clustering, indicating genetic heterogeneity among strains Figure 2, while still preserving signatures of relatedness within the local epidemiological context.

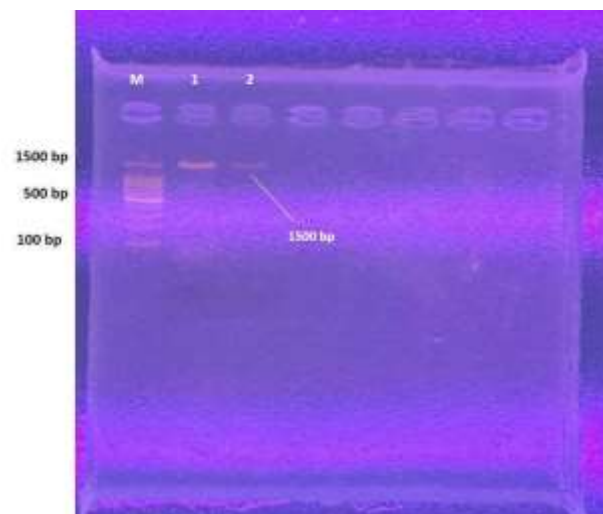


Fig 2: Gel electrophoresis genotyping of *K. variicola* 16srRNA gene sequences using the universal method (2% agarose, 80 volts). (M: MW 100-1500 bp DNA ladder).

Biogenic synthesis and characterization of Cr_2O_3 nanoparticles

Klebsiella variicola successfully mediated the formation of Cr_2O_3 nanoparticles from a 0.2M chromium precursor

in cell free supernatants Figure 3.

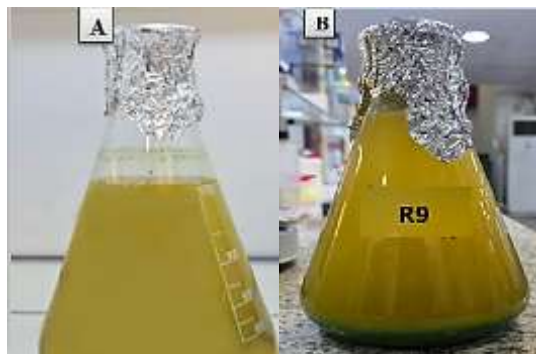


Fig 3: synthesis of Cr_2O_3 NPs in BHI broth using: A) before synthesis B) *Klebsiella variicola* at optimum conditions after addition of Cr_2O_3 .

UV-Visible spectra showed an absorption peak at 370 nm, confirming green synthesized Cr_2O_3 nanoparticle formation Figure 4.

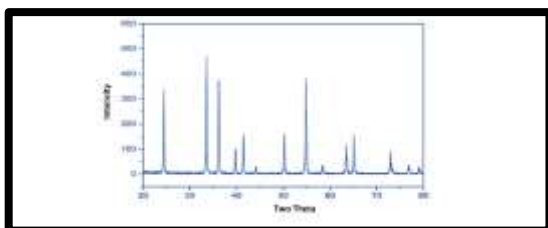


Fig 4: UV-visible spectroscopy analysis of Cr_2O_3 NPs synthesis by *Klebsiella variicola*

Field Emission Scanning Electron Microscopy (FESEM) analysis revealed was used to examine morphology and particle size. Cr_2O_3 NPs produced by *Klebsiella variicola* appeared predominantly spherical and homogeneous, with particle dimensions ranging from 54.03 to 75.72 nm Figure 5.

The FESEM analysis confirmed the successful biosynthesis of Cr_2O_3 nanoparticles with controlled morphology and nanoscale size, both of which are essential parameters affecting their physicochemical properties and potential antimicrobial activity.

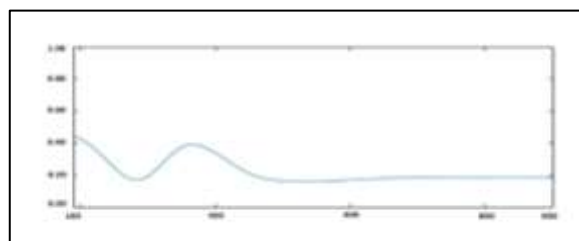


Fig 5: FESEM Micrograph of biogenic Cr_2O_3 nanoparticle synthesized by *Klebsiella variicola* showed Cr_2O_3 NPs with spherical well scattered with a size average of 74 .057nm. A: at 100 nm, B: at 500nm

X-ray diffraction (XRD): XRD patterns of both nanoparticle preparations were recorded and compared with the standard reference pattern for crystalline Cr_2O_3 . The diameter of nanoparticles it was 16 nm for *K. varricola*. The strong peak suggested the inclusion of bioorganic coupons/proteins in the nanoparticles throughout the manufacturing process (Figure 6). The diffraction peaks correlated well with the expected polymorph, indicating that the XRD data confirmed the elemental composition, crystalline structure, and presence of biomolecular capping agents.

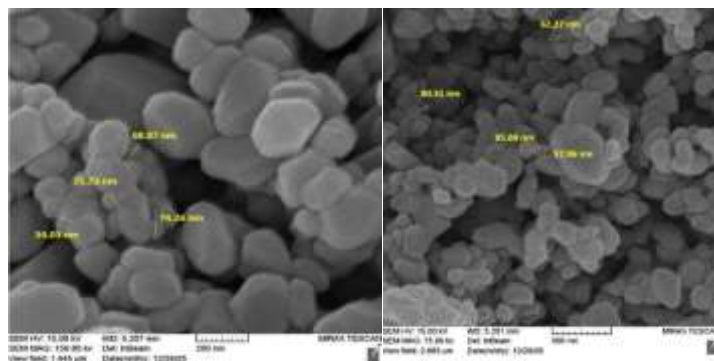


Fig 6: XRD analysis of Cr_2O_3 nanoparticles synthesized by *k.variicola* showing the structure and size.

Atomic Force Microscopy (AFM) was used to study the surface features and size of chromium oxide nanoparticles (Cr_2O_3 NPs) made by *k.variicola*. AFM provides detailed three dimensional images at a nanometer level, which helps to measure the height and roughness of nanoparticles accurately. The analysis showed that Cr_2O_3 nanoparticles from *k.varricala* were well distributed with an average diameter of about 8.1 nm. This data was supported by 3D AFM images and size distribution charts that confirmed the small size and

uniform distribution of the nanoparticles Figure 7. The AFM analyses indicated the presence of spherical and uniform nanoparticles. Variations in size may correspond to the different metabolic activities and secretion processes of the involved bacteria. AFM is recognized for its high spatial resolution and minimal sample preparation, allowing for analysis in conditions that closely resemble natural environments. These characteristics make AFM a valuable technique for characterizing biologically produced nanoparticles and their properties. In conclusion, AFM confirmed the successful production of nanoscale Cr_2O_3 nanoparticles by *k.varricola* bacterial species.

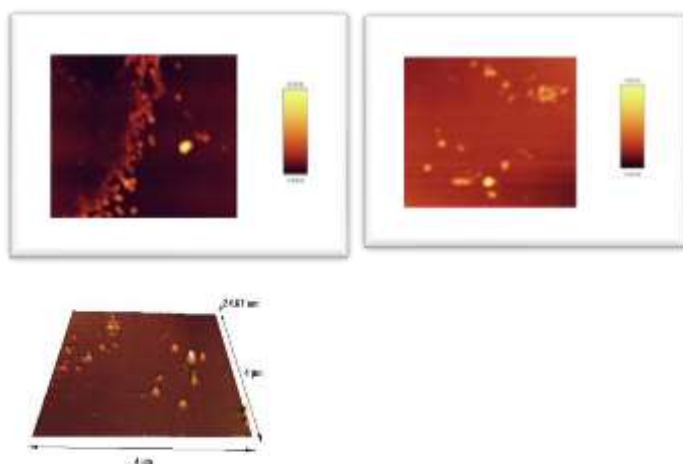


Fig 7: AFM analysis of biogenic Cr_2O_3 synthesis by *klebsiella variicola*

Antibacterial Effects of Cr_2O_3 NPs against Burkholderia cepacian

All isolated bacterial strains studied showed inhibition by chromium oxide (Cr_2O_3) nanoparticles at various concentrations (1000, 500, 250, and 125 $\mu\text{g}/\text{ml}$). In Gram-negative bacteria, the largest inhibition zone observed for Cr_2O_3 nanoparticles was 20 mm in *Burkholderia cepacia* at a concentration of 1000 $\mu\text{g}/\text{ml}$. Similarly, the antibacterial activity of biogenic Cr_2O_3 nanoparticles generated against *k. variicola* demonstrated inhibition of Gram-negative bacteria. As the concentration of Cr_2O_3 nanoparticles increased, the inhibitory effects became more pronounced, as illustrated in Figures 8 and Tables 1 [13].



Fig 8: Antibacterial activity of Cr_2O_3 NPs synthesized by *K. variicola* against *B. cepacia*. Well (1) contains 1000 $\mu\text{l}/\text{ml}$ from Cr_2O_3 , Well (2) 500 $\mu\text{l}/\text{ml}$, Well (3) 250 $\mu\text{l}/\text{ml}$, Well (4) 125 $\mu\text{l}/\text{ml}$,

Table 1: Antibacterial activity of chromium oxide nanoparticles synthesis by *K. variicola* against MDR *Burkholderia cepacia*

chromium oxide NP _s	Bacterial	Zone inhibition (mm)			
		125 μg /ml	250 μ g/ml	500 $\mu\text{g}/$ ml	1000 $\mu\text{g}/\text{ml}$
Cr_2O_3 green(<i>K. variicola</i>)	<i>Burkholderia cepacia</i>	12	16	18	20

Antioxidant activity of Cr_2O_3 NP

In the present study, the antioxidant activity of chromium oxide nanoparticles (Cr_2O_3 NPs) synthesized by *Klebsiella variicola* was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay.

The results demonstrated a clear concentration-dependent increase in antioxidant activity for types of nanoparticles. The inhibition percentage of nanoparticles synthesized by *Klebsiella variicola* showed an increase in inhibition from 38.8% to 59.1% over the same concentration range.

This gradual increase suggests that higher concentrations of nanoparticles provide more active surface sites capable of donating electrons or hydrogen atoms to neutralize DPPH free radicals, thereby enhancing the scavenging activity. These results indicate that synthesized Cr_2O_3 nanoparticles possess moderate to high antioxidant activity, making them promising candidates for medical and pharmaceutical applications. Similarly, as reported in previous studies, researchers have used selenium nanoparticles synthesized by *Klebsiella pneumoniae* [14].

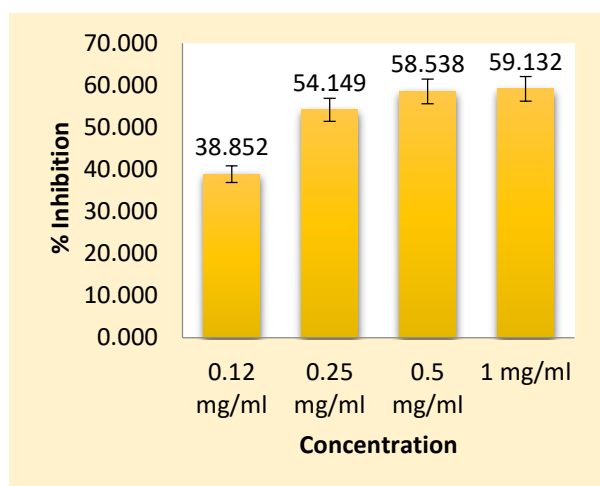


Fig 9: Antioxidant effect of Cr₂O₃ NPs synthesized by *Klebsiella variicola* in DPPH test.

Importantly, The results showed that chromium oxide nanoparticles produced by *k. variicola* did not cause hemolysis across all concentrations tested (125, 250, 500, and 1000 µg/ml) in the blood samples examined refer to Tables (2), indicating that under the conditions tested, Cr₂O₃ nanoparticles exhibit a favorable preliminary safety profile with respect to blood compatibility.

Table 2: chromium oxide NPs synthesized by *K. variicola* effect on hemolysis

Sample	Hemolysis %
Triton X-100(positive control)	100
PBS(negative control)	0
Blood with Cr₂O₃ 1000 µg/ml	0
Blood with Cr₂O₃ 500 µg/ml	0
Blood with Cr₂O₃ 500 µg/ml	0
	0

The present findings confirm that burn wounds are predominantly colonized by Gram-negative bacilli, with *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Burkholderia cepacia* representing the most clinically significant pathogens. The isolation of *B. cepacia* in 10% of cases is particularly noteworthy, as recent studies have emphasized its intrinsic multidrug resistance, and association with adverse outcomes in burn and

immunocompromised patients [15,16,17]. These observations are consistent with contemporary reports identifying *B. cepacia* complex as an emerging challenge in burn units worldwide. [18-20]

The successful green synthesis of Cr₂O₃ nanoparticles using *k. variicola* corroborates earlier work demonstrating that bacterial cell free supernatants can function as efficient biological platforms for metal oxide nanoparticle production [17, 21]. Differences observed in nanoparticle size and elemental composition between the two bacterial producers align with recent evidence that species specific metabolites and enzymatic systems critically influence nanoparticle nucleation, growth, and surface stabilization. Such tunability is increasingly recognized as advantageous for tailoring nanoparticles to targeted biomedical applications [21-24].

The concentration dependent inhibition of *B. cepacia* by biogenic Cr₂O₃ nanoparticles represents a key finding, given the pivotal role of *B. cepacia* in chronic burn wound infections and antibiotic tolerance. Previous studies have shown that metal oxide nanoparticles disrupt biofilm architecture through reactive oxygen species (ROS) generation, membrane damage, and interference with quorum sensing pathways [15,10]. The present results are in agreement with these mechanisms and suggest that green synthesized Cr₂O₃ nanoparticles may serve, either as standalone treatments or as adjuncts to conventional antibiotics [25].

In addition, the demonstrated antioxidant activity of the biogenic Cr₂O₃ nanoparticles supports a potential dual therapeutic role. Burn wounds are characterized by excessive oxidative stress, which impairs healing and exacerbates tissue damage [26-28]. The ability of Cr₂O₃ nanoparticles to scavenge free radicals may therefore contribute not only to antimicrobial efficacy but also to modulation of the local inflammatory microenvironment, as similarly reported for other biogenic metal oxide nanoparticles [18,11]. Importantly, the absence of hemolytic activity is consistent with recent hemocompatibility studies on green-synthesized nanoparticles, indicating a favorable preliminary safety profile [12,29].

Conclusions

Biological methods demonstrated that both *B. siamensis* and *K. variicola* are capable of synthesizing (Cr₂O₃)NPS. The Cr₂O₃ NPS exhibited excellent characteristics in Fourier transform infrared spectroscopy (FTIR), atomic force microscopy (AFM), X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM), and energy-dispersive X-ray spectroscopy (EDS). The research findings indicated that most of the tested extensively drug-resistant (XDR) isolates displayed multiple antibiotic resistance and were commonly isolated from clinical infections related to burns. Cr₂O₃ NPS demonstrated anti-biofilm activity against the pathogenic MDR strain *Burkholderia cepacia*.

The Cr₂O₃ NPS also exhibited antioxidant properties within an effective range, suggesting their potential to reduce free radical damage. Additionally, Cr₂O₃ NPS did not show any hemolytic effects.

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Author's Contributions

All authors had equal contribution to this research

Ethics

This study was conducted under approval by the medical ethics committee at the University of Kufa (2026). Verbal and written consent was provided by parents and agreement for publication was obtained from both participants and researchers.

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