

Biogenic Synthesis and Characterization of Copper Nanoparticles Using *Lactobacillus paracasei* and Their Potential for Diabetic Wound Healing

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ABSTRACT

Diabetes mellitus, characterized by chronic hyperglycemia, increases susceptibility to infections and impairs wound healing. Current treatment options often demonstrate limited efficacy or fail to provide a definitive cure. Nanotechnology presents a promising approach for the development of novel therapeutic materials, particularly in the field of medicine. Among various nanomaterials, copper nanoparticles have gained significant attention due to their unique physicochemical properties and potential biomedical applications. This study investigates the biogenic synthesis of CuNPs using the supernatant of *Lactobacillus paracasei* and evaluates their therapeutic potential. The biosynthesis of CuNPs through microbial-mediated processes offers an environmentally sustainable and cost-effective alternative to conventional chemical methods. Herein, CuNPs were synthesized using the supernatant of *L. paracasei*, exploiting its reducing and stabilizing properties. The synthesis was optimized using different concentrations of CuSO₄, with 0.1 M identified as the optimal concentration, as indicated by a color change from blue to dark brown or black. Characterization techniques, including UV–Vis spectroscopy, Fourier transform infrared spectroscopy, scanning electron microscopy, and X-ray diffraction, were employed to analyze the synthesized CuNPs. The UV–Vis absorption spectra exhibited a peak at 280 nm, confirming the formation of CuNPs, while SEM analysis validated their spherical morphology at the 0.1 M CuSO₄ concentration. The biosynthesized CuNPs demonstrated remarkable therapeutic

efficacy, including anticancer, antidiabetic, antimicrobial, antioxidative, and anti-inflammatory properties. Additionally, cytotoxicity assays were conducted to evaluate their potential anticancer activity against HepG2 liver cancer cell lines. These findings highlight the potential of *L. paracasei*-mediated CuNPs as promising therapeutic agents in healthcare and medicine, paving the way for future biomedical applications.

Introduction

Diabetes mellitus is a multifaceted metabolic condition characterized by persistent hyperglycemia due to defective insulin synthesis or action (Mohajan et al., 2023). It disrupts carbohydrate, fat, and protein metabolism, leading to long-term complications affecting microvascular and macrovascular systems, which may culminate in organ failure (Jose et al., 2024; Yu et al., 2024). Microvascular complications encompass nephropathy, retinopathy, and neuropathy, whereas macrovascular complications are associated with cardiovascular diseases, including coronary artery disease and stroke (Rawshani et al., 2017; Yavuz et al., 2022). The term "diabetes" was initially defined by Aretaeus of Cappadocia in the 2nd century AD, with Thomas Willis incorporating "mellitus" in the 17th century to signify the sweetness of diabetic urine (Karamanou et al., 2016). Research advancements, including Claude Bernard's work on liver glycogen metabolism and the 19th-century discovery of pancreatic involvement in diabetes by Minkowski and Mering, have significantly shaped understanding of the disease (Zajac et al., 2010; Baranowska et al., 2020). The breakthrough discovery of insulin by Banting and Best in 1921 revolutionized diabetes treatment, with biotechnology innovations such as synthetic human insulin further advancing management (Marshall et al., 2020; Riggs et al., 2021).

Globally, diabetes prevalence has surged, with an estimated 547 million individuals affected in 2022. This number is projected to rise to 644 million by 2030 and 784 million by 2045, underscoring its growing

burden (Hoogeveen et al., 2022). Pakistan, ranking among the top four countries for diabetes prevalence, anticipates an increase in diabetic cases from 4.3 million in 1995 to 14.6 million by 2025, with a significant rise in diabetic foot ulcers (Akhtar et al., 2019; Khan et al., 2024). Diabetes etiology is multifaceted, involving genetic predisposition, lifestyle choices, obesity, environmental pollutants, and infections such as rubella and Coxsackie viruses (Firdous et al., 2022; Zorena et al., 2022). Clinically, diabetes manifests through symptoms like excessive thirst, polyuria, fatigue, neuropathy, vision impairment, and slow wound healing (Mohajan & Mohajan, 2023; Kim et al., 2022). The disease primarily exists in three forms: Type 1 diabetes, Type 2 diabetes, and Gestational Diabetes Mellitus (GDM), which occurs during pregnancy and poses risks for both mother and child (Gantsgorn et al., 2023; Sweeting et al., 2022). Diabetes-induced complications span microvascular and macrovascular domains, leading to severe conditions such as diabetic retinopathy, nephropathy, neuropathy, and cardiovascular diseases (Shehzad et al., 2022; Bernardini et al., 2023). Effective diagnosis, as recommended by the World Health Organization, hinges on established glycemic thresholds, facilitating early detection and intervention (Deckers et al., 2006). As diabetes continues to escalate globally, research and advancements in medical management remain imperative to mitigate its impact. The skin microbiome plays a crucial role in wound healing, particularly in individuals with diabetes, where chronic wounds are prevalent. Research has shown that *Staphylococcus*

epidermidis, a common skin bacterium, secretes compounds that reduce edema and accelerate healing (White et al., 2023). In diabetic foot ulcers, infection occurs in over half of patients, with microbial populations influencing infection severity and progression. Deep cuts often harbor Staphylococci, while severe wounds are frequently associated with anaerobic gram-negative rods and Proteobacteria (Canchy et al., 2023). Understanding the microbial composition of chronic wound infections, including prevalent Staphylococcus and Streptococcus species in diabetics, is essential for developing effective treatment strategies (Huang et al., 2022). Wound healing is influenced by both local and systemic factors. Local factors such as temperature, infection, and oxygenation significantly impact tissue repair and inflammation (Tiwari & Pathak, 2023). Infections often lead to complications, necessitating the use of antimicrobial dressings like chitosan-based materials to promote healing (Maita et al., 2022). Adequate oxygenation is essential for cellular function and angiogenesis, thereby expediting wound repair (Bai et al., 2022). Systemic factors such as age, smoking, and obesity further affect healing capacity (Ateeq et al., 2022). Lactic acid bacteria (LAB), particularly Lactobacillus species, have demonstrated probiotic benefits in food fermentation and gut microbiota regulation (Asgher et al., 2020). The Lactobacillus casei group, comprising *L. paracasei* and *L. rhamnosus*, is widely utilized in dairy products and probiotics (Liu et al., 2023). Research has explored their taxonomic classification and probiotic potential, particularly their role in modulating microbial communities and metabolic by-products (Johansson et al., 2022; Ullah et al., 2023). The emergence of nanotechnology has significantly impacted medicine, particularly in wound healing applications. Nanoparticles (NPs), materials with sizes ranging from 1–100 nanometers, exhibit unique properties beneficial for targeted drug delivery, imaging, and antimicrobial

therapies (Gavali et al., 2023). Historically, nanotechnology was conceptualized by Richard Feynman in 1959 and further advanced by Norio Taniguchi (Bensaude & Simon, 2019). Green nanotechnology, utilizing plant extracts, algae, fungi, and bacteria for NP synthesis, offers a sustainable and eco-friendly alternative (Pandey, 2018). NPs can be synthesized through chemical, physical, and biological methods. Chemical synthesis employs metal precursors and stabilizers, while physical techniques such as ultrasonication and microwave irradiation facilitate NP production (Szczyglewska et al., 2023; Van et al., 2018). Biological synthesis, leveraging microorganisms and plant extracts, presents an environmentally benign approach (Karunakaran et al., 2023). Phytochemicals from plants and peptides from bacterial supernatants serve as reducing and stabilizing agents, yielding biocompatible NPs with biomedical applications (Guo et al., 2022; Shanmugam et al., 2023). Nanoparticles hold significant promise for wound healing due to their biocompatibility, antimicrobial properties, and ability to enhance tissue regeneration. Copper nanoparticles (CuNPs), collagen NPs, and MXene NPs have been incorporated into wound dressings to accelerate healing (Liang et al., 2023). Green-synthesized NPs, particularly those with antimicrobial properties, combat multidrug-resistant bacteria, making them valuable in treating chronic wounds and ulcers (Mendes et al., 2022; Aldakheel et al., 2023). Copper sulfate, historically used in South African traditional medicine, has demonstrated antimicrobial activity but poses toxicity risks if misused (Street et al., 2017; Mollick et al., 2011). Modern research highlights its potential in combating multidrug-resistant pathogens, underscoring the importance of controlled applications (Gamakaranage, 2018). While accidental exposure has led to burn wounds, understanding its local and systemic effects is essential for safe therapeutic use (Benhalima et al., 2019). This study

explores the interplay between microbiota, probiotics, nanotechnology, and their combined potential in enhancing wound healing processes.

Materials and Methods

Study site

This research was conducted at the Health Biotechnology Lab, Bioinformatics and Biotechnology Department, Government College University Faisalabad, Pakistan

Equipment

The majority of the equipment was provided by the Health Biotechnology Lab, Government College University Faisalabad (GCUF). The following apparatus were used for culturing retinal pigment epithelial cell lines: cell culture flasks, cell culture plates, disposable pipettes, gloves, paraffin tape, CO₂ incubator, laminar flow, inverted phase microscope, refrigerator, and centrifuge. Equipment used for bacterial culture and cell culture included pipettes, pipette tips, filter paper, vortex, Falcon tubes, ice box, test tubes, funnel, ELISA plate reader, measuring cylinder, tripod supports, weighing balance, 96-well plate, Petri plates, and streaking loop.

Chemicals

The majority of the chemicals were obtained from the Health Biotechnology Lab, Government College University Faisalabad (GCUF), with the rest being procured from other chemical manufacturers. Trypsin, fetal bovine serum, dimethyl sulfoxide, Dulbecco's Modified Eagle's Medium, and L-glutamine were utilized for the growth of HepG2 cell lines. Ethanol, nutrient agar, nutrient broth, distilled water, phosphate-buffered saline (PBS), and phosphoric acid (H₃PO₄) were used in the research.

Collection of *L. paracasei*

The *L. paracasei* culture used in this study was provided by the Microbiology Department of Government College University Faisalabad.

Bacterial Culture

L. paracasei was inoculated into an appropriate MRS medium (De Man–Rogosa–Sharpe agar) and incubated under optimal conditions (temperature, pH, and

aeration). The bacterial cultures were incubated at 37°C for 24 hours. The presence of *L. paracasei* was confirmed through biochemical assays. Following confirmation, bacterial inoculum was stored at -20°C to generate stocks for long term use.

Nanoparticle Preparation Using *L. paracasei* Supernatant

Copper oxide (CuO) nanoparticles were synthesized by dissolving 0.1 M copper sulfate (CuSO₄) in 25 ml of distilled water, which was stirred for 8 hours on a hot plate. Subsequently, 50 ml of bacterial supernatant was combined with the CuSO₄ solution and incubated overnight at room temperature. A color transition from blue to dark brown or black signified bioreduction and the formation of CuNPs. The pH was adjusted to 10.6 using sodium hydroxide (NaOH) while stirring continuously. The solution was then centrifuged at 6000 rpm for 30 minutes at room temperature, and the resultant pellet was washed with deionized water. This washing procedure was repeated 2–3 times to eliminate water-soluble impurities. The pellet was dried at 60°C for 24 hours, followed by calcination. The dry pellets were ground into fine CuNP powder for further bioactivity characterization and analysis.

Characterization of CuNPs

Ultraviolet Visible (UV-Vis) Spectrophotometer Analysis

Using UV-Vis spectrophotometry, the absorbance of CuNPs in the 200–800 nm range was measured. The reaction mixture was centrifuged at 6000 rpm, and the cell-free supernatant was analyzed using a UV-Vis spectrophotometer (Rifani et al., 2023).

Scanning Electron Microscopy (SEM)

Scanning electron microscopy was utilized to investigate the surface morphology and particle size of CuNPs. A drop of CuNP solution was dried on a hot plate at 100°C, mounted on an aluminum stub with carbon tape, and examined via SEM (Rostami et al., 2018).

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was conducted to identify functional groups involved in CuNP stabilization and reduction, using a PerkinElmer FTIR Spectrum-100 model with a resolution range of 400–4000 cm^{-1} (Mohamed & Kadium, 2022).

X-ray Diffraction (XRD) Analysis

XRD analysis was performed to determine the crystalline nature of CuNPs. The XRD instrument operated at 45 kV and 40 mA using Cu $K\alpha$ rays. The Debye-Scherrer formula was applied to calculate average particle size (Khan et al., 2017).

HepG2 Cell Lines for Cell Culture: The HepG2 cell line was maintained in DMEM enriched with 10% FBS and 1% L-glutamine. Cells were cultured in a humidified atmosphere at 37°C with 5% CO_2 . Trypsin (2–5 ml) was used to detach cells, which were then transferred to new culture flasks for further experiments.

Anticancer Activity of CuNPs (MTT Assay)

HepG2 cells were inoculated onto 96-well plates and incubated at 37°C with 5% CO_2 . After 24 hours, 2 μl of CuNPs were introduced, followed by incubation. MTT reagent was introduced and incubated for four hours. DMSO was used to solubilize formazan crystals, and absorbance was quantified at 630 nm with an ELISA plate reader (Younas et al., 2021).

Antidiabetic Activity of CuNPs (α -Glucosidase Assay)

The α -glucosidase inhibition assay was conducted in 96-well plates using PBS (negative control) and Acarbose (positive control). Various CuNP dilutions were prepared, and absorbance was measured at 405 nm after incubation (Chen et al., 2013).

Antioxidant Activity of CuNPs (DPPH Assay)

The DPPH test utilized Gallic acid as a positive control. CuNPs (10 μl) and DPPH were combined in a 96-well plate and incubated in darkness at 37°C for 30 minutes. Absorbance was measured at 490 nm (Chen et al., 2013).

Scratch Assay

A scratch experiment was performed with MCF7 cells in 12-well plates. A sterile 200 μl pipette tip was employed to produce a linear incision. Cells were subjected to various doses of CuNP and incubated for 24 hours. Images were captured at 0, 4, 8, 16, and 24 hours using an inverted microscope (Mumtaz et al., 2022).

Antimicrobial Assay

The antimicrobial activity of *L. paracasei*-mediated CuNPs was tested against *Staphylococcus aureus* and *Escherichia coli*. Bacterial cultures were cultivated on nutrient agar, and inhibition zones were quantified following incubation at 37°C. Gentamicin served as the positive control, while DMSO functioned as the negative control. The aforementioned approaches were systematically utilized to assess the synthesis, characterisation, and bioactivities of CuNPs.

Results

***Paracasei*-Mediated Biosynthesis of Cu-NPs**

Biogenic copper nanoparticles were produced utilizing the supernatant of *L. paracasei* as a bio-reductant. The selected microorganism has the potential and capability to biosynthesize Cu-NPs at a 0.1 M concentration of CuSO_4 salt. Salt concentrations lower or higher than 0.1 M were found to be ineffective for the biosynthesis of nanoparticles, as determined after repeated trials. Therefore, nanoparticle biosynthesis was carried out at 0.1 M salt concentration, mediated by the supernatant of *L. paracasei*, for the bio-reduction of copper ions into Cu-NPs. This process was indicated by a color change from blue to black after the synthesis of biogenic Cu-NPs.

Characterization of copper nanoparticles UV-Vis spectra

The bacterial supernatant and copper sulfate (CuSO_4) solution at a concentration of 0.1 M were placed at room temperature for 48 hours to ensure proper bio-reduction in the synthesis of fine-sized Cu-NPs. After incubation, a color change from dark blue to dark brown or black was observed, indicating the bio-reduction of metal ions

from the salt solution and confirming the synthesis of Cu-NPs. The solution was then subjected to centrifugation at 5000 rpm to obtain a pellet of nanoparticles. After several washings, the pellet was placed in a hot dry air oven overnight. The dried nanoparticles were collected from the bottom and the pellet was ground to make a

fine powder of Cu-NPs. The collected nanoparticles were analyzed using UV-visible spectrophotometry to confirm the absorbance range. Cu-NPs showed a maximum absorbance at 280 nm, as shown in Figure 1. The absorbance peak confirms the biosynthesis of Cu-NPs.

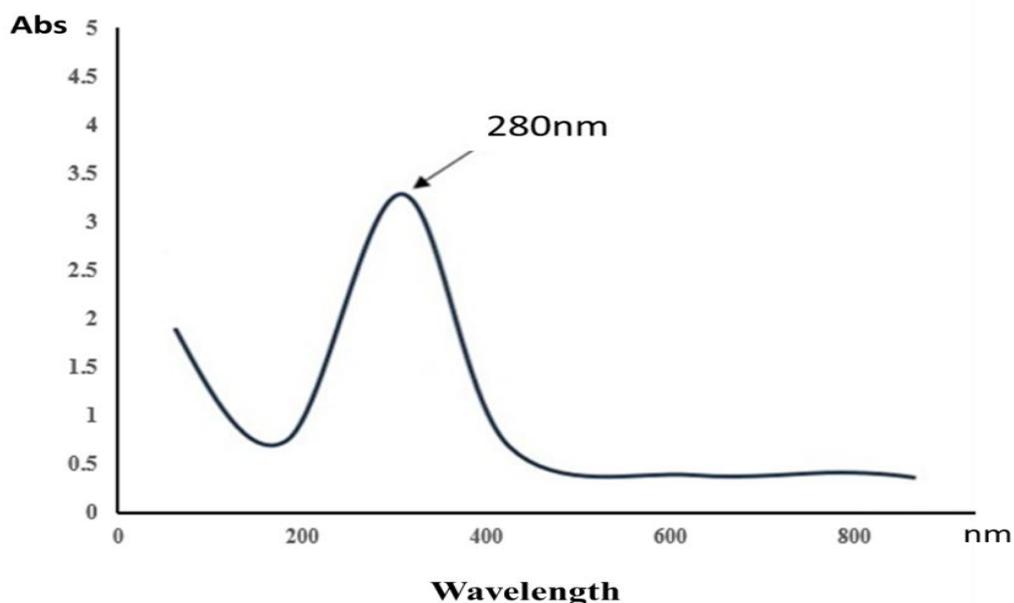


Figure 1. UV-Vis spectrum of Cu-NPs synthesized from *L. paracasei*

Scanning Electron microscope (SEM) Analysis

The shape and size of biogenic copper nanoparticles generated using a green technique from *L. paracasei* at a concentration of 0.1 M were examined. A

Scanning Electron Microscope (SEM) was employed for this purpose. Figure 2 shows the structural characteristics and surface features of the biogenic copper nanoparticles (Cu-NPs). The copper nanoparticles were spherical in shape.

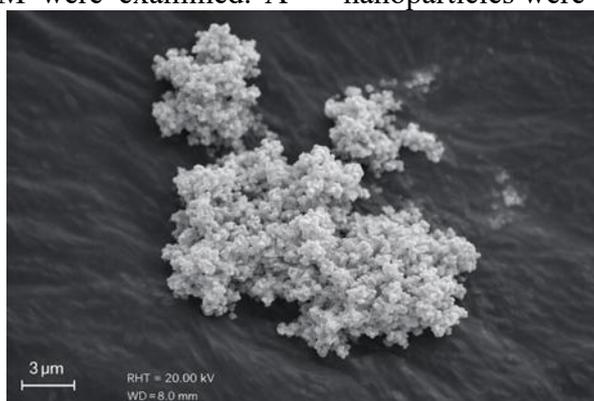


Figure 2. SEM images of Cu-NPs (0.1M) synthesized by using supernatant of *L. paracasei*

FTIR Analysis

In the FTIR spectrum of Cu nanoparticles synthesized using bacterial supernatant biomolecules, several characteristic peaks were observed. A large peak in the range of 3000–3840 cm^{-1} was

attributed to amine and amide functional groups, corresponding to O-H or bacterial protein membrane groups in $\text{Cu}(\text{OH})_2$, resulting from N-H bond stretching. Other peaks, such as those at 2817 cm^{-1} and 2754 cm^{-1} , corresponded to aliphatic C-H bonds,

while a peak at 2192 cm^{-1} was related to the $\text{N}=\text{C}=\text{S}$ group. An hint of $\text{C}=\text{C}$ bonds in aromatic rings or $\text{C}=\text{O}$ bonds in nucleic acids within the cell nucleus was detected at 1576 cm^{-1} . Additional peaks were present at 1044 cm^{-1} , corresponding to $\text{C}=\text{O}$ bonding in carboxylic acids, and at 1196 cm^{-1} , which corresponds to the bending vibration of the $-\text{CH}_3$ ester group or ether (Figure 3). These findings indicated that bioactive substances, such as amino acids, carboxylic acids,

nucleotides, and lipids, were present in the lactobacillus-assisted synthesis process prior to the selection of capping agents for copper oxide nanoparticles. The FTIR analysis validated the existence of many bioactive compounds in the supernatant of *L. Amino acids, nucleic acids, carboxylic acids, and lipids from paracasei* were crucial for the capping and stability of lactobacillus-assisted copper oxide nanoparticles (LA-CuONPs).

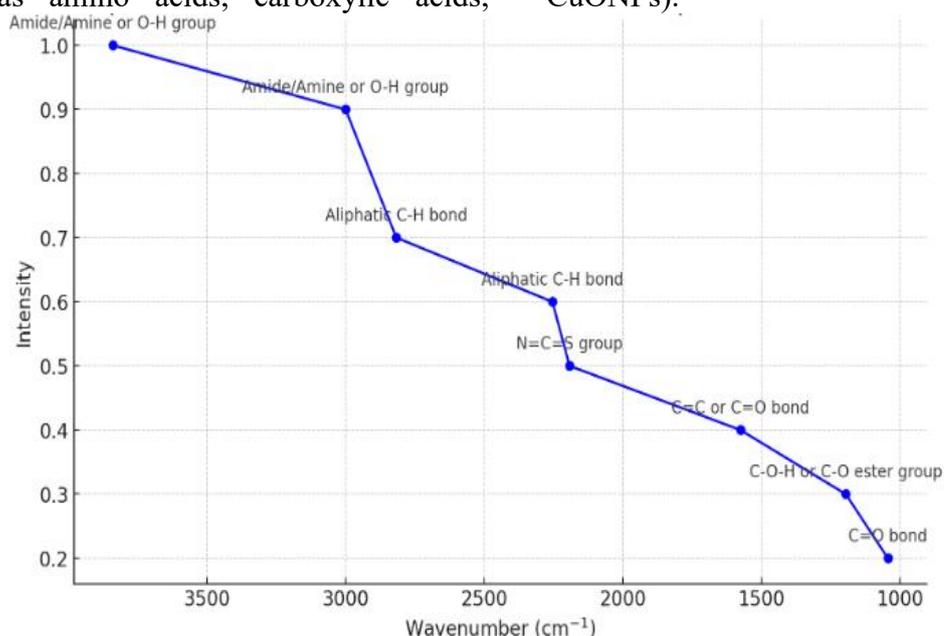


Figure 3. FTIR spectra of Cu-NPs created by using *L. paracasei* supernatant

MTT Assay to Evaluate Anticancer Activity

The anticancer, antiproliferative, or antitumor efficacy of copper nanoparticles (Cu-NPs) was assessed utilizing the MTT assay. Copper nanoparticles (Cu-NPs) were used at four distinct concentrations: $25\text{ }\mu\text{g/ml}$, $50\text{ }\mu\text{g/ml}$, $75\text{ }\mu\text{g/ml}$, and $100\text{ }\mu\text{g/ml}$ for cell treatment. Freshly produced Cu-NPs mediated by *L. paracasei* were suspended in DMSO (Dimethyl Sulfoxide) at different

dilutions to assess their anticancer or antitumor efficacy against HepG2 cell lines. Doxorubicin served as a positive control. All concentrations of Cu-NPs showed significant inhibition of HepG2 cancer cell lines. The maximum inhibition, 81.04%, was observed at $100\text{ }\mu\text{g/ml}$, while $25\text{ }\mu\text{g/ml}$, $50\text{ }\mu\text{g/ml}$, and $75\text{ }\mu\text{g/ml}$ resulted in inhibitions of 22.65%, 46.45%, and 64.78%, respectively, as shown in Figure 4.

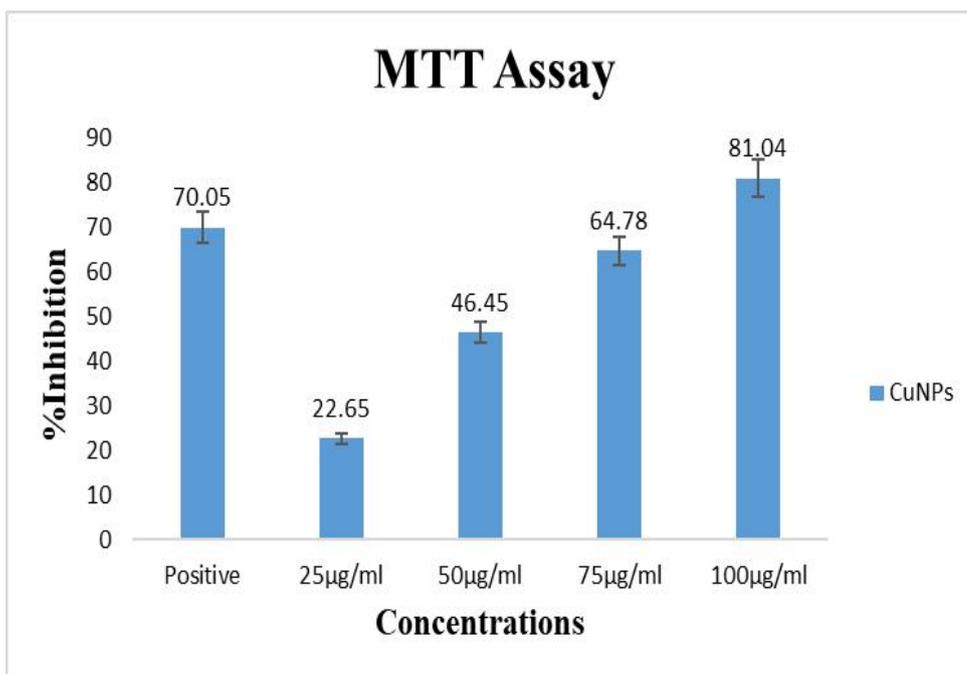


Figure 4. MTT assay at 490 nm showed the percentage inhibition of biogenic CuNPs mediated by *L. paracasei* against HepG2 cell lines

The anticancer or antitumor potential of copper sulfate nanoparticles (NPs) was evaluated using the MTT assay. Three different concentrations of copper sulfate solution were used, and cells were treated with concentrations of 25 µg/ml, 50 µg/ml, and 100 µg/ml. Copper sulfate at three

different dilutions was used to examine its anticancer or antitumor activity against HepG2 cell lines. The maximum inhibition, 81.12%, was obtained at 50 µg/ml, while 25 µg/ml and 100 µg/ml showed inhibition rates of 76.11% and 79.99%, respectively, as shown in Figure 5.

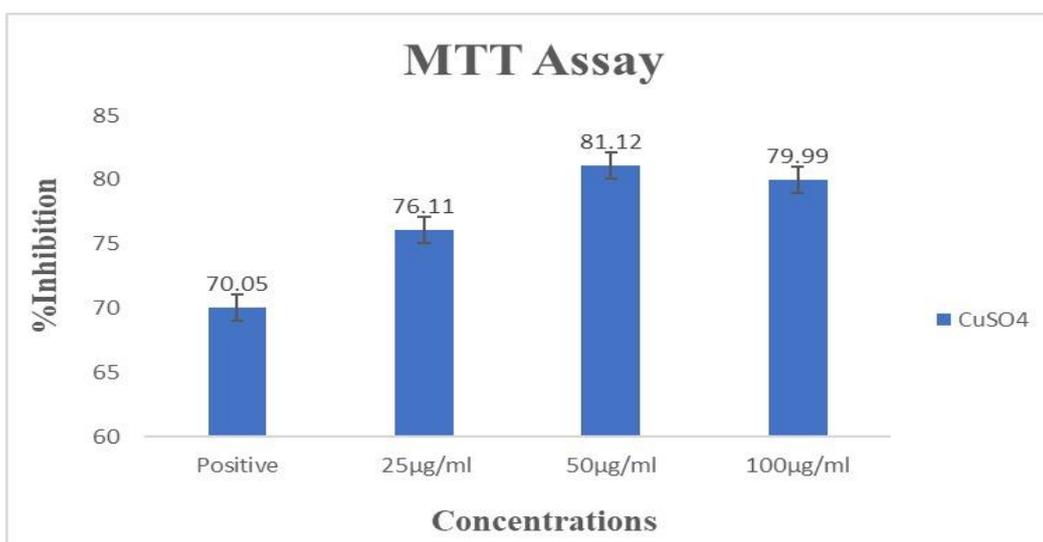


Figure 5. MTT assay at 490 nm showed the percentage inhibition of CuSO₄ NPs against HepG2 cell lines

α-Glucosidase Assay to Evaluate Antidiabetic Activity

To evaluate the antidiabetic potential of biogenically mediated Cu-NPs, an α-glucosidase assay was performed in a 96-

well plate. The α-glucosidase enzyme was utilized at a concentration of 0.5 U/ml, in conjunction with its substrate p-nitrophenyl-α-D-glucopyranoside at a concentration of 5 mM. Before treatment, Cu-NPs were

dispersed in dimethyl sulfoxide. Five distinct concentrations of Cu-NPs were produced to assess their antidiabetic efficacy, alongside the clinically employed positive control, acarbose. Acarbose is a clinically accessible medication with potential efficacy in diabetes management. All concentrations of Cu-NPs exhibited

substantial therapeutic benefits. Cu-NPs had the greatest inhibition percentage at 50 $\mu\text{g/ml}$, achieving 56.72% inhibition, whereas concentrations of 12.5 $\mu\text{g/ml}$, 25 $\mu\text{g/ml}$, and 100 $\mu\text{g/ml}$ resulted in inhibitions of 35.25%, 42.61%, and 42.34%, respectively, as seen in Figure 6.

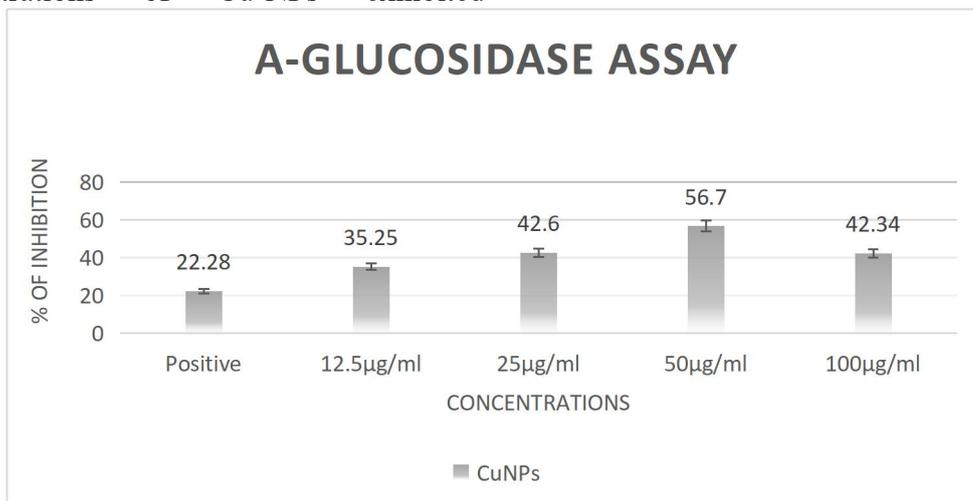


Figure 6. Analysis of antidiabetic potential of CuNPs synthesized from *L. paracasei* against α -glucosidase enzyme, along with acarbose as a standard positive control.

The antidiabetic potential of copper sulfate NPs was evaluated using a 96-well plate. The α -glucosidase enzyme at a concentration of 0.5 U/ml, along with its substrate p-nitrophenyl- α -D-glucopyranoside at a concentration of 5 mM,

was used. Three different concentrations of copper sulfate NPs were prepared to evaluate their antidiabetic potential, along with the clinically used positive control acarbose. Copper sulfate NPs showed the highest percentage of inhibition at 100 $\mu\text{g/ml}$, exhibiting 56.9%, while 25 $\mu\text{g/ml}$ and 50 $\mu\text{g/ml}$ showed inhibitions of 29.14% and 41.32%, respectively, as shown in Figure 7.

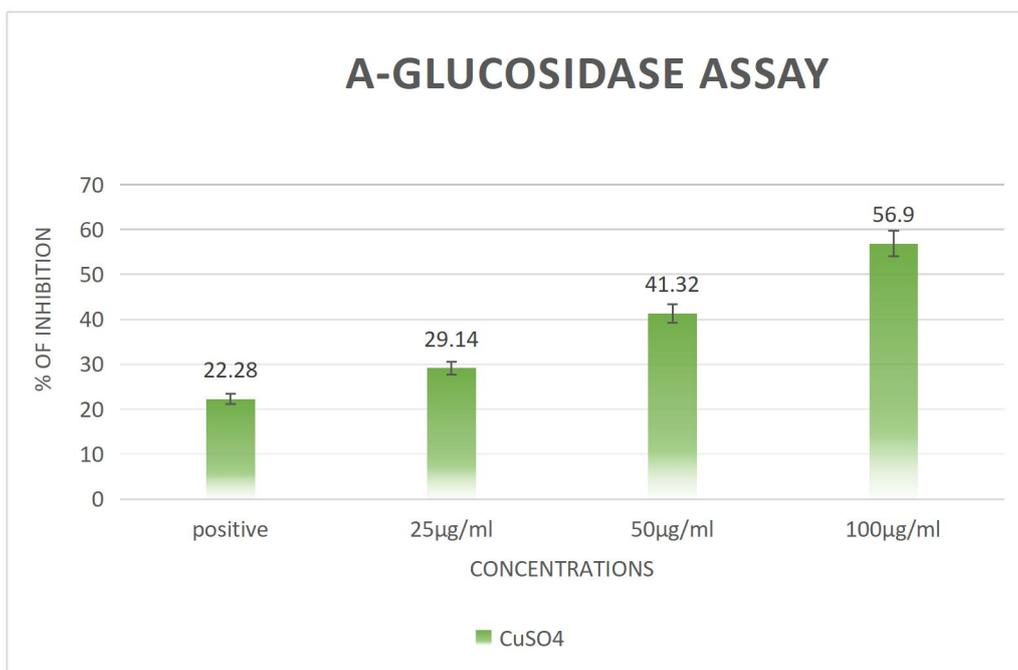


Figure 7. Analysis of antidiabetic potential of Copper Sulfate NPs against α -glucosidase enzyme, along with acarbose as a standard positive control.

DPPH Assay to Evaluate Antioxidant Activity

To assess the antioxidant capacity of Cu-NPs, they underwent the DPPH test. The DPPH reagent was solubilized in ethanol at a concentration of 0.3 mM, with ascorbic acid used at the identical concentration as DPPH. Ascorbic acid functioned as the positive control, whilst dimethyl sulfoxide acted as the negative control. Cu-NPs were

subjected to treatment at four distinct concentrations and suspended in DMSO, with positive and negative controls allocated to separate wells. Figure 8 illustrates that the peak free radical scavenging activity occurred at 25 μ g/ml, demonstrating 49.07% inhibition, whereas 12.5 μ g/ml, 50 μ g/ml, and 100 μ g/ml exhibited 40.13%, 45.66%, and 41.33% inhibition, respectively.

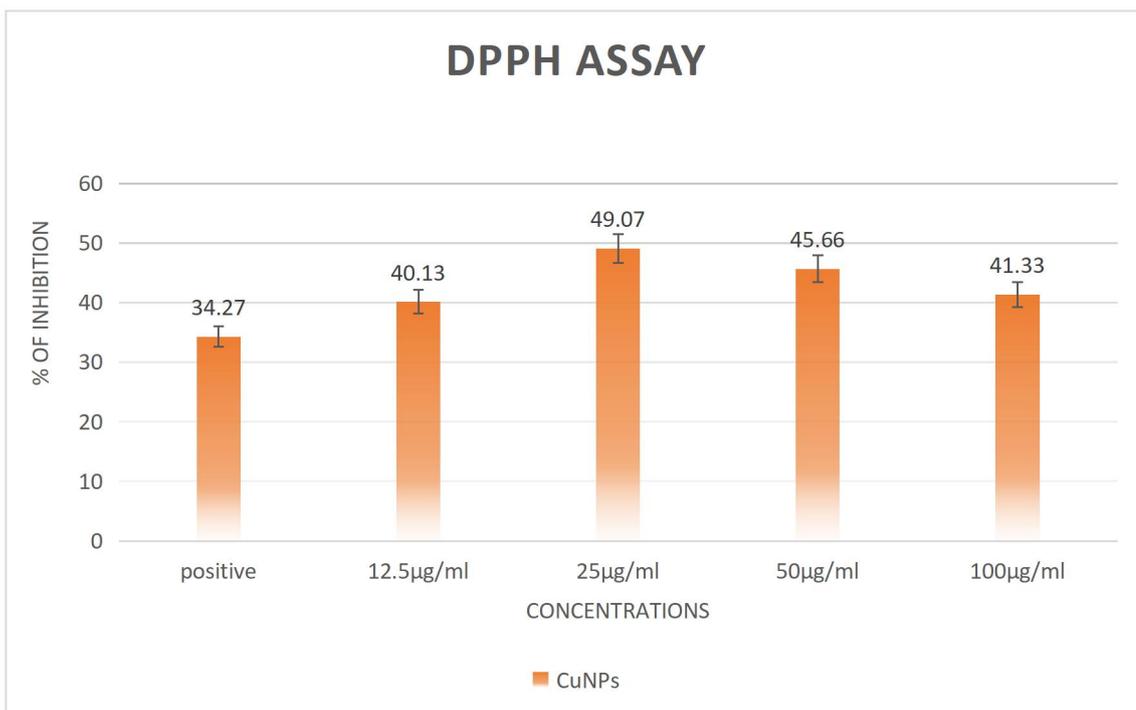


Figure 8. Scavenging capacity of CuNPs mediated by *L. paracasei* evaluated by DPPH assay

The antioxidant capacity of copper sulfate nanoparticles was assessed utilizing the DPPH test. The DPPH reagent was solubilized in ethanol at a concentration of 0.3 mM, with ascorbic acid utilized at an equivalent concentration to that of DPPH. Ascorbic acid functioned as the positive control, whilst dimethyl sulfoxide acted as the negative control. Three distinct

concentrations of copper sulfate nanoparticles were evaluated, with positive and negative controls in separate wells. Figure 9 demonstrated that the peak free radical scavenging activity occurred at 100 µg/ml, registering 72.59%, whereas 25 µg/ml and 50 µg/ml exhibited 49.37% and 59.48%, respectively.

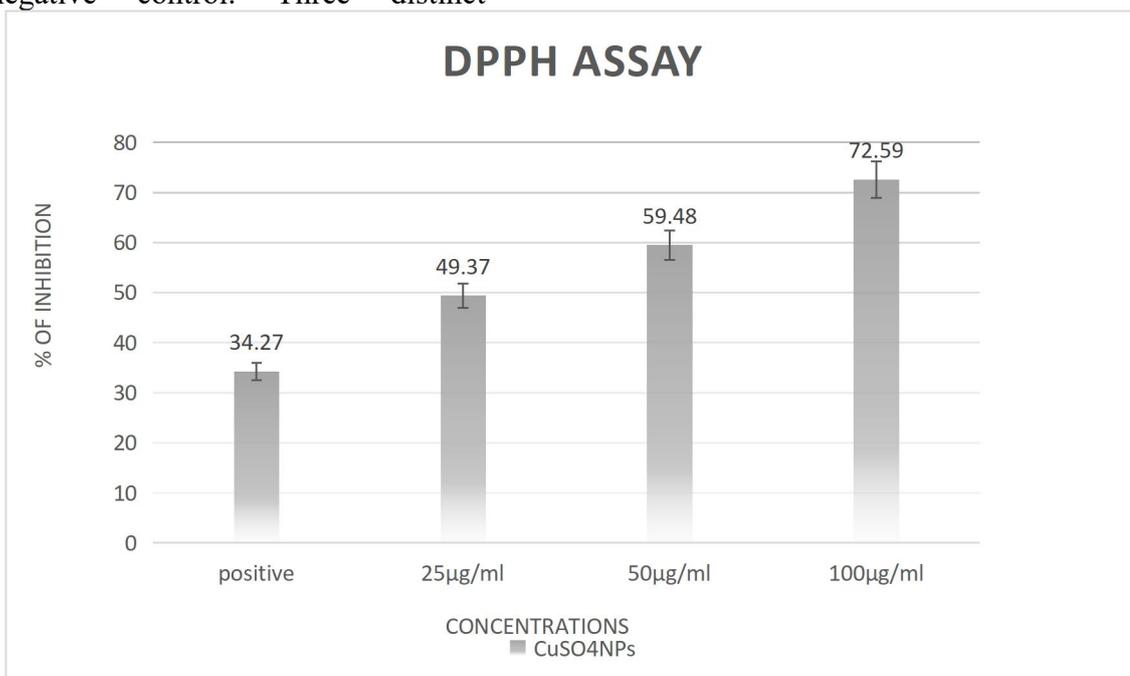


Figure 9. Copper sulfate nanoparticles was evaluated by DPPH assay

Well Diffusion Assay to Evaluate Antimicrobial Activity

The antimicrobial effects of copper sulfate nanoparticles and Cu-NPs mediated by *L. paracasei* were tested against *S. aureus* and *E. coli* at a concentration of 40 mg/ml. Gentamicin was used as the positive control, while dimethyl sulfoxide served as the negative control. It was found that *L. paracasei*-mediated Cu-NPs exhibited a zone of inhibition of 21 mm against *E. coli*,

whereas simple copper nanoparticles showed a zone of inhibition of 20 mm. On the other hand, simple copper nanoparticles exhibited a zone of inhibition of 20 mm against *S. aureus*, while *L. paracasei*-mediated Cu-NPs showed a zone of inhibition of 17 mm. Both types of nanoparticles displayed strong antimicrobial properties against both bacterial strains, as shown in Figure 10.

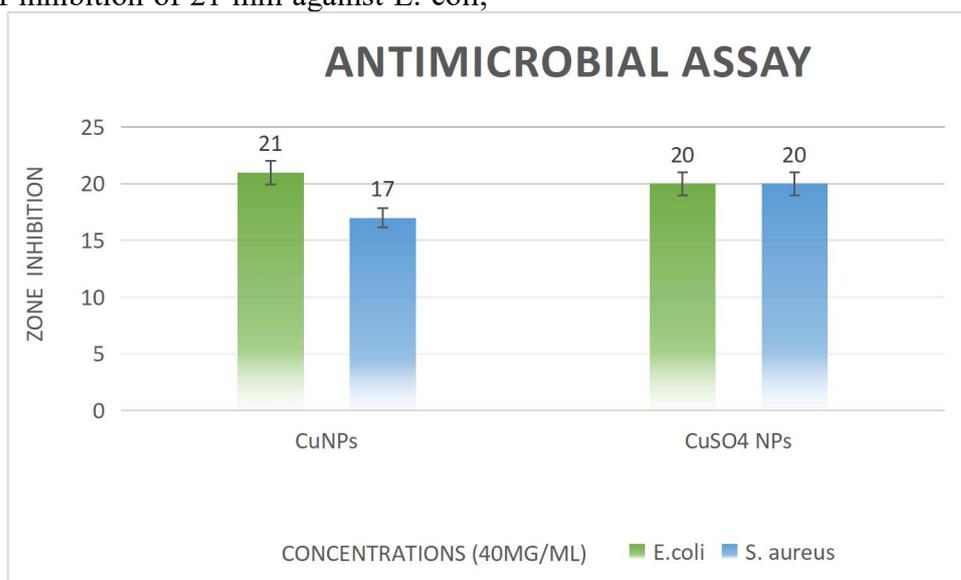


Figure 10. Antimicrobial assay showing zone inhibition of Cu-NPs mediated by *L. paracasei* and CuSO₄ nanoparticles

Discussion

In the present era of technological advancements, nanoparticles have emerged as a transformative force in biotechnology. Over recent years, nanotechnology has garnered significant attention from researchers worldwide due to its promising applications across diverse fields, including medicine, diagnostics, drug delivery, agriculture, and environmental sciences (Dos Santos et al., 2020; Rastogi et al., 2019). Among various nanoparticle synthesis approaches, green or biogenic synthesis has gained prominence due to its cost-effectiveness, non-toxicity, and environmental sustainability. Compared to chemically synthesized copper nanoparticles (CuNPs), biosynthesized CuNPs exhibit superior biocompatibility, rendering them

more suitable for biomedical applications (Bibi et al., 2023; Dahiya et al., 2023).

This study successfully synthesized bacterial-mediated copper nanoparticles using *L. paracasei* and evaluated their antidiabetic, free radical scavenging, antimicrobial, antitumor, and wound-healing activities. The optimal concentration of CuSO₄ for efficient nanoparticle synthesis was determined to be 0.1 M, as evidenced by a color change from blue to dark brown or black. The peptides present in the bacterial supernatant facilitated the bioreduction of metal ions, serving as capping agents for nanoparticle stabilization. The formation of CuNPs was confirmed through UV-Vis spectrophotometry, with an absorbance peak around 280 nm, consistent with prior studies (Dolati et al., 2023).

Differences in synthesis methods and reaction conditions can influence the peak absorbance range.

The physicochemical evaluation of produced CuNPs was conducted utilizing many analytical methods, such as scanning electron microscopy, UV-Vis spectrophotometry, and Fourier-transform infrared spectroscopy. SEM examination indicated that the CuNPs produced using *L. paracasei* supernatant at a 0.1 M copper salt concentration had a spherical shape, consistent with prior findings on bacterial-mediated nanoparticle production (BT et al., 2019; John et al., 2021). FTIR spectroscopy was utilized to identify functional groups involved in CuNP stabilization and bioreduction. The obtained spectra demonstrated distinct peaks at 3000–3840, 2817, 2754, 2192, 1576, 1044, and 1196 cm^{-1} , indicative of amide, aliphatic C–H, N=C=S, C=C (aromatic rings), and carboxylic acid groups, respectively. These findings suggest the presence of bioactive molecules such as carboxylic acids, amino acids, nucleotides, and lipids in the bacterial supernatant, contributing to nanoparticle capping and stabilization (Dolati et al., 2023; Singh et al., 2023). Cancer continues to be a primary cause of worldwide death, presenting considerable hurdles to traditional treatment modalities including chemotherapy, radiation, and surgery, which are frequently costly and linked to severe adverse effects. As a result, researchers are increasingly exploring alternative therapeutic strategies that offer enhanced efficacy, improved safety, and cost-effectiveness (Alayed et al., 2022). The anticancer efficacy of CuNPs was evaluated by an MTT test on HepG2 cell lines, demonstrating that CuNPs elicited substantial cytotoxic effects in a dose-dependent fashion. Peak inhibition occurred at a dose of 100 $\mu\text{g/ml}$, but a reduced concentration of 25 $\mu\text{g/ml}$ demonstrated little activity (22.65%). Notably, simple CuSO_4 nanoparticles demonstrated maximum inhibition at 50 $\mu\text{g/ml}$ (81.12%), suggesting that CuNPs-mediated cell death

may result from apoptosis, as previously reported (Rahbar Saadat et al., 2020; Wang et al., 2018).

Diabetes mellitus is a chronic endocrine condition marked by hyperglycemia resulting from insulin shortage or resistance, causing significant consequences that impact several organs. Diabetes is considered the third primary cause of death, behind cancer and cardiovascular illnesses (Roseline & Priya, 2023; Wang & Li, 2022; Won et al., 2021). The antidiabetic efficacy of CuNPs was evaluated using an α -glucosidase inhibition assay. CuNPs exhibited maximum inhibition at 50 $\mu\text{g/ml}$, whereas the lowest inhibition (35.25%) was observed at 12.5 $\mu\text{g/ml}$. Simple CuSO_4 nanoparticles demonstrated maximum inhibition at 100 $\mu\text{g/ml}$ (56.9%), confirming the potential of CuNPs in diabetes management.

Oxidative stress is a significant contributor to several metabolic illnesses, such as cancer and diabetes, resulting from the overproduction of reactive oxygen species and reactive nitrogen species. Although these species are crucial for cellular signaling, an imbalance in ROS levels may result in pathogenic diseases (Darby & Hewitson, 2016; Guo & DiPietro, 2010). The antioxidant activity of CuNPs was analyzed using the DPPH assay, demonstrating a significant free radical scavenging effect. The highest scavenging activity (49.07%) was observed at 25 $\mu\text{g/ml}$, while simple CuSO_4 nanoparticles exhibited maximum inhibition at 100 $\mu\text{g/ml}$ (72.59%) (Huligere et al., 2023; Talebian et al., 2023).

Chronic wounds, particularly in diabetic patients, present substantial therapeutic challenges due to impaired healing processes characterized by hypoxia, inflammation, and oxidative stress. Conventional treatment modalities often fail to promote effective wound repair, necessitating the exploration of novel therapeutic agents (Sandoval et al., 2022). The unique physicochemical properties of CuNPs, including their antimicrobial, anti-inflammatory, and antioxidant capabilities,

make them promising candidates for wound-healing applications. CuNPs have a high surface-to-volume ratio, enhancing their bioavailability and interaction with the wound site, thereby accelerating tissue regeneration and repair.

The antimicrobial properties of CuNPs were evaluated against *S. aureus* and *E. coli* using the antimicrobial assay. *L. paracasei*-mediated CuNPs exhibited a larger zone of inhibition against *E. coli* compared to simple CuNPs (20 mm), suggesting superior antimicrobial activity. However, against *S. aureus*, simple CuNPs (20 mm) showed greater inhibition than *L. paracasei*-mediated CuNPs (17 mm), indicating species-dependent variations in antibacterial efficacy. These findings highlight the efficacy of CuNPs as antibacterial agents against pathogenic microorganisms (Biju, 2023; Francis et al., 2023).

Overall, the study highlights the successful synthesis of biogenic CuNPs using *L. paracasei* and their multifaceted biomedical applications. The results emphasize the potential of CuNPs in cancer therapy, diabetes management, oxidative stress mitigation, wound healing, and antimicrobial treatments. Future research should focus on optimizing CuNP synthesis conditions, elucidating their precise molecular mechanisms, and evaluating them in vivo efficacy to facilitate clinical translation.

Conclusion

Biogenic synthesis of copper nanoparticles (CuNPs) using microbial methods, such as *L. paracasei* supernatant as a bio-reductant, offers an eco-friendly and cost-effective approach. The synthesis, confirmed by UV-Vis spectroscopy (280 nm), produced CuNPs with promising biomedical applications. These CuNPs exhibited anticancer activity against HepG2 cells, antidiabetic properties through α -glucosidase inhibition, free radical scavenging activity, antimicrobial efficacy, and enhanced wound healing. The research underscores the promise of *L. paracasei*-

mediated CuNPs as a multifaceted medicinal agent. Future investigations should concentrate on in vivo studies and the molecular processes behind the bioactivities of CuNP for therapeutic applications.

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