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Theme

Engineering Copper Oxide–DNA nanocomposites as Novel Antibacterial Nanomaterials Against Antimicrobial Resistance

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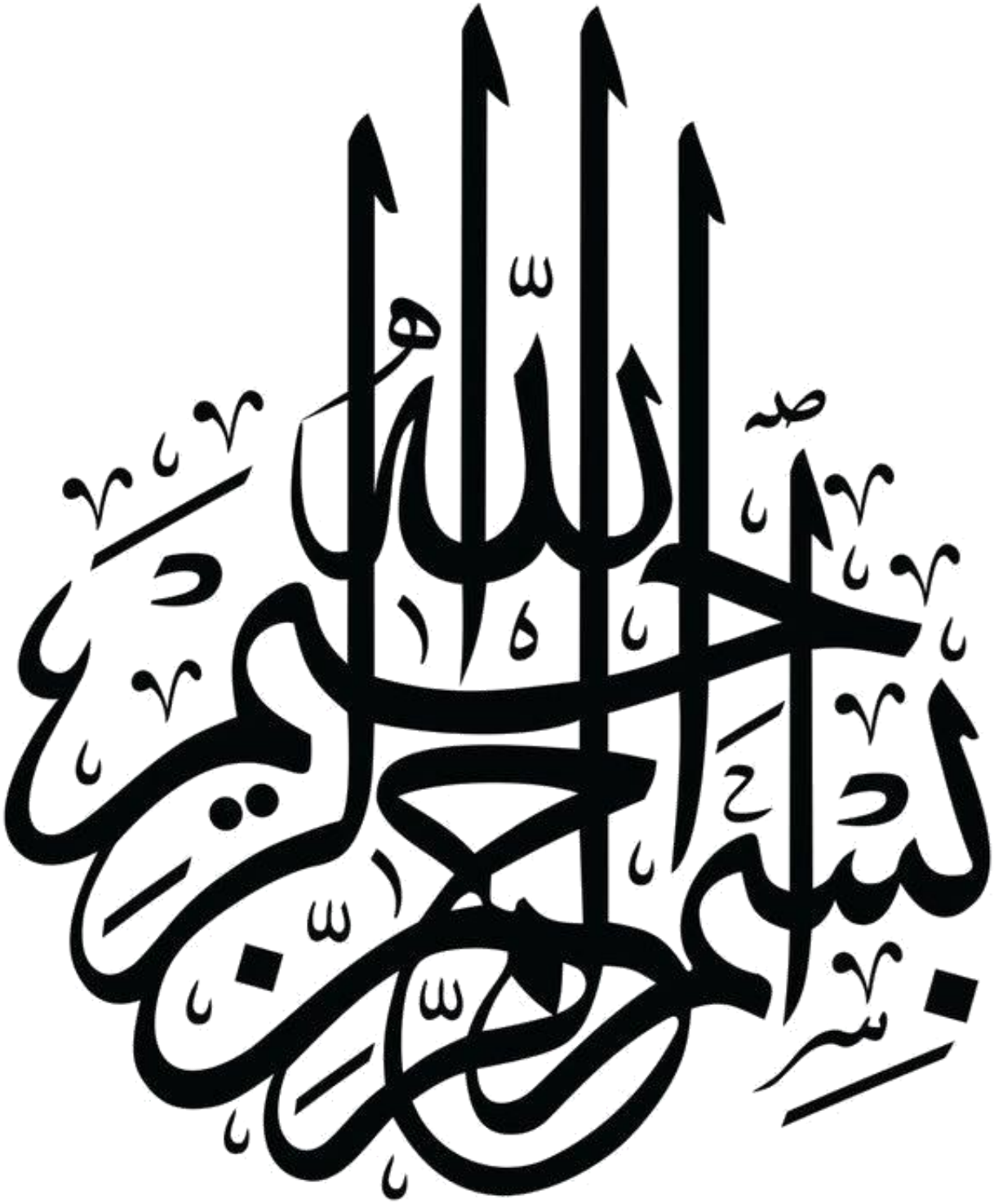
بسم الله الرحمن الرحيم

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Abstract

The rapid emergence of antimicrobial resistance (AMR) has become a major global health concern, reducing the effectiveness of conventional antibiotics and increasing the demand for alternative antimicrobial strategies. The aim of this work is try to overcome the problem of antibiotic resistance by developing a nano-product used as a selective antimicrobial agent. In this study, copper oxide nanoparticles (CuO NPs) were synthesized using a chemical reduction–precipitation method with ascorbic acid as a reducing agent. Furthermore, CuO-based nanocomposites functionalized with bacterial DNA extracted from Escherichia coli and Staphylococcus aureus were developed to investigate their potential as selective antibacterial agents. The synthesized Nano-composites were characterized using UV–Visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and scanning electron microscopy (SEM). UV–Vis analysis confirmed nanoparticle formation through characteristic absorption peaks, while FTIR spectra revealed the presence of Cu–O bonds and functional groups associated with bacterial DNA conjugation. XRD analysis confirmed the successful formation of crystalline Cu_2O structures, with crystallite sizes ranging from approximately 8.1 to 11.8 nm. SEM observations revealed aggregated nanoscale structures with heterogeneous morphologies, further confirming the formation of CuO–DNA nanocomposites. The biological activities of the synthesized nanoparticles were evaluated through antioxidant, anti-inflammatory, and antibacterial assays. CuO npsexhibited strong antioxidant activity, showing the lowest IC_{50} value (1.13 µg/mL), whereas Staphylococcus aureus-nanoparticles (S-CuNPs) demonstrated the highest anti-inflammatory activity, with an IC_{50} value of 0.5 µg/mL. Antibacterial activity, assessed using the disk diffusion method, revealed that DNA-functionalized nanoparticles exhibited enhanced antibacterial effects compared with CuO npsalone. The highest inhibition zone was observed for E-CuNPs against Escherichia coli at 40 mg/mL, while S-CuNPs showed superior activity against Staphylococcus aureus, suggesting possible strain-specific interactions between the nanocomposites and their corresponding bacterial targets. In addition, molecular docking studies demonstrated favorable interactions between Cu_2O nanoparticles and bacterial DNA gyrase-related proteins. The strongest binding affinity was observed for receptor 8BN6 (−4.82 kcal/mol), supporting the proposed antibacterial mechanism through interference with bacterial DNA replication processes. Overall, these findings indicate that DNA-functionalized copper oxide nanoparticles represent a promising and innovative strategy for to developing selective and efficient antibacterial nanomaterials capable of combating antibiotic-resistant bacteria.

Keywords:

Antimicrobial resistance, Copper oxide nanoparticles, Bacterial DNA, Biological activities, Molecular docking, Escherichia coli, Staphylococcus aureus.

الملخص

أصبح الظهور السريع لمقاومة مضادات الميكروبات (AMR) مصدر قلق صحي عالمي رئيسي، حيث أدى إلى تقليل فعالية المضادات الحيوية التقليدية وزيادة الحاجة إلى استراتيجيات بديلة لمكافحة الميكروبات. يهدف هذا العمل إلى المساهمة في التغلب على مشكلة مقاومة المضادات الحيوية من خلال تطوير منتج نانوي يُستخدم كعامل مضاد للميكروبات ذو انتقائية عالية.

تم توصيف الجسيمات النانوية المحضرة باستعمال عدة تقنيات، شملت مطيافية الأشعة فوق البنفسجية والمرئية (UV_VIS)، والتحليل بالأشعة تحت الحمراء(FTIR) ، وحيود الأشعة السينية(XRD) ، والمجهر الإلكتروني الماسح .(SEM) أكدت نتائج UV–Vis تكوّن الجسيمات النانوية من خلال ظهور قمم امتصاص مميزة، بينما أظهرت تحاليل FTIR وجود روابط Cu–O ومجموعات وظيفية مرتبطة بارتباط الحمض النووي البكتيري بالجسيمات النانوية. كما بين تحليل XRDتكوّن بنية بلورية من Cu_2O بأحجام بلورية تراوحت بين 8.1 و11.8 نانومتر، في حين كشفت صور SEM عن تراكيب نانوية متجمعة وغير منتظمة الشكل، مما يؤكد نجاح تكوين المركبات النانوية المرتبطة بالحمض النووي.

تم تقييم الأنشطة البيولوجية للجسيمات النانوية من خلال اختبارات النشاط المضاد للأكسدة، والمضاد للالتهاب، والنشاط المضاد للبكتيريا. أظهرت جسيماتCuO nps أعلى نشاط مضاد للأكسدة بقيمة IC_{50} بلغت 1.13µg/mL ، بينما سجلت الجسيمات المرتبطة بـ (SCuO NPs) Staphylococcus aureus أعلى نشاط مضاد للالتهاب بقيمة IC_{50} بلغت 0.5 .µg/mL أما اختبار النشاط المضاد للبكتيريا بطريقة الانتشار بالأقراص فقد أظهر أن الجسيمات النانوية المرتبطة بالحمض النووي البكتيري تمتلك فعالية أكبر مقارنة بالجسيمات النانوية وحدها، حيث سجلت ECuO npsأكبر منطقة تثبيط ضد Escherichia coli عند تركيز 40mg/mL ، في حين أظهرتSCuO nps فعالية أعلى ضد .Staphylococcus aureusوتشير هذه النتائج إلى احتمال وجود نوع من التوافق أو الانتقائية البيولوجية بين المركبات النانوية والبكتيريا المستهدفة.

كما أظهرت دراسة الالتحام الجزيئي (Molecular Docking) وجود تفاعلات جيدة بين جسيمات Cu_2O النانوية والبروتينات المرتبطة بإنزيمات DNA gyrase البكتيرية، حيث سُجل أقوى ارتباط مع المستقبل BN6 8بقيمة طاقة ارتباط بلغت4.82 kcal/mol– ، مما يدعم فرضية قدرة هذه الجسيمات على التأثير في عمليات تضاعف الحمض النووي البكتيري.

بشكل عام، تشير نتائج هذه الدراسة إلى أن الجسيمات النانوية الوظيفية المرتبطة بالحمض النووي البكتيري تمثل استراتيجية واعدة لتطوير مضادات بكتيرية نانوية انتقائية وفعالة قادرة على مواجهة البكتيريا المقاومة للمضادات الحيوية

List of Abbreviations

- **AMR:** Antimicrobial Resistance
- **ATCC:** American Type Culture Collection
- **ATP:** Adenosine Triphosphate
- **AWaRe:** Access, Watch, Reserve
- **BSA:** Bovine Serum Albumin
- **Cu nps:** Copper Nanoparticles
- **CuO :** Copper Oxide
- **DMSO:** Dimethyl Sulfoxide
- **DNA:** Deoxyribonucleic Acid
- **ECuO NPs:** Escherichia coli-associated Copper Nanoparticles
- **ESBLs :** Extended-Spectrum Beta-Lactamases
- **FRAP:** Ferric Reducing Antioxidant Power
- **FTIR:** Fourier Transform Infrared Spectroscopy
- **IC_{50}:** Half Maximal Inhibitory Concentration
- **IM:** Intramuscular
- **IV:** Intravenous
- **JCPDS:** Joint Committee on Powder Diffraction Standards
- **NPs:** Nanoparticles
- **PBS:** Phosphate Buffered Saline
- **PDB:** Protein Data Bank
- **ROS:** Reactive Oxygen Species
- **SCuO NPs:** Staphylococcus aureus-associated Copper Nanoparticles
- **SEM:** Scanning Electron Microscopy
- **TEM:** Transmission Electron Microscopy
- **UV–Vis:** Ultraviolet–Visible Spectroscopy
- **WHO:** World Health Organization
- **XRD:** X-ray Diffraction

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Worldwide, millions of people die from infectious disease agents (Choudhary et al., 2023), which belong to different classes of pathogens, including viruses, bacteria, fungi, and parasites (Maina et al., 2020). Bacteria are responsible for several life-threatening infectious diseases in humans, such as tuberculosis, cholera, tetanus, and pneumonia (Gupta et al., 2022). Therefore, bacterial diseases represent one of the leading causes of increased mortality rates, mainly due to the emergence of multidrug-resistant pathogenic microorganisms resulting from the overuse of conventional antimicrobial drugs, immunosuppressants, and other therapeutic agents (Qadri et al., 2023).

The development of antibiotics represents one of the greatest achievements of modern medicine, as these drugs have significantly reduced mortality associated with bacterial infections and improved the treatment of numerous diseases across all age groups and regions worldwide. However, the effectiveness of these therapies has become increasingly threatened by the rapid emergence and spread of antibiotic-resistant bacterial strains, making antibiotic resistance one of the most serious global health challenges today (Ventola, 2015). Early warnings regarding this issue date back to 1945, when Alexander Fleming and Howard Florey cautioned that the inappropriate use of antibiotics could lead to the emergence of resistant bacterial strains (Nathan, 2020). Over time, bacterial pathogens rapidly evolved and acquired multiple resistance mechanisms, while the discovery of new antibiotics slowed considerably (Theuretzbacher et al., 2020).

In addition, the misuse and overuse of antibiotics have accelerated this phenomenon, leading to reduced clinical efficacy and treatment failures, while also raising serious concerns about the long-term sustainability of current antimicrobial therapies. As a result, several pathogenic bacteria, including Mycobacterium tuberculosis, Staphylococcus aureus, Klebsiella pneumoniae, and Pseudomonas aeruginosa, have developed resistance to many available antibiotics. In this context, the World Health Organization has warned of a potential “post-antibiotic era,” in which common infections and minor injuries could once again become life-threatening (Wilson et al., 2020).

Nanobiotechnology, which integrates biology, medicine, and nanotechnology, is significantly transforming modern healthcare by improving approaches in diagnosis, treatment, and disease prevention (Malik et al., 2023). In this context, nanomaterials have gained considerable attention due to their unique physical, chemical, and biological properties, which make them highly valuable for biomedical applications (Ahmed & Othman, 2024). Various synthesis strategies have been developed for the production of nanomaterials, including chemical, physical, and biological (green) methods. Among these, green synthesis has emerged as a promising and sustainable approach because it utilizes

biological resources and minimizes the use of toxic chemicals, leading to safer and more environmentally friendly production processes (Chopra et al., 2022; El-Khawaga et al., 2023). Moreover, microorganisms, particularly bacteria, have demonstrated strong potential in nanoparticle biosynthesis due to their natural metabolic capabilities and efficiency (Talebian et al., 2023). Among different nanomaterials, copper nanoparticles (CuO NPs) have attracted increasing interest because of their diverse properties, including catalytic, anticancer, coating, and antibacterial activities, making them promising candidates for a wide range of biomedical and industrial applications (Naz et al., 2023).

In the first part of this study, the general issue of increasing bacterial infections and the growing problem of antibiotic resistance was addressed, along with an overview of proposed strategies to mitigate this challenge. In the second part, the study focused on the in vitro evaluation of the biosynthesis of copper nanoparticles (CuO NPs) using Staphylococcus aureus and Escherichia coli, aiming to investigate their potential application as targeted antibacterial agents against the studied strains within a more specific and precise therapeutic approach.

First part

Bibliographic synthesis

I

Escherichia coli

and

Staphylococcus aureus

Bacterial infections are among the most common causes of human diseases worldwide and remain a major public health concern due to their high morbidity and mortality rates. These infections are caused by pathogenic bacteria capable of invading the human body, multiplying rapidly, and disrupting normal physiological functions. They can affect different organs and systems, leading to a wide range of diseases, from mild skin infections to severe and life-threatening conditions such as pneumonia, tuberculosis, meningitis, cholera, and sepsis. The transmission of bacterial pathogens may occur through contaminated food and water, direct contact, airborne droplets, or environmental exposure. Despite the remarkable progress achieved in modern medicine and antibiotic therapy, bacterial infections continue to pose significant challenges because of the increasing emergence of antibiotic-resistant strains, which reduce treatment effectiveness and complicate infection control strategies (Ventola, 2015). Therefore, continuous research is essential to develop innovative therapeutic approaches and more effective antimicrobial agents to combat bacterial pathogens and reduce their global health impact.

1) Escherichia coli

1.1. Description

E. coli is a facultative anaerobic, rod-shaped bacterium of straight, rod-shaped, nonsporing, non-acid fast bacilli that exist in singles and pairs. Cells are typically rod-shaped, around 1 μm long, 0.35 μm wide, and 0.6–0.7 μm in volume. It belongs to the coliform family (M. Basavaraju, B.S. Gunashree 2022). Most strains are harmless and part of the normal intestinal flora, but some pathogenic serotypes can cause serious Infections (Cobo-Simón et al., 2023).

1.2. Classification of Escherichia coli (Faner et al.,2017)

- **Domain:** Bacteria
- **Phylum:** Pseudomonadota
- **Class:** Gammaproteobacteria
- **Order:** Enterobacterales
- **Family:** Enterobacteriaceae
- **Genus:** Escherichia
- **Species:** E. Coli

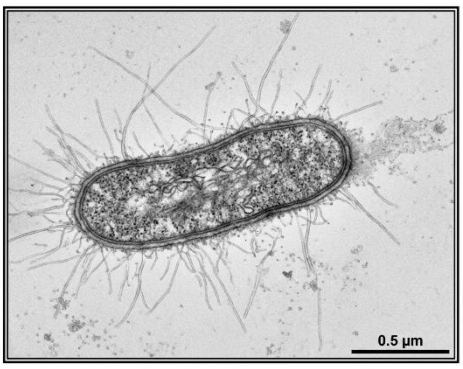

Figure 01 : E. Coli (Jauffred et al., 2017)

1.3. Pathogenicity of E. coli

Pathogenic E. coli is divided into two groups: extraintestinal pathogenic E. coli (ExPEC) and intestinal pathogenic E. coli (InPEC) (Sora et al., 2021). It can be categorized into various pathotypes (Table 01), depending on the presence of specific virulence factors, mechanisms of infection, tissue tropism, interactions with host cells, and clinical symptômes (Pokharel et al., 2023) hogenicity of E. coli

Table 01 : E. coli pathotypes and their associated symptoms (Geurtsen et al., 2022)

Pathotypes of *E. coli*	Clinical symptoms
Enteropathogenic *E. coli* (EPEC)	A cause of acute and prolonged diarrhea in infants
Enterohemorrhagic *E. coli* (EHEC)	Can cause hemorrhagic colitis and hemolytic uremic syndrome (HUS)
Enterotoxigenic *E. coli* (ETEC)	A major cause of travelers' diarrhea
Enteroaggregative *E. coli* (EAEC)	A cause of acute and chronic diarrhea
Diffusely adherent *E. coli* (DAEC)	Associated with watery diarrhea in young children
Enteroinvasive *E. coli* (EIEC)	A cause of dysentery and watery diarrhea
Adherent-Invasive *E. coli* (AIEC)	It has been associated in the pathogenesis of inflammatory bowel disease (IBD)
Uropathogenic *E. coli* (UPEC)	A common cause of urinary tract infections (UTI)
Neonatal meningitis *E. coli* (NMEC)	A top cause of neonatal meningitis
Septicemia-associated *E. coli* (SEPEC)	bacteremia and sepsis
Avian pathogenic *E. coli* (APEC)	Can cause severe respiratory and systemic infections in poultry

2) Staphylococcus aureus

2.1. Description

Staphylococcus aureus is a Gram-positive, spherical bacterium arranged in clusters. It has an average diameter of about 1 µm, is non-motile, and does not form spores. Some strains possess a polysaccharide capsule that enhances adhesion and helps the bacterium evade the host immune system.

In aerobic conditions, it forms smooth, creamy, opaque colonies with a characteristic golden-yellow pigmentation. These colonies typically reach about 4 mm in diameter after incubation, which is a distinctive feature of the species. (Baron, 1996).

2.2. Classification

- **Domain:** Bacteria
- **Division:** Bacillot
- **Class:** Bacilli
- **Order:** Caryophanales
- **Family:** Staphylococcaceae
- **Genus:** Staphylococcus
- **Species:** Staphylococcus aureus

Authority Rosenbach, 1884 (Baron, 1996)

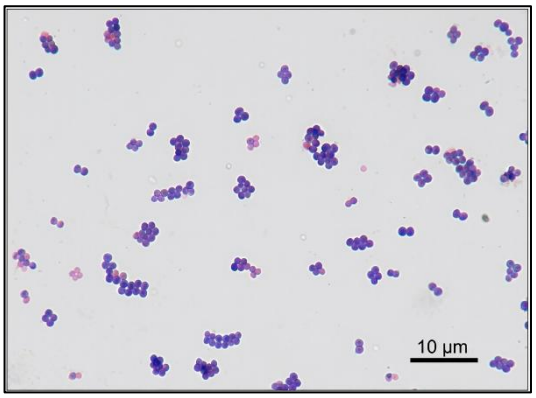

Figure 02 : (Malgorzat Mnich, 2022)

2.3. Pathogenicity of Staphylococcus aureus

The pathogenicity of Staphylococcus aureus is mainly attributed to its ability to produce a wide range of virulence factors, including enzymes and toxins that facilitate tissue invasion and immune evasion. The bacterium secretes several enzymes such as coagulase, fibrinolysin, hyaluronidase, proteases, phosphatases, and deoxyribonuclease. These enzymes contribute to the degradation of host tissues and extracellular barriers, thereby promoting bacterial dissemination within the host. In addition, S. aureus produces multiple toxins, including enterotoxins, staphylolysins, and leukocidins, which are responsible for its cytotoxic effects. These toxins can damage host cells and disrupt immune responses. Of particular importance is the Panton–Valentine leukocidin (PVL), which is associated with severe infections and targets leukocytes, leading to their destruction. The genes encoding this toxin are often carried by bacteriophages integrated into the bacterial genome, which may contribute to increased virulence in certain strains (Kobayashi, Malchow, & DeLeo, 2015).

II

Antibiotic

1) Definition of antibiotic

Antibiotics are bioactive compounds used to inhibit or kill bacterial growth. They may be naturally produced by microorganisms or obtained through synthetic and semi-synthetic methods. Their action is based on targeting essential bacterial processes such as cell wall synthesis, protein synthesis, and nucleic acid replication (Encyclopedia Britannica, 2026).

2) Classification of Antibiotics

Antibiotics can be classified according to several criteria, including their mechanism of action, chemical structure, spectrum of activity, and their clinical role in antimicrobial stewardship. This multidimensional classification helps to better understand their therapeutic applications and optimize their use in the context of increasing antimicrobial resistance (Shi et al., 2023)

2.1. Classification based on mechanism of action

Antibiotics are primarily grouped according to the bacterial target they affect:

- **Inhibitors of cell wall synthesis:**

  These antibiotics block the formation of the bacterial cell wall, leading to cell lysis and death. Examples include β-lactams (penicillins, cephalosporins, carbapenems) and glycopeptides. They are generally bactericidal (IJCSRR, 2024).

- **Inhibitors of protein synthesis:**

  They act on bacterial ribosomes (30S or 50S subunits), preventing protein production. This group includes tetracyclines, macrolides, and aminoglycosides, and may be either bacteriostatic or bactericidal depending on the class (Shi et al., 2023).

- **Inhibitors of nucleic acid synthesis:**

  These interfere with DNA replication or RNA transcription. Examples include fluoroquinolones and rifampicin, and they are typically bactericidal (IJCSRR, 2024).

  Antimetabolites:

  These inhibit essential metabolic pathways such as folic acid synthesis.
  Sulfonamides and trimethoprim are the main examples, usually acting as bacteriostatic agents (IJCSRR, 2024).

  Cell membrane disruptors:

  They damage bacterial membrane integrity, causing leakage of cellular contents. Polymyxins are the main representatives and are bactericidal (Recent reviews, 2024).

2.2. Classification based on spectrum of activity

Broad-spectrum antibiotics: active against a wide range of Gram-positive and Gram-negative bacteria, used when the pathogen is unknown (WHO, 2024).

Narrow-spectrum antibiotics: target specific bacterial groups and are preferred to reduce selective pressure and resistance development (WHO, 2024).

2.3. Classification based on chemical structure

Antibiotics can also be grouped into structural families such as β-lactams, tetracyclines, macrolides, aminoglycosides, fluoroquinolones, and sulfonamides. These groups often share similar mechanisms of action, and more than 200 antibiotics can be classified into major structural families (Butler & Capon, 2025; Shi et al., 2023).

2.4. Modern clinical classification (AWaRe – WHO)

The World Health Organization introduced the AWaRe classification to guide rational antibiotic use:

- Access: first-line antibiotics with lower resistance potential
- Watch: antibiotics with higher resistance risk, requiring careful use
- Reserve: last-resort antibiotics for multidrug-resistant infections

This system is widely used in antimicrobial stewardship programs to reduce antibiotic resistance globally (WHO, 2023–2024)

3) Routes of Administration of Antibiotics

The route of administration is a key factor that influences the effectiveness, bioavailability, and tissue distribution of antibiotics in the body (Brunton et al., 2023; WHO, 2024). The choice of the appropriate route depends on the severity of infection, the pharmacokinetic properties of the drug, and the clinical condition of the patient.

3.1. Oral route:

The oral route is the most commonly used due to its convenience and suitability for mild to moderate infections. Antibiotics administered orally must be stable in the gastrointestinal tract and efficiently absorbed into the bloodstream. Common examples include amoxicillin, tetracycline, and ciprofloxacin. However, their bioavailability may be affected by factors such as gastric pH, food intake, and intestinal absorption (Brunton et al., 2023).

3.2. Intravenous (IV) route:

Intravenous administration allows direct delivery of antibiotics into the bloodstream, ensuring rapid distribution and immediate therapeutic effect. It is mainly reserved for severe or life-threatening infections such as sepsis. This route provides 100% bioavailability and precise control of plasma drug concentration (WHO, 2024).

3.3. Intramuscular (IM) route:

The intramuscular route enables gradual absorption of antibiotics into systemic circulation and is used when oral administration is not feasible. Examples include certain penicillins and aminoglycosides. However, it may be associated with local pain at the injection site (Brunton et al., 2023).

3.4. Topical route:

Topical antibiotics are applied directly to the skin or mucous membranes for the treatment of localized infections. They are widely used in dermatological and ophthalmological conditions in the form of creams, ointments, or eye drops (Ventola, 2023).

3.5. Inhalation route:

Inhaled antibiotics are used in respiratory infections, particularly in chronic conditions such as cystic fibrosis. This route allows high local drug concentration at the site of infection while minimizing systemic side effects (WHO, 2024).

3.6. Other specialized routes:

In specific clinical situations, alternative routes such as rectal, subcutaneous, or intrathecal administration may be used, particularly when conventional routes are not suitable or when targeting the central nervous system is required (Brunton et al., 2023).

III

Antimicrobial Resistance

Global Impact of Antimicrobial Resistance

Antimicrobial resistance (AMR) is currently recognized as one of the most serious global public health threats, compromising the effectiveness of antibiotics and making many bacterial infections increasingly difficult to treat, (Salam et al., 2023).

Recent global estimates indicate that approximately 4.95 million deaths were associated with bacterial antimicrobial resistance in 2019, highlighting the enormous burden of this problem worldwide (Albukhari, 2025).

Among these cases, about 1.27 million deaths were directly attributable to antimicrobial-resistant infections, demonstrating that resistance itself plays a critical role in mortality outcomes. The global burden of antimicrobial resistance is now considered comparable to or even greater than that of major infectious diseases such as HIV/AIDS and malaria. When deaths associated with antimicrobial resistance are considered collectively, AMR could rank among the leading causes of death worldwide, following only major cardiovascular diseases (Murray et al., 2022).

The impact of antimicrobial resistance is not evenly distributed across the world; sub-Saharan Africa and other low- and middle-income regions experience the highest mortality rates, reflecting disparities in healthcare access, surveillance, and antibiotic stewardship. In addition, certain infectious syndromes contribute disproportionately to the burden of resistance-related deaths, particularly lower respiratory infections, bloodstream infections, and intra-abdominal infections, which together account for a large proportion of AMR-associated mortality (Nathan & Cars, 2014).

Several bacterial pathogens are responsible for the majority of these deaths, notably Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa, which are recognized as major drivers of antimicrobial resistance worldwide. Furthermore, projections suggest that if no effective global interventions are implemented, antimicrobial resistance could cause up to 10 million deaths annually by 2050, underscoring the urgent need for coordinated strategies to control its spread (Ntim et al., 2025).

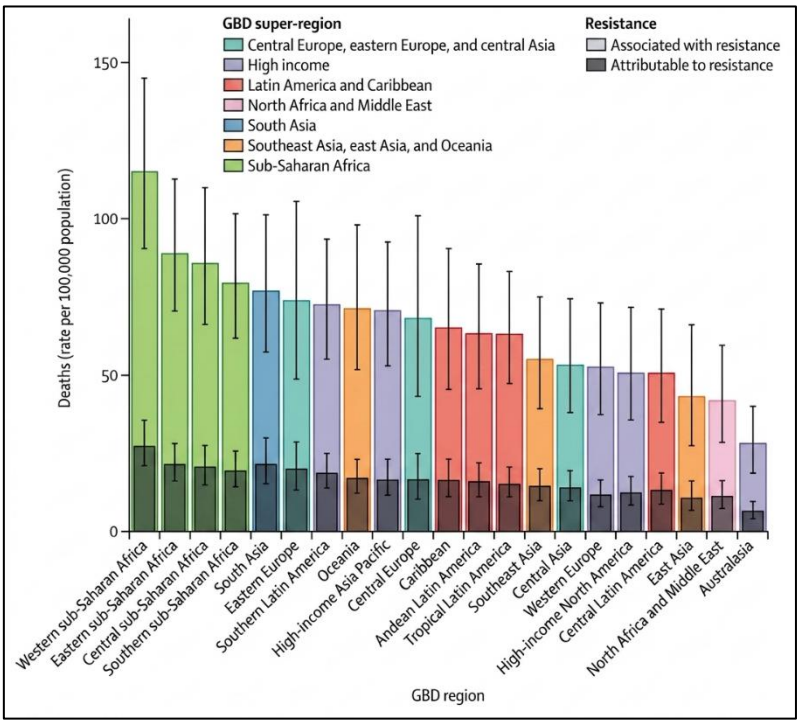

Figure 03 : All-age rate of deaths attributable to and associated with bacterial antimicrobial resistance by GBD. (Albukhari, 2025).

1) Causes of Antibiotic Resistance

The increasing spread of antimicrobial resistance is driven by a combination of scientific, economic, and behavioral factors. Following the end of the golden era of antibiotic discovery between the 1930s and 1960s, the development of new antibiotics has significantly slowed down due to scientific challenges and high research costs, limiting the availability of effective treatments against resistant pathogens, as described by (Salam et al., 2023). In addition, many pharmaceutical companies have reduced their investment in antibiotic research, as these drugs are typically used for short durations and are less profitable compared to medications for chronic diseases. The misuse and overuse of antibiotics in human medicine, including inappropriate prescriptions and self-medication, further contribute to the emergence of resistant bacterial strains by increasing selective pressure (Muteeb et al., 2023). Moreover, the extensive use of antibiotics in agriculture and animal production, particularly for growth promotion and disease prevention, accelerates the development and dissemination of resistance. The circulation of substandard or counterfeit drugs also plays a role in reducing treatment efficacy, thereby facilitating the survival and adaptation of resistant bacteria. Finally, the global spread of resistant microorganisms is enhanced by factors such as international travel, population mobility, and inadequate infection control measures, making antimicrobial resistance a rapidly expanding worldwide problem (Singh et al., 2024)

2) Mechanisms of Bacterial Resistance to Antibiotics

Bacteria have developed a wide range of mechanisms that enable them to survive exposure to antibiotics. These mechanisms include enzymatic inactivation of antibiotics, modification of the antibiotic molecule, alteration of the drug target, decreased permeability of the bacterial membrane, and active efflux of antibiotics from the cell. Among these mechanisms, enzymatic resistance represents one of the most important strategies used by bacteria to counteract antibiotic pressure, contributing significantly to the emergence and spread of resistant strains (Vivekananda et al., 2025). Enzymatic inactivation may occur through several biochemical processes such as hydrolysis, group transfer reactions, and redox reactions, all of which lead to the loss of antibiotic activity. One of the most well-known examples is the production of β-lactamases, enzymes capable of hydrolyzing the β-lactam ring found in penicillin and related antibiotics, thereby neutralizing their antibacterial effect. Interestingly, these enzymes are believed to have existed in nature before the clinical use of penicillin, suggesting that antibiotic production and resistance mechanisms have co-evolved in microbial ecosystems. In

addition to enzymatic degradation, bacteria can also develop resistance through structural modifications of antibiotic targets, particularly alterations in penicillin-binding proteins (PBPs), which reduce the affinity of β-lactam antibiotics for their targets (Liu et al., 2018) Furthermore, bacteria may chemically modify antibiotics through processes such as acetylation, phosphorylation, or adenylation, which inactivate the drug molecule and prevent its interaction with the bacterial target . The spread of resistance genes through horizontal gene transfer and mobile genetic elements, including plasmids carrying enzymes such as TEM, SHV, and extended-spectrum β-lactamases (ESBLs), further accelerates the dissemination of resistance among bacterial populations (Schwarz, Cloeckaert, & Roberts, 2006)

3) Strategies to Combat Antimicrobial Resistance

Several strategies have been proposed to combat antimicrobial resistance and overcome the limitations of conventional antibiotic therapies. These approaches include the use of combination therapy, targeting resistance mechanisms, and the development of alternative therapeutic strategies. Combination therapy, which involves the use of two or more antibiotics, has proven effective in enhancing antibacterial activity and reducing the likelihood of resistance development in addition, the use of β-lactamase inhibitors in combination with β-lactam antibiotics has been shown to restore the efficacy of these drugs against resistant bacterial strains. Targeting resistance-related enzymes also represents a promising strategy, as inhibiting these enzymes can prevent the inactivation of antibiotics (Muruga Iyan & Iskandar, 2022). Furthermore, alternative therapeutic approaches such as antimicrobial peptides (AMPs) have demonstrated strong antibacterial activity and the ability to inhibit biofilm formation. Bacteriophage therapy has also emerged as a highly specific strategy that utilizes viruses to selectively infect and eliminate bacterial pathogens. The use of phytochemicals and natural compounds offers another promising avenue, as these substances can act either as direct antimicrobial agents or as adjuvants to enhance antibiotic activity. Additionally, advanced genetic tools such as CRISPR-Cas systems provide innovative methods to target and eliminate antibiotic resistance genes within bacterial populations (Arora, 2025). Other strategies include anti-virulence approaches, which aim to reduce bacterial pathogenicity without directly killing the bacteria, as well as quorum sensing inhibition, which interferes with bacterial communication and biofilm formation. Drug delivery systems, including liposomes and other carriers, have also been developed to improve antibiotic targeting and effectiveness. Among these emerging approaches,

nanotechnology has gained considerable attention, as nanoparticles can act both as antimicrobial agents and as efficient drug delivery systems, enhancing antibiotic activity through multiple mechanisms and reducing the likelihood of resistance development (Thomas et al., 2026).

4) Role of Nanoparticles in Combating Antibiotic Resistance

Nanoparticles exhibit unique physicochemical properties, including their small size and high surface-to-volume ratio, which enable them to interact efficiently with bacterial cells. One of the primary mechanisms underlying their antibacterial activity is the generation of reactive oxygen species (ROS), which induce oxidative stress within bacterial cells and lead to damage of essential biomolecules. These reactive species, such as hydrogen peroxide (H_2O_2), can penetrate the bacterial membrane and disrupt intracellular components. In addition, ROS contribute to lipid peroxidation, resulting in loss of membrane integrity and leakage of cellular contents. Nanoparticles can also release metal ions, such as Ag^+, which enter bacterial cells and interfere with vital biological processes. These ions interact with functional groups in proteins, particularly sulfhydryl (–SH) groups, leading to enzyme inactivation and disruption of metabolic pathways (Parmar & Sharma, 2022). Furthermore, nanoparticles can induce damage to bacterial DNA, inhibiting its replication and transcription. They also affect cellular respiration, leading to reduced ATP production and eventual cell death. Due to their nanoscale size, particularly in the range of 1–12 nm, nanoparticles are capable of penetrating the bacterial cell wall and interacting directly with intracellular targets. Inside the cell, they can simultaneously affect multiple components, including membranes, proteins, and genetic material. Moreover, nanoparticles can alter gene expression and protein synthesis, further impairing bacterial survival. Importantly, the ability of nanoparticles to target multiple sites within bacterial cells makes it extremely difficult for bacteria to develop resistance against them (Kalu et al., 2026).

IV

Nanotechnology

IV- Nanotechnology

Nanotechnology is a rapidly developing interdisciplinary field that focuses on the manipulation and control of matter at the nanoscale, typically ranging between 1 and 100 nm. At this scale, materials exhibit unique physical and chemical properties that are significantly different from their bulk counterparts, mainly due to the increased surface-to-volume ratio and the emergence of quantum effects (Iravani, 2014; Rai et al., 2009).These nanoscale properties result in enhanced reactivity, improved electrical and optical behavior, and increased biological activity, making nanomaterials highly suitable for a wide range of applications. In recent years, nanotechnology has gained considerable attention in various scientific fields, particularly in medicine, biotechnology, environmental science, and materials engineering (Zhang et al., 2010; Slavin et al., 2017). In the biomedical domain, nanomaterials have shown great potential in drug delivery systems, diagnostic tools, cancer therapy, and antimicrobial treatments. Their small size allows them to penetrate biological membranes and interact directly with cellular components, which enhances their therapeutic efficiency (Pelgrift & Friedman, 2013; Huh & Kwon, 2011). Among the different types of nanomaterials, metallic nanoparticles have attracted significant interest due to their strong antimicrobial properties. Silver nanoparticles (AgNPs), zinc oxide nanoparticles (ZnONPs), copper nanoparticles (CuO NPs), and magnesium-based nanoparticles have demonstrated effective activity against a wide range of pathogenic microorganisms, including bacteria, fungi, and viruses (Gudkov et al., 2024; Ren et al., 2009).The antimicrobial activity of these nanoparticles is mainly attributed to several mechanisms, such as the generation of reactive oxygen species (ROS), disruption of microbial cell membranes, interaction with intracellular proteins and enzymes, and damage to genetic material. These multiple mechanisms make nanoparticles particularly effective against antibiotic-resistant bacteria (Slavin et al., 2017; Rai et al., 2009). Furthermore, recent studies have emphasized the importance of developing environmentally friendly synthesis approaches for nanoparticles. Green synthesis methods, which utilize biological agents such as plant extracts and microorganisms, have emerged as promising alternatives to conventional chemical methods due to their low toxicity, cost-effectiveness, and sustainability (Iravani, 2014; Ahmed et al., 2016).

Therefore, nanotechnology represents a promising strategy for the development of innovative antimicrobial agents capable of overcoming the limitations of traditional antibiotics and addressing the growing problem of microbial resistance.

1) General Properties of Nanoparticles

Nanoparticles exhibit unique physicochemical properties that clearly distinguish them from their bulk counterparts. These properties arise mainly from their extremely small size and high surface area, which significantly influence their chemical reactivity, optical behavior, and biological interactions (Rai et al., 2009; Slavin et al., 2017). One of the most important characteristics of nanoparticles is their high surface-to-volume ratio. As particle size decreases to the nanoscale, a large proportion of atoms becomes exposed at the surface, leading to increased surface energy and enhanced chemical reactivity. This property allows nanoparticles to interact more efficiently with surrounding molecules, including biological macromolecules such as proteins, enzymes, and nucleic acids (Gudkov et al., 2024; Zhang et al., 2010). The high surface-to-volume ratio also plays a crucial role in antimicrobial activity. Nanoparticles can easily attach to microbial cell membranes, penetrate the cell wall, and disrupt essential cellular processes. This increased interaction enhances their ability to inhibit or kill microorganisms, making them highly effective against pathogenic bacteria (Slavin et al., 2017; Ren et al., 2009). In addition to surface properties, nanoparticles exhibit unique optical properties that depend on their size, shape, and composition. For example, metal nanoparticles such as silver and gold display surface plasmon resonance, which contributes to their applications in biosensing and imaging (Rai et al., 2009). Nanoparticles also show enhanced chemical reactivity compared to bulk materials. This increased reactivity is due to the presence of unsaturated atoms on the surface, which can easily participate in redox reactions and generate reactive oxygen species (ROS). These ROS play an important role in antimicrobial mechanisms by inducing oxidative stress in microbial cells (Gudkov et al., 2024; Slavin et al., 2017) Furthermore, nanoparticles possess significant biological activity, as their small size allows them to cross biological barriers and interact directly with intracellular components. This property makes them particularly useful in biomedical applications such as drug delivery and antimicrobial therapy (Huh & Kwon, 2011; Pelgrift & Friedman, 2013). Another important property is the surface charge (zeta potential), which influences the stability of nanoparticles in suspension and their interaction with biological membranes. Positively charged nanoparticles tend to interact more strongly with negatively charged bacterial cell walls, enhancing their antimicrobial effectiveness (Zhang et al., 2010). Overall, the unique physicochemical properties of nanoparticles make them promising candidates for a wide range of applications, particularly in the development of novel antimicrobial agents capable of overcoming bacterial resistance.

2) Green Chemistry in Nanotechnology

The concept of green chemistry has become increasingly important in nanotechnology, particularly in the synthesis of metal nanoparticles. Green synthesis methods aim to reduce the use of hazardous chemicals and minimize environmental pollution while producing stable and biologically active nanoparticles. Biological materials such as plant extracts and microorganisms can act as natural reducing and stabilizing agents during nanoparticle synthesis. These environmentally friendly approaches are widely investigated for producing nanoparticles with biomedical applications (Iravani, 2014). Recent studies also highlighted the importance of green nanoparticles in medicine and antimicrobial applications, where copper, zinc, and magnesium nanoparticles exhibit promising biological activities (Derouiche et al., 2024).

3) Copper Nanoparticles

Copper nanoparticles (CuO NPs) are metallic nanostructures that have attracted significant scientific interest due to their unique physicochemical properties and wide range of applications in various fields, particularly in biomedicine and antimicrobial therapy. These nanoparticles exhibit remarkable catalytic, electrical, optical, and antimicrobial properties, which make them suitable for numerous industrial and biomedical applications (Ren et al., 2009; Gudkov et al., 2024). One of the main advantages of copper nanoparticles is their relatively low cost and high availability compared to noble metal nanoparticles such as silver and gold. This makes CuO npsa promising alternative for large-scale applications, especially in developing countries where cost-effective solutions are required (Singh et al., 2018; Banerjee et al., 2024). Copper nanoparticles have demonstrated strong antibacterial activity against a wide range of pathogenic microorganisms, including both Gram-positive and Gram-negative bacteria such as Escherichia coli and Staphylococcus aureus. Their effectiveness is attributed to multiple mechanisms of action that target different cellular components, making it difficult for bacteria to develop resistance (Ren et al., 2009; Slavin et al., 2017).

4) Mechanisms of Antibactrial Activity of Copper Nanoparticles

One of the primary antibacterial mechanisms of CuO npsis the generation of reactive oxygen species (ROS), which induce oxidative stress within bacterial cells. This oxidative stress can damage cellular components such as lipids, proteins, and DNA, ultimately leading to cell death. In addition, copper nanoparticles can directly interact with the bacterial cell membrane, causing structural damage, increased permeability, and leakage of intracellular contents (Gudkov et al., 2024; Rai et al., 2009). Furthermore,

CuO npscan release copper ions (Cu^{2+}), which play a crucial role in their antimicrobial activity. These ions can bind to proteins and enzymes, disrupting their normal function and interfering with essential metabolic processes within the bacterial cell. Copper ions may also interact with nucleic acids, leading to DNA damage and inhibition of replication (Ren et al., 2009). Another important feature of copper nanoparticles is their ability to inhibit biofilm formation. Biofilms are structured communities of bacteria that are highly resistant to antibiotics. By disrupting biofilm formation, CuO npsenhance their effectiveness against persistent infections (Slavin et al., 2017). In addition to their antimicrobial properties, copper nanoparticles are also widely studied in green synthesis approaches. Eco-friendly methods using plant extracts and microorganisms have been developed to produce CuO npswith improved stability and reduced toxicity, making them more suitable for biomedical applications (Iravani, 2014; Ahmed et al., 2016). Despite their promising potential, the use of copper nanoparticles requires careful consideration of their potential toxicity and environmental impact. Therefore, further studies are needed to optimize their synthesis, dosage, and application to ensure safe and effective use in medical and industrial fields (Gudkov et al., 2024).

Second part

Experimental part

I

Materials and Methods

1) Microorganisms

The bacterial strains used in this study were obtained in October 2025 from the Pasteur Institute of Algiers (Algeria). Four reference strains were selected based on their clinical relevance and standard identification codes: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *Streptococcus pneumoniae* ATCC 49619. Following their acquisition, the bacterial strains were transported under appropriate conditions using a suitable transport medium to preserve their viability and integrity. Upon arrival, they were maintained and stored in the microbiology laboratory of El Nour, located in the city center of El Oued, under optimal laboratory conditions to ensure their stability and suitability for subsequent experimental procedures.

2) Synthesis of Copper Nanoparticles (CuO NPs)

Copper oxide nanoparticles (CuO NPs) were synthesized using a chemical reduction followed by a precipitation method (Ahlem et al., 2025). Briefly, 2 g of copper precursor was dissolved in 200 mL of distilled water under continuous stirring. Separately, 2 g of ascorbic acid was dissolved in 10 mL of distilled water. The copper solution was maintained under constant stirring at 40 °C with a 1500 rpm. Subsequently, ascorbic acid solution was added to the reaction mixture. After a few minutes, a color change from blue to a characteristic copper-red was observed, indicating the formation of copper nanoparticles (Cu NPs) as a result of the reduction process .The pH of the solution was then adjusted and maintained in an alkaline range, promoting the oxidation of copper nanoparticles and the formation of copper nanoparticles (CuO NPs).After 4 hours of reaction, the samples were subjected to centrifugation for 20 minutes. The obtained precipitate was washed twice with distilled water and once with ethanol to remove impurities, and then dried in an electric oven at 70 °C to obtain the final nanoparticle product.

2.1. DNA Extraction Method

Bacterial DNA was extracted using a simple heat lysis method (Dashti et al., 2009). Briefly, 1 mL of bacterial culture was transferred into a sterile microcentrifuge tube and centrifuged at 10,000 rpm for 5 minutes to obtain a bacterial pellet. The supernatant was carefully discarded, and the pellet was washed with 1 mL sterile distilled water. The suspension was vortexed and centrifuged again under the same conditions, after which the supernatant was discarded. Subsequently, 100 µL of sterile distilled water was added to the pellet and vortexed to ensure complete resuspension.

The mixture was then subjected to heat treatment at 95–100 °C for 10 minutes to lyse the bacterial cells and release genomic DNA. Immediately after heating, the tubes were placed on ice for 5 minutes to enhance DNA stability. The lysate was then centrifuged at 12,000 rpm for 5 minutes to remove cellular debris. The supernatant containing the extracted DNA was carefully transferred into a new sterile microcentrifuge tube. Finally, the extracted DNA was stored at −20 °C until further use.

2.2. Synthesis of CAD nanocomposite

Coupling between copper nanoparticles (CuO NPs) and bacterial DNA was performed to obtain a CuNP–DNA nanocomposite by using method of (Kim et al., 2019) . Briefly, 1000 µL of the extracted DNA solution was added to 50 mL of distilled water containing 500 mg of CuO NPs.The mixture was thoroughly homogenized and maintained under continuous stirring at 500 rpm to ensure efficient interaction between the nanoparticles and DNA molecules. The reaction was allowed to proceed for 4 hours at room temperature. After incubation, the samples were centrifuged to separate the formed nanocomposite from the supernatant. The resulting CuNP–DNA nanocomposite was then washed twice with distilled water to remove any unbound or loosely attached materials. In this study, three experimental samples were prepared: a sample of CuO npscoupled with *Staphylococcus aureus* DNA, a sample coupled with *Escherichia coli* DNA, and a control sample containing CuO npsalone without DNA. Finally, all obtained samples were stored at 4 °C for further characterization and analysis.

2.3. CAD Characterization

A variety of techniques were employed to elucidate the characteristics of the green-synthesized copper nanoparticles (CuO NPs). Ultraviolet–visible (UV–Vis) spectroscopy was used to confirm nanoparticle synthesis using a PG Instruments T80 spectrometer. The characterization was performed on three samples: CuO NPs–*E. coli* (200–800 nm), Cu NPs–*S. aureus* (260–340 nm), and CuO nps(260–340 nm), with distilled water used as a reference blank. Fourier-transform infrared (FT-IR) spectroscopy (Agilent Technologies) was carried out to identify the involvement of bioactive phytochemicals from the plant extract in the reduction and stabilization of the nanoparticles. The morphology and size distribution of the nanoparticles were analyzed using Scanning Electron Microscopy (SEM) (Zeiss). The crystalline structure and grain size were determined by X-ray diffraction (XRD) analysis using a diffractometer (PROTO® AXRD Benchtop), within a 2θ range of 10–80°, employing Cu Kα radiation (λ = 1.5406 Å).

3) Biological activities

3.1. Antioxidant Activity

For the FRAP assay, the principle is based on the ability of antioxidant compounds present in the sample to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) in a reaction medium, leading to the formation of a colored complex whose intensity reflects the reducing power of the sample. The procedure involves taking 500 µl of the sample, adding 1.25 mL of buffer solution (0.2 M, pH 6.6), and incubating for 20 minutes in a water bath at 50°C. After incubation, 1.25 ml of aqueous TCA solution (10%) is added to stop the reaction, followed by centrifugation at 3000 rpm for 5 minutes. Then, 1.25 ml of the supernatant is mixed with 1.25 ml of distilled water and 250 µl of $FeCl_3$ (0.1%). The absorbance is read at 700 nm against a blank. The results are expressed as IC_{50} after calculating the inhibition percentage values according to (Oyaizu 1986).

$$IP(\%) = 100 - \left(\frac{ODcontrôle}{ODsample}\right) \times 100$$

3.2. Anti-inflammatory Activity

The principle of this assay is based on the ability of the tested sample to inhibit protein denaturation, which is commonly associated with inflammatory processes. In this method, bovine serum albumin (BSA) is used as a model protein, and its structural denaturation is induced by heat and acidic conditions. The presence of anti-inflammatory compounds in the sample can stabilize the protein structure and prevent aggregation, thereby reducing turbidity, which is measured spectrophotometrically.
The procedure involves adding different concentrations (10–100 µg/mL) of the sample to a 1% bovine serum albumin (BSA) solution, followed by incubation for 30 minutes at room temperature. The pH of the solution is then adjusted to 2 by dropwise addition of concentrated HCl. After incubation, the mixture is heated at 72°C for 30 minutes, and all tubes are subsequently cooled for 10 minutes. The turbidity of the solution is measured at a wavelength of 660 nm, with diclofenac used as the standard (Vennila et al., 2018). The results are expressed as IC_{50} values after calculating the percentage inhibition.

$$IP(\%) = \left(\frac{A\ contrôle - A\ sample}{A\ controle}\right) \times 100$$

3.3. Antibacterial Activity

The antibacterial activity of copper nanoparticles (CuO NPs) was evaluated against *Escherichia coli (ATCC 25922), Streptococcus pneumoniae (ATCC 49619), Staphylococcus aureus* (ATCC 25923), and *Pseudomonas aeruginosa* (ATCC 27853) using the standard disc diffusion assay .Bacterial strains were cultured in nutrient broth for 24 hours at 37 °C. Subsequently, active cultures were inoculated onto Mueller–Hinton agar plates using sterile swabs (Moroda et al., 2025) . Sterile discs were then impregnated with 20 µL of copper nanoparticle solution (CuO NPs), as well as nanoparticles associated with E. coli and Staphylococcus aureus (CuO NPs–*E. coli* and CuO NPs–*S.aureus*), in addition to nanoparticles alone (NPs only), all dissolved in dissolved in dimethyl sulfoxide (DMSO) at concentrations of 5, 10, 20 and 40 and mg/mL .The impregnated discs were placed onto the surface of the agar plates and incubated at 37 °C. Gentamicin and ciprofloxacin were used as reference antibiotics, while for Streptococcus pneumoniae, a third antibiotic, levofloxacin, was also included. The antibacterial activity was assessed by measuring the diameters of the inhibition zones around the discs**.**

4) IN SILICO study

Molecular Docking

The molecular docking study of Copper(I) oxide was performed to evaluate its potential interaction with bacterial DNA gyrase and topoisomerase-related proteins. The cubic structure of the Cu_2O nanoparticle was designed according to the XRD results, with an estimated size of 8.9 nm, using BIOVIA Materials Studio software. The docking analysis was carried out using AutoDock. Four bacterial target receptors were selected from the Protein Data Bank (PDB), including topoisomerase ATPase inhibitor of *Staphylococcus aureus* (PDB ID: 3TTZ), DNA gyrase of *Streptococcus pneumoniae* (PDB ID: 4MOT), DNA gyrase B of *Escherichia coli* (PDB ID: 5L3J), and DNA gyrase B ATPase of *Pseudomonas aeruginosa* (PDB ID: 8BN6). The binding affinity between the Cu_2O nanoparticle and the selected receptors was evaluated based on the calculated binding energy values (kcal/mol).

II

Results

1) biosynthesis and Characterization of copper nanoparticles

The current investigation demonstrates that the incorporation of ascorbic acid into the copper sulfate solution induced a distinct chromatic transformation from light blue to copper-red, thereby indicating the successful synthesis of copper nanoparticles, while the color changed to dark green upon their integration with

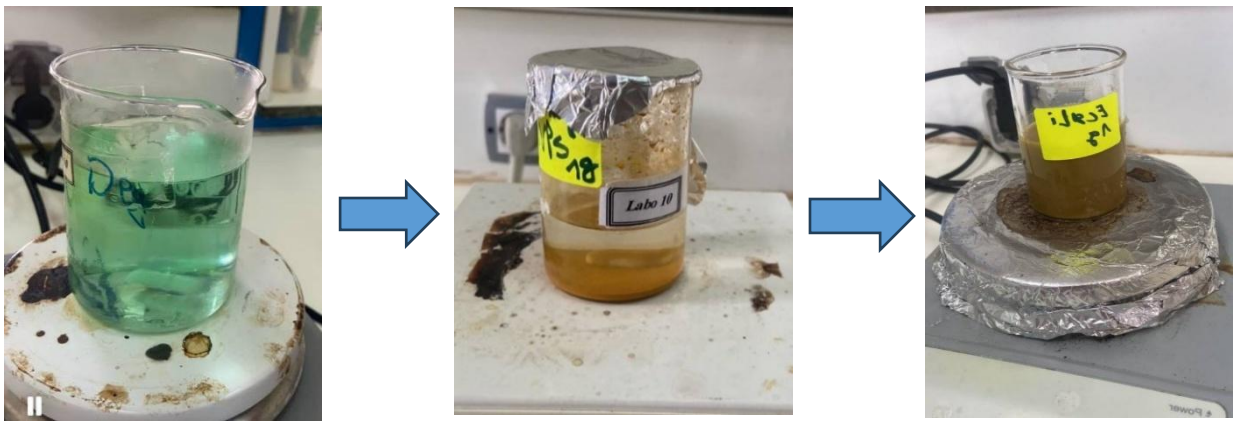

Figure 04: Color change during the formation of copper nanoparticles

1.1. Infrared analysis

Figures 05 showed photographs of the FTIR spectroscopy recorded for CuO nps, CuO nps-S.aureus and CuO nps-E.coli *(* 06, 07) respectively. The presence of the band at 600cm-^{1} in both observed samples is indicative of the characteristic bands associated with the Cu-O bond.

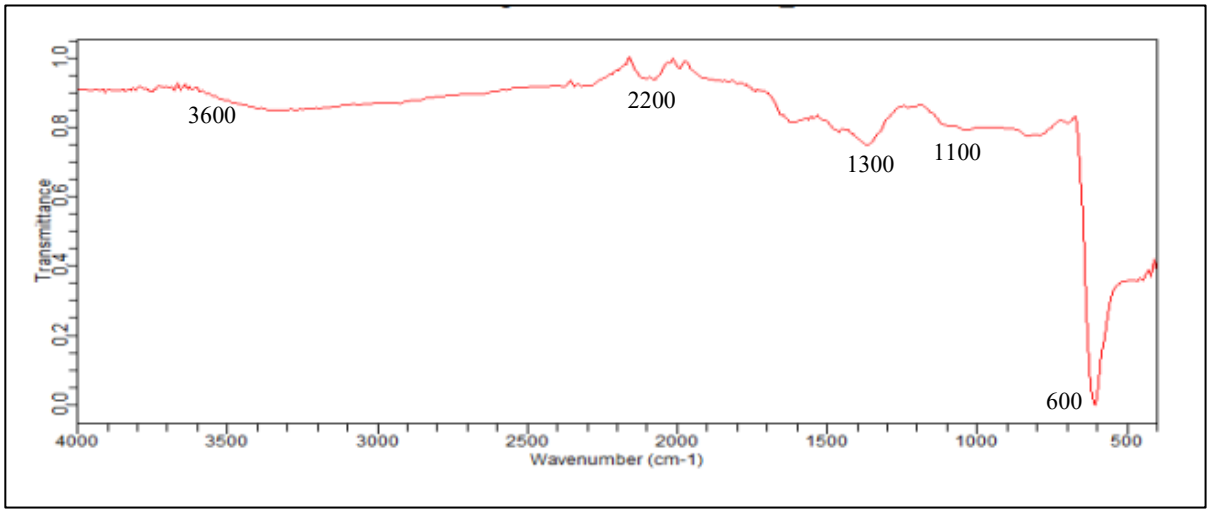

Figure 05: FTIR spectrum of CuO nps

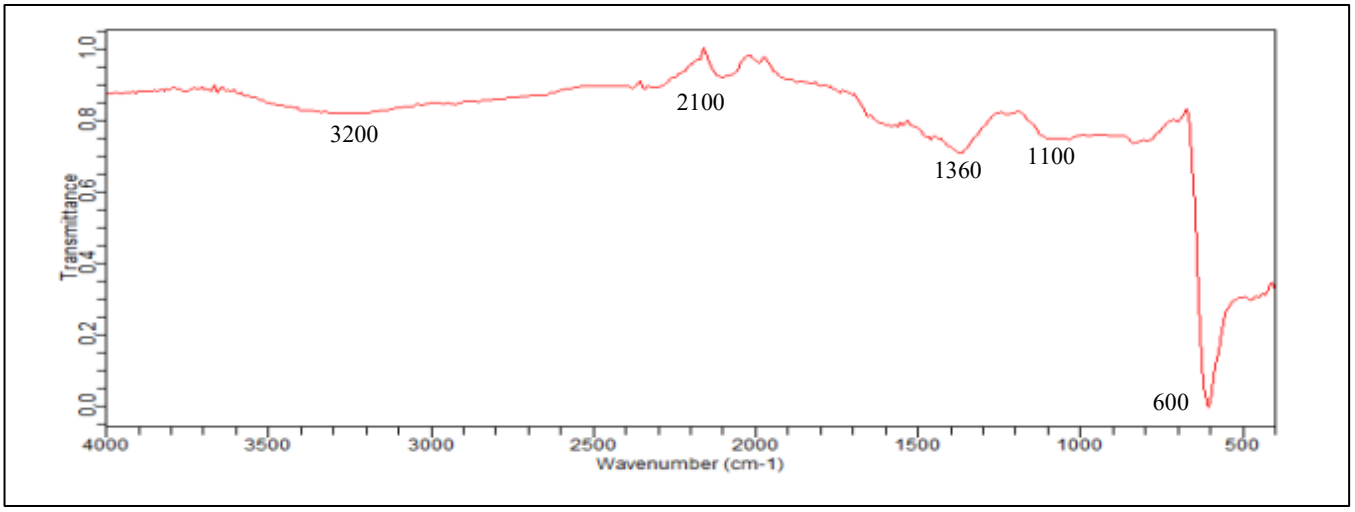

Figure 06: FTIR spectrum of CuO nps-E.coli

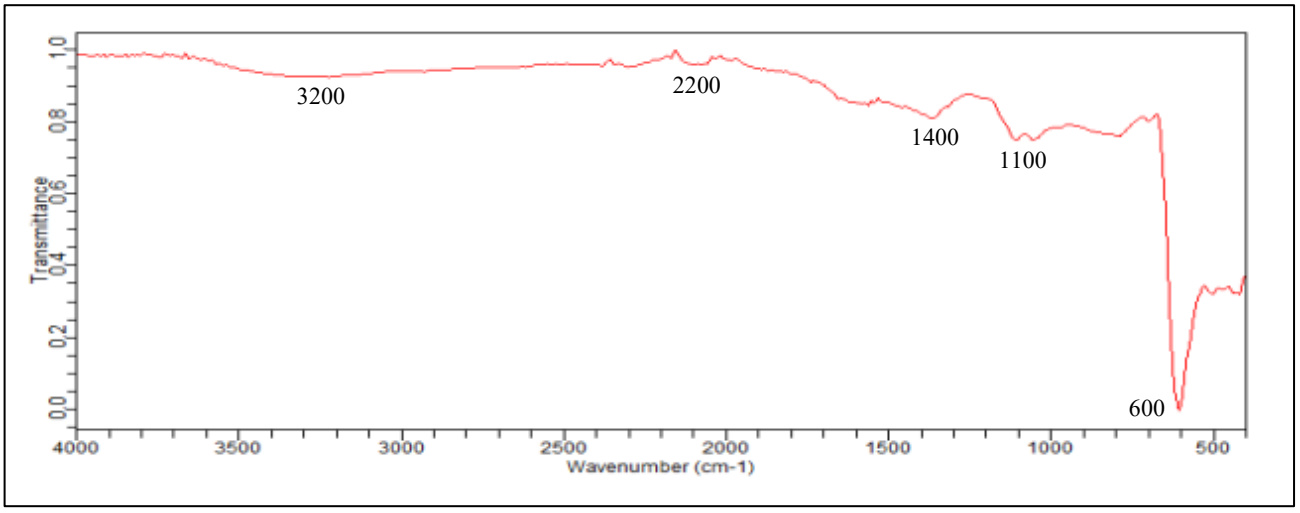

Figure 07: FTIR spectrum of SCuO nps-S.aureus

1.2. UV-VIS spectroscopy

The UV-Vis spectrum of CuO nps08 synthesized from E. coli and S. aureus absorption spectra is shown in figure 09, 10 and 6, respectively. The production of CuO npswas confirmed by a maximum absorption at 250 nm and 300 nm for CuO nps, CuO nps-E.coli and CuO NPs-*S. aureus*, respectively

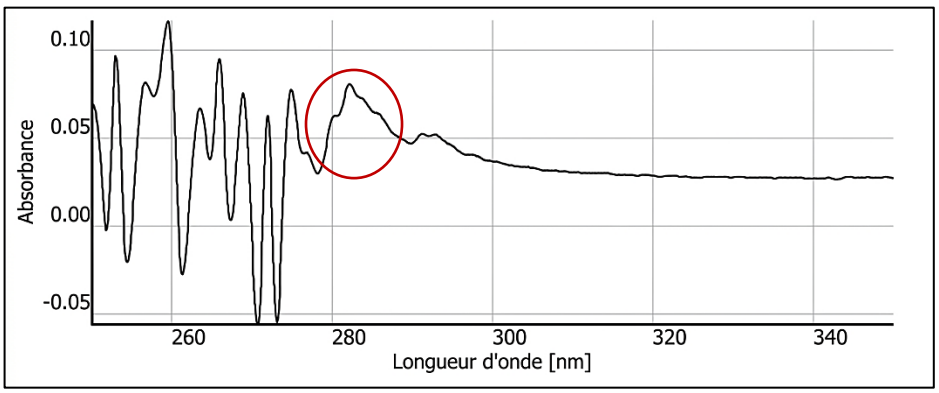

Figure 08: UV-Vis spectrum of -CuO NPs

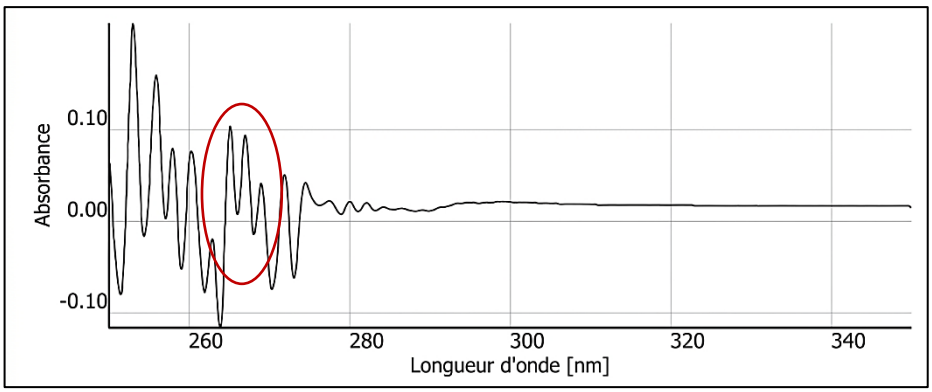

Figure 09: UV-Vis spectrum of -CuO nps-E.coli

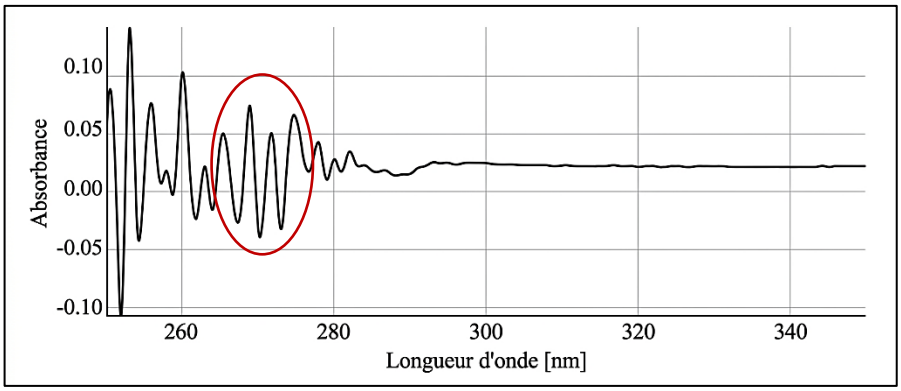

Figure 10: UV-Vis spectrum of CuO nps-S.aureus

1.3. X- ray Diffraction analysis

The X-ray diffraction (XRD) pattern of Cu_2O exhibits diffraction peaks located at $2\theta \approx 29.00°$, 36.42°, 42.29°, 61.36°, and 73.72°, which are indexed to the (110), (111), (200), (220), and (311) crystallographic planes of Cu_2O, respectively. These results are in good agreement with the standard JCPDS card No. 05-0667, confirming the formation of a cubic cuprite structure. The absence of additional peaks related to metallic copper (Cu) or copper oxide (CuO) indicates that the sample is single-phase with good crystallinity, The estimated crystallite size was found to be ~9.1 nm. FIGURE (11)

The XRD pattern of Sample 2 (Cu NPs-*E.coli*) reveals the coexistence of two crystalline phases. The diffraction peaks observed at $2\theta \approx 29.49°$, 36.42°, 42.29°, 61.36°, and 73.72° are attributed to the Cu_2O phase (JCPDS No. 05-0667). In addition, the appearance of extra peaks at 43.44°, 50.60°, and 74.25° corresponds to the (111), (200), and (220) planes of metallic copper (JCPDS No. 04-0836), indicating the formation of Cu nanoparticles. The absence of characteristic CuO peaks suggests that this phase is not formed. These results confirm the formation of a biphasic Cu_2O/Cu system due to partial reduction of Cu^{2+} ions , The calculated crystallite size was ~8.1 nm, FIGURE (12)

The XRD pattern of (Cu NPs-*S.aureus*) shows similar peak positions to those of Sample 2, confirming the coexistence of Cu_2O and metallic Cu phases. However, a noticeable increase in the intensity of the peaks associated with metallic copper is observed, suggesting enhanced crystallinity and/or a higher fraction of the Cu^0 phase. This behavior indicates a more advanced reduction process of Cu^{2+} species i the crystallite size calculated from the same diffraction peak increased to ~11.8 nm nto Cu^0, leading to the formation of more abundant or better-crystallized copper nanoparticles. FIGURE (13)

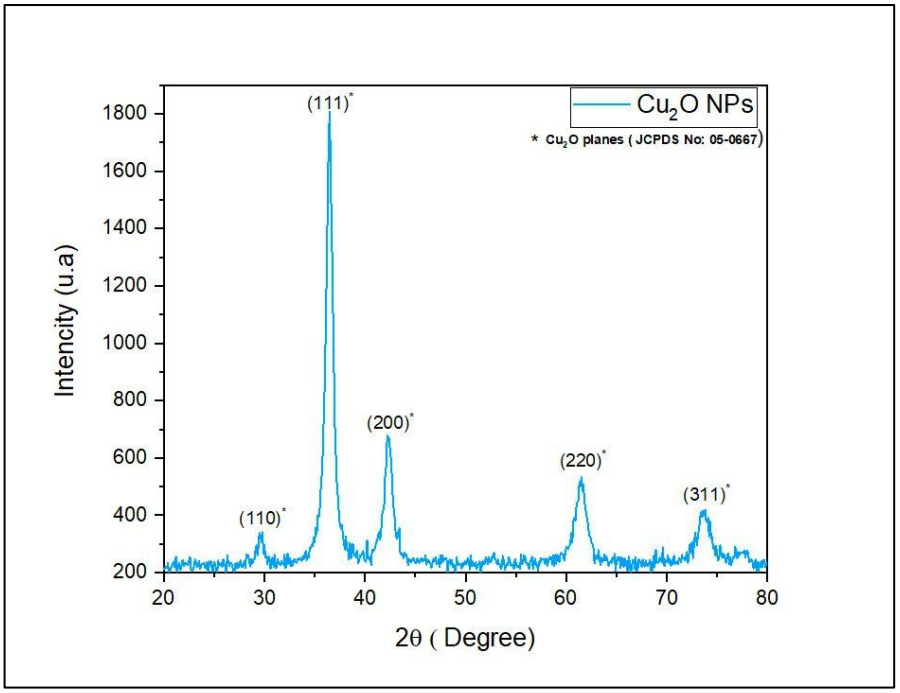

Figure 11: XRD patterns of CuO nps(Cu_2O)

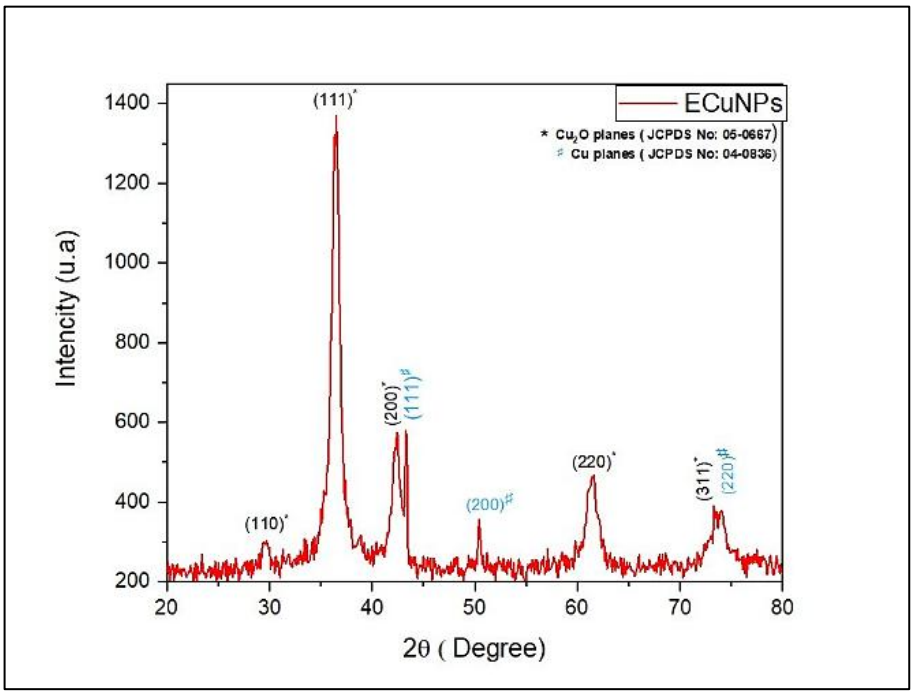

Figure 12: XRD patterns CuO nps(Cu_2O)-E.coli

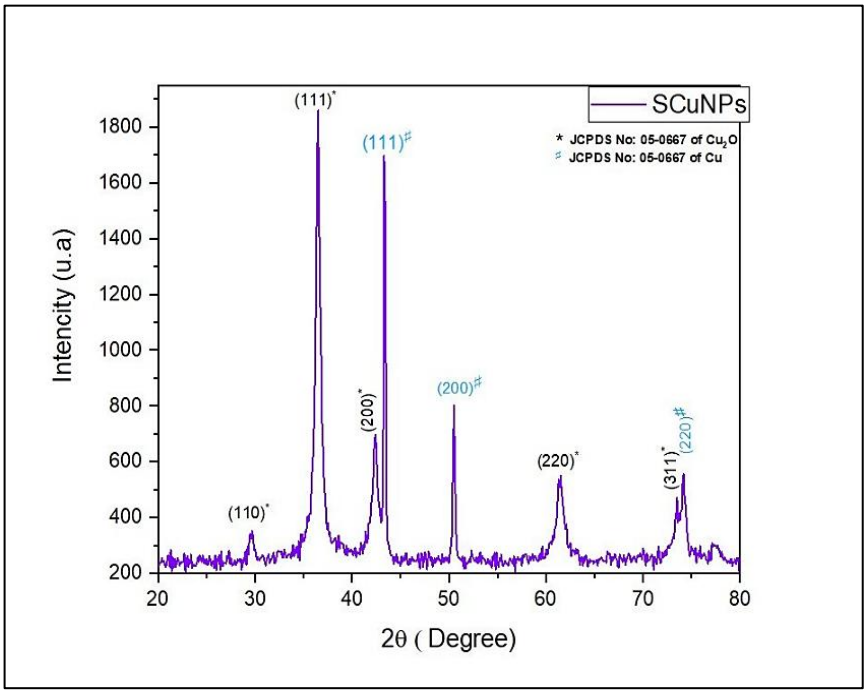

Figure 13 : XRD patterns CuO nps(Cu_2O)-S.aureus

1.4. Scanning electron microscopy

The SEM micrographs of the synthesized Cu nanoparticles and Cu–DNA nanocomposites revealed aggregated and irregularly shaped structures distributed over the sample surface. The nanoparticles appeared as compact agglomerates with rough and porous morphology. In addition, small granular particles surrounding the larger aggregates were observed, confirming the formation of nanoscale Cu particles. Figure 14 The SEM images of the nanocomposite samples showed more heterogeneous and compact structures compared to CuO npsalone. Furthermore, elongated and clustered structures were observed in some regions, indicating the successful formation of Cu–DNA nanocomposites figure 15 et 16

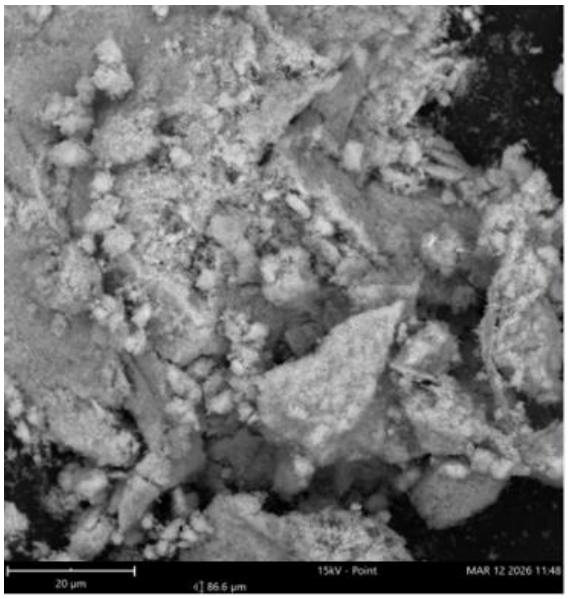

Figure 14: Scanning electron microscopy images of CuNP

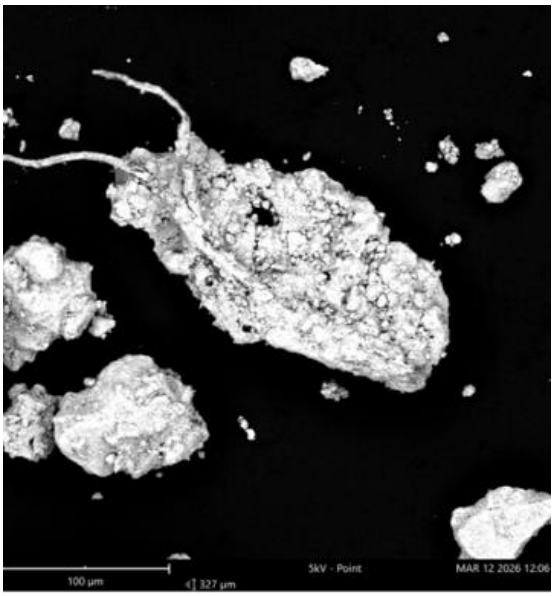

Figure 15: Scanning electron microscopy images of CuO nps-E.coli

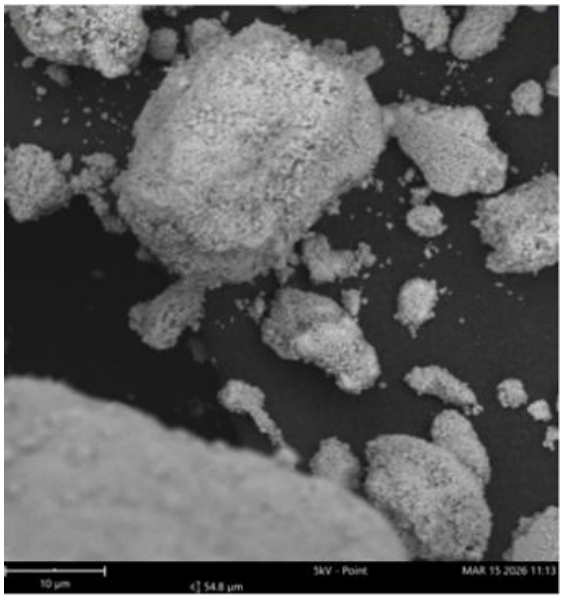

Figure 16: Scanning electron microscopy images of CuO nps-S.aureus

2) Biological activities

2.1. Antioxidant activity

The antioxidant activity of CuO NPs, CuO nps-S.aureus, CuO nps-E.coli, and acide ascorbique, was evaluated based on their IP values. The obtained results showed that CuONPs recorded the lowest IC_{50} value of . ug/mL, followed by both CuO nps-S.aureus and CuO nps-E.coli, which exhibited similar IC_{50} values of . ug/mL. In contrast, acide ascorbique recorded the highest IC_{50} value of 8.84 ug/mL under the same experimental conditions

2.2. Anti-inflammatory Activity

The anti-inflammatory activity of CuO NPs, CuO nps-E.coli , CuO nps-S.aureus, and diclofenac was evaluated based on their IC_{50} values. The obtained results showed that CuO nps-S.aureus recorded the lowest IC_{50} value of , mg/mL, followed by CuO npswith an IC_{50} value of , ug/mL. ECuO nps-E.coli exhibited an IC_{50} value of , ug/mL, while diclofenac showed the highest IC_{50} value of , ug/mL under the same experimental conditions,

2.3. Antibacterial Activity

Table 02 illustrates the antibacterial activity of copper DNA-nanoparticle conjugates (CuO nps-S.aureus and CuO nps-E.coli), along with three standard antibiotics, against four Gram-positive and Gram-negative bacterial strains. The antibacterial activity was evaluated by measuring the diameters of the inhibition zones formed around the discs. The largest inhibition zone was recorded against Escherichia coli at a concentration of 40 mg/ml for *CuO nps-E.coli* (table 02), indicating a strong antibacterial effect against this strain. On the other hand, *CuO nps-S.aureus* exhibited the highest antibacterial activity against *Staphylococcus aureus* compared to the other tested strains.

Table 02: Inhibition zone values (mm) of the tested bacterial strains and their corresponding antibiotics.

	E. coli		**P. aeruginosa**		**S. aureus**		**K. pneumoniae**	
Concentration (µg/ml)	/	/	/	/	/	/	/	/
CuO NPs	/	/	/	/	/	/	/	/
CuO nps-E.coli	/	/	/	/	/	/	/	/
SCuO nps-S.aureus	/	/	/	/	/	/	/	/
Ciprofloxacine	1,3 ± 32		1,5± 35		0,8± 20		0,7± 19	
Gentamicin	0,9±18		1,2± 22		0,6±16		0,5± 15	

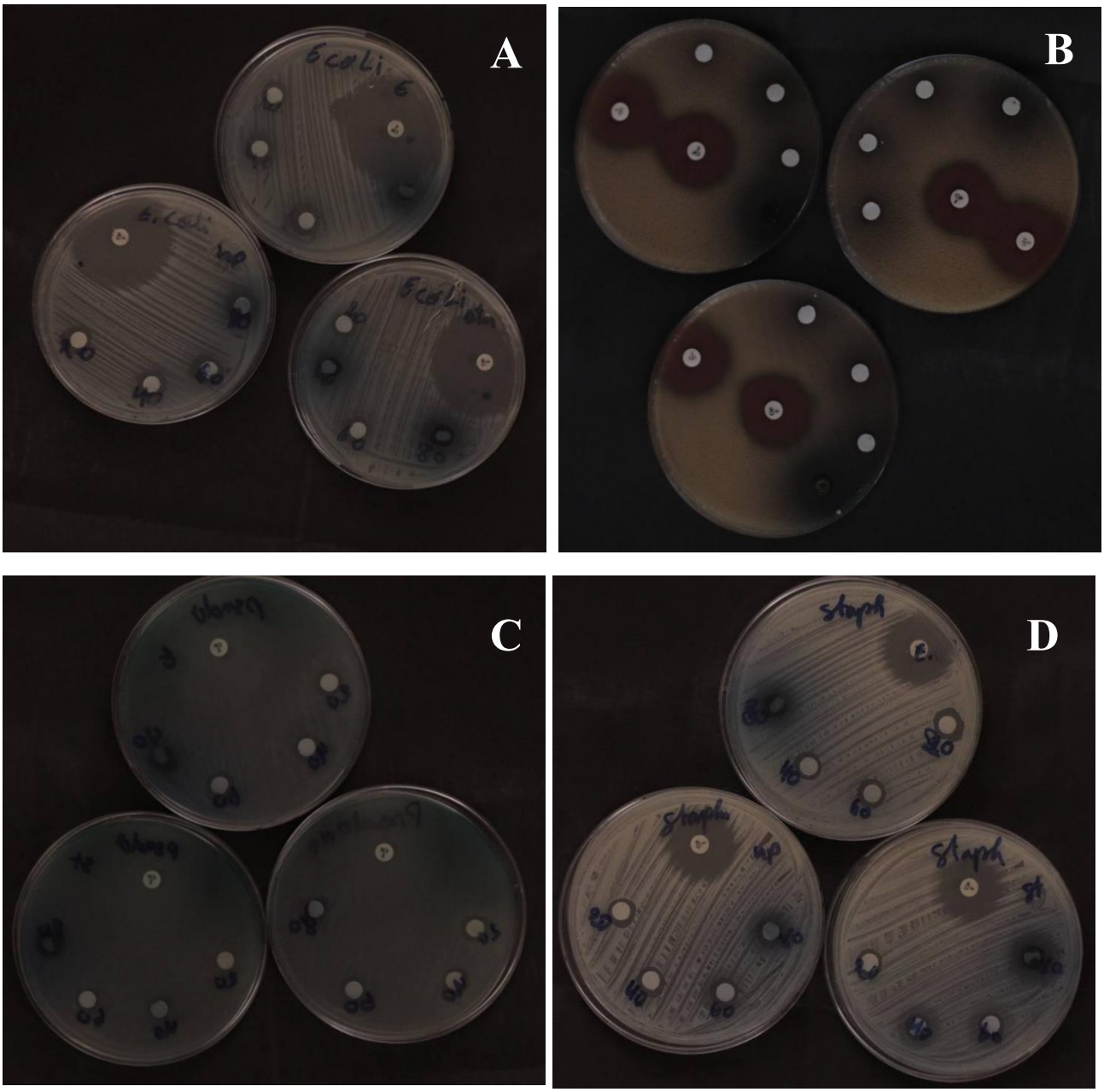

Figure 17: Anti-bacterial activity of Cu NPs, CuO nps-E.coli *and* CuO nps-S.aureus against,(A) *E.coli* (B) , *K. pneumoniae* (C) , *P. aeruginosa* , (D) , *S. aureus*

3) Molecular Docking

The molecular docking results demonstrated the ability of CuO nps-E.coli to interact with bacterial receptors associated with DNA gyrase and topoisomerase enzymes, with binding energy values ranging from −3.63 to −4.82 kcal/mol. These findings suggest the potential antibacterial activity of the nanoparticles through their interaction with essential enzymes involved in bacterial DNA replication and cell division. In addition, the relatively close binding energy values obtained with the different receptors indicate the possibility of interaction with multiple bacterial targets, which may support the antibacterial effect of CuO NPs-*E. coli* . Although molecular docking studies involving nanoparticles are generally more complex than those involving conventional small molecules, the obtained results remain promising and support the biological potential of these nanoparticles. shown in Table 03:

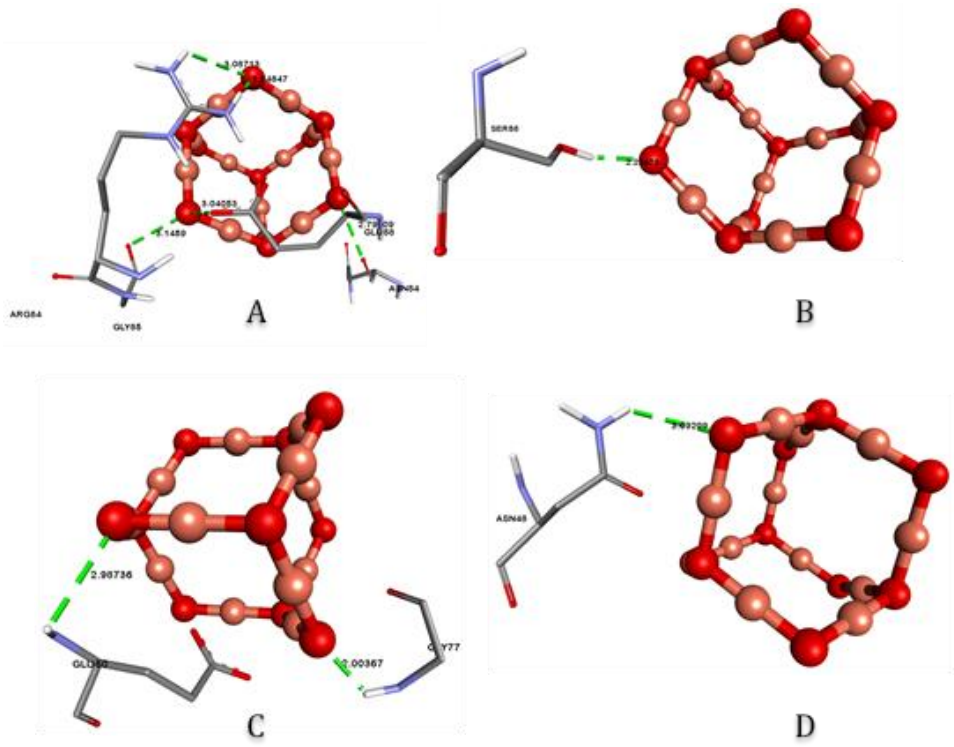

Figure 18: The visualization of interactions of Cu_2O nanoparticle with targeted receptors in three-dimensional. A) Cu_2O-3TTZ, B) Cu_2O-4MOT, C) Cu_2O-5L3J, and D) Cu_2O-8BN6

III

Discussion

1) Characterization of nanoparticles

The technique employed for the synthesis of nanoparticles plays a crucial role in determining the physicochemical and biological properties of the resulting particles. The selected synthesis approach significantly influences characteristics such as particle size, surface morphology, stability, and biological activity. Several parameters, including reaction time, reagent concentration, temperature, pH, and the nature of the reducing or stabilizing agents, must be carefully optimized to obtain nanoparticles with desirable properties

The initial detection of CuO npsproduction was indicated by a noticeable change in visual color, and UV–Visible spectroscopic analysis in this study confirmed the formation of copper-based nanoparticles. Our results showed absorption peaks at 250 nm and 300 nm for CuO npsand CuO nps-E.coli, respectively, indicating successful nanoparticle formation. These findings are consistent with previous studies. In particular, Ssekatawa et al. (2022) reported UV–Visible absorption spectra with surface plasmon resonance peaks ranging from 272 to 286 nm, confirming the presence of CuO nanoparticles, which is in agreement with our results. Similarly, Shanmugapriya et al. (2022) observed an absorption peak at 330 nm, confirming the biosynthesis of CuO NPs. The variation in absorption peaks may be attributed to differences in nanoparticle size, shape, concentration, and synthesis conditions. positions among different studies may be attributed to differences in synthesis procedures, phytochemical composition of plant extracts, oxidation state of copper, and the size and morphology of the synthesized nanoparticles. In addition, surface interactions between nanoparticles and biological molecules may influence their optical properties and absorption behavior performed to confirm the successful formation of copper nanoparticles and their interaction with bacterial genetic material. The FTIR spectrum of CuO npsshowed a characteristic absorption band in the region 520–600 cm^{-1}, corresponding to Cu–O vibrations, which confirms the successful synthesis of copper nanoparticles. Similar FTIR absorption bands around 602 cm^{-1} were also reported in plant-mediated synthesized copper nanoparticles, supporting the characteristic Cu–O vibration region associated with CuO npsformation (Saran et al., 2018). Similarly, the spectra of CuO NPs- *E.coli* and CuO nps-S.aureus also exhibited strong absorption bands within the same region, indicating the presence of Cu–O bonds and confirming nanoparticle formation. In addition, broad absorption bands observed around 3400 cm^{-1} were attributed to N–H stretching vibrations, while the peaks detected between 1630 and 1600 cm^{-1} corresponded to carbonyl (C=O) and amide functional groups. Most importantly, absorption bands

observed in the region 1000–1100 cm^{-1} were assigned to phosphate (P–O) stretching vibrations, characteristic of nucleic acids. These findings suggest the successful association of copper nanoparticles with the genetic material of *Escherichia coli* and *Staphylococcus aureus*

The X-ray diffraction (XRD) analysis of sample A confirms the formation of a pure Cu_2O phase. The diffraction peaks observed at 2θ values of approximately 29.00°, 36.42°, 42.29°, 61.36°, and 73.72° were indexed to the (110), (111), (200), (220), and (311) crystallographic planes of cubic cuprous oxide Cu_2O (JCPDS No. 05-0667). These results are consistent with those reported by (Khanna et al. 2023), where the absence of metallic Cu and CuO peaks confirmed the formation of pure Cu_2O nanocrystals.
This is further supported by (Chen et al. 2015)., who reported that only Cu_2O diffraction peaks were observed without any signals corresponding to Cu or CuO, confirming the formation of a single-phase structure. In contrast, the XRD pattern of sample B (Cu NPs-*E.coli*) exhibits additional peaks at approximately 43.44°, 50.60°, and 74.25°, corresponding to the (111), (200), and (220) planes of metallic copper with a face-centered cubic (fcc) structure (JCPDS No. 04-0836). Similar peaks were reported (Khanna et al. 2023) at 43.39°, 50.49°, and 74.18°, confirming the formation of Cu^0 nanoparticles. The coexistence of Cu_2O and Cu phases indicates a partial reduction of Cu^{2+} ions during the synthesis process, leading to a biphasic Cu/Cu_2O nanostructure.

Furthermore, the crystallite size obtained in this study (8.1–11.8 nm) is significantly smaller than previously reported values, where (Chen et al.2015) observed Cu_2O structures with an average size of approximately 120 nm, and Amer and Awwad reported copper nanoparticles with an average size of about 18 nm. This reduction in size suggests that the synthesis method used in this work promotes efficient nucleation and limits particle growth, likely due to strong stabilization effects of the reducing medium, resulting in well-dispersed nanoparticles with controlled nanoscale dimensions.

SEM analysis revealed that the synthesized Cu nanoparticles exhibited an aggregated morphology with irregularly shaped clusters distributed over the sample surface. The particles appeared as dense agglomerates with rough and porous surfaces, indicating the formation of nanoparticle assemblies. Small granular particles surrounding the larger aggregates were also bserved, confirming the nanoscale nature of the synthesized Cu nanoparticles. These observations are consistent with the findings of (Singh et al. 2023), who reported that Cu nanoparticles exhibited irregular and aggregated morphologies with rough surfaces due to the high surface energy of nanoparticles. (Singh, J. et al 2023).

Green synthesis and characterization of Cu nanoparticles with antibacterial activity. In the nanocomposite samples, SEM micrographs showed more compact and heterogeneous structures compared to CuO npsalone. The nanoparticles appeared embedded within matrixlike structures, which may indicate interactions between Cu nanoparticles and bacterial DNA. Additionally, elongated and irregular structures were observed after the coupling process, suggesting the successful formation of Cu–DNA nanocomposites. Similar results were described by (Khan et al. 2023), who demonstrated that metal nanoparticle–DNA composites exhibit heterogeneous and compact morphologies due to interactions between nanoparticles and biomolecules.

The study reported that DNA coupling may promote particle aggregation and structural stabilization. (Khan, I. et al. 2023). Interaction of metal nanoparticles with DNA: structural and biological perspectives. The SEM images also demonstrated non-uniform particle distribution and different particle sizes and shapes. This aggregation behavior may be attributed to the high surface energy of Cu nanoparticles and intermolecular interactions occurring after the coupling process. Comparable observations were reported by (Banerjee et al.2024), who found that Cu-based nanocomposites frequently exhibit agglomerated structures with heterogeneous morphology, which may enhance their interaction with bacterial cells and improve antibacterial activity. (Banerjee, A. et al.2024).

2) Biological activities

Regarding the anti-inflammatory activity, it was evaluated using the protein denaturation inhibition assay. The results showed that Staphylococcus aureus-associated copper nanoparticles (CuO nps-S.aureus) exhibited the highest anti-inflammatory activity, with an IC_{50} value of 0.5 µg/mL, followed by CuO npswith an IC_{50} value of 0.54 µg/mL, indicating a strong ability to inhibit protein denaturation. In contrast, CuO nps-E.coli showed relatively lower activity with an IC_{50} value of 1.07 µg/mL, while Diclofenac, used as a reference standard, exhibited an IC_{50} value of 3.5 µg/mL. It is well established that lower IC_{50} values indicate higher anti-inflammatory activity, demonstrating that the synthesized nanoparticles showed stronger anti-inflammatory potential than Diclofenac, particularly CuO nps-S.aureus , which exhibited the highest efficacy among all tested samples.

In comparison with previous studies, copper oxide nanoparticles reported by (Chellathurai et al. 2024) showed protein denaturation inhibition ranging from 50% to 78%, confirming their ability to stabilize protein structures and reduce inflammatory responses. These findings are consistent with those reported by (Almehizia et al. 2023),

who demonstrated that copper oxide nanoparticles exhibit significant anti-inflammatory activity through protein denaturation inhibition, with an IC_{50} value of 7.13 ± 0.03 µg/mL. In addition,(Angajala et al. 2014) reported that copper nanoparticles are able to inhibit protein denaturation in a concentration-dependent manner, further supporting the anti-inflammatory potential of copper-based nanomaterials .Overall, the obtained results demonstrate the superiority of Staphylococcus aureus-mediated copper nanoparticles (CuO nps-S.aureus) compared to previous studies, as they exhibited the lowest IC_{50} value, reflecting the highest anti-inflammatory activity.

This enhanced performance suggests that biological modification of nanoparticles using bacteria may significantly improve their bioactivity by enhancing surface properties and increasing interactions with target proteins.

Regarding the antioxidant activity evaluated using the FRAP assay, copper nanoparticles (CuO NPs) exhibited the highest reducing capacity among the tested samples, recording the lowest IC_{50} value of 1.13 µg/ml, indicating the strongest antioxidant activity. In contrast, both CuO nps-S.aureus and CuO nps-E.coli showed an identical IC_{50} value of 2.06 µg/ml, reflecting good antioxidant activity, although lower than that of CuO NPs. As for the reference standard vitamin C, it recorded an IC_{50} value of 8.84 µg/ml. It is well established that lower IC_{50} values are associated with higher antioxidant activity, confirming the superior efficiency of CuO npscompared to the other tested samples .Similar findings have been reported in previous studies using the FRAP assay, where synthesized nanoparticles exhibited concentration-dependent reducing activity associated with strong antioxidant potential. In the study of (Ahmad et al. 2022), the increase in absorbance values with rising concentration reflected enhanced reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), confirming the electron-donating ability and strong reducing power of the synthesized nanoparticles.

These results are also consistent with those reported by (Frahtia et al. 2025), who investigated the biosynthesis of copper nanoparticles using Phragmites australis roots. Their study demonstrated antioxidant activity in the FRAP assay with an IC_{50} value of 131 µg/ml. The lower values obtained in the present study indicate that CuO NPs, as well as CuO nps-E.coli and CuO nps-S.aureus , exhibit higher reducing capacity compared to the previously reported study, reflecting the strong biological activity of the synthesized nanoparticles.

Antibacterial Activity

This study is based on the hypothesis that copper oxide nanoparticles (CuO NPs), when functionalized with bacterial DNA, may exhibit enhanced antibacterial activity against the corresponding bacterial strain .This potential enhancement could be attributed to the increased interaction between the nanoparticles and the target bacteria, as well as possible interference with bacterial genetic material.

The antibacterial activity of CuO nps-S.aureus, CuO NPs, and CuO nps-E.coli was evaluated against four bacterial strains, namely *Staphylococcus*, *Escherichia coli*, *Pseudomonas,* and *Pneumococcus*, using the disk diffusion method at two concentrations (20 and 40). The results showed that the nanocomposite exhibited higher antibacterial activity compared to CuO npsalone against all tested strains. It was also found that the antibacterial effect was concentration-dependent, with higher activity observed at a concentration of 40. Notably, the nanocomposite (CuO nps-E.coli) showed a stronger effect against *Escherichia coli* (E. coli), where the highest antibacterial activity was recorded The antibacterial activity of copper nanoparticles (CuO nps) was evaluated in our study at a concentration of 40 mg, where inhibition zones ranging from 10 to 12 mm were recorded against both *Staphylococcus aureus* and *Escherichia coli*. These values indicate a notable antibacterial efficacy of the nanoparticles in inhibiting bacterial growth, which is consistent with the findings reported by (Ijaz et al.), where copper oxide nanoparticles (CuO NPs) exhibited antibacterial activity against *Staphylococcus aureus* at a concentration of 5 mg, with an inhibition zone of 10 ± 0.11 mm.

This similarity in results supports the hypothesis that copper-based nanoparticles, whether in the form of CuO NPs, possess a strong ability to inhibit bacterial growth even at different concentrations. The comparable inhibition zone values further suggest that nanoscale copper is an effective antibacterial agent.

This antibacterial activity can be partially explained by the ability of CuO nps/CuO npsto generate reactive oxygen species (ROS), such as hydroxyl radicals (•OH), superoxide anions (O_2^-), and hydrogen peroxide (H_2O_2), which induce oxidative stress within bacterial cells. This stress leads to the damage of essential cellular components, including lipids, proteins, and nucleic acids, ultimately contributing to the inhibition or destruction of bacterial growth. Moreover, the concentration-dependent antibacterial effect is consistent with ROS-mediated mechanisms, where the activity of nanoparticles increases with higher concentrations, as confirmed in recent studies such as (Radulescu et al. 2025).

The results of our study showed that the copper-based nanocomposite exhibited antibacterial activity at a concentration of 40 mg, with inhibition zones of 17 mm against Escherichia coli and 16 mm against *Staphylococcus aureus.* When comparing these findings with those reported by (Moroda et al.,) who used biosynthesized CuO nanoparticles prepared using Rosmarinus officinalis leaf extract, a clear similarity in antibacterial performance is observed, where inhibition zones reached 20.3 ± 0.30 mm at 50 mg and 17.8 ± 0.15 mm at 25 mg.

This similarity despite differences in concentrations suggests that the nanocomposite used in our study exhibits higher efficiency, as it achieved considerable antibacterial activity at a lower concentration (40 mg) compared to the reference study, which required higher doses to obtain comparable results. This improvement can be explained by the presence of a biological component in both systems. In particular, the incorporation of functional DNA (fDNA) into nanoparticles imparts selective recognition and targeting capabilities toward a wide range of biological targets, including bacterial cells, thereby enhancing their interaction with the biological environment (Li et al., 2019). Moreover, this integration improves the structural stability of nanoparticles and reduces their aggregation, leading to enhanced biological performance. In this context, our results showed that the particle size of CuO nps(9.15 nm) was larger than that of CuO nps-E.coli (8.09 nm), indicating improved dispersion and stability upon incorporation of the biological agent. In addition, fDNA possesses several favorable characteristics, including small size, low immunogenicity, and ease of chemical modification, which further enhance biocompatibility and nanoparticle performance (Li et al., 2019). Collectively, these properties contribute to the enhanced antibacterial activity of the nanocomposite compared to conventional nanoparticles

The results of the present study demonstrated that nanocomposites functionalized with the genetic material specific to each bacterial strain exhibited higher antibacterial activity compared to copper nanoparticles alone, thereby supporting the main hypothesis of this work, which is the possibility of developing a more selective antibacterial system through the incorporation of bacterial genetic material into nanoparticles. Indeed, CuO npsshowed the highest antibacterial activity against Escherichia coli, with inhibition zones reaching 15 mm and 17 mm at concentrations of 20 and 40 mg, respectively, while CuO nps-S.aureus exhibited enhanced antibacterial activity against *Staphylococcus aureus* compared to conventional CuO NPs. This correspondence between the type of nanocomposite and its matching bacterial strain suggests that the biological component

improved the recognition and interaction between nanoparticles and the target bacteria, leading to enhanced antibacterial efficiency.

Furthermore, these findings suggest that the incorporation of DNA improved nanoparticle stability and reduced aggregation, thereby increasing the available surface area for interaction with bacterial cells and enhancing nanoparticle adhesion or penetration through the bacterial membrane. As a result, the antibacterial performance of the nanocomposites was significantly improved. On the other hand, although the highest activities were observed against the bacterial strains corresponding to the incorporated genetic material, the nanocomposites also exhibited noticeable antibacterial effects against other bacterial strains such as *Pseudomonas* and *Pneumococcus*. This indicates that the activity of these systems is not limited to selective targeting only, but rather combines enhanced specificity with the broad-spectrum antibacterial properties of copper nanoparticles, including oxidative stress induction and membrane disruption, resulting in an efficient and versatile antibacterial effect

3) Molecular Docking:

Among the studied receptors, the strongest interaction was observed with receptor 8BN6, with a binding energy value of −4.82 kcal/mol, indicating a more stable interaction between Cu_2O nanoparticle and the target protein. This may suggest the potential ability of the nanoparticles to interfere with enzymatic activities associated with bacterial DNA processing. Receptors 5L3J (−4.28 kcal/mol) and 3TTZ (−4.23 kcal/mol) also exhibited relatively similar binding affinities, reflecting a consistent interaction pattern with proteins associated with DNA gyrase. The similarity between these values supports the possibility that the nanoparticles may interact with multiple bacterial targets involved in DNA replication and cell division. On the other hand, receptor 4MOT showed the weakest interaction with a binding energy of −3.63 kcal/mol, which may indicate lower binding stability compared with the other receptors. Although the obtained binding energies remain relatively moderate, they are still considered promising given the complex nature of molecular docking studies involving nanoparticles compared with conventional small molecules.

These findings are consistent with previous molecular docking studies targeting DNA gyrase B using copper-based nanocomposites, where quercetin-functionalized nanoparticles demonstrated strong interactions with this enzyme, with binding energies reaching −10.151 kcal/mol, supporting the hypothesis that copper nanoparticles may affect enzymes associated with bacterial DNA replication (Aziz et al., 2025). Furthermore, these results are in agreement with previous studies suggesting that CuO npsmay target DNA gyrase B as one of the possible mechanisms underlying their antibacterial activity. A recent study reported high binding affinities between CuO npsand GyrB, with docking scores of −8.368 and −8.797 kcal/mol, and proposed that GyrB inhibition may contribute to antibacterial activity through interference with pathways related to bacterial DNA replication (Sazak et al., 2023).

Conclusion

The present study highlighted the promising potential of copper oxide nanoparticles (CuO NPs) and DNA-functionalized nanocomposites as innovative antibacterial agents against pathogenic bacteria associated with antimicrobial resistance. The successful synthesis of CuO nanoparticles was confirmed through several physicochemical characterization techniques, including UV–Vis spectroscopy, FTIR, XRD, and SEM analyses, which demonstrated the formation of stable nanoscale crystalline structures with suitable morphological and structural properties.

The obtained results demonstrated that coupling copper nanoparticles with bacterial DNA significantly enhanced their biological activities compared with CuO nanoparticles alone. The synthesized nanocomposites exhibited strong antioxidant and anti-inflammatory activities, with SCuO npsshowing the highest anti-inflammatory effect, while CuO npsdisplayed remarkable antioxidant capacity. Furthermore, the antibacterial assays confirmed that DNA-functionalized nanoparticles possessed enhanced inhibitory activity against bacterial strains, particularly against the corresponding bacterial species used during their preparation. ECuO npsexhibited the highest activity against *Escherichia coli,* whereas SCuO npswere more effective against *Staphylococcus aureus*, suggesting a possible selective interaction between the nanocomposites and target bacteria.

In addition, molecular docking analysis supported the antibacterial potential of Cu_2O nanoparticles through their interaction with bacterial DNA gyrase and topoisomerase-related proteins involved in DNA replication and cell division. The observed binding affinities indicate that these nanoparticles may interfere with essential bacterial enzymatic processes, thereby contributing to their antibacterial effect.

Overall, this study demonstrates that DNA-functionalized copper oxide nanoparticles represent a promising nanobiotechnological strategy for the development of efficient and potentially selective antimicrobial systems. The integration of biological molecules with nanoparticles may improve nanoparticle stability, enhance bacterial targeting, and increase biological performance. These findings open new perspectives for the design of alternative therapeutic approaches capable of combating multidrug-resistant bacteria. Nevertheless, further investigations, including toxicity evaluation and in vivo studies, remain necessary to confirm their safety, biocompatibility, and potential clinical applications**.**

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ANNEX

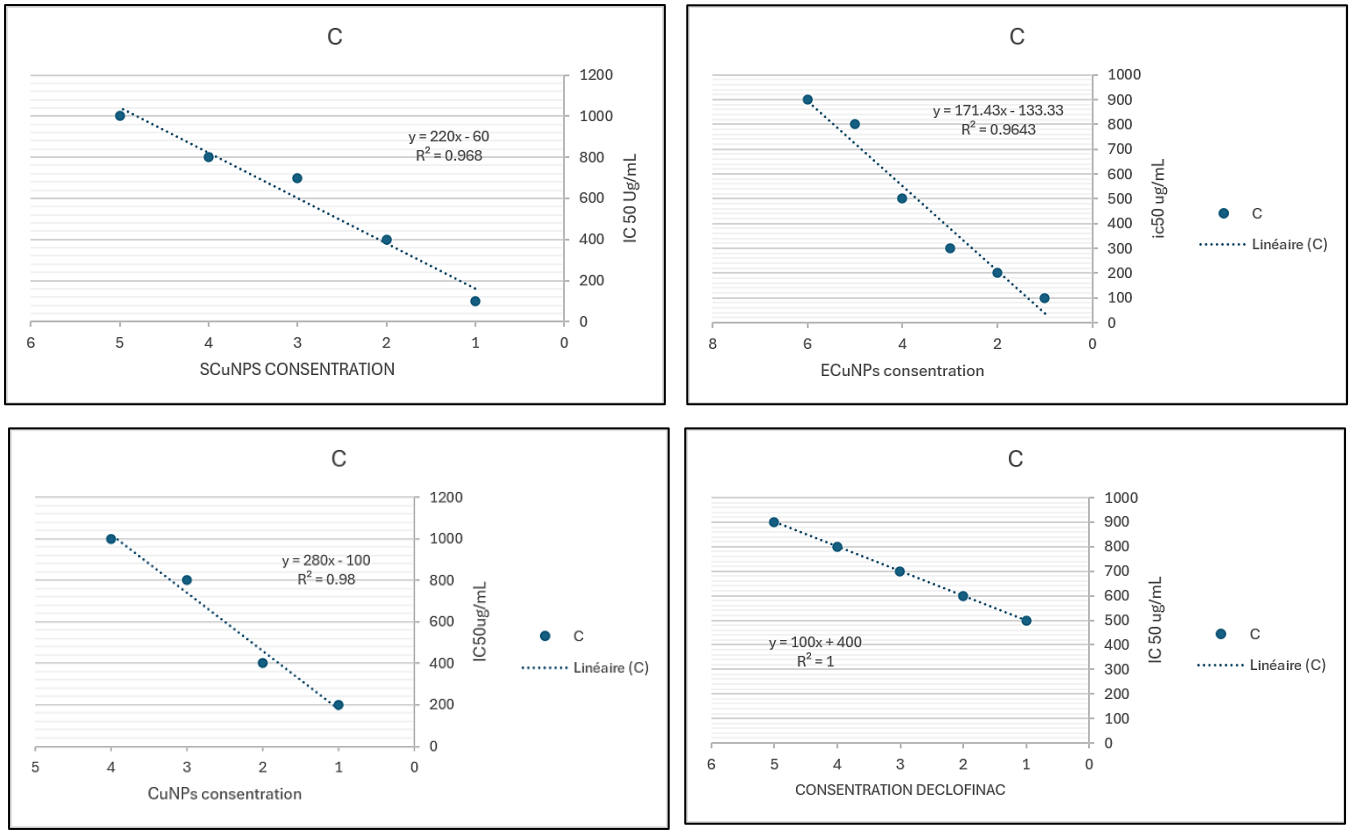
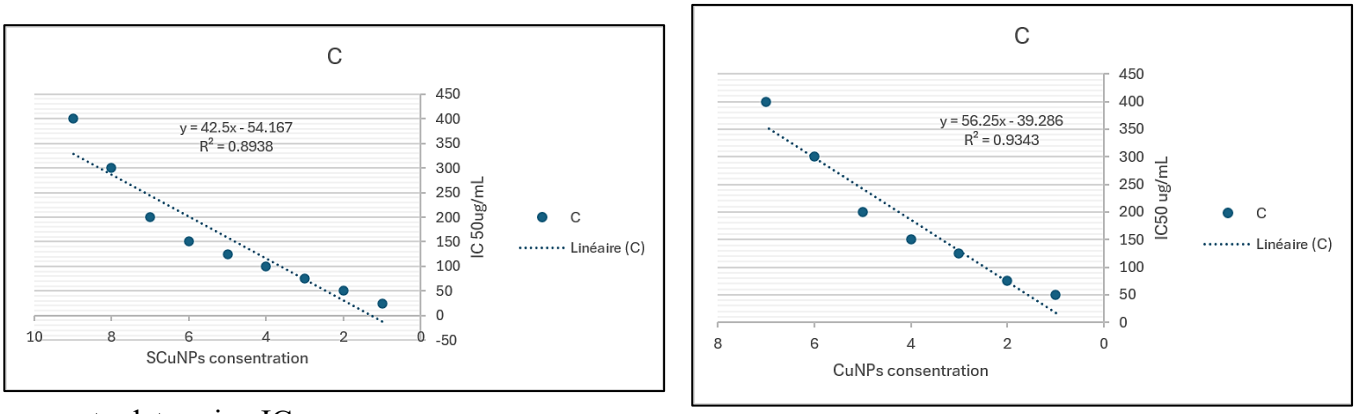
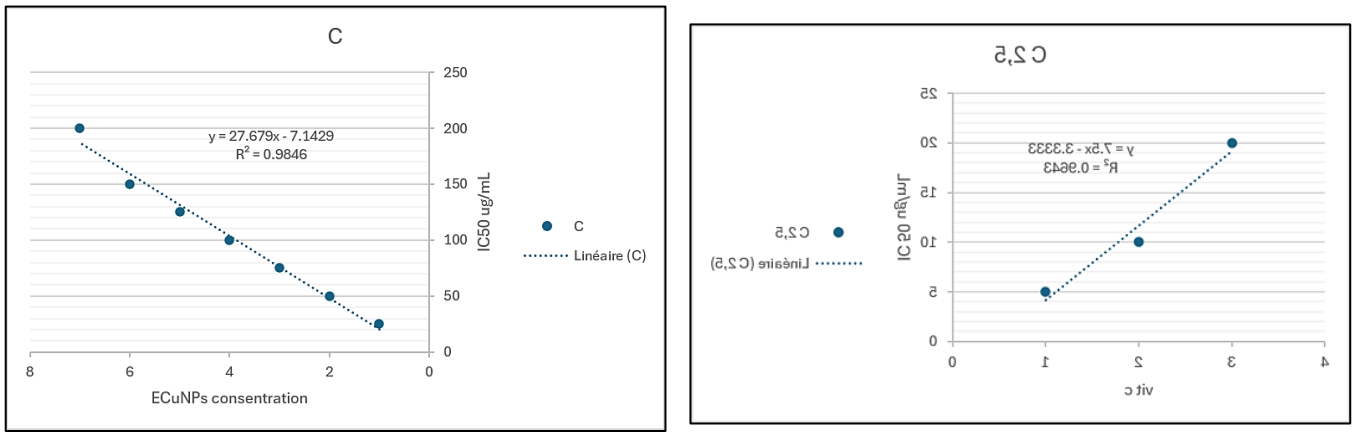


Figure 1: Percentage of BSA inhibition as a function of concentrations of SCuNP, ECuNP and declofinac



to determine IC



Percentage of FRAP radical inhibition as a function of concentrations of SCuNP, ECuNP and ascorbic acid to determine IC50