GREEN SYNTHESIS OF COPPER DOPED SILVER NANOPARTICLES, AND THEIR ANTI-MICROBIAL ACTIVITY

Project report submitted in fulfillment of the

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Master of Science

in

Biotechnology

By

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CANDIDATE'S DECLARATION

I hereby declare that the work presented in this report entitled "**GREEN SYNTHESIS OF COPPER DOPED SILVER NANOPARTICLES, AND THEIR ANTI-MICROBIAL ACTIVITY**" in fulfillment of the requirements for the award of the degree of Master of Science in Biotechnology submitted in the Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology, Waknaghat is an authentic record of my own work carried out over a period from July 2023 to May 2024 under the supervision of **Dr. Abhishek Chaudhary, Assistant Professor, Department of Biotechnology and Bioinformatics.**

I also authenticate that I have carried out the above-mentioned project work under the proficiency stream biotechnology.

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

Bishal Tiwari, 225111009

This is to certify that the above statement made by the candidate is true to the best of my knowledge.

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Dated:

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
FTIR	Fourier Transform Infrared
g	Gram
mM	Milli molar
mg	Milligram
ml	Milliliter
mm	Millimeter
$AgNO_3$	Silver Nitrate
$CuSO_4$	Copper Sulphate pentahydrate
NP	Nanoparticles
AST	Anti-Microbial Susceptibility Test
E.coli	*Escherichia coli*
S. aureus	*Staphylococcus Aureus*
AgNP	Silver Nanoparticles
CuNP	Copper Nanoparticles
ROS	Reactive Oxygen Species
TEM	Transmission Electron Microscopy
SEM	Scanning electron microscope

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Abstract

Silver nanoparticles are utilized in the medicinal industry as a medication delivery system or as an antibacterial agent. This work used the green synthesis process with *Eclipta Alba* to create copper doped silver nanoparticles . Their antibacterial activity was investigated in vitro against bacteria and fungus, the synthesized nanoparticles were subjected to UV-VIS spectroscopy that confirmed the formation of NPs an at absorption peak of 350nm. Antimicrobial activity was investigated by agar disc diffusion method (AST) which confirmed the synergistic antimicrobial activity against bacterial strains like *E coli* and *S aureus*, Antioxidant Assays like DPPH radical scavenging test and ABTS method tests were also performed under lab conditions to confirm desired properties in synthesized nano formulations.

Therefore, the primary goal of the current work was to develop, copper doped silver nanoformulations, which show synergistic effect on drug resistant bacterial strains, and can potentially be used as a antimicrobial agent,

KEYWORDS: Silver, Copper, Nanoparticles, Doping, Antimicrobial Nanoformulaation.

CHAPTER-1

INTRODUCTION

Green synthesis emerges as a pivotal approach in nanobiotechnology, leveraging natural resources to produce nanomaterials in an eco-friendly and cost-effective manner. This method, unlike traditional synthesis that often uses hazardous chemicals, utilizes plant extracts or microorganisms, marking a significant advance in the development of safer, biocompatible nanotechnologies [1]. Particularly, silver nanoparticles, recognized for their antibacterial, antiviral, and anti-inflammatory properties, are at the forefront of this innovative field. Their unique capabilities make them an invaluable asset in various biomedical applications [1]. The process of doping silver nanoparticles with copper further enhances their properties and stability, spotlighting the role of green synthesis in creating high-performance nanomaterials that are both effective and environmentally sustainable [1].

These green synthesized, copper-doped silver nanoparticles, achieved through methods like plant extract-mediated synthesis, offer several advantages including reduced toxicity, cost-effectiveness, and scalability. Their biocompatibility and controlled release capabilities extend their applicability across a broad spectrum, from drug delivery systems and cancer treatment to environmental remediation and energy storage. As we delve into the realm of nanobiotechnology, understanding these nanoparticles' synthesis, mechanism, and potential applications becomes essential. Their promise for the future of healthcare and industry illustrates the transformative power of green synthesis in nanotechnology, showcasing an eco-conscious path to innovation [1].

Historical Background of Nanoparticle Synthesis

Nanotechnology, a term first coined by Norio Taniguchi in 1974, has roots that trace back much further in history, intertwining with the practices and discoveries of ancient civilizations [12]. The journey from these early innovations to modern scientific applications illustrates a rich history of development and understanding at the nanoscale.

Early Historical Instances

1.Ancient Uses of Nanoparticles: Artisans in prehistory utilized nanoparticles unknowingly, evident in artifacts like the Roman Lycurgus Cup, which exhibits dichroic glass properties, and the lusterware pottery of Mesopotamia [13].

2.Greek Foundations: The theoretical underpinnings of nanotechnology can be seen back to the Greeks and the philosopher Democritus., who conceptualized atoms as indivisible components of matter [12].

- Development Through the Ages

1.19th Century Insights: Michael Faraday, in 1857, was among the first to describe the unique optical properties of nanometre-scale metals, laying a foundational understanding of nanoscale phenomena [14].

2.Evolution of Terminology: The term "ultrafine particles" was initially used to describe these minute particles; however, by the 1990s, the term "nanoparticle" became more prevalent, reflecting a refined understanding of their distinct characteristics [14].

- Newer Synthesis Approaches

1. Strategies both top-down and bottom-up: The top-down and bottom-up approaches are presented as crucial methods for creating nanostructures. The former involves breaking down bulk materials, while the latter gathers structures atom by atom or molecule by molecule. [12].

2.Diverse Fabrication Techniques: Techniques have evolved to include mechanical methods like grinding in ball mills, breakdown of biopolymers to produce fibre-like nanoparticles, and pyrolysis for turning precursor substances into solid particles [14].

3.Innovative Production Methods: Radiolysis and wet chemical processes are modern methods that utilize gamma rays and chemical reactions to produce nanoparticles, respectively [14].

- Safety and Environmental Considerations

1.Potential Risks: Nanoparticles present unique challenges such as their ability to penetrate cell membranes due to their high surface-to-volume ratio, posing potential medical and environmental risks [14].

2.Surface Modifications: The properties of nanoparticles can be significantly altered by coating them with various substances to enhance their stability, solubility, and activity, which is crucial for safe and effective application [14].

This historical perspective not only highlights the ancient origins of nanotechnology but also underscores the significant advancements that have shaped its development into a cornerstone of modern science and industry

Understanding Green Synthesis

Green Synthesis Techniques and Their Advantages

1.Biological Pathways and Living Cells

Green synthesis of nanoparticles utilize the living matter for synthesis of nanoparticles offers a more efficient and cost-effective approach compared to traditional methods. This method leverages the natural capabilities of cells to produce nanoparticles, bypassing the need for high energy consumption and toxic chemicals [2].

2.Plant Extracts and Phytochemicals

Plants are a wealthy source of auxiliary metabolites, such as phenolics, terpenoids, polysaccharides, and flavonoids, which have solid oxidation-reduction capabilities. These properties make them perfect for the green blend of nanoparticles, especially through plant extract-mediated union [2]. This strategy not as it was utilizing the inborn properties of plant extricates but too advances to utilize of agro-industrial bio-waste, which can be changed into high-value items like biodiesel and utilitarian lipids [1].

Environmental and Economic Benefits

1.Cost-Effectiveness and Low Toxicity

The use of green synthesis for producing nanoparticles is recognized for its cost-effectiveness and low toxicity. Methods such as bio-fabrication utilizing agro-industrial bio-wastes offer a sustainable alternative to physical and chemical synthesis methods, which often involve harmful substances [1].

2.Sustainability and Pollution Reduction

Green synthesis methods are crucial for promoting sustainable technologies. By utilizing eco-friendly, non-toxic, and sustainable approaches, these methods help reduce pollution and encourage the use of renewable resources [3]. Moreover, the synthesis process can convert biodegradable waste into useful nanomaterials, thus addressing environmental challenges associated with waste management [8].

Technological Applications and Innovations

1.Characterization and Biofunctionalization

Various advanced characterization tools like UV-Visible, XRD, and SEM are employed to analyse the properties of synthesized nanoparticles. Additionally, biofunctionalization techniques involve attaching specific biomolecules to nanoparticles to enhance their properties and tailor them for specific applications such as catalysis and medicinal uses [3].

2.Applications in Diverse Sectors

Green-synthesized nanoparticles have found applications across multiple sectors, including electronics, agriculture, and medicine. These nanoparticles are particularly valuable in healthcare for their roles in drug delivery systems and as potential treatments for various diseases [5][7].

Mechanism of Antimicrobial Action

Copper Nanoparticles and Antimicrobial Mechanisms

1.Generation of (ROS)

Copper-containing nanoparticles (NPs) contribute to antimicrobial effects by generating ROS. These ROS are capable of damaging microbial cell structures, including cell walls of bacterial membranes, leading to cell death [15].

2.Disruption of Metalloproteins

Copper NPs interfere with the normal functions of microbial cells by replacing or binding to the primary cofactors in metalloproteins. This disruption hampers the metabolic processes essential for the survival of microorganisms like Streptococcus mutans and Candida albicans [15].

3. Release of Copper Ions

The antimicrobial action of copper-containing nanoparticles is significantly enhanced by releasing of copper ions. These ions damage the cell membrane, disrupt the electron transport chain, and further generate ROS, contributingto the broad-spectrum antimicrobial properties observed in copper nanomaterials [16].

4.Synergistic Effects with Silver Nanoparticles

When combined with silver nanoparticles (Ag NPs), (CuO NPs) enhance the antibacterial effect significantly. This synergy results from the faster dissolution of Ag^+ ions in the presence of Cu^{2+} and the reduced binding of Ag^+ by proteins in the incubation media [17].

- Factors Influencing Antimicrobial Activity

1.Physical and Chemical Properties

The antimicrobial property of copper nanoparticles is influenced by various factors including size, morphology, and the physical conditions such as temperature and pH of the surrounding medium. These factors affect how the nanoparticles interact with microbial cells [15].

2.Environmental Conditions

The presence of wet or dry conditions and the composition of the surrounding medium can alter the effectiveness of copper nanoparticles. These environmental factors play a pivitol role in determining the interaction dynamics between the nanoparticles and microbial cells [15].

- Applications and Testing Methods

1.Broad Spectrum Antimicrobial Activity

Copper nanomaterials have demonstrated effective antimicrobial activity against a variety of organisms including bacteria (both Gram-positive and Gram-negative), fungi, and algae. This broad-spectrum activity makes them suitable for diverse applications in biomedicine and environmental restoration [16].

2.Testing and Evaluation

The effectiveness of copper nanomaterials is typically assessed through various methods such as the disc diffusion test, minimum inhibitory concentration (MIC), and (MBC). These tests help in determining the concentrations necessary to inhibit or kill microorganisms effectively [16].

This detailed exploration into the mechanisms and factors influencing the antimicrobial action of copper-doped silver nanoparticles highlights their potential in enhancing nanobiotechnology applications, particularly in areas requiring stringent microbial control.

- Antimicrobial Activity Mechanism
- Efficacy Against Resistant Bacteria

1.Improved Antimicrobial Properties: Green-synthesized nanoparticles (NPs) have illustrated noteworthy victory in hindering the development of different microorganisms, acting as powerful antimicrobial operators. Their viability is especially outstanding in situations where microscopic organisms have created resistance to conventional anti-microbials [10].

2.Broad-Spectrum Antioxidant Capabilities: In expansion to their antimicrobial properties, these nanoparticles too show solid antioxidant exercises, which contribute to their generally adequacy in combating microbial diseases [10].

• Application in Antibiotic-Resistant Strains

1. Predominant Execution Against Safe Microbes: The antibacterial exercises of nanoparticles are particularly articulated in strains of microscopic organisms that have created resistance to existing anti-microbial medications. This makes them priceless in the battle against drug-resistant diseases, advertising a modern road for helpful intercession [21].

- Applications in Healthcare and Industry
- Biomedical and Environmental Applications

1.Environmental Remediation and Photocatalysis: Plant-mediated copper oxide nanoparticles, prepared from various plant extracts, have demonstrated effectiveness in environmental remediation and photocatalysis [2]. These nanoparticles contribute to catalytic induced reduction, sensing, storing energy, and various organic transformations, making them versatile in ecological applications [2].

2.Biomedical Uses: Silver chloride nanoparticles, developed through green synthesis, are utilized in creating innovative biomedical applications. These applications leverage the unique properties of silver nanoparticles to enhance healthcare technologies [2].

Agricultural Enhancements

1.Nanofertilization and Pest Control: In the agricultural sector, green synthesized nanoparticles are instrumental in delivering nutrients efficiently to crops, controlling pests, and improving food quality [1]. This not only boosts crop yield but also ensures the production of healthier food products.

2.Photovoltaics and Energy Storage: The role of these nanoparticles extends to enhancing the efficiency and performance of solar cells and energy storage devices. This application is critical in promoting sustainable energy solutions [1].

Industrial Applications

1.Bio-nanosorbents and Bio-nano catalysts: Derived from agro-industrial wastes, bio-nano sorbents and bio-nano catalysts exhibit significant catalytic activity. Their utility spans various sectors, including healthcare and industry, where they aid in processes like waste treatment and chemical transformations [1].

2.Air Pollution Control: Utilizing green synthesized nanoparticles in air pollution control demonstrates their capacity to improve environmental health. These nanoparticles effectively remove pollutants from the air, contributing to cleaner and safer atmospheric conditions [1].

Broad Spectrum Applications

1.Catalysis and Space Industries: The wide range of applications of green synthesized nanoparticles includes catalysis, environmental management, cosmetics, drug delivery, healthcare, optics, and even space industries. These nanoparticles are tailored to meet the specific needs of each industry, showcasing their adaptability and importance [10].

2.Restorative and Mechanical Innovations: Silver nanoparticles (AgNPs) are especially famous for their assorted applications in therapeutic and mechanical advances due to their special physical, chemical, and natural properties. These incorporate antifungal, antibacterial, antiviral, anti-infectious, wound mending, and anti-inflammatory properties, which are useful at moo concentrations [11].

Safety and Efficacy

1. Decreased Harmfulness in Natural Applications: Thinks about including creature models have appeared that green synthesized nanoparticles show lower poisonous quality compared to their chemically synthesized partners. This viewpoint is significant for guaranteeing the security of nanoparticles utilized in natural applications [22].

CHAPTER- 2

REVIEW OF LITERATURE

Amalgamation and Applications of Silver Nanoparticles (AgNPs) have been broadly utilized in different restorative areas due to their antimicrobial and anticancer properties, their part in wound repair, bone recuperating, and as antibody adjuvants. They are too utilized as against-diabetic operators and in biosensors [20]. The union of AgNPs can be accomplished through different strategies, counting physical, chemical, and organic courses. Physical union includes mechanical and vapor-based forms, whereas chemical union regularly includes the decrease of silver particles to silver molecules. Organic strategies use microorganisms or plant extricates to encourage the blend [20].

Development of Copper Doped Silver Nanoparticles

Copper doped silver nanoparticles speak to a critical headway in the field of nanotechnology, combining the advantageous properties of both copper and silver. The expansion of copper upgrades the steadiness and reactivity of silver nanoparticles, which increments their viability in different applications such as catalysis, detecting, and biomedicine [21]. The union of copper nanoparticles frequently includes the decrease of copper nitrate utilizing hydrazine monohydrate as a reducer, with preformed silver nanoparticles acting as catalysts. This strategy altogether diminishes the response time and comes about in the arrangement of copper {22].

- Environmental and Safety Considerations

The potential for large-scale use of copper-based nanomaterials, including copper-doped silver nanoparticles, has raised important environmental and safety concerns. These concerns are driven by the high redox activity and reported toxicity of these nanomaterials from in vitro studies, emphasizing the need for careful consideration and management of their environmental impact [23].

- Historical Significance of Copper's Antimicrobial Properties

The antimicrobial properties of copper have been recognized for centuries, a fact underscored by the enduring efficacy of copper surfaces in places like New York's Grand Central Terminal, where they continue to exhibit antimicrobial effects even after hundreds of years [21]. This historical use underscores the application of copper and copper-doped nanoparticles in contemporary applications, combining ancient wisdom with modern technology.

- Comparative Analysis of Antimicrobial Efficacy

Copper-based nanomaterials exhibit remarkable antibacterial properties, especially against formidable pathogens such as methicillin-resistant *Staphylococcus aureus* [27]. In particular, the incorporation of copper into nanoparticle formulations significantly enhances their antimicrobial effectiveness. For instance, a study utilizing copper doped nanoparticles combined with glass ionomer cement and metronidazole showed superior antimicrobial efficacy against *Staphylococcus aureus* and *Streptococcus mutans* compared to other formulations [28].

Synergistic Effects of Cu and Ag Nanoparticles

1.The synergistic action of coupled copper nanoparticles (Cu NPs) and silver nanoparticles (Ag NPs) greatly amplifies their antibacterial activity [29].

2. Because Ag NPs have a strong interaction with proteins and polysaccharides found in microbial cell walls, this synergy is especially efficient against fungi and a variety of bacterial species, such as *S. aureus* and *E. coli*.[29].

3.The combination of positively charged copper oxide (CuO) nanoparticles with silver nanoparticles has been shown to enhance the antibacterial effect of silver up to six times, indicating a potent synergistic interaction [30].

Comparative Efficiency of Nanoparticle Combinations

Nanocomposite Efficiency Against Bacteria

CuO/Ag and ZnO/Ag nanocomposites both achieved the same MIC of 0.25 mg/ml against *E. coliand S. aureus* [31].

CuO/Ag nanocomposites demonstrated approximately 98.8% efficiency against Gram-positive bacteria and 98.7% against Gram-negative bacteria. In comparison, ZnO/Ag nanocomposites showed slightly lower efficiencies of 91.7% and 89.3%, respectively [31].

The enhanced antimicrobial activity of copper-doped silver nanoparticles against a diverse range of bacteria, including those resistant to antibiotics, underscores the potential of these nano formulations in addressing the challenge of antibiotic resistance [12]. Further research into the synergistic effects of these nanoparticles with antibiotics is recommended to fully exploit their antimicrobial properties [32]. Additionally, the specific mechanisms through which these synergistic effects occur warrant deeper investigation to optimize the design and application of these nano formulations in healthcare settings [30] [32].

Comparative Analysis with Conventional Nanoparticles

Antimicrobial Efficacy Comparison

1.Copper Nanomaterials' Antimicrobial Activity: Copper nanomaterials are known for their antimicrobial properties against a variety of organisms like bacteria, fungi, and algae. The effectiveness of these nanomaterials varies depending on the species of microbe and the conditions of the experiment [26].

2.Enhanced Toxicity in Cancer Cells: AgCu-NP nanoparticles have shown significant toxicity to lung cancer NCI- 1975 cells, a feature not observed with Ag-NP or Cu-NP alone [33].

- Comparative Cost and Application Efficiency

In addition to being very effective in the biomedical domain, copper-based nanoparticles are also well-suited for use in solar energy conversion and electrochemical sensors. Their cheaper manufacturing costs and conductive qualities make them a competitive substitute for other noble metals. [27].

- Dental Applications: GIC Combined with Nanoparticles

Efficiency Against Dental Caries Microbes: When copper doped silver nanoparticles (TVE-CuNPs) were compared with conventional silver nanoparticles (AgNPs) in combination with GIC and metronidazole, both groups demonstrated enhanced antimicrobial efficiency against Staphylococcus aureus and Streptococcus mutans [28].

Impact on Compressive Strength: The addition of nanoparticles did not adversely affect the compressive strength of the dental cement, indicating that the integration of these nanoparticles can be done without compromising the material strength [28].

Potential for Improved Dental Restorations: The superior antimicrobial efficacy observed suggests potential for developing more effective dental restorations that could prevent caries lesions more efficiently [28].

These comparisons highlight the distinct advantages of copper doped silver nanoparticles over conventional nanoparticles, showcasing their broader applicability and enhanced efficacy in various settings.

Green Synthesis Methods

Overview of Green Synthesis

Green synthesis of copper-doped silver nanoparticles utilizes natural resources, including plants, microorganisms, or enzymes, which not only supports environmental sustainability but also enhances the biocompatibility of the nanoparticles [34]. The process involves various factors such as temperature, pH, reaction time, and the volume of biological extracts, which all play crucial roles in determining the size, shape, and physicalfunctions of the synthesized nanoparticles [22] [25].

- Key Factors in Green Synthesis

1.Temperature and pH: These are critical factors that influence the nucleation and growth phases of nanoparticle synthesis, affecting their stability and morphology [22].

2.Reaction Time and Volume of Reagents: Longer reaction times and the appropriate volumes of reagents can lead to more uniform nanoparticles with desired properties [22].

3.Biological Extracts: The type and concentration of biological extracts used can significantly impact the reduction potential and stabilization of nanoparticles [34].

- Green Synthesis of Copper Nanoparticles

Several plants have been identified as potent sources for the synthesis of copper nanoparticles. Notable among these are:

Hageniaabyssinica L. leaf extract Citrus medica Linn. juice Syzygiumguineense extract

These plants are utilized for their inherent phytochemicals, which act as reducing and stabilizing agents during the nanoparticle synthesis process [22].

- Case Studies in Green Synthesis

Morindacitrifolia Leaf Extract: This extract has been used to synthesize (CuO NPs), which are highly stable and exhibit a sphere-like shape with sizes ranging from 20 to 50 nm. The

CuO NPs demonstrated strong antimicrobialproperty against both Gram-positive and Gram-negative bacteria, as well as notable antifungal effects [35].

Parthenium hysterophorus Aqueous Extract: This plant extract is another example used in the synthesis of CuO NPs, highlighting the versatility and efficacy of plant-based synthesis methods [36].

- Advantages of Green Synthesis

The synthesis of copper-doped silver nanoparticles through green methods offers several advantages:

Environmental Sustainability: Utilizes renewable resources and reduces the environmental impact associated with conventional chemical synthesis methods.

Cost-effectiveness: Lower production costs due to the use of naturally available materials.

Simplicity and Safety: Processes are generally simpler and safer, reducing the need for toxic chemicals and high energy inputs [37].

- Potential Applications

Copper-doped silver nanoparticles produced using environmentally friendly processes have special optical, electrical, and physical characteristics that make them useful for a variety of applications, such as biomedicine, sensing, and catalysis [34]. They are especially useful in medical and healthcare applications because of their improved antibacterial and antifungal properties.

Copper-doped silver nanoparticles are produced using environmentally friendly synthesis techniques, which not only supports global sustainability goals but also improves the nanoparticles' functional characteristics and makes them appropriate for a wide range of applications. [34] [37].

Antimicrobial Mechanisms

Understanding the Interaction with Bacterial Cell Walls

Copper-doped silver nanoparticles inhibit bacterial growth through various mechanisms, including direct interactions with bacterial cell walls. These interactions disrupt cellular processes and generate ROS, which are crucial in the antimicrobial action of these nanoparticles [32].

- Role of (ROS)

Cu and Ag nanoparticles' production of ROS is a major factor in their antibacterial action. Within microbial cells, these ROS induce oxidative stress, which results in cell damage and death. Copper nanoparticles have strong antibacterial capabilities, as evidenced by their capacity to produce reactive oxygen species (ROS), substitute native cofactors in metalloproteins, and impair a variety of cellular processes. [39].

- Impact of Nanoparticle Size and Shape

The antimicrobial potential of nanoparticles is significantly influenced by their size and shape. Smaller nanoparticles with specific geometric configurations, such as truncated triangular or spherical shapes, exhibit enhanced antimicrobial properties compared to larger particles or those with different shapes [29].

- Synergistic Effects of Cu and Ag Nanoparticles

The combination of Cu and Ag nanoparticles produces a synergistic effect that enhances their antimicrobial capabilities. This synergy is particularly effective against both Gram-negative and Gram-positive bacteria, increasing cell permeability and leading to more effective microbial inactivation [30].

- Surface Treatment and Chemical Agents

The surface treatment of nanoparticles with chemical agents or coatings can significantly enhance their antimicrobial properties. These treatments stabilize the nanoparticles against aggregation and enhance their interaction with microbial cells, leading to improved antimicrobial efficacy [29].

- Differential Susceptibility of Bacteria

Different bacteria exhibit varying levels of susceptibility to Cu and Ag nanoparticles. Some bacteria are resistant to these nanoparticles, which necessitates a deeper understanding of the mechanisms behind this resistance and strategies to overcome it [29].

- Mechanisms of Copper Nanoparticles

Copper nanoparticles exert their antimicrobial effects through several mechanisms, including the release of copper ions, induction of oxidative stress, and direct interaction with microbial cells. These interactions disrupt the integrity of microbial cell walls and membranes, leading to cell death [38] [39].

- Enhanced Antimicrobial Properties of Nanoalloys

Synthetic nanoalloys of copper and silver, such as AgCu, display enhanced antimicrobial properties compared to individual nanoparticles. These nanoalloys benefit from the combined effects of both metals, including increased stability and reduced toxicity, making them effective against a broad spectrum of bacteria [38] [29].

- Photocatalytic and Photothermal Effects

Certain Cu and Ag nanoparticles exhibit unique photocatalytic and photothermal effects that contribute to their antimicrobial activity. For example, CuS nanoparticles display strong photothermal effects, which can be harnessed to enhance their antimicrobial capabilities [29].

- Biomedical Applications

The antimicrobial properties of Cu and Ag nanoparticles have significant implications for biomedical applications. These nanoparticles are used in various products, including wound dressings, biosensors, and medical devices, where their ability to inhibit microbial growth is crucial [5] [6].

- Future Research Directions

Further research is needed to elucidate the precise mechanisms through which copper-doped silver nanoparticles exert their antimicrobial effects. Understanding these mechanisms will enable the optimization of nanoparticle formulations for enhanced antimicrobial efficacy and reduced toxicity [12] [20].

- Influence of Copper Doping

Copper doping in silver nanoparticles significantly enhances their antimicrobial properties and alters their physical characteristics, making them more effective and versatile in various applications. This section explores the influence of copper doping on silver nanoparticles, highlighting the synergistic effects, modifications in properties, and specific biomedical applications.

- Synergistic Effects Against Bacteria

1.Increased Antimicrobial Activity: Copper oxide (CuO) and silver (Ag) nanoparticles work in concert to produce a synergistic effect that greatly increases their antibacterial activity against a variety of bacteria, including types that are resistant to antibiotics [10].

2. Mechanism of Synergy: The production of Copper+ ions, increased dissolution of Ag+ from Ag nanoparticles, and decreased Ag+ binding by proteins in the presence of Cu2+ are all thought to be responsible for this increased activity [10].

3. Quantitative Improvement: Research indicates that the combination of CuO and Ag nanoparticles has an antibacterial impact that is more than five times stronger than the combined effects of each component employed alone.[10].

- Modifications in Nanoparticle Properties

1.Changes in Optical, Electrical, and Chemical Properties: Copper doping can modify the silver nanoparticles' optical, electrical, and chemical characteristics, improving their suitability for certain uses including biomedicine and sensing [21].

2.Dependency Factors: The size and form of the silver nanoparticles, the synthesis technique, and the concentration of copper are some of the variables that determine how much these modifications are altered. [21].

- Biomedical Applications of Copper-Doped Nanoparticles

1.Toxicity to Cancer Cells: Silver-copper alloy nanoparticles (AgCu-NP) have shown specific toxicity to breast cancer MCF-7 cells at certain concentrations, while exhibiting no harmful effects on healthy cells [13].

2.Wound Healing: Copper-doped nanoparticles facilitate wound healing by triggering the production of (ROS), which play a crucial role in killing bacteria and promoting the healing process [22].

3.Enhanced Healing Materials: Innovations such as Cu-BG/ESM membranes, which incorporate copper-containing glass ceramic disks on natural eggshell membranes, have shown to reduce E. coli viability and enhance healing quality and time both in vitro and in vivo [12].

4.Antimicrobial Films: Polycaprolactone films doped with CuONPs have demonstrated the ability to inhibit MRSA within 24 hours, showcasing their potential in preventing infections in medical settings [18].

These insights into the influence of copper doping on silver nanoparticles underline the importance of further research to optimize these nano formulations for enhanced efficacy and reduced toxicity in medical and other applications [18] [21].

Characterization Techniques

Overview of Characterization Techniques

The characterization of copper-doped silver nanoparticles is crucial for understanding their physical, chemical, and optical properties [12]. Various techniques are employed to analyse these properties comprehensively.

- Common Methods Used

1.UV-Vis Spectrophotometry: This procedure is utilized to survey the optical properties of nanoparticles. For copper oxide nanoparticles, the UV-Vis spectra ordinarily appear a SPR band at 340 nm [16].

2.X-ray Diffraction Examination (XRD): XRD makes a difference in deciding the crystalline structure of nanoparticles. For illustration, the monoclinic crystalline structure of CuO nanoparticles has been affirmed through XRD [16].

3.Fourier Change Infrared Spectroscopy (FTIR): FTIR examination can recognize useful bunches on the nanoparticle surface, such as the characteristic Cu-O extending band at 522 cm^-1 in CuO nanoparticles [16].

4. (SEM) and (TEM): These strategies give point by point pictures of the nanoparticle morphology. SEM and TEM micrographs of CuO nanoparticles have appeared them to be about circular with an normal molecule estimate of 59.99 nm [16].

5.Dynamic Light Scrambling (DLS): DLS is utilized to degree the measure dissemination and agglomeration state of nanoparticles. It shown consistency in CuO nanoparticles in spite of a few agglomeration [16].

6.Energy-Dispersive X-ray Spectroscopy (EDX): This method is pivotal for basic examination, making a difference in the recognizable proof of copper in the nanoparticles [5].

- Advanced Characterization Techniques

In addition to the standard methods, several advanced techniques are also utilized:

1. Auger Electron Spectroscopy (AES) and Low-energy Ion Scattering (LEIS): These methods are used for surface chemical analysis [5].
2. Zeta Potential Analysis: This technique assesses the surface charge of nanoparticles, which is important for understanding their stability in various mediums [25].
3. Scanning Tunnelling Microscope (STM) and Atomic Force Microscope (AFM): These are used for observing the surface topography at the atomic level [17].

- Importance of Characterization

Characterization techniques not only help in understanding the basic properties of nanoparticles but also assist in tailoring them for specific applications. The stability of copper oxide(I) nanoparticles, for instance, is influenced by the fluid's chemistry, including pH and ionic strength [7]. Therefore, comprehensive characterization is essential for optimizing the nanoparticles for their intended use [17].

Table 1: Characterization Techniques and Their Applications

Technique	Property Analyzed	Application Example
UV-Vis Spectrophotometry	Optical Properties	Determining plasmonic resonance [16]
X-ray Diffraction (XRD)	Crystalline Structure	Confirming crystalline phases [16]
(FTIR)	Surface Chemistry	Identifying functional groups [16]
(SEM)	Morphology	Imaging particle shape and size [16]
(TEM)	Detailed Morphology	High-resolution particle imaging [16]
Dynamic Light Scattering (DLS)	Size Distribution	Assessing agglomeration state [16]
Energy-Dispersive X-ray (EDX)	Elemental Composition	Elemental analysis [5]
Zeta Potential	Surface Charge	Stability analysis in various media [25]

These characterization methods are integral to developing effective copper-doped silver nanoparticles, ensuring they meet the required standards for various applications in fields such as medicine, environmental science, and technology.

Current Applications in Healthcare

Antimicrobial Applications in Healthcare

Copper-doped silver nanoparticles have demonstrated significant effectiveness against a variety of pathogens, including drug-resistant bacteria like MRSA, and viruses such as MERS, H1N1, and SARS-CoV-2 [2]. The mechanisms by which these nanoparticles exert their effects include altering pH levels and membrane channels in microbial cells, producing ROS that cause critical damage to cellular structures [2]. These properties make copper-doped silver nanoparticles highly valuable in developing antimicrobial coatings for medical devices, wound dressings, and other healthcare applications where infection control is crucial [12].

- Dental Healthcare Innovations

Dental materials are increasingly being enhanced with copper nanoparticles to improve their antibacterial capabilities and guard against conditions including implantitis, secondary caries, and stomatitis. Advanced dental care materials with enhanced mechanical qualities and antibacterial efficiency include copper-nanoparticle-incorporated acrylic resin and copper-doped mesoporous bioactive glass nanosphere acrylic resin. [26].

- Cancer Therapy and Drug Delivery Systems

The potential of copper-doped silver nanoparticles in cancer therapy has been highlighted by their enhanced toxicity to cancer cells, such as MCF-7 breast cancer cells, compared to treatments using silver or copper nanoparticles alone [13]. Moreover, copper nanoparticles serve as effective drug vehicles, providing targeted detection and therapeutic capabilities, which are essential for treating diseases at their source with minimal side effects [7].

Table 2: Healthcare Applications of Copper-Doped Silver Nanoparticles

Application Area	Description	References
Antimicrobial Coatings and Wound Dressings	Used in medical devices and wound care products to prevent infections by destroying harmful microbes.	[12]
Dental Materials	Enhance antimicrobial properties and mechanical strength in materials like fillings and coatings to prevent oral diseases.	[26]
Cancer Therapy	Utilized in targeted cancer treatments due to their ability to induce cytotoxicity specifically in cancer cells.	[13]
Drug Delivery Systems	Serve as carriers for drugs, enhancing the precision and efficiency of treatment delivery.	[7]

- Antiviral and Antibacterial Properties

Cu and Ag nanoparticles have been studied extensively for their antiviral and antibacterial capabilities. They are used in various medical applications to combat bacterial and viral infections, including those resistant to traditional treatments [7]. The biosynthesized CuONPs, in particular, have shown promising results against both gram-positive and gram-negative bacterial strains [17].

- Enhancements in Bone and Tissue Regeneration

Copper nanoparticles have been employed in products designed to facilitate tissue regeneration and improve bone regeneration. Examples include copper-based substances like nano copper-nonstoichiometric dicalcium silicate and copper-doped biphasic calcium phosphate, which are used in bone substitution and to enhance anti-inflammatory effects [26].

These diverse applications illustrate the broad potential of copper-doped silver nanoparticles in enhancing healthcare outcomes across various domains, from infection control to advanced therapies for chronic diseases.

Future Perspectives and Research Directions

Optimization of Synthesis Processes

1. Uniform Doping and Nanoparticle Control: Research should focus on optimizing the synthesis process for copper-doped silver nanoparticles to ensure uniform doping and precise control over the physical properties of the nanoparticles [12].

- Environmental and Safety Evaluations

1.Assessment of Environmental Impact: It is crucial to study the environmental implications of copper-doped silver nanoparticles, particularly their potential toxicity to aquatic life, behaviour under various environmental conditions, and patterns of degradation or accumulation [12].

2.Human and Ecological Safety: Evaluating the safety of these nanoparticles is essential, including their potential toxicity to humans and other organisms and their capacity to cause allergic reactions or other adverse effects [12].

- Regulatory and Economic Considerations

1.Development of Guidelines: Addressing regulatory considerations is necessary, including establishing guidelines for the use and disposal of copper-doped silver nanoparticles [12].

2.Cost-Effectiveness Analysis: The economic viability of these nanoparticles should be assessed by comparing their synthesis, characterization, and application costs with their effectiveness and durability against other antimicrobial agents [12]

Table 3: Degradation Efficiency of Biosynthesized Nanoparticles

Parameter	Condition	Degradation Efficiency (%)
Temperature	65℃	98.43
Dosage of CuO NPs	50 mg	98.43
Concentration of Rifampicin	10 mg/L	98.43
pH of Rifampicin Solution	2	98.43
Reaction Time	8 min	98.43

Green Synthesis of Copper Doped Silver Nanoparticles

General Process of Green Synthesis

Green synthesis of copper-doped silver nanoparticles (Ag-Cu NPs) involves environmentally friendly methods that utilize natural resources to reduce and stabilize nanoparticles. The process is outlined in the following steps:

1.Preparation of Plant Extract: Initially, a plant extract or biomolecule is prepared. In reported studies, Duranterecta fruits extract has been used effectively at ambient conditions [64}.

2.Preparation of Metal Salt Solutions: Solutions of silver nitrate and copper nitrate are prepared separately to serve as the source of metal ions [50].

3.Mixing and Reaction: The prepared plant extract or biomolecule is then mixed with the precursor salt solutions. This mixture undergoes a reaction where the metal ions are reduced [50].

4.Reduction and Capping: The metal ions in the solution are reduced to form nanoparticles. Simultaneously, the biomolecules from the extract act as capping agents, stabilizing the formed nanoparticles [50].

5.Characterization: The final step involves characterizing the synthesized nanoparticles to evaluate their size, shape, and chemical composition [50].

- Applications of Ag-Cu Nanoparticles

Ag-Cu NPs synthesized through green methods are notable for their applications across several fields. These nanoparticles are utilized in:

1. Catalysis: Enhancing the rate of chemical reactions due to their unique surface properties [50].
2. Sensing: Used in biosensors and chemical sensors for detecting various biological and chemical species [50].
3. Biomedicine: Applied in medical diagnostics and treatments, leveraging their antimicrobial and therapeutic properties [50].

By employing green synthesis methods, copper-doped silver nanoparticles are produced in a way that is not only environmentally sustainable but also effective for practical applications in catalysis, sensing, and biomedicine [50][64].

Key Properties of Copper Doped Silver Nanoparticles

Copper-doped silver nanoparticles (Ag-Cu NPs) have garnered attention in nanobiotechnology due to their distinctive properties, which are significantly enhanced by the presence of both copper and silver. These nanoparticles are not only biocompatible but also capable of controlled substance release, making them highly suitable for various applications [49][50].

- Unique Physical and Chemical Properties

1.Size and Shape Control: The synthesis process allows precise control over the size and shape of Ag-Cu NPs, which directly influences their physical and chemical properties. This control is crucial for tailoring nanoparticles for specific applications [50].

2.Enhanced Antimicrobial Activity: Ag-Cu NPs exhibit strong antimicrobial properties against a wide array of microorganisms, including bacteria, fungi, and algae. Their effectiveness varies with the microbial species and the conditions of the experimental setup [56].

3.Shape-Dependent Properties: These nanoparticles can vary greatly in nano-size and form, which affects their distribution and surface-area-to-volume ratio. Such properties are critical for their function in biophysical and chemical applications [66].

- Influence of Synthesis Conditions

Copper Doping Concentration: The amount of copper integrated during the synthesis affects the nanoparticles' effectiveness, particularly in antimicrobial applications. Higher copper concentrations generally enhance the bactericidal activity against specific bacteria strains [66].

Particle Size and Shape: The dimensions and morphology of Ag-Cu NPs are determined during the synthesis phase, impacting their stability, reactivity, and surface chemistry [75][76].

- Biomedical and Environmental Applications

1. Biocompatibility: Due to their non-toxic nature, Ag-Cu NPs are suitable for various biomedical applications, including drug delivery systems and therapeutic agents. Their ability to interact benignly with biological systems enhances their utility in medical applications [49].
2. Environmental Applications: The unique properties of Ag-Cu NPs also make them effective in environmental applications, such as in sensors and catalysts, which benefit from their reactive surface properties [50].

These key properties highlight the versatility and potential of copper-doped silver nanoparticles in both scientific research and practical applications, spanning from healthcare to environmental management.

Comparison with Conventional Nanoparticle Synthesis

Conventional vs. Green Synthesis Methods

Conventional nanoparticle synthesis often employs methods that may not be environmentally friendly or biocompatible. These methods typically involve:

1.Use of High Temperatures and Vacuum Conditions: Many conventional techniques require extreme conditions, which can be energy-intensive and unsafe for both the environment and the researchers involved [51].

2.Application of Harsh Chemicals: Hazardous chemicals are commonly used in processes like chemical precipitation and sol-gel methods, posing risks to health and the environment [51][55].

3.Variability in Nanoparticle Properties: Techniques such as thermal decomposition and mechano-chemical methods can lead to nanoparticles with diverse sizes and morphologies, which may not always be desirable for specific applications [52].

In contrast, green synthesis methods offer several advantages that address these issues:

1. Lower Energy Consumption: Green synthesis typically occurs at ambient temperatures and pressures, significantly reducing the energy requirements [51].
2. Non-toxic Chemicals: Biocompatible agents like plant extracts or biodegradable polymers are used, minimizing environmental and health risks [53][54].
3. Controlled Synthesis: Green methods provide better control over the size, shape, and composition of nanoparticles, enhancing their application in areas like drug delivery and biosensing [53][62].

- Economic and Safety Considerations

1. The economic and safety aspects of nanoparticle synthesis are crucial for sustainable development. Conventional methods often involve:
2. High Costs and Energy Demands: The need for specialized equipment and high energy inputs makes conventional synthesis expensive and less sustainable [55].
3. Potential Toxicity: The presence of residual hazardous chemicals on nanoparticles can lead to biocompatibility issues, limiting their use in medical applications [56].
4. Green synthesis, however, uses cost-effective and safe materials, such as:
5. Biodegradable Materials for Surface Engineering: These materials not only ensure the stability of nanoparticles but also reduce toxicity, making nanoparticles safer for medical applications [53].
6. Economic Efficiency: Utilizing natural and readily available materials reduces costs and promotes the scalability of nanoparticle production [51].

- Technological and Application Differences

Both conventional and green synthesis methods have their unique technological applications and challenges:

Conventional Techniques: Methods like laser ablation and ion implantation are highly precise, making them suitable for creating complex materials. However, they require sophisticated technology and are not always scalable [51][65].

Green Techniques: While simpler and more environmentally friendly, green synthesis must continually evolve to match the efficiency and precision of conventional methods in applications such as electronics and heavy industry [51].

Overall, while conventional synthesis methods have been foundational in the development of nanotechnology, green synthesis is gaining prominence due to its environmental and health benefits, along with advancements that increasingly meet the technical demands of various industries.

Antimicrobial Activity of Copper Doped Silver Nanoparticles

Mechanisms of Antimicrobial Action

Copper-doped silver nanoparticles (Ag-Cu NPs) exhibit significant antimicrobial properties through various mechanisms. These nanoparticles release silver and copper ions, which are known to disrupt bacterial cell membranes and interfere with essential cellular processes [50]. Additionally, the presence of copper enhances the bactericidal effects by generating (ROS) and disrupting metalloproteins by replacing or binding their native cofactors [67].

- Synergistic Effects with Antibiotics

Research has demonstrated that biosynthesized silver nanoparticles can effectively enhance the antibacterial activity of antibiotics such as gemifloxacin. This synergy is particularly effective against both gram-positive Staphylococcus aureus and gram-negative *Escherichia coli*, showcasing a potential route for combating antibiotic-resistant bacterial strains [64]. Moreover, the combination of copper oxide (CuO) and silver nanoparticles has been shown to increase the antibacterial effectiveness up to six times compared to their individual effects [68].

- Specificity and Efficiency in Different Conditions

The effectiveness of Ag-Cu NPs is influenced by several factors including the concentration of nanoparticles, the contact time with microorganisms, and the specific strains of bacteria or fungi involved. These factors can significantly affect the outcomes of antimicrobial tests, such as disc diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays [56]. The antimicrobial activity also varies with the microbial species, highlighting the need for tailored approaches in different experimental setups [56].

- Enhanced Antimicrobial Activity

Copper-doped silver nanoparticles have shown higher bactericidal activity compared to silver nanoparticles alone. This enhanced activity is likely due to the synergistic action of silver and copper ions within the microbial cells, leading to more effective disruption of cellular functions [18][50][52]. Additionally, the unique properties of these nanoparticles, such as their increased surface area and different crystal structures, contribute to their higher antimicrobial efficiency [19].

- Applications in Microbial Control

Due to their potent antimicrobial properties, Ag-Cu NPs are utilized in various applications beyond medical treatments. They are employed in environmental remediation, such as in the catalytic degradation of pollutants and in sensing applications for detecting harmful microbial and chemical species [53]. This broad range of applications underscores the versatility and importance of copper-doped silver nanoparticles in both healthcare and environmental management.

By understanding these mechanisms and factors affecting the antimicrobial activity of Ag-Cu NPs, researchers can further optimize these nanoparticles for specific applications, potentially leading to more effective strategies in combating microbial resistance and enhancing public health safety.

Characterization of Copper Doped Silver Nanoparticles

Techniques Employed in Characterization

Various advanced techniques are utilized to characterize copper-doped silver nanoparticles (Ag-Cu NPs), ensuring detailed analysis of their physicochemical properties:

1UV-Visible Spectrophotometry: This strategy is significant for distinguishing the surface plasmon reverberation (SPR) of Ag-Cu NPs, demonstrating nanoparticle arrangement [64][69][70].

2.X-Ray Diffraction (XRD): XRD makes a difference in deciding the crystalline structure of the nanoparticles. The crystallite estimate can be calculated from XRD information utilizing the Debye-Scherrer condition, giving bits of knowledge into the molecule estimate dispersion [64][69].

3.Fourier Change Infrared Spectroscopy (FTIR): FTIR examination is utilized to recognize the chemical holding and atomic structure, affirming the nearness of utilitarian bunches that stabilize the nanoparticles [64][69].

4. TEM: TEM offers high-resolution pictures that uncover the measure, shape, and morphology of the nanoparticles, vital for understanding their blend and potential applications [64][69][70].

5.SEM: SEM is utilized to watch the surface morphology and geology of Ag-Cu NPs, giving basic information on their basic characteristics [64][69].

6.Dynamic Light Diffusing (DLS): DLS measures the hydrodynamic measure and solidness of nanoparticles in arrangement, which is imperative for applications in biomedicine and natural sciences [64][70].

7.Energy-Dispersive X-Ray Spectroscopy (EDX): EDX examination is pivotal for natural investigation, affirming the composition and virtue of Ag-Cu NPs [64][69][70].

8.Auger Electron Spectroscopy (AES) and Low-Energy Particle Scrambling (LEIS): These procedures give surface compositional investigation and are basic for understanding the natural composition of the peripheral layers of the nanoparticles [69].

Summary of Characterization Methods

The table below summarizes the key characterization techniques used for copper-doped silver nanoparticles, highlighting their respective roles and contributions to nanoparticle analysis:

Table 4: Summarizing the various characterization techniques

Technique	Purpose	Importance
UV-Visible Spectrophotometry	Identifies SPR of nanoparticles	Essential for confirming nanoparticle formation
X-Ray Diffraction (XRD)	Determines crystalline structure	Helps calculate particle size using Debye-Scherrer equation
Fourier Transform Infrared Spectroscopy (FTIR)	Analyzes chemical bonding and structure	Confirms presence of stabilizing functional groups
TEM	Provides high-resolution images of size and shape	Crucial for detailed morphological analysis
SEM	Observes surface morphology	Important for structural analysis
Dynamic Light Scattering (DLS)	Measures hydrodynamic size and stability	Vital for applications requiring stable nanoparticle dispersions

Energy-Dispersive X-Ray Spectroscopy (EDX)	Elemental analysis	Confirms composition and purity
Auger Electron Spectroscopy (AES)	Surface compositional analysis	Provides detailed surface composition data
Low-Energy Ion Scattering (LEIS)	Analyzes outermost layer composition	Essential for surface-specific studies
Technique	Purpose	Importance
UV-Visible Spectrophotometry	Monitors the surface plasmon resonance, indicating nanoparticle formation	Essential for confirming successful synthesis
X-Ray Diffraction (XRD)	Determines the crystalline structure and phase composition	Helps in understanding the structural properties
Fourier Transform Infrared Spectroscopy (FTIR)	Identifies functional groups and chemical bonding	Crucial for confirming the capping and stability of nanoparticles
TEM	Provides detailed images of nanoparticle morphology	Key in analyzing shape, size, and distribution
Dynamic Light Scattering (DLS)	Measures the hydrodynamic diameter and stability of nanoparticles in suspension	Important for assessing the dispersibility and stability

Green Synthesis Methods for Copper-Doped Silver Nanoparticles

Step-by-Step Green Synthesis Process

The green synthesis of copper-doped silver nanoparticles (Ag-Cu NPs) involves a series of steps, each crucial for achieving high-quality nanoparticles with desired properties. Below is a detailed description of each step:

1.Preparation of Plant Extract or Biomolecule: Initially, a specific plant extract or biomolecule is prepared. This extract acts as a reducing and capping agent, essential for the synthesis of Ag-Cu NPs. The choice of plant or biomolecule depends on its availability and the specific chemical properties it offers for the reduction process [72].

2.Preparation of Metal Salt Solutions: Separate solutions of silver nitrate (AgNO3) and copper nitrate (Cu(NO3)2) are prepared. These solutions provide the metal ions needed for nanoparticle formation. The concentration of these solutions can vary depending on the desired size and concentration of the nanoparticles [72].

3.Mixing of Plant Extract/Biomolecule with Precursor Salt Solutions: The prepared plant extract or biomolecule is then mixed with the solutions of AgNO3 and Cu(NO3)2 in a specific ratio. This step is critical as the ratio determines the final composition and properties of the nanoparticles [72].

4.Reduction and Capping: During this stage, the Ag+ and Cu2+ ions in the solution are reduced to their nanoparticle forms. The plant extract or biomolecule not only facilitates this reduction but also caps the nanoparticles, preventing them from agglomerating and ensuring their stability in the solution [72].

5.Characterization: After synthesis, the Ag-Cu NPs are characterized using various techniques. Methods such as UV-Visible spectroscopy, X-ray diffraction (XRD), and TEM are employed to analyse the size, shape, and other physicochemical properties of the nanoparticles. This characterization is essential to confirm the successful synthesis of nanoparticles and to evaluate their potential applications [72].

Properties and Characterization of Copper-Doped Silver Nanoparticles

Green synthesized copper-doped silver nanoparticles (Ag-Cu NPs) are utilized extensively in environmental remediation, demonstrating significant effectiveness in various applications. These include antimicrobial activities, catalytic activities, the removal of pollutant dyes, and heavy metal ion sensing. The properties of these nanoparticles, influenced by their synthesis method, play a crucial role in their functionality and effectiveness in these applications [75].

- Applications in Environmental Remediation

1.Antimicrobial Activity: Ag-Cu NPs are known for their potent antimicrobial properties, which make them suitable for use in water treatment and surface sterilization processes.

2.Catalytic Activity: These nanoparticles act as catalysts in the breakdown of environmental pollutants, aiding in the detoxification of hazardous substances.

3.Removal of Pollutant Dyes: They are effective in adsorbing and breaking down dyes from industrial wastewater, which is a significant step towards mitigating water pollution.

4.Heavy Metal Ion Sensing: Ag-Cu NPs are employed in sensors that detect harmful levels of heavy metals in water, ensuring safer drinking water and adherence to environmental safety standards.

Applications in Drug Delivery and Therapeutic Agents

Copper-doped silver nanoparticles (Ag-Cu NPs) have illustrated noteworthy potential in the field of biomedicine, especially in sedate conveyance frameworks and restorative operators. The one of kind properties of these nanoparticles permit for improved interaction with organic frameworks, making them successful carriers for drugs and dynamic restorative agents.

Drug Conveyance Systems

1.Large Particular Surface Zone: Copper nanoparticles are utilized as successful medicate nanocarriers due to their huge particular surface zone, which encourages the conjugation with different biomolecules [88].

2.Controlled Sedate Discharge: Copper-based nano definitions are known to hinder cancer cell expansion and give controlled discharge of anticancer drugs, upgrading the adequacy of the treatment whereas minimizing side impacts [88].

3.Overcoming Sedate Resistance: Copper nanoparticles can avoid cancer cell metastasis and address medicate resistance by bypassing P- glycoprotein intervened sedate efflux transporters, which are regularly a critical boundary in chemotherapy [88].

- Therapeutic Agents

1. Anticancer Properties: Ag-Cu NPs exhibit preferential toxicity towards cancer cells, such as breast cancer cells, compared to healthy cells. This selective toxicity is crucial for reducing the adverse effects on normal cells during cancer treatment [87].
2. Synergistic Antimicrobial Effects: When combined with silver nanoparticles, copper-based nanoparticles show enhanced antibacterial properties, which can be utilized in preventing infections in medical settings [88].
3. Dental Applications: These nanoparticles have also shown potential in improving the properties of dental materials and preventing oral infections, which is vital for dental health and hygiene [84].

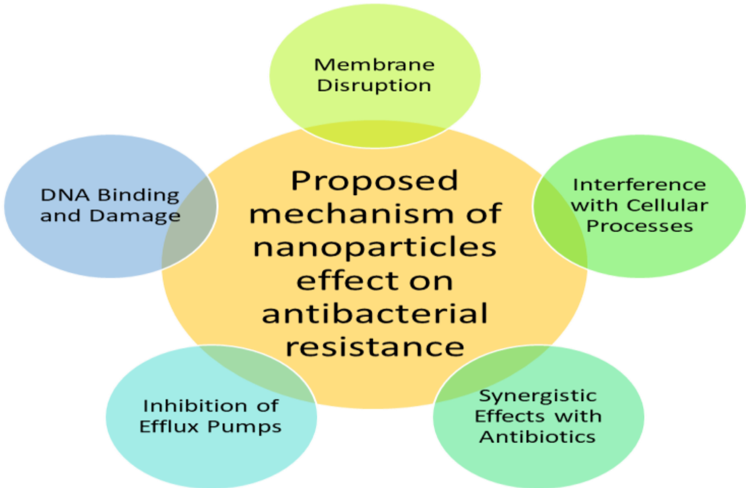


Fig 1- Showing different mechanism by which nanoparticles exert antibacterial effect

Table 5: Application and mechanism of doped NP

Application	Mechanism	Benefits
Drug Delivery	Large surface area and controlled release	Efficient drug delivery and reduced side effects
Anticancer Therapy	Preferential toxicity and overcoming resistance	Targeted cancer treatment with fewer normal cell impacts
Antimicrobial and Dental Applications	Synergistic effects and improved material properties	Enhanced infection control and dental material performance

Principles of Green Synthesis

Overview of Eco-Friendly Synthesis Approaches

Green synthesis of nanoparticles employs natural biological systems, avoiding the use of harmful chemicals and high-energy processes typical of traditional methods. This approach not only emphasizes sustainability but also enhances the biocompatibility of the nanoparticles [20].

- Key Components and Processes

1.Utilization of Plant and Microbial Extracts:

Various plant extracts and microorganisms such as bacteria, yeast, fungi, and algae are used as substrates to reduce metal salts to nanoparticles. This process leverages the natural reducing power of these biological systems [87].

For example, the use of Eucalyptus camaldulensis and Terminalia arjuna extracts facilitates the synthesis of silver nanoparticles by acting as both reducing and capping agents [78].

2.Active Molecules in Synthesis:

Active molecules present in these biological systems, such as proteins, enzymes, and other metabolites, play a crucial role in reducing metal ions and stabilizing the formed nanoparticles. These molecules determine the morphology, size, and functional properties of the nanoparticles [81].

The specific interactions between these molecules and metal ions lead to the formation of nanoparticles with desired properties[89].

- Advantages of Green Synthesis

Environmental Sustainability: By using naturally sourced materials and energy-efficient processes, green synthesis minimizes environmental impact [87].

Cost-Effectiveness: Reduces the need for expensive and hazardous chemicals, making the process more economical [88]. Enhanced Biocompatibility: Nanoparticles produced via green synthesis are generally more compatible with biological systems, making them ideal for medical and pharmaceutical applications [87].

Characterization Techniques

1. Green nanoparticles (GNPs) are characterized using a variety of techniques to confirm their structure, composition, and stability:
2. X-ray Diffraction (XRD) and Fourier Transform Infrared Spectrum (FT-IR) provide information on the crystal structure and chemical bonds [75].
3. UV-visible Absorption Spectrum helps in understanding the optical properties due to surface plasmon resonance [75].
4. Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM), and Atomic Force Microscopy (AFM) are used to analyse the morphology and topography at the nanoscale [75].

The 12Principles of Green Chemistry

Adhering to these principles ensures that the synthesis process is as environmentally benign as possible. These guidelines help in reducing the toxicity of byproducts, enhancing the efficiency of reactions, and minimizing the exposure of hazardous substances to both humans and the environment [87].

By integrating these principles and methods, green synthesis of nanoparticles not only addresses the limitations of traditional synthetic methods but also opens new pathways for advancements in nanotechnology, particularly in the fields of medicine, electronics, and environmental science [86][87].

- Antimicrobial Activity of Copper Nanoparticles
- Mechanism of Antimicrobial Action

Copper nanoparticles (CuNPs) exhibit potent antimicrobial properties primarily through the generation of (ROS). These ROS led to the oxidation of lipids and proteins and cause significant DNA damage within microbial cells [88]. Additionally, CuNPs disrupt the normal functions of metalloproteins by replacing or binding their native cofactors, further inhibiting microbial growth [88].

- Impact on Microbial Structures

The antimicrobial activity of CuNPs extends to the destruction of cell walls and membranes. This disruption is crucial as it compromises the integrity of microbial cells, leading to their eventual death [23]. The interaction of CuNPs with cellular proteins and DNA further ensures a broad-spectrum efficacy against various microorganisms [88].

- Influence of Physical Properties

The effectiveness of CuNPs against microbes is influenced by their size, shape, and the environmental conditions such as temperature and pH. Smaller nanoparticles have a higher surface area to interact with microbial cells, enhancing their antimicrobial actions [88].

Environmental factors like humidity and the presence of other ions can also affect the activity of CuNPs [88].

1. Enhancement Through Doping-Doping CuNPs with other metals such as Mg, Zn, or Ce can significantly enhance their antimicrobial properties. These modifications aid in the controlled release of $Cu2+$ ions, which are critical for the antimicrobial efficacy of the nanoparticles [88].
2. Spectrum of Antimicrobial Activity-CuNPs have demonstrated significant inhibitory effects against a wide range of microorganisms, including bacteria such as Escherichia coli, Staphylococcus aureus, and fungi like Candida albicans. Their activity varies with the microbial species and the specific conditions of the experimental setup [80][88].
3. Application in Biofilm Prevention-In addition to their use in suspensions, CuNPs can be incorporated into various biocompatible materials like chitosan and hydrogels. These composites are particularly useful in medical applications where they prevent the formation of biofilms by pathogenic bacteria on medical devices and implants [88].

4. Antifungal and Antibacterial Assays-The antimicrobial efficacy of CuNPs has been quantitatively assessed using various methods such as the disc diffusion test, minimum inhibitory concentration (MIC), and counting colony-forming units. These methods help in determining the effective concentrations needed for microbial inhibition and eradication [78].
5. Toxicity Against Drug-Resistant Strains-Green synthesized CuNPs have shown promising results against drug-resistant strains of bacteria and fungi, indicating their potential as alternative antimicrobial agents in scenarios where traditional antibiotics fail [78][87].
6. By harnessing these properties, CuNPs hold the potential to revolutionize the field of antimicrobial treatments, providing a robust defence against a variety of pathogenic microorganisms.

- Antimicrobial Activity of Silver Nanoparticles
- Mechanism of Antimicrobial Action

Silver nanoparticles (AgNPs) are renowned for their potent antimicrobial properties, primarily due to their ability to disrupt bacterial membranes and interfere with DNA and protein processes. This disruption is facilitated through the release of Ag+ ions, which are highly reactive and contribute to the destruction of microbial structures [24]. Additionally, AgNPs enhance microbial control by capitalizing on the generation of ros, which further damages bacterial cells [89].

- Synergistic Effects with Other Metals

The incorporation of bimetallic nanoparticles, specifically those combining silver (Ag) and copper (Cu), enhances antibacterial effectiveness while maintaining low cytotoxicity. These bimetallic nanoparticles (BNPs) exhibit a synergistic antibacterial mechanism, involving the simultaneous release of Ag+ and Cu2+ ions. This dual release disrupts bacterial membranes more effectively, interferes with DNA replication, and induces oxidative stress within the bacterial cells [89].

- Influential Factors on Antibacterial Efficacy

The antibacterial efficacy of AgNPs, particularly when used in combination with copper nanoparticles (CuNPs), is influenced by several factors. These include the ratio of silver to copper, the size and shape of the nanoparticles, and the presence of carriers or other materials that may affect their delivery and stability [24].

Broad-Spectrum Antibacterial Activity

AgNPs have illustrated noteworthy antibacterial movement against a wide run of microscopic organisms, counting both Gram-negative and Gram- positive strains, as well as multidrug-resistant strains. This broad-spectrum movement makes AgNPs especially important in different applications over the therapeutic and healthcare divisions, where they can be utilized to treat or anticipate diseases [90].

• Applications in Therapeutic and Healthcare Products

Due to their successful antimicrobial properties and relative biocompatibility, silver nanoparticles are progressively being utilized in a assortment of therapeutic and healthcare items. These applications incorporate, but are not constrained to, wound dressings, coatings for therapeutic gadgets, and added substances in items to anticipate bacterial growth [90].

- Antimicrobial Assays and Experimental Evidence

The effectiveness of AgNPs in inhibiting bacterial growth and biofilm formation has been confirmed through various assays. Studies using the well diffusion method have shown that AgNPs can create zones of inhibition around the bacteria, effectively preventing their growth and the formation of harmful biofilms [83]. Additionally, biosynthesized AgNPs have shown to work in synergy with antibiotics like Gemifloxacin, enhancing their antibacterial activity [85].

- Safety and Biocompatibility Concerns

While silver nanoparticles are beneficial in numerous applications, their safety, especially in long-term use scenarios such as dental and orthodontic treatments, remains a concern. Although AgNPs can induce oxidative stress and impair mitochondrial function, they also exhibit anti-inflammatory properties and are generally biocompatible with human cells such as fibroblasts and keratinocytes. Studies indicate that silver accumulated in the body from medical applications can mostly be cleared after 8 weeks, suggesting a manageable level of risk when used appropriately [90].

Silver Nanoparticles: Synthesis, Characterization, and Applications

Characterization Techniques

- UV-Vis Spectrophotometry and X-Ray Diffraction

Silver nanoparticles (AgNPs) are commonly characterized using UV-visible spectrophotometry, which confirms their synthesis through the detection of surface plasmon resonance bands. This technique, along with X-ray diffraction (XRD), provides insights into the crystalline structure of the nanoparticles. Specifically, AgNPs synthesized using Eugenia roxburghii DC extract exhibit an average crystalline size of approximately 35 nm [78].

- High-Resolution TEM

High-resolution TEM offers detailed imagery that confirms the nanoscale size and shape of the AgNPs. The images reveal that these nanoparticles are un-agglomerated spheres with a hydrodynamic diameter between 70 and 90 nm [90].

- Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy is utilized to identify the functional groups involved in the nanoparticle synthesis, providing a chemical fingerprint that indicates which biomolecules are responsible for reducing the silver ions and stabilizing the nanoparticles [73].

- Stability and Optical Properties

The zeta potential of biosynthesized AgNPs is typically around -37.8 mV, indicating a high degree of stability, which is essential for various applications [76]. The distinct optical properties of AgNPs, characterized by a peak at 420 nm in UV-Vis spectroscopy, further underline their utility in areas requiring precise optical performance [90].

- Applications of Silver Nanoparticles

Silver nanoparticles have a broad spectrum of applications due to their unique properties. Their potent antibacterial activity makes them ideal for use in medical and healthcare products, such as wound dressings and coatings for medical devices. Moreover, their optical properties are leveraged in sensors and imaging devices, where precision is crucial [79].

- Environmental and Industrial Uses

In the environmental sector, AgNPs are employed in applications such as wastewater remediation, where they help decontaminate water by breaking down harmful pollutants like naphthalene [3]. Additionally, their photocatalytic properties are utilized in the degradation of dyes, such as methyl red, showcasing their potential in industrial waste management [79].

- Antioxidant Activities

The DPPH free radical scavenging activity of AgNPs indicates their capability as antioxidants. This property is particularly valuable in the development of products aimed at combating oxidative stress in biological and environmental systems [79].

By synthesizing and characterizing silver nanoparticles through green methods, not only are their beneficial properties harnessed, but it also aligns with the principles of sustainability and eco-friendliness. This approach ensures that the applications of AgNPs are as environmentally responsible as they are diverse and effective.

Copper Nanoparticles: Synthesis, Characterization, and Applications

Synthesis and Characterization Techniques

- UV-Vis Absorption and X-Ray Diffraction

Copper nanoparticles (CuNPs) synthesized using green methods exhibit distinct characteristics identified through various techniques. UV- Vis spectroscopy reveals a surface plasmon resonance peak at 679 nm, indicative of the nanoparticles' optical properties [10]. X-ray diffraction analysis provides insight into the crystal structure, showing diffraction peaks that correspond to the face-centred cubic (fcc) crystallographic planes, confirming the crystalline nature of these nanoparticles [75].

- SEM

SEM analysis of CuNPs reveals their spherical but agglomerated shape with sizes ranging between 51.26 and 56.66 nm. This detailed imaging helps in understanding the morphological attributes which are crucial for their functional applications [80].

- Environmental and Biomedical Applications
- Wastewater Remediation

Studies have demonstrated the effectiveness of CuNPs in wastewater treatment, particularly in the removal of naphthalene, a polycyclic aromatic hydrocarbon. The removal efficiency is notably high, with Azadirachta indica synthesized nanoparticles achieving up to 98.81% removal [73]. This underscores the potential of CuNPs in environmental pollution control.

- Antibiotic Pollution Remedy

In the context of environmental remediation, CuO nanoparticles synthesized from Parthenium hysterophorus aqueous extract have shown promising results in degrading antibiotics like rifampicin. The optimal conditions for this process include a temperature of 65℃ and a specific nanoparticle dosage, resulting in a degradation efficiency of 98.43% [77].

- Photocatalytic Degradation of Dyes

The photocatalytic properties of CuNPs are leveraged in the degradation of methylene blue under sunlight, achieving up to 90% degradation efficiency. This application is vital in addressing industrial dye pollutants in water bodies [76].

- Industrial and Construction Applications
- Building Material Enhancement

Copper nanoparticles are utilized in construction to enhance building materials. By reducing the roughness of steel surfaces, CuNPs contribute to improved resistance to corrosion, enhanced thermal conductivity, and better heat transfer properties, making them valuable in building and construction sectors [83].

Flame Spray Pyrolysis

The scalable production of copper oxide nanoparticles through flame spray pyrolysis illustrates the versatility of synthesis methods. This technique allows for the control of nanoparticle size by adjusting flame temperature, residence time, and the concentration of the liquid precursor, catering to various industrial needs [83].

- Broad Spectrum of Applications

Copper nanoparticles find applications across a diverse range of fields including biomedicine, pharmaceuticals, bioremediation, and cosmetics. Their structural properties and biological effects make them particularly effective in life sciences, offering new avenues for innovation in drug delivery systems, therapeutic agents, and diagnostic tools [83].

- Comparative Analysis of Cu and Ag Nanoparticles

In the comparative analysis of Cu and Ag nanoparticles, it is crucial to consider their respective efficiencies in environmental applications, such as pollutant removal. Studies have shown a notable difference in the adsorption capacities of nanoparticles synthesized using different plant extracts. Specifically, copper nanoparticles synthesized using Azadirachta indica have demonstrated a higher removal rate of pollutants, achieving up to 98.81% efficiency. In contrast, nanoparticles synthesized using Coriandrum sativum exhibited a slightly lower efficiency, with an adsorption power of 95.29% [73]. This variance highlights the influence of the biological source material on the nanoparticles' properties and their subsequent environmental applications.

Case Studies and Experimental Evidence

Sub-Acute Toxicity Evaluation

In a case study focusing on the safety of nanoparticles, sub-acute toxicity evaluations were conducted. It was observed that nanoparticles administered at a dosage of 200 µg/kg presented signs of progressive onset of toxicity [90].

- Characterization of Synthesized Nanoparticles

The nanoparticles synthesized were extensively characterized using a combination of techniques:

UV-Vis Spectroscopy: Utilized to dissect the optical properties of the nanoparticles [79].

Fourier Change Infrared Spectroscopy (FTIR): Illustrated that all utilitarian bunches display in the algal extricate taken part in the diminishment and stabilization of the nanoparticles [79].

X-Ray Diffraction (XRD): Affirmed the crystalline nature of the nanoparticles, appearing a face-centred cubic (FCC) geometry [22]. SEM: Given point by point pictures appearing the morphology of the biogenically synthesized metal nanoparticles [79].

Antimicrobial Viability Testing

The antimicrobial efficency of the synthesized nanoparticles was thoroughly tried using:

1.Agar Well Plate Strategy: This strategy was utilized to degree the zone of hindrance, which evaluates the adequacy of the nanoparticles against microscopic organisms [79].

2.Broth Weakening Test: Utilized to decide the MIC, assist surveying the nanoparticles' viability in restraining microbial development [79].

- Adsorption Process Studies

Kinetic and equilibrium studies were applied to explore the adsorption processes involving nanoparticles. These studies are crucial for understanding how nanoparticles interact with various substances, which is essential for applications such as pollutant removal [73].

- Antimicrobial Mechanisms of Nanoparticles
- Disruption of Microbial Cell Membranes

Nanoparticles, particularly those synthesized through green methods, exhibit a primary mechanism of antimicrobial action by disrupting microbial cell membranes. The nanoparticles interact with the lipid bilayer of bacterial cells, causing structural damage that leads to cell lysis and death. This interaction is facilitated by the unique surface properties and small size of nanoparticles, which allow them to attach to and penetrate the bacterial cell walls effectively [80].

- Release of Metal Ions
- Another noteworthy antimicrobial component is the discharge of metal particles from nanoparticles, such as silver (Ag^+) and copper (Cu^{2+}). These particles are profoundly responsive and can disturb fundamental cellular capacities by collaboration with

proteins and proteins inside the microbial cells. The particles can associated with the respiratory chain and vitality generation, driving to the inactivation of bacterial development and propagation [84].

- • Generation of (ROS)
- Nanoparticles can actuate oxidative stretch in microbial cells by producing (ROS). These ROS, counting free radicals such as hydroxyl radicals and hydrogen peroxide, harm cellular components like DNA, proteins, and lipids, eventually causing cell passing. The oxidative push component is especially successful against a wide range of microorganisms, upgrading the antimicrobial viability of nanoparticles [84].
- • Interaction with DNA and Proteins
- Nanoparticles can specifically associated with DNA and proteins, driving to the hindrance of imperative cellular forms such as replication, translation, and interpretation. This interaction anticipates organisms from increasing and performing vital capacities, contributing altogether to their antimicrobial activity. The little measure and tall surface region of nanoparticles encourage near interaction with these macromolecules, upgrading their capacity to disturb microbial life cycles [84].

Table 6: Mechanism of action NP on microorganism

Mechanism	Description	Impact on Microbes
Disruption of Cell Membranes	Nanoparticles damage the lipid bilayer, causing cell lysis.	Leads to physical destruction of microbial cells.
Release of Metal Ions	Reactive metal ions from nanoparticles disrupt cellular functions.	Inhibits growth and reproduction of microbes.
Generation of (ROS)	Induces oxidative stress in microbes, damaging vital cellular components.	Causes cell death through oxidative damage.
Interaction with DNA and Proteins	Nanoparticles interfere with essential processes like DNA replication and protein synthesis.	Stops microbial cell functions, preventing survival.

CHAPTER- 3

MATERIALAND METHOD

Materials- Silver Nitrate, Copper Sulphate, Dried Eclipta leaves, DPPH, ABTS from (Himedia Lab.), Muller Hinton Agar, Muller Hinton Broth, E.coli.(ATCC 25922), S. aureus(ATCC 23235), Deionized water (Millipore Q.) All solvents used in this study are of analytical grade.

1. Preparation of plant extract

- Fresh young leaves of E. alba were collected.
- They were weighed,
- Washed under running tap water to remove any dust and dirt,
- Further washed with distil water,
- They were then allowed to shade dry for 14 days,
- Then the dried leaves were weighed,
- Boiled in distil water at 60oC for 30 mins
- Filtered using Wattman no 1 paper and stored at 4^oC for further use.

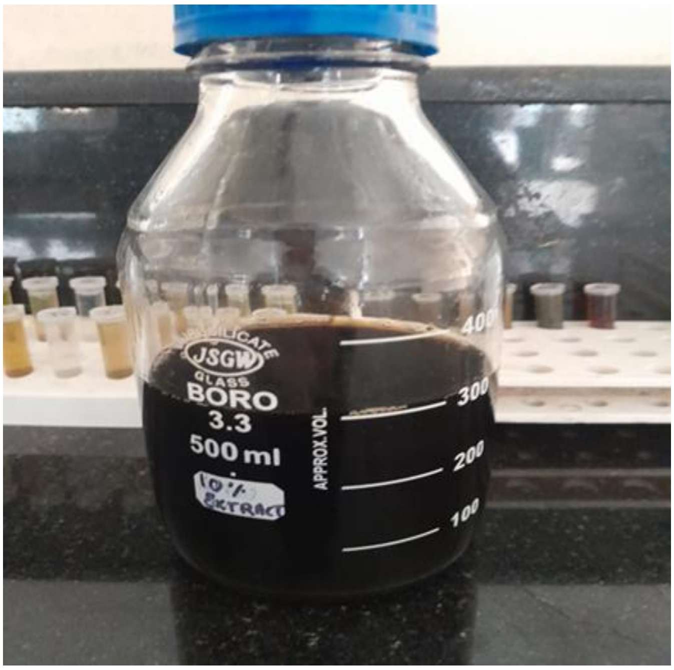


Fig 2- Synthesised Plant Extract

2. Synthesis of Nanoparticles.

- To synthesis doped nanoparticles, firstly only, silver and copper nanoparticles were made using the 10% plant extract. Their parameters wee optimized.
- Afte that the conditions and parameters were optimized for the doped nanofomulations.

To synthesise the nano formulations, 0.1M $AgNO_3$was used, it was made by weighing, 0.169g of $AgNO_3$in 10ml, and similarly 5mM of $CuSO_4$was used, which was prepared by weighing, 0.024g of $CuSO_4$in 10ml of distil water.

Further then 1ml of the above made 0.1M $AgNO_3$was taken and along with it 330 ul of 5mM of $CuSO_4$was taken and mixed in a beaker, to this then twice the volume of 10% Plant extract was added, and this reaction was then kept at an orbital shaker which was set to 40^oC, 117 RPM for 30 mins. After the incubation, the solution was transferred to a falcon and stored for further uses.

- To note, all the solutions used for synthesis, i.e. the plant extract and copper and silver salts were kept chilled and the order of addition were silver, followed by copper and then twice the volume of plant extract, and also the orbital shaker used when set to 40^oC goes from a range of 40-45^oC.

Physical and Chemical Characterization:

UV-VISIBLE SPECTROSCOPY

Using a Thermo Fisher Scientific Spectrophotometer, the UV-visible absorption spectrum of the samples was produced in order to investigate the optical absorption characteristics of the photocatalyst. Understanding the spectral characteristics of Cu and Ag nanoparticles was made possible by spectroscopy, that were recorded at constant room temperature within the wavelength of 200-800 nm

Bioactive properties determined by:

ANTIOXIDANT ASSAY

The free radical scavenging test to check the capacity of copper-doped silver nano formulations was evaluated using the standard 2,2-diphenyl-1-Picrylhydrazyl DPPH assay. It is a free radical method based on electron transfer). The antioxidant's IC50 was determined. To determine the radical scavenging activity (RSA) formula RSA (%) = [(AcontrolAsample)/Acontrol] /100 is used.

The antioxidants are oxidized by the free radical ABTS. It is a cyan-coloured (bluish-green) reagent that becomes colourless when an antioxidant is applied. Antioxidant activity is assessed as a function of colour change intensity. The 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS radical scavenging test was used to test the antioxidant activity of copper doped silver nano formulations. UV-Vis spectrometer was used to measure the absorbance at 734 nm (Shimadzu, Japan). The antioxidant's IC50 was determined. To determine the radical scavenging activity (RSA) formula RSA (%) = [(AcontrolAsample)/Acontrol] /100 is used

ANTIBACTERIAL ACTIVITY

Microbial growth culture parameters and the Antibiotic Sensitivity Test (AST) was performed using Kirby Bauer Method at different concentrations and the zone of inhibition was calculated using diameter.

CHAPTER- 4

RESULTS AND DISCUSSIONS

The copper doped silver nano formulation was prepared from the method mentioned in the above section, and a characteristic double peak or double hump was visible.

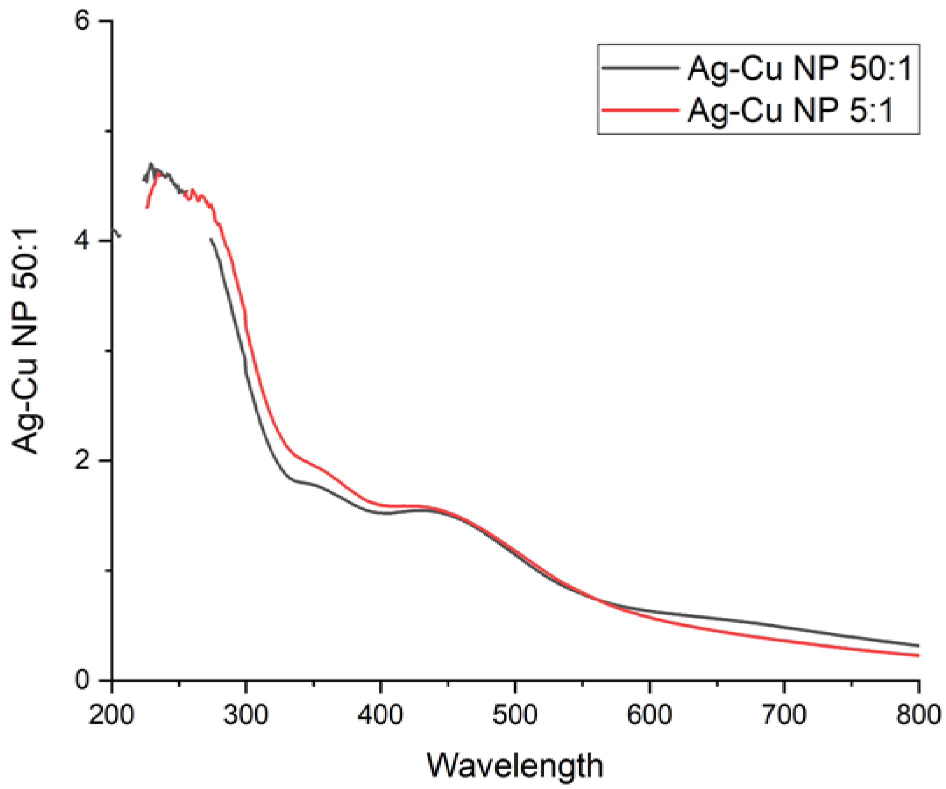
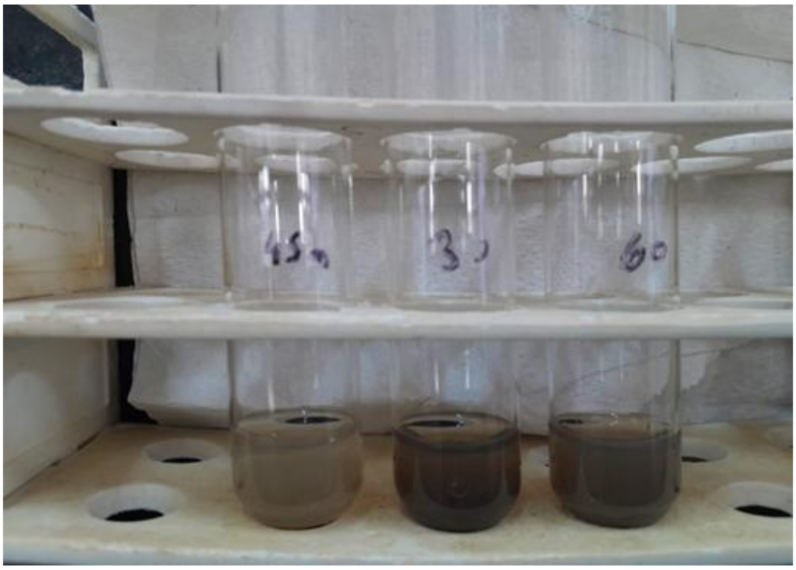


Fig 3- Synthesised copper doped silver nanoparticles

Optimization of nanoparticles

Time of reaction

The copped doped silver nanoparticles were optimized and starting from the time of reaction, it was observed that the only results were visible at 30 mins of reaction, any other time more, or less, the peaks were not visible.



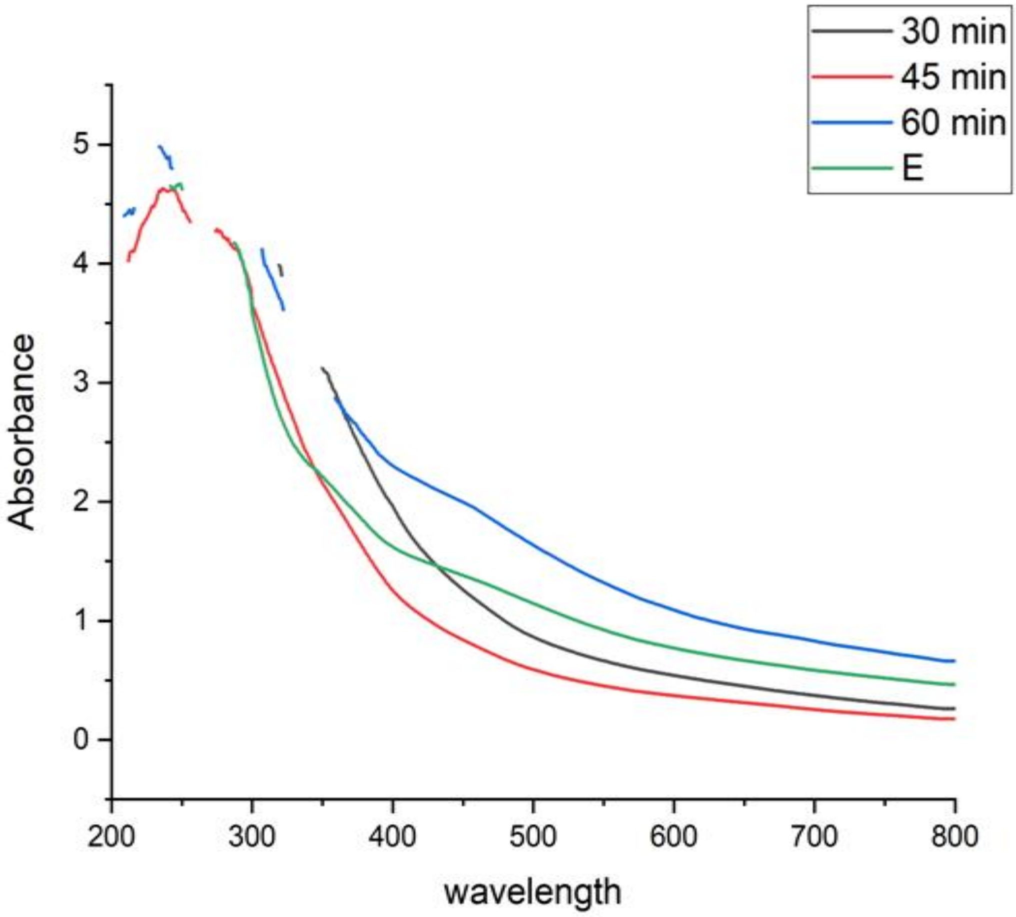
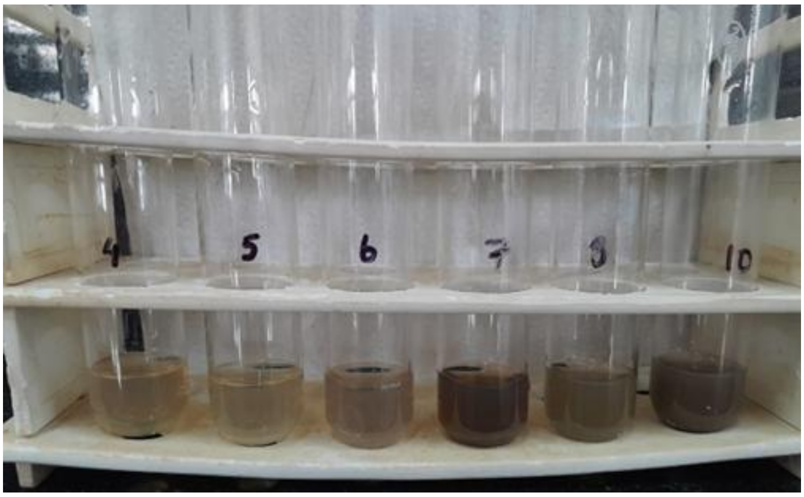


Fig 4- Showing the colour photo of the reactions at different time, and below that the attached spectrum of the following samples.

- Different pH

The reactions were kept at different pH to optimize, and it was then noted and seen that the best reaction and peak was observed at neutral (~6.7 pH)



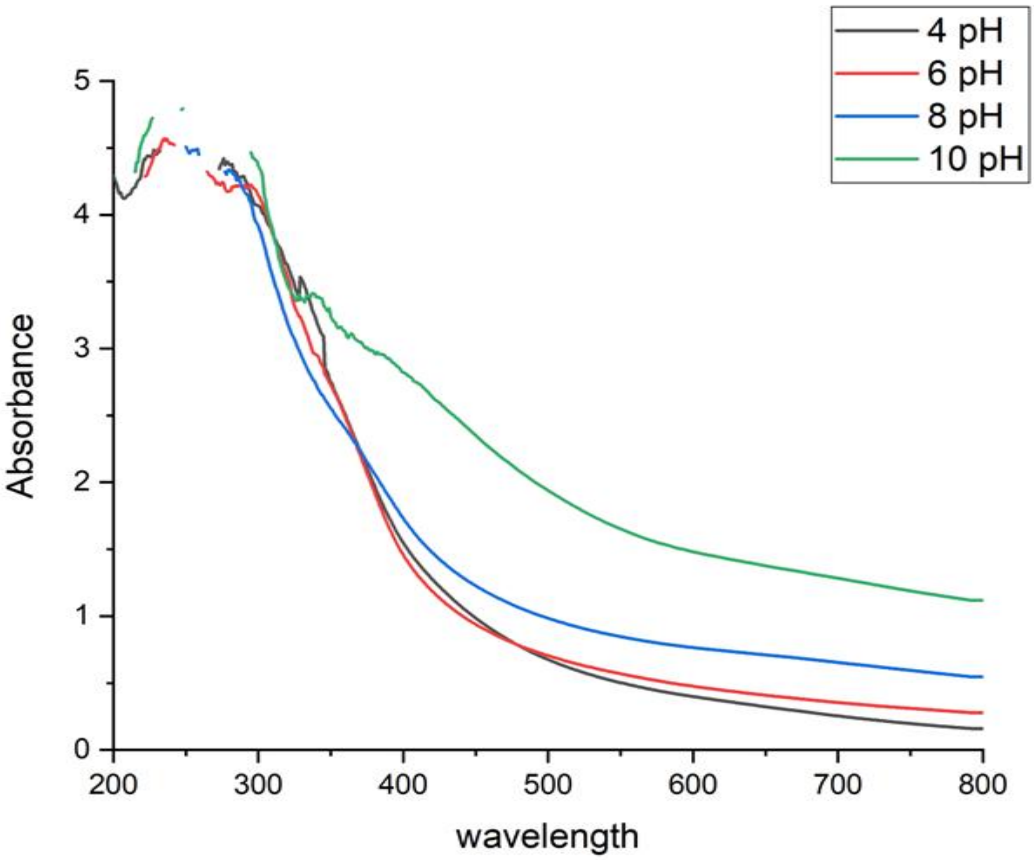


Fig 5- showing the colour photo of the reaction at different pH, and the attached graph

- **Optimization of concentration**

The concentration of salts was optimized and then in those optimized concentrations, the ratio of the $AgNO_3$ and $CuSO_4$ was optimized, and was noted and seen, that the peak was only observed at 50:1 of $AgNO_3$: $CuSO_4$

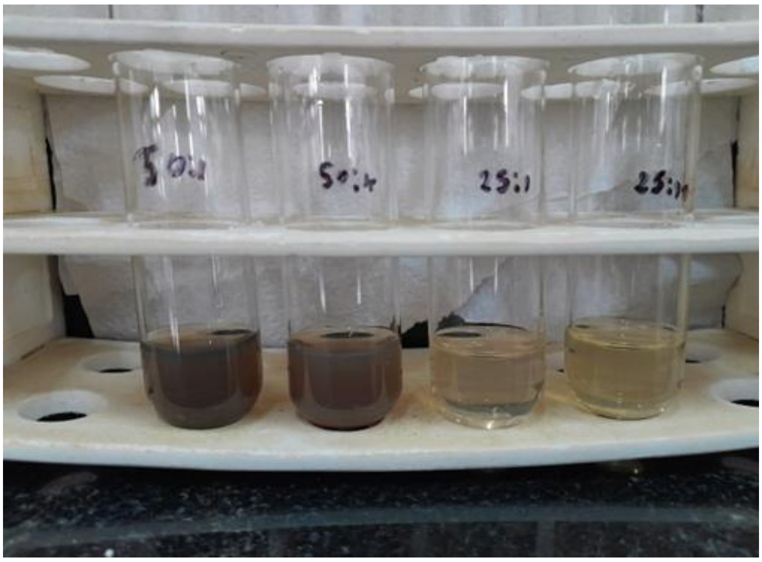


Fig 6- Optimization of concentration of salts and their attached graph

- Optimization of temperature.

The reactions were made and the kept at various different temperature and it was noted that the best reaction was seen at 40^{o}C, and the best peak was then seen at 40^{o}C reaction.

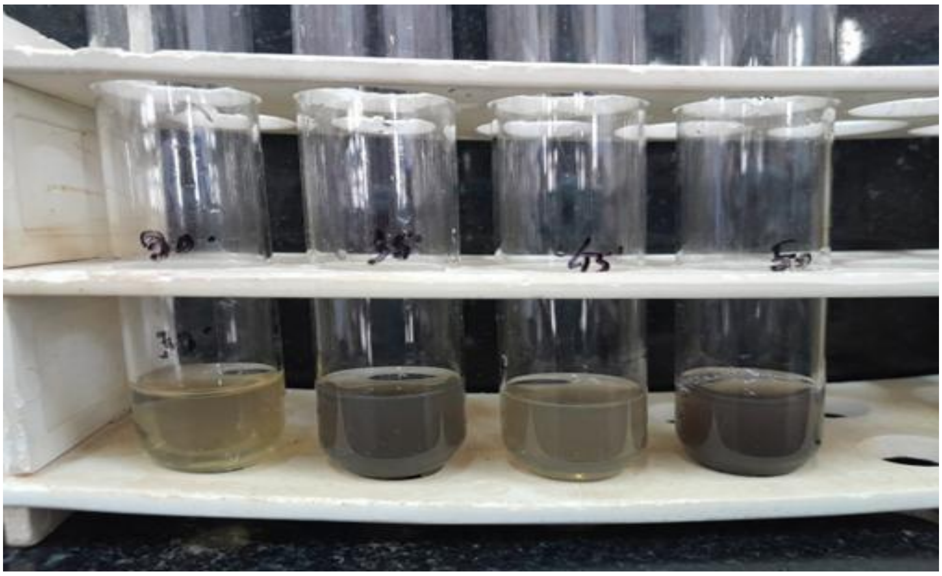


Fig 7- Optimization of temperature of reaction, and the attached graph of the reaction

- Anti-Microbial Susceptibility Test.

E. coli and S. aureus were inoculated overnight at Muller Hinton Broth, and then were spread in Muller Hinton Agar plates, and then the copper doped silver nanoparticles were loaded 20ul, along with pure Cu and Ag nanoparticles, and also with $CuSO_4$ and $AgNO_3$and plant extract, which were all diluted to same concentration (working concentration).

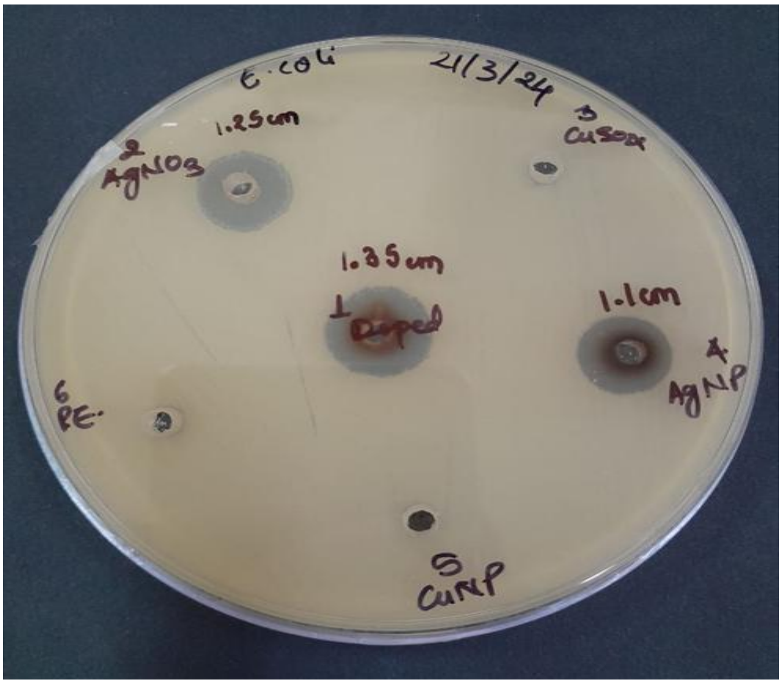


Fig 8- AST on *E. coli*, showing various Dia of different samples.

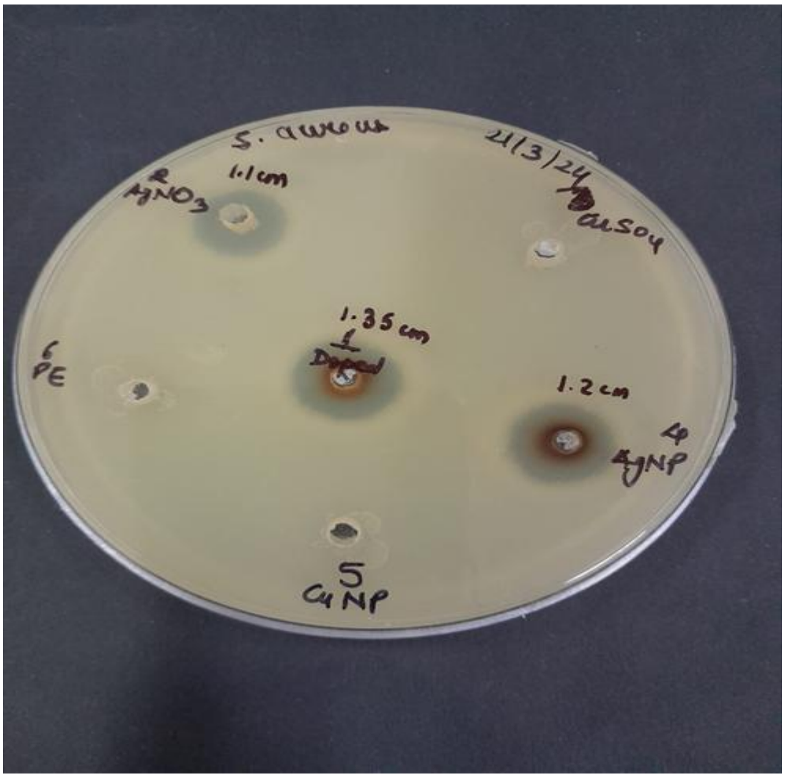


Fig 9- AST on *S. aureus* showing various Dia of different samples.

CHAPTER 5-

CONCLUSIONS

The synthesised nano formulation, showed a double hump, or a double peak indicating the early presence of copper and silver. With the time of reaction, the colour of the solution turned dark brown, which is typically that of silver NP, the colour turned such due to the major part being silver. This Synthesised nano formulation was then used up for further testing, and to access their various potentials and applications.

The use of *Eclipta Alba* plant extract adds as a green synthesis method, and green synthesis is a much preferred method in recent times. Since plant extracts have secondary metabolites like flavonoids, terpenes etc., they act as reducing as well as stabilizing agents, and help in reduction and stabilizing the NP, reducing the need for chemicals, which in turns reduce the toxic byproducts generally associated with the chemical synthesis.

The synthesised nano formulation was initially characterized by UV-vis spectroscopy, which showed the double peak or double hup at 300-400 nm these double peaks indicate the presence of copper and silver, but further characterization remains to be done for conformation. The pure copper and silver neoformations showed a peak at 250nm and 420 nm respectively for both metals, the shift in the peak further confirms the synthesis of a doped system.

The synthesised doped nano formulation was checked for its antimicrobial efficacy, using *E.coli* and *S. aureus*, the test was performed by the Disc Diffusion method, and wells were made out in the media after spreading the bacterial cultures, and 20 μL of the working concentration of samples were added, It was observed that the well having AgNO3 and AgNP showed an inhibition zone, along with the doped system. The inhibition zone was bigger and wider in the doped system, conforming the synergistic effect of copper and silver on the nano formulation.

The synthesisednano formulation can have various potential application, like-

1. Wound Healing- The nano formulation can be used along with or loaded on other biomaterials, like hydrogel which can enhance the wound healing properties of the utilized biomaterials.
2. Anti-Microbial Agent- The nano formulation can be used as ananti-microbial agent and can be used in fighting bacterial infections and to combat anti-microbial resistance which is a huge problem in today's scenarios.
3. Drug Delivery system- The formulation can be used as a drug delivery system and can be loaded with drugs to enhance their efficacy, and to inject and deliver the drug better.
4. Water purification- The formulation can be added to unsafe and undrinkable water, and due to presence of copper and silver, they can act as antimicrobial agents and kill the microbes that are in the water, making it relatively safer to drink.
5. Dye Degradation- The nano formulation can be used to degrade the various harmful dyes, which are used in the industries, that are just released in the environment, polluting it, these dyes can be degraded by the action of nanoparticles.

In the future, these synthesisednano formulations, can be utilized to check the above mentioned, various potential applications. More work is needed in these directions, as there are research articles on the synthesis of doped formulation, but very few papers are there in the subject of doped nano formulations, which are synthesised using the green route.

In conclusion, the synthesisednano formulation was optimised under various parameters, which are mentioned, and were tested for anti-microbial efficiency, although the, doped nano formulation holds many possible outcomes, due to the restriction of time and knowledge, on the above mentioned was possible. In future, more work needs to be done on this field to gain better and deeper insights, on the mechanism of actions of the nanoformulation, how they work, and what are their mechanism of actions.If time permits further, I would like to continue my work and do further research's on this.

Various different in vitro and in vivo studies can be conducted and performed to check other different applications of the nano formulations.

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