Green Synthesis and Characterization of Copper Nanoparticles Using *Justicia gendarussa*: Antimicrobial Activity and Comparative Evaluation for Wastewater Remediation

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by

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CANDIDATE'S DECLARATION

I, **BIDISHNA BASUMATARY**, Roll No. **2K23/MSCBIO/16** hereby certify that the work which is being presented in the thesis entitled **"Green Synthesis and Characterization of Copper Nanoparticles Using *Justicia gendarussa*: Antimicrobial Activity and Comparative Evaluation for Wastewater Remediation"** is in partial fulfilment of the requirement for the award of the Degree of Master of Science, submitted by me to the **Department of Biotechnology, Delhi Technological University, Delhi-42** is an authentic record of my own work carried out during the period from January 2025 to June 2025 under the supervision of **Prof. Jai Gopal Sharma.** The matter presented in the thesis has not been submitted by me for the award of any degree of this or any other Institute.

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CERTIFICATE BY THE SUPERVISOR

It is certified that **BIDISHNA BASUMATARY,** (2K23/MSCBIO/16) has carried out her research work presented in this thesis entitled **"Green Synthesis and Characterization of Copper Nanoparticles Using *Justicia gendarussa*: Antimicrobial Activity and Comparative Evaluation for Wastewater Remediation"** for the award of Degree of Masters of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi under my supervision. The thesis embodies results of original work, and studies are carried out by the student herself, and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

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ABSTRACT

The growing load of environmental pollution and antimicrobial drug resistance calls for the creation of eco-friendly and multi-functional remediation technologies. In this report, copper nanoparticles (CuNPs) were **green synthesized** using the aqueous leaf extract of the medicinal plant ***Justicia gendarussa,*** which contains bioactive molecules. Various methods of synthesizing plant extract and copper sulfate salt ratios were tried to achieve stable nanoparticles' best synthesis, and the 4:1 ratio was most effective as a combination based on naked eye observation, FTIR spectroscopy, and UV-Visible spectroscopy. The characterization techniques confirmed that stable CuNPs were synthesized effectively, showing evidence of phytochemicals as **reducing and capping agents.**

The **antimicrobial activity** of as-synthesized CuNPs was tested against specific bacterial strains by routine disk diffusion assays. The composition 4:1 showed astounding antibacterial activity and its potential as a bioactive nanomaterial. Parallel comparative tests were also carried out in tandem between as-synthesized CuNPs and Effective Microorganism (EM) technology for evaluating their relative treatment efficiency of synthetic wastewater. The physicochemical parameters of pH, Total Dissolved Solids (TDS)/ Electrical Conductivity (EC), Nitrate / Sulfate Analysis (SA), Chloride Content, and BOD were compared pre-treatment and post-treatment. The findings revealed that CuNPs ensured total microbial inhibition and decontamination, surpassing EM in water quality improvement in some areas.

This study reports that green-synthesized CuNPs from ***Justicia gendarussa*** show a promising, sustainable solution for environmental remediation and antimicrobial activities. The dual potential of the nanoparticles provides the opportunity for further modifying them into large-scale, **sustainable remediation technologies** in contaminated ecosystems.

LIST OF PUBLICATIONS

1. A paper entitled **“Bionanotechnology-Driven systems for Environmental Studies: Innovations in Monitoring and Remediation”** was presented at the 3rd International Conference on "Advanced Functional Materials & Devices for Sustainable Development" (AFMD-2025) organised by Department of Physics under the aegis of IQAC, ARSD College, University of Delhi, India during March 03-05, 2025 in hybrid mode.
   The review paper is accepted for further proceedings.

2. Presented a paper entitled **“Toward Smart PGPR Systems for Millets: Bioinoculant Technology for Climate-Smart Agriculture”** at the International Conference on Renewable, Environment and Agriculture(ICREA) held in Ajmer, India on 01 June, 2025.
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LIST OF SYMBOLS, ABBREVIATIONS

°C: Degree Celsius

Cu: Copper

NP: Nanoparticles

EM: Effective Microorganisms

FTIR: Fourier Transform Infrared Spectroscopy

UV-Vis: Ultraviolet (UV)-visible spectroscopy

ZOI: Zone of Inhibition

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CHAPTER 1

INTRODUCTION

1.1 Background and Significance of Nanotechnology and Nanoparticles

Nanotechnology is a fast-growing interdisciplinary technology that functions on the atomic and molecular level, which is usually between **1 and 100 nanometres**. It employs concepts of physics, chemistry, biology, and engineering for the **manipulation of matter** at the **nanoscale** for various applications (Khan et al., 2019). These converging disciplines have developed novel technologies with high potential for application in medicine, energy, electronics, and environmental science. One of the most encouraging advances in nanotechnology is the **synthesis and application of nanoparticles**, especially metal nanoparticles, because of their superior physicochemical characteristics and high surface-area-to-volume ratio (Singh and Mehta, 2016).

Nanoparticles have size-dependent particular properties, such as increased chemical reactivity, optical properties, thermal conductivity, and mechanical strength, which are significantly different from their bulk states (Sahoo et al., 2020). These properties make them extremely versatile in a variety of applications. Gold and silver nanoparticles, for example, are extensively studied for their biomedical implications, including **drug delivery, imaging, and antimicrobial actions**. Concurrently, iron and copper nanoparticles have become well-known for their application in environmental remediation processes like the degradation of organic contaminants and heavy metals from wastewater (Rai and Ingle, 2012).

Metal nanoparticles are of special interest to green chemistry and environmental nanotechnology because they can interact with pollutants at the molecular level, catalyze redox reactions, and even degrade recalcitrant pollutants that traditional methods cannot remove (Kumar and Yadav, 2021). In addition, their use for water purification is also good for **global sustainable development** and **cleaner production technologies**. Adding metal nanoparticles to water purification systems and bioremediation systems not only introduces efficiency but also eco-compatibility, particularly if synthesized by using green, plant-based, or microbial methods (Ahmed et al., 2016).

The technology is progressing fast, and scientists are searching for effective and eco-friendly methods of synthesizing nanoparticles. Green methods of synthesis involving plant extracts and microbes are especially promising for the synthesis **of stable, bio-compatible nanoparticles** with minimal or no use of toxic chemicals (Iravani, 2011). These findings have led to new areas in environmental biotechnology with metal nanoparticles synthesized by living organisms as cheap, efficient, and eco-friendly alternatives to traditional remediation agents.

For this purpose, the current research considers **biosynthesis of copper nanoparticles from plant extracts**, their effectiveness in the **treatment of synthetic wastewater**, and how they compare to traditional microbial treatments like **Effective Microorganisms (EM)**. The research intends to add to the list of research activities in nanobiotechnology-based water treatment systems for the advantage of sustainable environmental management systems.

1.2 Significance of Copper Nanoparticles (CuNPs)

Copper is a transition metal with superior electrical and thermal conductivity, catalytic activity, and relatively affordability compared to noble metals such as silver and gold. In the nanoscale, copper possesses superior attributes such as increased surface area, great reactivity, and even enhanced electron transfer ability, making copper extremely useful in many scientific and industrial applications (Gawande et al., 2016). Copper nanoparticles (CuNPs), for example, have emerged as an extremely effective class of engineered nanomaterials with multirelevance applications.

CuNPs have been of significant interest in many areas such as **catalysis, electronics, biomedicine, and environmental engineering**. In catalysis, CuNPs are cheap catalysts used in carbon-carbon coupling reactions, hydrogenation reactions, and detoxification of contaminants (Nasrollahzadeh et al., 2019). In electronics, copper high conductivity and nanometer-scale size make it suitable for printed circuits, conductive inks, and nano-interconnects (Chouhan et al., 2020). Biomedically, CuNPs possess extremely high antimicrobial, antioxidant, and anti-inflammatory activities, and they are promising candidates for wound healing, biosensing, and drug delivery systems (Vinod et al., 2021).

Ecologically, CuNPs have proven to be of importance in purification of water because of their **antimicrobial and catalytic activity**. They are ideal for the treatment of wastewater since they can degrade organic pollutants and suppress microbial growth (Ruparelia et al., 2008). Due to cost-effectiveness and being eco-friendly compared to silver nanoparticles, copper is a more viable candidate for remediation in large scales (Iravani et al., 2014). The antibacterial effectiveness of CuNPs results from the ability of CuNPs to produce reactive oxygen species (ROS) and degrade the microbial cell membranes, whereas their catalytic property promotes the degradation of toxic substances into less toxic products (Ren et al., 2009).

Drawing inspiration from the present work, emphasis is given to the **green synthesis of CuNPs through plant extracts**, which not only excludes toxicants but also benefits from the reducing and capping property of plants. Biologically synthesized CuNPs are tested for their dual-function potential of **antimicrobial and catalysis** in synthetic wastewater treatment as an environmentally friendly alternative to chemical treatment.

1.3 Problems with Traditional Nanoparticle Synthesis Methods

Synthesis of nanoparticles has long been based on **chemical and physical techniques** like chemical reduction, sol-gel processing, hydrothermal treatment, and physical vapor deposition. These techniques have provided controlled nanoparticle synthesis with defined size, shape, and functionality and are highly efficient in research and industry (Pérez-López et al., 2017). Even though the processes are very effective, they have quite a significant number of disadvantages, especially if quantified in terms of environmental security, sustainability, and cost effectiveness.

Chemical reduction, the most common method, in most cases employs metal salts which are reduced from solution by such reducing agents as sodium borohydride or hydrazine. Although it produces nanoparticles of low size distribution, the method employs very **hazardous and toxic chemicals** that are risky to human life as well as the environment (MubarakAli et al., 2011). Equally**, sol-gel processing** through system transformation of liquid "sol" into a solid "gel" phase is very good in material control homogeneity but has complex reaction conditions and precursors that are perhaps not environmentally friendly (Suriati et al., 2012).

Hydrothermal synthesis, although suitable for crystalline nanomaterial growth in high-pressure and high-temperature conditions, needs high-end hardware and has a huge energy footprint. **The physical vapor deposition (PVD)** method, widely employed in coating technology, also needs high vacuum conditions, high-end equipment, and is economically not efficient for bulk application, particularly in Third World countries (Goyal et al., 2020).

In addition, most of these conventional processes culminate in the formation of **toxic byproducts and non-biodegradable wastes**, which complicate their use in environmentally friendly processes like agriculture, biomedicine, and water treatment (Iravani, 2011). Moreover, utilization of **multi-step purification procedures** and post-synthesis treatments necessary to stabilize nanoparticles contributes to costs and complexity.

Owing to the limitations, more emphasis has been placed recently on **green and biological synthesis** pathways that are safer, more convenient, and environmentally friendly. Green synthesis pathways, for example, do not require corrosive chemicals and are conducted at mild conditions to yield phytochemically stabilized and capped nanoparticles. These ecofriendly routes are being considered as promising alternatives in applications wherein human and environmental protection is best prioritized.

1.4 Emergence and Benefits of Green Synthesis

The green synthesis approaches have been a major development in nanotechnology, with green and sustainable technologies providing alternatives for the synthesis of nanoparticles instead of conventional technologies. Green synthesis is in line with green chemistry and its focus on **using non-toxic reagents, renewable sources of material, energy efficiency, and the creation of minimal amounts of toxic byproducts** (Anastas and Warner, 1998). These approaches are increasingly being accepted as part of the direction towards the achievement of sustainability, particularly in fields that involve applications in biomedicine, agriculture, and environmental remediation.

Green synthesis of nanoparticles employs biotic systems, ranging from plant extracts, bacteria, fungi, and algae, as capping and reducing agents. Among these, **plant-mediated synthesis** has been given maximum significance because of ease of process, scalability, and general availability of phytochemicals with high bioreduction potential (Mittal et al., 2013). In comparison to chemical syntheses, which typically are obtained under harsh conditions and caustic solvents, green synthesis can be obtained under ambient conditions with aqueous plant extracts and hence limiting energy input as well as environmental risk (Iravani, 2011).

One of the major advantages of plant-mediated synthesis is the dual role of phytochemicals in plants. The extracts of plants contain **phenolics, flavonoids, alkaloids, terpenoids, and proteins** as **bioreducing agents** to convert metal ions to their nanometallic form and as capping and stabilizing agents against agglomeration and oxidation of the nanoparticles (Ahmed et al., 2016). This bi-directional performance increases the biocompatibility and stability of the nanoparticles, and they are best applied in medical and environmental applications.

Green-synthesized nanoparticles are **nontoxic, stable**, and have **enhanced interaction with the biological system**, which is very important for their efficiency in antimicrobial treatments and pollutant degradation processes. In addition, the utilization **of renewable vegetable feedstocks** and **low-cost protocols** renders green synthesis economically viable for large-scale industrial production in resource-poor environments (Kuppusamy et al., 2016). These characteristics in combination render green synthesis an attractive means of preference for research and industries aiming to integrate nanotechnology into sustainable development aims.

1.5 Presentation of Justicia gendarussa

Justicia gendarussa is a small, evergreen medicinal shrub under the Acanthaceae family. It is also referred to as "Willow-leaved Justicia" and has extensive distribution in the world's tropical and subtropical areas, particularly in India and Southeast Asia. It typically grows in wet, shaded areas and has linear-lanceolate leaves and minute purplish flowers (Sharma et al., 2022). *J. gendarussa* has been utilized in numerous systems of medicine such as Ayurveda, Siddha, and Unani due to its analgesic, anti-inflammatory, antipyretic, and anti-rheumatic activities (Thirugnanasambantham et al., 2021).

Justicia gendarussa's phytochemical screening has yielded a wealth of bioactive compounds that **include flavonoids, alkaloids, tannins, phenolics, and saponins**

(Chandran et al., 2019; Rautela et al., 2020). Flavonoids and phenolic acids are significant for having **better reducing and stabilizing abilities** that are needed for the biosynthesis of nanoparticles. They are able to decrease metal ions to nanoparticles, in addition to capping and stabilizing nanoparticles to render them biocompatible and prevent them from aggregating (Mohanta et al., 2022; Raj et al., 2020).

The selection of J. gendarussa for this investigation lies in both **its ethnobotanical potential and the phytochemical potential** to produce nanoparticles. *J. gendarussa* is relatively **less investigated** compared to *Azadirachta indica* or *Ocimum sanctum*, which are better researched in nanobiotechnology, presenting a **novel source of bioactive compounds**. In addition, its **non-toxic nature**, ease of accessibility, and established **antimicrobial and antioxidant activities** make it a viable contender for green CuNP synthesis that has uses in wastewater treatment and the regulation of microorganisms (Pathania et al., 2023; Johncy et al., 2022).

Recent research has also established phytochemical content of the reducing agent as crucial in the determination of plant-mediated synthesis of CuNPs, and J. gendarussa's abundance in flavonoid and polyphenolic content makes it a potent bio-reducing as well as stabilizing agent (Senthil and Govindasamy, 2021). That makes it a logical choice for this research whose objective is to confirm its ability to synthesize stable CuNPs with potential antimicrobial as well as catalytic functions for environmental purpose.

1.6 Antimicrobial Resistance and the Requirement for New Agents

Antimicrobial resistance (AMR) has become the biggest 21st-century global health challenges. Misuse and overuse of antibiotics in medicine, agriculture, and animal husbandry have encouraged resistant microbial strains to develop at an alarming rate, making most traditional drugs ineffective (WHO, 2020). Based on the World Health Organization, as stated, AMR would cause 10 million deaths every year by 2050 if not contained, threatening human health, food security, and economic stability (Murray et al., 2022).

Increased emergence of multi-drug resistant (MDR) pathogens like *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* has significantly put an end to the effectiveness of first-line antibiotics, and new treatment paradigms have never been more needed (Dadgostar, 2019). Nanotechnology is rapidly emerging as a promising future for next-generation antimicrobial development against this epidemic.

Among all types of nanoparticles, **copper nanoparticles (CuNPs)** have been of immense interest owing to their **broad-spectrum antimicrobial activity**, **affordability**, **and lower likelihood of resistance development** (Hashem et al., 2021). CuNPs function through several mechanisms that distinguish them from traditional antibiotics. CuNPs produce **reactive oxygen species (ROS)**, destabilize microbial cell membranes, and inhibit intracellular constituents like DNA and proteins (Vinod et al., 2021; Alias et al., 2020). These multimodal therapies minimize the likelihood of resistance development compared to conventional antibiotics that often hit single molecular targets.

Additionally, CuNPs have been reported to be effective against a broad spectrum of **Gram-positive and Gram-negative bacteria**, fungi, and some viral diseases (Ren et al., 2019). They have also been reported to be effective as combinational therapy whereby CuNPs would augment the activity of already present antibiotics via synergistic effects (Abo-Zeid et al., 2020).

In environmental and biomedical applications, CuNPs provide a **green and efficient means of disinfection**, especially in wastewater treatments, surface coatings, wound dressing, and antimicrobial garments (Pathania et al., 2023). Addition to these systems not only prevents microbial infection but also meets the increasing need for environmentally friendly antimicrobial technologies.

Due to the increased demand to combat AMR and the specific benefits of CuNPs, a study into their green synthesis and use in **antimicrobial water treatment systems** is a pressing and critical research path.

1.7 Wastewater Remediation: Novel Challenges and Nanotechnology Solutions

Water pollution is a new global issue with the fast pace of industrialization, urbanization, and excessive use of agrochemicals. **Organic dyes, toxic heavy metals like lead, cadmium, and mercury, residues from pharmaceuticals, and pathogenic microorganisms** are some of the frequent water pollutants present in wastewater (Sharma et al., 2021). Textile dyeing factories, tanneries, chemical processing industries, and domestic sewage are the primary sources of water pollution. In most of the developing nations, wastewater is not treated or is partially treated before discharge into natural waters, thus contributing to further ecosystem degradation and severe public health impacts (Patel et al., 2020).

Traditional wastewater treatment technologies namely, **chemical precipitation, coagulation-flocculation, activated sludge processes, and sand filtration** are inadequate in eliminating complex or emergent pollutants. The above technologies are generally plagued by **high operating costs, energy needs, and secondary pollution issues like toxic sludge** (Subramani and Westerhoff, 2020). Traditional systems are also generally not capable of treating **low-concentration micropollutants**, persistent organic pollutants, and antibiotic-resistant bacteria (Huang et al., 2021).

In this regard, **nanotechnology provides promising avenues** for wastewater treatment based on the distinct surface chemistry and high reactivity of nanomaterials. Nanoparticles have the capacity to **adsorb, catalyze, and redox-mediate molecular-level conversions** more efficiently than traditional methods (Chaudhary et al., 2023). Of the various nanomaterials, **copper nanoparticles (CuNPs)** are of specific interest given their multifunctionality, low cost, and antimicrobial property.

CuNPs have useful applications in **dye degradation, heavy metal adsorption, and microbial inactivation**. Their catalytic action drives the oxidative degradation of dyes like methylene blue and malachite green by Fenton-like reactions (Vimala et al., 2022). CuNPs have also shown great adsorption capacity for heavy metal ions because they possess high surface areas and ion exchange capacities (Wang et al., 2019). Furthermore, their antimicrobial activity provides a function to eliminate pathogenic bacteria and fungi in contaminated water sources, hence giving a **complete remediation platform** (Salem et al., 2021).

The incorporation of CuNPs synthesized through green methods into wastewater treatment is especially important as it is consistent with sustainable values. Biosynthesized CuNPs will be **biocompatible, stable, and less toxic** than CuNPs synthesized through chemical methods (Pathania et al., 2023). In general, CuNPs present a **sustainable and effective means** of overcoming the challenges currently faced by wastewater remediation, particularly in resource-limited and high-risk environments.

1.8 Problem Statement

Although the amount of literature concerning the area of nanotechnology continues to increase, **green and sustainable synthesis** of metal nanoparticles is still a major challenge, particularly with real-world applications towards environmental remediation. The traditional chemical reduction and physical methods for synthesizing copper nanoparticles (CuNPs) typically utilize toxic reagents, require a high energy source, and produce toxic byproducts (Chaudhary et al., 2023; Subramani and Westerhoff, 2020). These characteristics not only pose environmental and safety issues but also hinder the scalability and application of such kinds of nanoparticles in areas like water treatment, agriculture, and public health.

While green synthesis may prove to be a promising alternative, presently, it remains under-explored and is concentrated on relatively few economically valuable, highly studied plant species. There is still a very big gap for exploration of less-well-characterized but **phytochemically richer medicinal plants** for their potential uses in nanoparticle synthesis. *Justicia gendarussa*, herein, is **a locally available, underexploited plant** that contains a high level of flavonoids, phenolics, and alkaloid compounds that have been shown to possess the capacity to serve as effective **capping and reducing agents** in green CuNPs synthesis (Sharma et al., 2022; Johncy et al., 2022). Additionally, comparative studies that explore the antimicrobial and catalytic capability of green-synthesized CuNPs against traditional biological solutions (e.g., Effective Microorganism or EM solutions) in actual utilization, such as treatment of wastewater, are not available. This is a key knowledge gap since water contamination and the problem of antimicrobial resistance continue to be major issues worldwide, and effective, affordable, and environmentally friendly strategies are needed (Murray et al., 2022; Pathania et al., 2023).

Hence, this current research seeks to fill this gap by creating a **plant-mediated green synthesis protocol of CuNPs from *Justicia gendarussa* leaf extract** and testing its **remediation and antimicrobial activity against conventional EM technology**. The protocol is anticipated to help realize the achievement of developing sustainable nanotechnology practice and widen the application of innovative phytochemical resources to environmental biotechnology.

1.9 Objectives of the Study

The major aim of the present work is to formulate a green and efficient method for the synthesis of copper nanoparticles using Justicia gendarussa leaf extract and analyze their physicochemical attributes, antimicrobial activity, and feasibility in the treatment of wastewater.
For this purpose, the following goals have been designated:

1.9.1 Water extraction of total phytochemical compounds from *Justicia gendarussa* leaf.

1.9.2 Qualitative phytochemical screening of *Justicia gendarussa* leaf extract to identify the presence of major bioactive compounds (flavonoids, saponins, tannins, terpenoids, glycosides, sterols, etc.).

1.9.3 Green synthesis of copper nanoparticles (CuNPs) using *Justicia gendarussa* leaf extract.

1.9.4 Characterization of biosynthesized copper nanoparticles using UV-Vis spectroscopy and FTIR analysis.

1.9.5 Evaluation of antioxidant activity of biosynthesized copper nanoparticles from *Justicia gendarussa*.

1.9.6 Antibacterial activity testing of copper nanoparticles synthesized using *Justicia gendarussa* extract against selected microbial strains.

1.9.7 Comparative analysis between Effective Microorganisms (EM) and EM-CuNPs for wastewater remediation efficiency.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Overview of Green Synthesis of Metal Nanoparticles

2.1.1 Plant Sources Used for Green Synthesis of Metal Nanoparticles

Green synthesis of metal nanoparticles (MNPs) is an environmentally friendly process compared to the traditional physical and chemical procedures because it is cost-effective and environmentally safe. Out of all biological compounds, plant extracts have been widely investigated to prepare a vast array of MNPs ranging from silver (Ag), gold (Au), copper (Cu), zinc oxide (ZnO), and iron (Fe) nanoparticles.

Silver nanoparticles (AgNPs) have been one of the most researched, utilizing plants such as *Azadirachta indica, Asparagus racemosus,* and *Ocimum sanctum* for their synthesis because they are rich in flavonoids, terpenoids, and phenolic acids (Ahmed et al., 2016; Singh and Mehta, 2016).

Gold nanoparticles (AuNPs) have been successfully prepared from *Terminalia catappa, Aloe vera,* and *Lantana camara,* among many others, because their biomolecule-structure enables them to stabilize and reduce gold ions (Mittal, Chisti and Banerjee, 2013).

Copper nanoparticles (CuNPs)**,** more difficult to stabilize, have also been synthesized from different plant extracts like *Camellia sinensis* and *Moringa oleifera* and *Justicia gendarussa* (Rautela, Rani and Debnath, 2020; Hashem et al., 2021; Johncy, Asha and Jayanthi, 2022). Copper is of special interest as it possesses catalytic and antimicrobial activity.

Zinc oxide nanoparticles (ZnONPs) *and* ***iron oxide nanoparticles (FeONPs)*** have also been prepared using green approaches. *Calotropis procera* and *Lawsonia inermis* extracts, for instance, have resulted in ZnONPs and FeONPs synthesis with success, respectively (Kuppusamy et al., 2016; Raj et al., 2020).

The broad variety of plants studied indicates the metabolic adaptability of plants towards metal ion reduction and stabilization, and this points to a high potential for customizable nanoparticle synthesis based on the target application.

2.1.2 Plant-Mediated Synthesis Mechanism of Nanoparticles

The plant-mediated MNP synthesis via green synthesis represents biochemical interactions of a complex nature. The overall mechanism involves metal ion **reduction, capping**, and **stabilization** by a range of **phytochemicals** such as flavonoids, polyphenols, alkaloids, terpenoids, and proteins.

The reduction is typically triggered by the reduction of metal ions (e.g., Cu^{2+}, Ag^{+}, Au^{3+}) to zerovalent state by the **phenolic compounds, ascorbic acid**, and other **antioxidants** present in the extract. The flavonoids and terpenoids, with electron-donating nature, reduce metal salts (Mohanta et al., 2022; Chandran, Meena and Barupal, 2019).

After reduction, capping agents, most commonly polysaccharides, proteins, and tannins, are attached to the surface of the nanoparticle, preventing them from agglomeration and oxidation. The particle size, shape, and dispersity of the nanoparticles depend on phytochemical concentrations, pH of the reaction, temperature, and concentration of metal ions (Iravani, 2011; Kumar and Yadav, 2021).

In the instance of Justicia gendarussa, its phytoconstituents defined therein, gendarusin A, flavonoids, and alkaloids, are speculated to be reducing as well as stabilizing agents (Sharma et al., 2022; Thirugnanasambantham, Ramachandran and Subramanian, 2021).

This process has the added benefit of functionalizing nanoparticles with bioactive compounds inherently found in plant extracts for greater **biocompatibility and biological activity** (Pathania et al., 2023).

2.2 Synthesis and Applications of Copper Nanoparticles Synthesized Green (CuNPs)

Plant extract-mediated biogreen synthesis of copper nanoparticles (CuNPs) has attracted global attention because of the eco-friendliness, simplicity, and low cost involved in this process. Compared to chemical or physical processes of synthesis, there is no use of toxic reducing agents or high energy requirements for plant-mediated synthesis. Various parts of plants like leaves, stem, bark, seeds, and flowers have been employed effectively as reducing and stabilizing agents.

For example, *Camellia sinensis* (green tea) leaf extract was utilized by Rautela et al. (2020) for the synthesis of CuNPs with outstanding antioxidant and antibacterial activities. Hashem et al. (2021) also illustrated CuNPs synthesis using *Moringa oleifera* leaf extract with high antimicrobial and cytotoxic activities against cancer cells. Phytochemical constituents in these plant extracts—flavonoids, phenolics, terpenoids, and alkaloids—are responsible for performing dual roles as a reducing and capping agent, which supports stable nanoparticle formation (Iravani, 2011).

Justicia gendarussa, a medicinal plant, has also been used in the synthesis of CuNP. Thirugnanasambantham et al. (2021) and Johncy et al. (2022) synthesized CuNPs with efficient antifungal and antibacterial activities. The CuNPs were well shaped and stable due to the high content of secondary metabolites in the leaf extract.

Green-synthesized CuNPs have found uses in numerous applications. They have been used as potent catalysts for the degradation of environmental pollutants like pesticides and dyes in catalysis (Raj et al., 2020). As sensors, CuNPs have been applied in nanocomposites and thin films to detect heavy metal and organic pollutants through electrochemical means (Pathania et al., 2023). Biomedically, they are thoroughly investigated for multi-drug-resistant (MDR) pathogenic antimicrobial properties, anticancer potentials, and wound healing process (Mittal et al., 2013; Hashem et al., 2021).

In addition, CuNPs prepared from *Azadirachta indica* (neem) were found to possess enhanced larvicidal and antioxidant activities (Kuppusamy et al., 2016), and those prepared from *Ziziphus mauritiana* leaf demonstrated excellent photocatalytic degradation of organic dyes (Kumar and Yadav, 2021). These findings are indicative of the multi-functional nature of CuNPs and their excellent utility in biomedical, agrochemical, and environmental applications.

Although promising, challenges are associated with stability, scalability, and reproducibility of green-synthesized CuNPs. The content of phytochemicals from the plant, extraction process, and reaction conditions immediately affect nanoparticle yield and quality (Mohanta et al., 2022). Therefore, method standardization and profound mechanistic investigations are a necessary condition for taking the field ahead.

2.3 Antimicrobial Action of Copper Nanoparticles

Copper nanoparticles (CuNPs) are found to be powerful antimicrobial agents since they possess the potential to get involved with microbial membranes, produce reactive oxygen species (ROS), and interfere with intracellular activities. Due to the high surface-area-to-volume ratio, they are highly reactive and even more effective compared to bulk copper or copper ions alone in pure form.

2.3.1 Mechanism of Antimicrobial Action

Antimicrobial action of CuNPs is through multiple mechanisms. Mainly, CuNPs can bind to microbial cell walls and cause membrane integrity damage resulting in enhanced permeability and leakage of intracellular material (Laha et al., 2020). It is very active in Gram-negative bacteria in which there is a distinct interactive site offered by the outer membrane. Secondarily, CuNPs produce ROS like hydroxyl radicals and superoxide anions, resulting in oxidative stress, protein denaturation, and lipid peroxidation (Vinay et al., 2021). Third, CuNPs can penetrate microbial cells and bind to DNA and enzymes, causing DNA breakage, replication inhibition, and inactivation of the enzyme (Abo-Neima et al., 2022).

These multi-targeting mechanisms prevent microbial resistance from developing, making the hypothesis possible that CuNPs can replace traditional antibiotics.

2.3.2 Overview of Antimicrobial Research with Green Synthesised CuNPs

Several research studies have confirmed the antimicrobial potential of green-synthesized CuNPs. For example, Singh et al. (2018) green-synthesized CuNPs with *Azadirachta indica* (neem) extract, which showed great inhibition zones against *Escherichia coli, Staphylococcus aureus,* and *Candida albicans*. El-Naggar et al. (2020) also synthesized CuNPs with *Ocimum basilicum* extract, which inhibited effectively Gram-negative as well as Gram-positive bacteria such as *Bacillus subtilis* and *Klebsiella pneumoniae*.

CuNPs prepared from *Justicia gendarussa* were found to have strong antibacterial and antifungal activity, with very good activity against *Pseudomonas aeruginosa* and *Aspergillus niger* (Johncy et al., 2022). The above studies reveal that phytochemicals from plant extracts not only help in the synthesis of the nanoparticles but also increase their antimicrobial activity through synergism.

2.3.3 Comparison of Activity Against Gram-Positive vs. Gram-Negative Bacteria

The effectiveness of CuNPs may vary in Gram-negative and Gram-positive bacteria due to differences in cell wall composition. Gram-negative bacteria like *E. coli* and *P. aeruginosa* have a high lipopolysaccharide outer membrane that might be more likely to be disrupted by CuNPs. Gram-positive bacteria like S. aureus lack an outer membrane but have a thicker peptidoglycan layer and are otherwise sensitive to CuNPs.

For instance, Khatami et al. (2018) found that green-synthesized CuNPs were more effective against *E. coli* (Gram-negative) than against *S. aureus* (Gram-positive) because of the simple penetration of CuNPs through the thin peptidoglycan layer of Gram-negative bacteria. Equivalence in activity against both has been reported with the use of very strong phytochemical-concentrated plant extracts, though (Rautela et al., 2020).

These observations suggest that though Gram-negative bacteria in general are more susceptible to CuNP-induced damage, the quality of synthesis route and plant extract significantly influences overall antimicrobial activity.

2.4 Application of Nanoparticles for Wastewater Treatment

The growing load of organic contaminants, dyes, heavy metals, and microbes in wastewater necessitates the use of higher order treatment technologies. Among them, nanotechnology has been recognized as an effective tool, with different nanoparticles (NPs) demonstrating great potential towards wastewater treatment owing to their expanded surface area, tunable properties, and multifunctionality.

2.4.1 Nanoparticles Used for Wastewater Treatment

Different nanoparticles have been employed for the removal of different types of contaminants from wastewater:

- **Iron oxide nanoparticles** (e.g., magnetite, maghemite) are widely used for the adsorption of heavy metals and the removal of arsenic, lead, and chromium due to their magnetic nature and high metal ion affinity (Chowdhury et al., 2012).

- **Titanium dioxide (TiO_2) nanoparticles** are the most active photocatalysts for dye and organic pollutant degradation under UV light or sunlight (Sharma et al., 2016).
- **Carbon nanomaterials** like carbon nanotubes (CNTs), graphene oxide (GO), and activated carbon nanocomposites are renowned for their high adsorption ability for dyes, phenolic compounds, and drugs (Ali et al., 2019).
- **Silver (AgNPs), copper (CuNPs),** and **zinc oxide (ZnO NPs)** metal nanoparticles possess excellent antimicrobial activity and catalytic oxidative degradation of organic contaminants (Mittal et al., 2021).

The nanoparticles are usually blended in membranes, composites, or supported on porous substrates to facilitate performance and recyclability in wastewater treatment processes.

2.4.2 Use of CuNPs for Wastewater Treatment

Copper nanoparticles (CuNPs), especially those prepared through green pathways, are currently well known for their multi-functionality in water wastewater treatment. CuNPs are highly effective in degrading toxic organic dyes like methylene blue, rhodamine B, and malachite green through catalytic and photocatalytic activities (El-Naggar et al., 2020). They are also high in heavy metal binding capacity for metals like lead (Pb^{2+}) and cadmium (Cd^{2+}) and help in efficient removal through adsorption (Siddiqui et al., 2021). Besides, CuNPs also exhibit excellent antimicrobial activity, which enables the inactivation of the pathogens present in contaminated water. For example, CuNPs prepared from *Azadirachta indica* were reported to inactivate coliform bacteria in wastewater samples (Thakur and Jain, 2021).

The dual feature of CuNPs as catalysts and adsorbents renders them versatile agents in the **primary detoxification** and **secondary disinfection** stages of water purification.

2.4.3 Mechanisms Involved

The prominent processes through which CuNPs and other nanoparticles operate in treating wastewater are:

- **Adsorption**: CuNPs adsorb pollutants such as heavy metals and dyes on their surface by electrostatic attraction, van der Waals forces, or coordination bonding.

Plant capping agent functional groups tend to strengthen the interaction.

- **Catalysis**: CuNPs have the ability to catalyze redox reactions to degrade complex organic pollutants into less harmful or biodegradable compounds. CuNPs can be used to catalyze Fenton-like reactions with the help of hydrogen peroxide or solar irradiation.
- **Oxidation/Reduction**: CuNPs are involved in electron transfer reactions that reduce toxic metal ions (e.g., Cr^{6+} to Cr^{3+}) or oxidize organic molecules. Redox activity is the basis for pollutant transformation.

  These mechanisms would be synergistic in action, enabling CuNPs to simultaneously target several classes of pollutants in a single-step treatment.

2.5 Studies Involving *Justicia gendarussa* Extract

Justicia gendarussa, or Gandarusa, or willow-leaved justicia, is a medicinal shrub belonging to the family Acanthaceae and has been utilized for centuries as a traditional medicine in Southeast Asian traditional medicine and the Indian subcontinent. Although scant direct information regarding its use in the synthesis of nanoparticles is documented, abundant reports speak of its abundance of phytochemicals and diverse set of pharmacological activities and thus, as a potential candidate lead for green nanotechnology applications.

2.5.1 Phytochemical Components of Justicia gendarussa

Various studies have reported the diversity of bioactive phytochemicals in *Justicia gendarussa*. Flavonoids (such as gendarusin A and B), alkaloids, phenolics, saponins, and steroids are some of the components, many of which were shown to exhibit reducing and capping properties required for the green synthesis of metal nanoparticles (Rastogi et al., 2015; Singh et al., 2014). Flavonoids and polyphenols, however, have been assigned antioxidant and antimicrobial effects, serving a dual purpose during the formation of nanoparticles as reducing agents and stabilizers (Vasanth et al., 2017).

Along with these, the plant has aromatic acids and lignans, which can add to redox chemistry in nanoparticle formation even more. These biomolecules can also facilitate bioreduction of metal salts and provide surface functionality for targeted applications in uses like catalysis or biomedicine.

2.5.2 Biological Activities of Justicia gendarussa

Though limited direct research has been done on nanoparticle synthesis using Justicia gendarussa, the plant has been greatly researched based on its vast biological activities. These are:

- **Antibacterial and antifungal activities**: Water and ethanol extracts have been shown to be active against Gram-positive and Gram-negative bacteria (Pratiwi et al., 2020), which indicates its promise in antimicrobial nanomaterials in the future.
- **Antioxidant activity**: DPPH and ABTS assays have been found to show good radical scavenging activity, which is due to flavonoid and phenolic content (Widodo et al., 2018).
- **Anti-inflammatory and analgesic activity**: Anti-inflammatory activity of the leaf extract was reported in preclinical studies, which may be mediated via inhibition of prostaglandin synthesis (Naik et al., 2013).
- **Antifertility activities**: The plant is seen for its contraceptive effect in males, i.e., by the effect of gendarusin A, wherein it affects sperm function (Hikmatullah et al., 2012).

Such activities indicate the availability of bioactive compounds with high activity that can also facilitate green synthesis of nanoparticles by serving as bioreducing and biofunctionalizing agents.

2.5.3 Potential of *Justicia gendarussa* in Nanoparticle Synthesis

Despite a lack of immediate visible evidence for the use of *Justicia gendarussa* in metal nanoparticle synthesis, phytochemical diversity and reported bioactivities of the plant indicate broad potential for such application. With access to polyphenols, flavonoids, and alkaloids, preeminent functional groups in successful nanoparticle synthesis pathways the plant extract ought to be capable of reducing metal ions efficiently to stable nanoparticles. In addition, its antioxidant and antimicrobial activities would be synergistically improved in the metallic nanoparticle form (e.g., CuNPs or AgNPs), which would find potential applications in multifunctional wastewater treatments, biomedical therapeutics, and environmental monitoring.

Investigating *Justicia gendarussa* green nanoparticle synthesis may not only value this under-exploited plant species but also provide a greener option in nanotechnology-based pollution and disease control measures.

2.6 Comparative Studies in Wastewater Remediation

The treatment of wastewater, especially from industrial and domestic sources, is an essential process for environmental sustainability and public health. Over the years, numerous remediation techniques have been developed, ranging from conventional biological treatment methods to advanced oxidation processes (AOPs) and nanotechnology-based solutions. Comparative assessments of these technologies help identify the most efficient, sustainable, and cost-effective approaches.

2.6.1 Traditional and Conventional Wastewater Treatment Methods

Conventional wastewater treatment primarily involves physical (sedimentation, filtration), chemical (coagulation, chlorination), and biological (activated sludge, trickling filters) processes. Biological treatments are widely used due to their cost-effectiveness and ability to degrade organic pollutants; however, they are often inefficient in removing heavy metals, persistent organic pollutants (POPs), and dyes (Ahmad et al., 2019).

Chemical methods like coagulation-flocculation and advanced oxidation (e.g., ozonation, Fenton's reaction) can be more effective in degrading non-biodegradable compounds but involve high operational costs and may generate secondary pollutants (Ghosh et al., 2021).

2.6.2 Nanoparticle-Based Wastewater Remediation Techniques

Nanoparticle-based techniques particularly those using metal and metal oxide nanoparticles have emerged as highly efficient alternatives. Their high surface area-to-volume ratio, surface reactivity, and catalytic potential allow them to degrade or adsorb pollutants at much lower dosages compared to traditional materials. Among these, copper nanoparticles (CuNPs), silver nanoparticles (AgNPs), titanium dioxide (TiO_2), iron oxides (Fe_3O_4), and zinc oxide (ZnO) have shown significant promise (Ahmed and Haider, 2020).

These nanomaterials exhibit mechanisms such as:

- Adsorption: capturing heavy metals, dyes, and organic molecules on the nanoparticle surface.
- Catalysis: accelerating degradation reactions under ambient conditions.
- Redox activity: facilitating oxidative breakdown of toxic compounds.
- Antimicrobial effects: eliminating pathogens without promoting antibiotic resistance (Sharma et al., 2023).

2.6.3 Comparative Studies Between Nanoparticle-Based and Conventional Methods

Recent comparative studies have demonstrated the superior performance of nanomaterials over traditional methods:

- **Dye removal**: CuNPs and TiO_2 nanocomposites have achieved >90% degradation of azo dyes such as methyl orange and methylene blue under visible or UV light, outperforming conventional activated carbon or alum treatments (Zhou et al., 2018; Kumar et al., 2022).
- **Heavy metal removal**: Iron oxide nanoparticles showed higher removal efficiencies for arsenic and lead (above 95%) compared to lime precipitation or ion exchange resins (Alnuaimi et al., 2020).
- **Pathogen inactivation**: AgNPs and CuNPs demonstrated faster and broader antimicrobial action against E. coli and S. aureus compared to chlorination or UV exposure, with added benefits of long-term activity and lower residual toxicity (Jain et al., 2020).
- **Combined systems**: Hybrid technologies incorporating nanoparticles into traditional methods (e.g., nanocomposite membranes or bio-nano filters) have also enhanced performance, enabling multi-pollutant removal with reduced energy and chemical input (Singh et al., 2021).

While nanoparticles provide higher efficiency and multifunctionality, concerns such as nanoparticle leaching, toxicity, and environmental persistence must be addressed. Thus, eco-friendly, green-synthesized nanoparticles (e.g., plant-based CuNPs) are gaining traction for sustainable wastewater remediation.

2.7 Identified Knowledge Gaps

In spite of significant growth and development of green nanotechnology for environmental management, certain relevant gaps in knowledge continue to remain, notably the synthesis and application of copper nanoparticles (CuNPs) with underutilized plant material and the utilization of their potentiality for the treatment of wastewater. Thorough review of literature on sections 2.1-2.6 identifies the following specific gaps this research seeks to bridge:

2.7.1 Limited Use of Untapped Medicinal Plants in CuNP Synthesis

The majority of studies were centered on some commonly used plants (e.g., Azadirachta indica, Ocimum sanctum, Camellia sinensis) for green CuNPs synthesis. Yet, plants such as Justicia gendarussa, though infused with bioactive phytochemicals (alkaloids, flavonoids, phenols), are neither explored nor unexplored whatsoever for nanoparticle synthesis. No thorough reports existed for green CuNPs synthesis using J. gendarussa, a critical research gap to examine its bioreduction and stabilizing capacities.

2.7.2 Lack of Mechanistic Understanding of CuNP-Biological Interactions

While the antimicrobial activity of CuNPs has been amply proven, the mechanisms—mainly the plant-mediated nanoparticle morphology, stability, and bio-properties—are not yet clearly understood. Not much research has been carried out on how various phytoconstituents regulate the functioning of nanoparticles, particle size distribution, or reactivity, more so for green-synthesized systems.

2.7.3 Lack of Understanding on CuNPs in Real-Life Wastewater Treatment

While some studies have reported the effectiveness of green-synthesized CuNPs for removal of certain pollutants or pathogens, hardly any of them have analyzed their performance in real wastewater samples or against conventional treatment systems (e.g., EM technology, conventional biological treatments). Moreover, limited studies have correlated CuNP antimicrobial properties with pollutant degradation under real environmental conditions.

2.7.4 Few Comparative Studies between Conventional and Green Nanomaterials

Although nanoparticle-based technologies have shown promise, few comparative assessments of green CuNPs with respect to traditional wastewater treatment systems regarding cost-effectiveness, sustainability, and performance are available. There is a need for comparison to scale up from the laboratory to the field scale.

2.7.5 Need for Integrated Assessment of Remediation Efficiency and Safety

The majority of literature currently available is centered on pollutant removal efficiency or antimicrobial activity but few of them incorporate the two parameters. Additionally, no evidence is available on testing the ecotoxicological effect of green CuNPs and temporal stability of their remediation efficacy.

The present study endeavors to fill these knowledge gaps by:

- Exploring *Justicia gendarussa* as a novel plant resource for the synthesis of green CuNPs.
- Physicochemical characterization of CuNPs synthesized to define the role of plant phytochemicals.
- Antimicrobial and efficiency of wastewater treatment by CuNPs in simulated as well as actual wastewater samples.
- Comparative assessment of CuNP treatment efficiency with EM technology in wastewater treatment.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials Required

3.1.1 Raw Materials

1. Plant Material
2. Dried or fresh leaves of *Justicia gendarussa*
3. Distilled water (aqueous extraction)

3.1.2 Glassware and labware.

1. Beakers (50, 100, and 250 mL)
2. Conical flasks
3. Test tubes
4. Tube rack
5. Measuring cylinders
6. Funnels
7. Glass rods
8. Pipettes
9. Watch glass
10. Measuring spoons
11. Filter paper (Whatman No. 1)
12. Mortar and pestle for leaf grinding
13. Centrifuge tubes (if centrifugation is employed)

3.1.3 Chemicals and Reagents (Phytochemical Screening)

1. Test reagents
2. Polyphenols 10% ferric chloride ($FeCl_3$)
3. Flavonoids 10% sodium hydroxide ($NaOH$)

4. Saponins Olive oil
5. Tannins 1% ferric chloride solution
6. Terpenoids Chloroform, concentrated sulfuric acid (H_2SO_4)
7. Glycosides Glacial acetic acid, ferric chloride, and concentrated sulfuric acid
8. Carbohydrates Molisch's reagent is concentrated sulfuric acid
9. Anthraquinones 10% ammonia solution
10. Sterols Concentrated sulfuric acid
11. Coumarins 10% sodium hydroxide

3.1.4. For the biosynthesis of copper nanoparticles (CuNPs)

1. Copper sulfate ($CuSO_4 \cdot 5H_2O$) - 0.1 M solution
2. NaOH solution (0.1 M)
3. *Justicia gendarussa* aqueous extract (derived from leaves)
4. Water bath (to maintain 80 °C)
5. Ice bath (for colder temperatures)
6. Mechanical shaker or magnetic stirrer

3.1.5. For characterization

1. UV-VIS. Spectrophotometer
2. FTIR Spectrometer
3. Quartz cuvettes (UV-Vis)
4. Sample vials

3.1.6. For Antibacterial Testing: Mueller -Hinton's Agar plates

1. Sterilized Petri dishes.
2. Bacterial strains (e.g., E. coli, S. aureus) - Incubator at 37°C
3. A sterile spreader.
4. Antibiotic discs (Control)
5. Micropipettes with sterile tips
6. Sterile saline solution (dilutions)

3.1.7. Wastewater Remediation Test: Effective Microorganism (EM) Solution

1. EM -CuNPs produced from Justicia gendarussa.
2. Wastewater samples (domestic source) - pH and turbidity meter
3. COD/BOD Kits
4. Treatment beakers and flasks - Shaking incubator/stirrer

3.2 Sample Collection

Justicia gendarussa is the plant species used in this study and was collected from the campus of **Delhi Technological University (DTU), New Delhi**. The campus, recognized for its wide and well-kept green landscape, is home to a diverse range of medicinal and attractive plants. Justicia gendarussa leaves were specially chosen because of their renowned phytochemical and medicinal properties.

Figure 3.1: *Justicia gendarussa*

Figure 3.2: Dried leaves of *Justicia gendarussa*

To ensure the highest phytochemical efficacy, only healthy and mature leaves were collected early in the morning. The collected leaves were cleaned thoroughly with distilled water to remove surface dust and pollutants before being air-dried in hot air for a few hours at 60-70 degrees Celsius. After being thoroughly dried, the plant material was ground into fine powder with a mortar and pestle and stored in airtight containers for future experimental operations.

Figure 3.4: Fine powder of *Justicia gendarussa*

Figure 3.3: Grinding process

3.3 Making a Standard Solution of the Sample

To prepare the standard solution, **10-20 grams** of *Justicia gendarussa* leaf powder were accurately weighed. It is then added to 100 mL of distilled water in a conical flask. To ensure efficient extraction of the phytochemical contents, the mixture was continuously stirred on a magnetic stirrer for **60 to 90 minutes** (approx.) at room temperature.

Following vigorous mixing, the extract was filtered using Whatman No.1 filter paper to eliminate plant residues and produce a clear filtrate. This filtrate served as the standard aqueous extract solution for all subsequent tests, including phytochemical analysis, biological activity testing, and green extraction of copper nanoparticles (CuNPs).

3.4 Phytochemical Screening and Biosynthesis of Copper Nanoparticles Using *Justicia gendarussa*

The presence or absence of phytochemicals such as flavonoids, saponins, tannins, terpenoids, polyphenols, glycosides, sterols, carbohydrates, coumarins and anthraquinones in *Justicia gendarussa* extracts were analyzed using standard laboratory procedures. Chemical tests were done on aqueous extracts and powdered plant material using conventional phytochemical screening techniques(Pates et al., 2024)(Abdullahi et al., 2023)(Kumar et al., 2023).

3.4.1 Test for Polyphenols

Ferric chloride assay was used. 1 mL of aqueous extract was diluted with 2 mL distilled water, then 2 mL of 10% $FeCl_3$ was added. Blue or green coloration indicates presence of phenols or polyphenols.

3.4.2 Test for Flavonoids

Alkaline reagent test was used. Few drops of 10% NaOH was added to 2 mL of aqueous extract. Intense yellow color developed indicates presence of flavonoids.

3.4.3 Test for Saponins

Frothing test was employed. 3 mL of aqueous extract was diluted with 5 mL distilled water and shaken vigorously. 3 drops of olive oil was added to the froth, agitated and examined for emulsion formation. Persistent froth formation indicates presence of saponins.

3.4.4 Test for Tannins

Ferric chloride test was used. 3 mL of aqueous extract was mixed with 3 mL distilled water and 2 drops of 1% ferric chloride. Brownish green or blue-black color indicates tannins.

3.4.5 Test for Terpenoids

Salkowski test was done. 3 mL aqueous extract was mixed with 2 mL chloroform and 2 mL concentrated H_2SO_4. Reddish-brown interface coloration indicates terpenoids.

3.4.6 Test for Glycosides

Keller-Killiani test was used. 0.5 mL extract was mixed with 2.5 mL distilled water, 1 mL glacial acetic acid with ferric chloride and 1 mL concentrated H_2SO_4. Reddish-brown layer or bluish-green upper layer indicates glycosides.

3.4.7 Test for Carbohydrates

Molisch's test was done. 2 mL extract was treated with 1 mL Molisch's reagent and few drops of concentrated H_2SO_4. Purple-reddish ring appeared indicates carbohydrates.

3.4.8 Test for Anthraquinones

1 mL of aqueous extract was treated with 10% ammonia solution. Pink coloration indicates anthraquinones.

3.4.9 Test for Sterols

1 mL of concentrated H_2SO_4 was added to 2 mL of aqueous extract. Red coloration indicates sterols.

3.4.10 Test for Coumarins

3 ml of 10% sodium hydroxide was added to 2 ml of aqueous extract. Yellow color formation indicates coumarins.

3.5 Biosynthesis of Copper Nanoparticles (CuNPs)

Deionized water was used to make a solution of 0.1 M copper sulfate to form a blue solution.

This was combined with the extract of *Justicia gendarussa* in the correct volume ratio. Subsequently, 0.1 M sodium hydroxide was added under stirring. Colour change from blue to green was noticed immediately at low temperature, and then heating at 80°C in a water bath. Reddish-brown colour at the end revealed the reduction to copper nanoparticles.

3.5.1 Preparation of Stock Solution of Copper Sulphate Pentahydrate

For the biosynthesis of copper nanoparticles (CuNPs), **a 0.1 M stock solution of copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$)** was prepared.

To make **100 mL of 0.1 M solution**, **2.497 grams** of $CuSO_4 \cdot 5H_2O$ were accurately weighed and dissolved in 100 mL of **distilled water**. The solution was then stirred continuously using a magnetic stirrer until complete dissolution was achieved and forming a clear **blue-colored solution**.

This freshly prepared copper sulfate solution served as the **metal precursor** in the nanoparticle synthesis and was stored in a clean, tightly sealed amber bottle to avoid contamination and photodegradation(Javed et al., 2020; Patil et al., 2021).

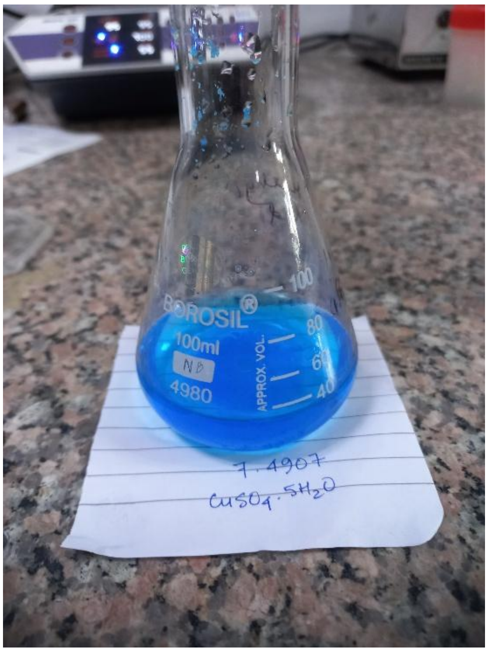

Figure 3.5: *CuSO4.5H2O* Solution

3.5.2 Preparation of Different Ratios of Plant Extract and Copper Sulfate Solution

To evaluate the effect of varying the proportion of *Justicia gendarussa* aqueous extract to copper sulfate solution on the biosynthesis and stability of copper nanoparticles (CuNPs), four distinct **extract-to-metal salt ratios** were prepared. The volume ratios were selected to understand how the concentration of phytochemicals influences the reduction efficiency and stabilization of the nanoparticles.
The ratios prepared were:

Sample 1: 1:2 (Plant Extract : $CuSO_4$ Solution)
Sample 2: 2:1 (Plant Extract : $CuSO_4$ Solution)
Sample 3: 3:1 (Plant Extract : $CuSO_4$ Solution)
Sample 4: 4:1 (Plant Extract : $CuSO_4$ Solution)

3.5.3 Procedure to prepare different volume ratios:

For each formulation, accurately measured volumes of *Justicia gendarussa* aqueous extract and 0.1 M copper sulfate solution were mixed in clean, sterile conical flasks. The combinations were as follows:

Table 3.1: Procedure to prepare different volume ratios

Sample	Plant Extract (mL)	$CuSO_4$ Solution (mL)	Ratio
1	5	10	1:2
2	10	5	2:1
3	15	5	3:1
4	20	5	4:1

After mixing, the solutions were vigorously stirred in magnetic stirrer at room temperature for 20-30 minutes to allow the bioactive components (flavonoids, tannins, polyphenols, and terpenoids) to interact with the copper ions.

Observations:

- A color change that is visible from blue to **green to reddish-brown** was exhibited, showing the decrease in copper ions and CuNPs formation.
- The intensity and speed of the color change were inconsistent with different ratios, but were faster for higher concentrations of the extract and stabilized the particles better.

This approach facilitated reproducible copper nanoparticle synthesis and facilitated comparison in ratios to ascertain the best conditions for future **spectroscopic characterization, antioxidant activity, antibacterial tests, and wastewater treatment experiments.**

Purpose of Ratio Variation:

- A greater ratio of plant extract content (e.g., 4:1) will have more reducing and stabilizing agents, which can result in smaller-sized nanoparticles with greater stability.
- Lower extract concentration (e.g., 1:2) could lead to incomplete reduction or bigger particles because of the lack of capping agents.
- Comparisons are made to determine the optimum ratio of nanoparticle yield, stability, and bioactivity.

3.5.4 Procedure for obtaining Nanoparticles:

***Mixing*:** The appropriate volumes of *Justicia gendarussa* extract and 0.1 M $CuSO_4$ solution were mixed in sterile conical flasks.

***Initial Color Observation*:** Immediately upon adding the copper sulfate solution to the plant extract, a **visible color change** occurred:

- The **initial greenish-brown color** of the plant extract turned to a **bluish-green hue,** which indicates interaction between the copper ions and the phytochemicals.

- This shift suggested the onset of **bio-reduction**, where compounds like flavonoids, tannins, and polyphenols began converting Cu^{2+} ions to copper nanoparticles.

Figure 3.6:
Plant extract filtrate

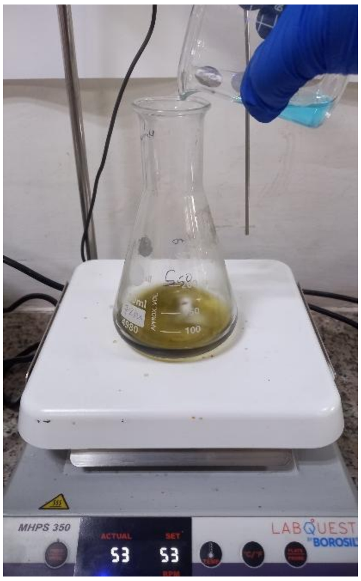

Figure 3.7:
Addition of *CuSO4.5H2O*
Solution

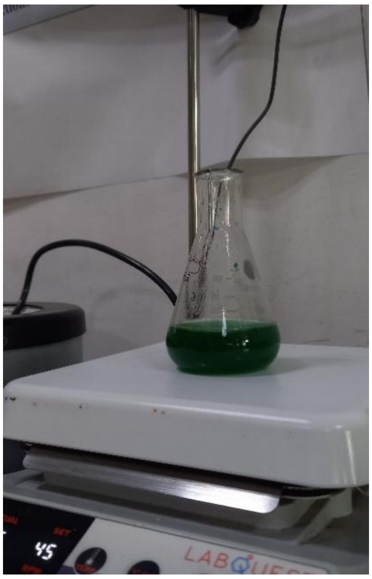

Figure 3.8: Bluish-green
hue Solution

***Stirring*:** The mixtures were stirred vigorously using a magnetic stirrer for **30 minutes** (approx) at room temperature to facilitate proper interaction and initial reduction.

***Incubation*:** After stirring, the solutions were allowed to rest at **room temperature for another 30 minutes** for further bio-reduction and nucleation.

***Centrifugation*:** The reaction mixtures were centrifuged at **7000 rpm for 15 minutes at 5°C** to pellet down the synthesized nanoparticles.

***Separation*:** The supernatant was discarded, and the resulting reddish-brown pellet (indicating CuNP formation) was collected carefully.

Drying: The nanoparticle pellets were dried in a **hot air oven at 80°C** until completely and uniformly dried. The dry CuNPs were then stored in sterile, airtight containers for further characterization and application.

Figure 3.9: Dried nanoparticle pellets

3.6 Anti-Microbial Test

3.6.1 Disk diffusion method

Antimicrobial Activity Testing of *Justicia gendarussa* Copper Nanoparticles Using Disk Diffusion Method:

1. Preparation of *Justicia gendarussa* Copper Nanoparticles

Synthesized copper nanoparticles of *Justicia gendarussa* were precisely weighed. Stock solutions were made by dissolving the nanoparticle in an appropriate sterile solvent (e.g., ethanol or distilled water) with varying concentration. Vortex mixing was rigorously performed to get proper and consistent dispersion.

2. Preparation of Bacterial Culture

Pure bacterial cultures of test strains (Gram-positive and Gram-negative) were obtained. *Brevibacillus brevis, Achromobacter, and Escherichia coli* were some of the most frequent test microorganisms.

Bacterial cultures were incubated on Nutrient Agar at 37°C for 18–24 hours.

Bacterial suspensions were made by picking 3–5 isolated colonies and suspending them in sterile broth or saline.

3. Preparation of Agar Plate

Mueller-Hinton agar (MHA) was prepared according to the manufacturer's instructions.

Sterile Petri dishes were filled with molten MHA to a level of about 4 mm and then left to solidify.

37°C was used to incubate the agar plates for 30 minutes with lids left slightly open for drying of the surface.

4. Plate Inoculation

Spreading of standardized bacterial suspension evenly over the surface of the MHA plates was done using a sterile glass spreader.

The inoculated plates were dried in air for 10–15 minutes before discs were added.

5. Nanoparticle Application

Sterile 5 mm filter paper discs were treated with known quantities of *Justicia gendarussa* copper nanoparticle suspensions of different concentrations with the help of a micropipette.

Control disks with sterile solvent alone and positive control disks with reference antibiotics were also kept.

Disks were pressed lightly for good contact and aseptically placed on the inoculated agar surface with sterile forceps. Disks should be separated by at least 24 mm. A total of maximum 4-6 disks per 90 mm Petri dish.

6. Incubation

Plates were incubated for 18–24 hours at 37°C under aerobic conditions. Plates were kept upside down so that condensation will not interfere with the surface.

7. Measurement of Inhibition Zones

Following incubation, plates were checked for inhibition zones around the disks.

Inhibition zone diameters were taken using a ruler or digital caliper across the broadest point, in millimeters.

8. Results Interpretation

Large inhibition zones were related to increased antimicrobial activity of the nanoparticles.

Zone diameters were compared to those of antibiotic and solvent controls.

Dose-response was evaluated, wherein there were larger inhibition zones from increased concentrations of nanoparticles.

This qualitative study gave some information regarding the antimicrobial action of the nanoparticles.

9. Key Points

Aseptic practice was strictly followed during conducting the process.

Tripling of experiments was conducted to make them replicable.

The wide range of nanoparticle concentrations was experimented upon to determine the most effective antimicrobial action.

Nanoparticles were well characterized to make sure that they had consistent size, shape, and composition.

Growth conditions such as temperature, incubation time, and media were made constant in all plates.

3.7 Methodology for Treatment of Wastewater using Effective Microorganisms and *Justicia gendarussa* Copper Nanoparticles (EM-CuNPs)

3.7.1 Preparation of Synthetic Wastewater Sample:

For the sake of simulation of actual domestic wastewater conditions, a synthetic wastewater sample was purposely prepared from a combination of organic and inorganic wastewaters commonly contained in domestic effluents.

Materials Used:

- Detergent Powder (2g): Employed to simulate surfactant materials in domestic wastewater.
- Rotten Tomato (1 medium-sized): Employed as common organic food waste carrying biological load.
- Incense Stick Waste (Ash and residue): Used to mimic charred organic waste.
- Flower Vase Water (250 mL): Provided naturally decaying plant material and bacteria present in stagnant water.

Procedure:

The above components were blended together in 500 mL distilled water in 1 L beaker. The mixture was shaken furiously for 10 minutes to homogenize. The resulting mixture was left at room temperature without disturbance for 1 hour for partial sedimentation and stabilization of components. This sample was the untreated synthetic wastewater and was taken as the control for further treatment and tests.

3.7.2 Treatment Procedures

Two processes were utilized to test their efficacy in remediating the synthetic wastewater:

Effective Microorganism (EM) Technology treatment

- EM solution was prepared in the laboratory
- The synthetic wastewater was aliquoted to 250 mL portions.
- Each aliquot was treated with 10% (v/v) of activated EM solution.
- The 10 minutes of shaking and incubation at room temperature (around 27°C) for 48 hours in a covered container were used to allow the microbial activity to occur.
- The incubated mixture was filtered using Whatman No.1 filter paper to eliminate suspended matter and then kept at 4°C for testing.

EM-CuNPs Treatment (Effective Microorganism with Justicia gendarussa Copper Nanoparticles)

- *Justicia gendarussa* extract-based copper NPs were synthesized following the protocol laid out in the previous sections.
- The EM solution was combined with CuNPs in 1:1 volume ratio to create the treatment complex.
- 10% (v/v) of the EM-CuNP complex was added into another 250 mL aliquot of the synthetic wastewatert.
- The treatment mixture was incubated and well-shaken under identical conditions to EM-alone treatment for 48 hours.
- The treated sample after incubation was filtered and refrigerated after treatment for further analysis.

3.7.3 Physicochemical Characterization of Wastewater Samples

All the three samples — untreated, EM-treated, and EM-CuNP treated underwent extensive physicochemical characterization to establish the change in water quality after treatment.

Parameters Examined:

All physicochemical parameters were accurately determined under laboratory conditions using certified available test kits and standard procedures for ensuring data accuracy and reliability.

The tests were conducted in systematic and controlled conditions under observation using well-calibrated instruments.

Color and Odor

Manual examination of color and odor of water samples was conducted through visual and smelling inspection in order to detect obvious differences between before and after treatment. This was used in the measurement of the aesthetic and sensory character of the water.

pH

Alkalinity or basicity of each sample was ascertained using a calibrated electronic pH meter. This gave information on hydrogen ion concentration, which may influence chemical and biological activity in wastewater.

Total Dissolved Solids (TDS) and Electrical Conductivity (EC)

TDS and EC were measured using a digital TDS-EC meter. TDS is a measure of the concentration of material in solution in the total, and EC is a measure of the electrical conductivity of the sample, an ionic strength proxy.

Nitrate / Sulfate Analysis (SA)

Nitrate and sulfate levels were determined using commercially purchased colorimetric test kits and/or the spectrophotometric method. These parameters aid in the calculation of nutrient and inorganic pollution within the water samples.

Chloride Content

Chloride level was determined by silver nitrate titration or commercial chloride-specific test kits. Elevated levels of chloride would indicate domestic or industrial effluent contamination.

Biological Oxygen Demand (BOD_1 Day Test)

Instead of the regular five-day operation, a one-day operation of the BOD test was used to estimate directly the quantity of the biodegradable organic content of the synthetic wastewater and its treated forms.

Figure 3.10: A water quality analyzer by *hach*

Figure 3.11: Test of conductivity

3.7.4 Comparative Analysis

- Untreated wastewater was compared to all the EM and EM-CuNP-treated sample parameters to measure the improvement.
- Percentage reductions in BOD, COD, turbidity, and TDS were calculated to assess treatment efficiency.
- The synergistic effect of CuNPs on the facilitation of microbial degradation and metal ion scavenging was of top priority in the EM-CuNP treatment.

3.7.5 Implication

This technique permitted comparative examination of conventional biological wastewater treatment (EM) and new hybrid technology based on nanotechnology (EM-CuNPs). Improved treated water parameters described in the present work indicate the potential use of nanomaterials isolated from *Justicia gendarussa* as an active component of environmental remediation systems.

Additional confirmation through microbial load tests and scaling studies would determine the practicality of the system for decentralized wastewater treatment programs.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Zone of Inhibition Results (in mm)

Table 4.1 Zone of Inhibition Results (in mm)

Plate	Bacterial Strain	Control (Ampicillin)	CuNP 1:2	CuNP 2:1	CuNP 3:1	CuNP 4:1
1	*Bacillus brevis* (Plate 1)	8 mm	19 mm	23 mm	–	–
2	*Bacillus brevis* (Plate 2)	8 mm	–	–	15 mm	24 mm
3	*Achromobacter*	12 mm	0 mm	0 mm	0 mm	26 mm
4	*Escherichia coli*	12 mm	0 mm	5.1 mm	0 mm	10 mm

The entire diameter (in millimeters) of the clear area surrounding each disk, including the disk itself, was measured with a digital caliper to determine the zone of inhibition. The measurements were collected after 24 hours of incubation at 37°C in accordance with CLSI recommendations.

4.1.1 Observations & Interpretation:

Bacillus brevis:

- The all CuNP samples were at least more antibacterial active than ampicillin.
- CuNP 2:1 (23 mm) and 4:1 (24 mm) ratios exhibited maximum inhibition, exhibiting maximum nanoparticle dispersion and bioavailability at these ratios.

- Deduction: CuNPs prepared at high extract ratios are very potent against B. brevis, marking broad-spectrum Gram-positive activity.

Figure 4.1: ZOIs in plate 1 of *B.Brevis*

Figure 4.2: ZOIs in plate 2 of *B.Brevis*

Achromobacter:

- CuNPs at 1:2, 2:1, and 3:1 showed no inhibitory action.
- All except the 4:1 ratio were ineffective (26 mm zone), with the exception of ampicillin (12 mm).
- This indicates concentration-dependent or capping agent–dependent activity, perhaps requiring additional coating of phytochemicals to reach this Gram-negative strain's outer membrane.

Figure 4.3: ZOIs in plate of *Achromobacter*

Escherichia coli:

- Trace to moderate activity. Only 2:1 (5.1 mm) and 4:1 (10 mm) inhibited.
- Ampicillin is still more effective overall (12 mm), but CuNP 4:1 has good results.
- Preventing partial resistance or reduced permeability towards E. coli by the tested formulations of nanoparticles, and optimization is necessary.

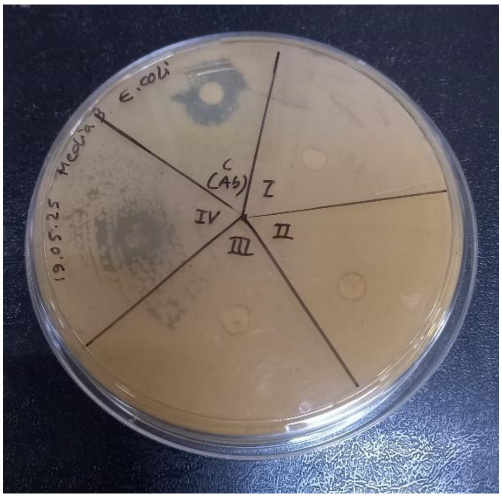

Figure 4.4: ZOIs in plate of *E.coli*

4.1.3 General Trends:

- CuNP 4:1 showed the highest antibacterial activity against all the tested strains even against Gram-negative bacteria where the others did not succeed.
- This confirms the hypothesis that increased plant extract to metal ion ratio results in improved capping, stabilization, and activity.
- No single CuNP ratio proved to be the best for all, but the best range was with 4:1.

4.2 FTIR Results

4.2.1 FTIR spectra of CuNp (1:2)

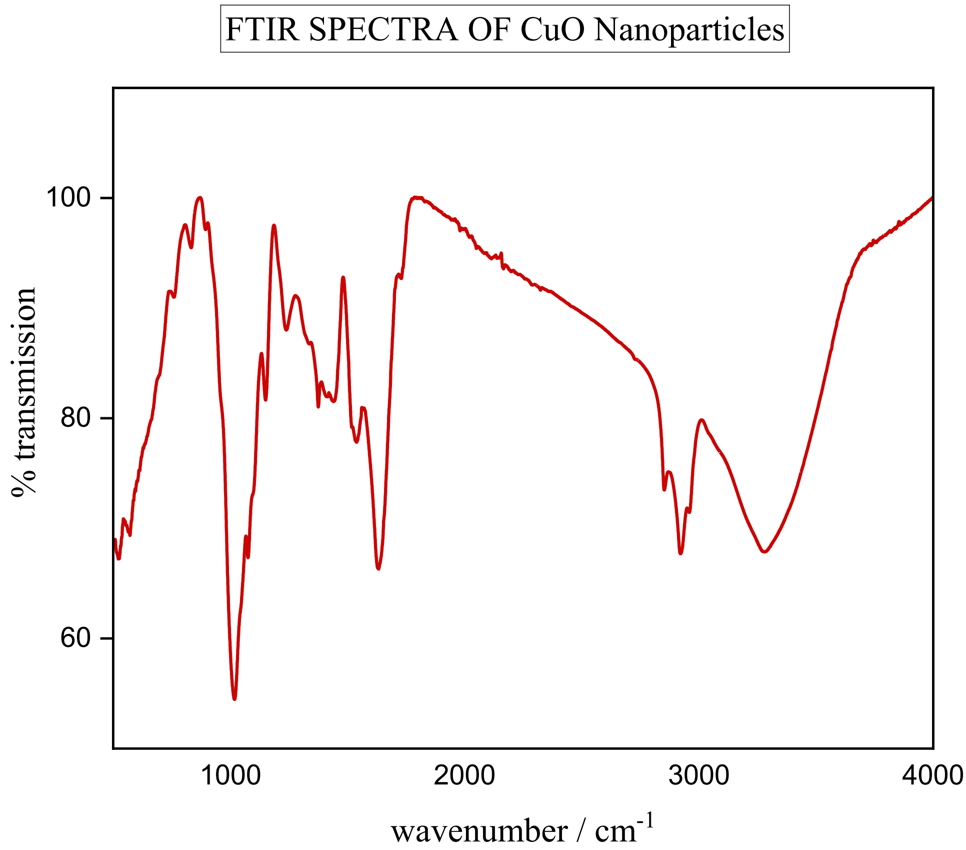

Figure 4.5: 1 FTIR spectra of CuNp (1:2)

The IR spectrum primarily reflects **phytochemicals** (from the extract) and possibly **adsorbed surface groups** that helped reduce and stabilize the CuNPs.

Signals related to the following is studied:

- **O–H, N–H** (alcohols, phenols, amines)
- **C=O** (carbonyls, carboxylic acids)
- **C=C / C–O** (aromatics, ethers)
- **Cu–O or Cu-related modes** (if present at lower frequencies, <600 cm^{-1})

Key IR Peaks and Their Interpretations:

Table 4.2: Key IR Peaks of FTIR Spectra of CuNp (1:2) and Their Interpretations

Wavenumber (cm^{-1})	Likely Vibration & Assignment
~3400	Broad O–H stretch (alcohols/phenols or water)
~2920	C–H stretch (alkyl groups from plant extract)
~1640	C=O stretch (carbonyls or amide I from proteins)
~1380	C–H bend (methyl groups or phenolics)
~1240–1040	C–O stretch (ethers, alcohols, flavonoids)
~800–600	Aromatic ring deformation or metal–oxygen bonds
~500–400	Possible Cu–O stretching (indicative of metal–oxygen interactions)

Notes:

- The **broad O–H band around 3400 cm^{-1}** suggests the presence of polyphenols or water adsorbed on the surface.
- The **C=O and C–O bands** confirm the presence of **flavonoids, proteins, or tannins** from the plant extract, likely responsible for **reducing Cu^{2+} to Cu^{0}** and capping the nanoparticles.
- Signals below **600 cm^{-1}** may indicate **Cu–O bonding**, especially if oxidized Cu species (CuO, Cu_2O) are present.

4.2.2 FTIR spectra of CuNp (2:1)

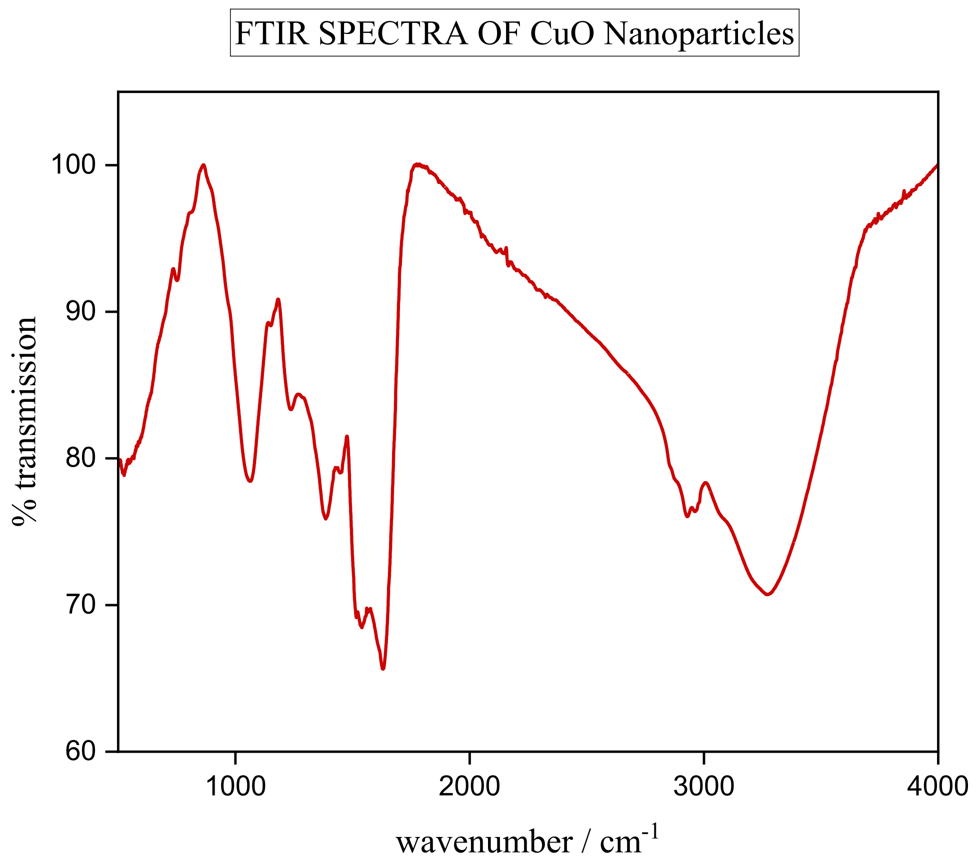

Figure 4.6: 1 FTIR spectra of CuNp (2:1)

CuNp of 2:1 ratio shows **lower intensity of organic signals**, suggesting limited reduction and stabilization. The plant extract likely didn't provide enough reducing or capping phytochemicals.

Table 4.3: Key IR Peaks of FTIR Spectra of CuNp (2:1) and Their Interpretations

Wavenumber (cm^{-1})	**Interpretation**
~3400	Broad O–H stretch from alcohols/phenols (weak) — indicates fewer capping agents.
~2920	Aliphatic C–H stretch — possibly from plant lipids or terpenoids.
~1640	C=O stretch from proteins or flavonoids — moderate, implies partial capping.
~1380	C–H bending — from methyl groups, basic plant structure.
~1050–1200	C–O stretch from alcohols or ethers — indicates some polyphenol presence.
~500–600	Weak Cu–O vibrations — suggests limited CuNP formation or weak surface binding.

4.2.3 FTIR spectra of CuNp (3:1)

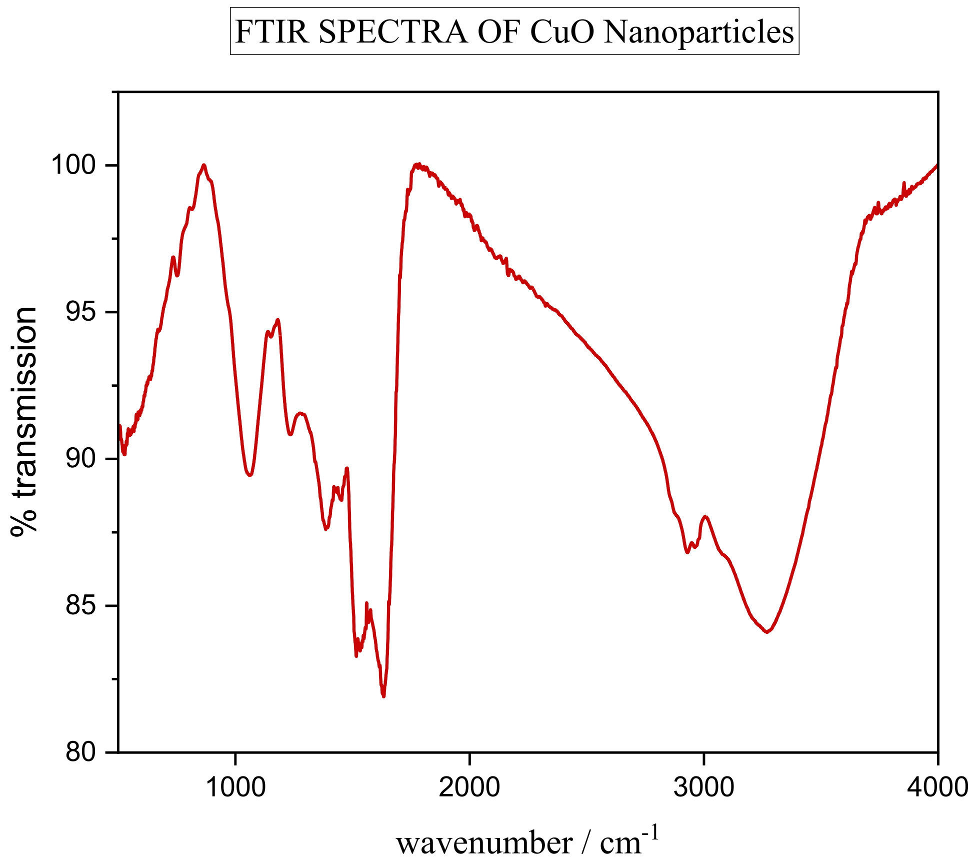

Figure 4.7: 1 FTIR spectra of CuNp (3:1)

CuNp of 3:1 ratio has **improved phytochemical signals**, indicating more effective **reduction of Cu^{2+}** and **better capping**. This batch likely formed more stable and smaller nanoparticles than Batch 2.

Table 4.4: Key IR Peaks of FTIR Spectra of CuNp (3:1) and Their Interpretations

Wavenumber (cm^{-1})	Interpretation
~3400	Broad O–H stretch — stronger than Batch 2; more phenolic compounds involved.
~2920	C–H stretch from aliphatic chains — present, from plant metabolites.
~1630–1650	Strong C=O stretch — indicates increased protein or flavonoid content.
~1380	Strong C–H bending — from more abundant plant compounds.
~1050–1200	Very strong C–O stretching — increased flavonoid and ether content.
~450–600	Noticeable Cu–O peaks — clear indication of CuNP surface interactions.

4.2.4 FTIR spectra of CuNp (4:1)

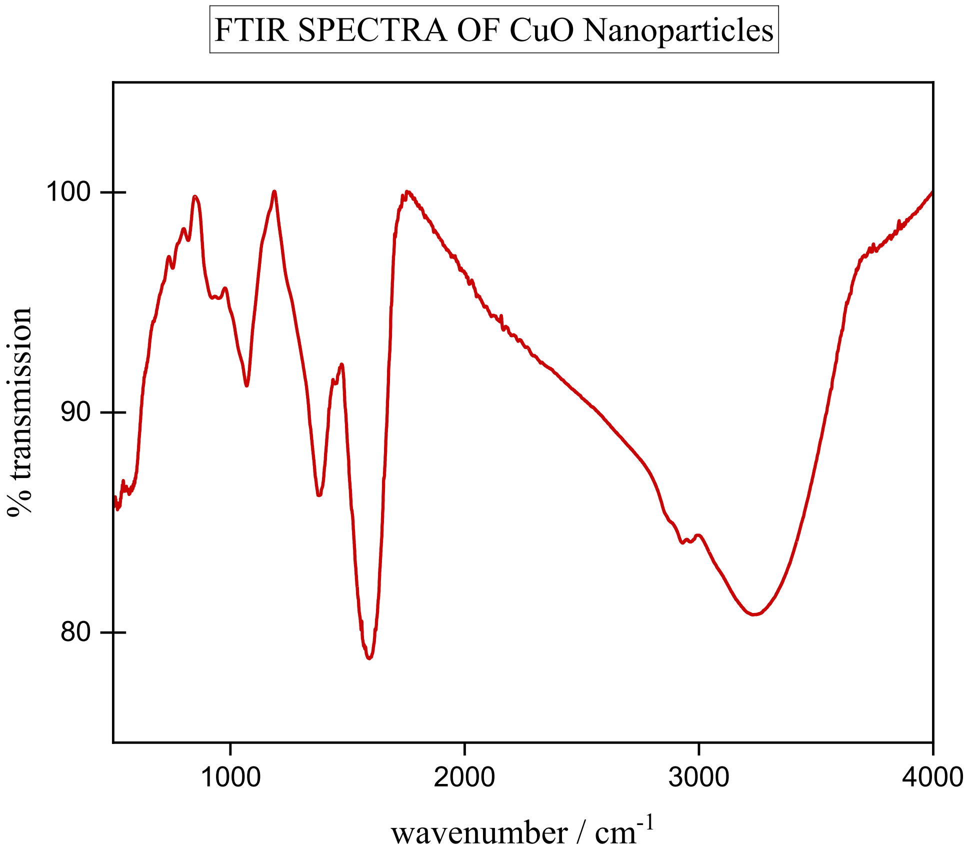

Figure 4.8: 1 FTIR spectra of CuNp (4:1)

CuNp of 4:1 ratio shows the **most intense and well-defined peaks**. High extract ratio leads to **stronger reduction**, **denser capping**, and **clear Cu–O bonding**, indicating highly stabilized copper nanoparticles.

Table 4.5: Key IR Peaks of FTIR Spectra of CuNp (4:1) and Their Interpretations

Wavenumber (cm^{-1})	**Interpretation**
~3400	Very broad and intense O–H — strong polyphenol and alcohol content.
~2920	Consistent C–H aliphatic signal.
~1630–1650	Strongest C=O stretch — maximum presence of reducing/capping agents like flavonoids.
~1380	Strong methyl group bending — more phytochemicals present.
~1050–1200	Highest intensity C–O — heavy flavonoid, carbohydrate, or ether involvement.
~400–600	Most defined Cu–O peaks — confirms substantial CuNP formation with strong surface bonding.

Overall Conclusion:

CuNp (1:2): Instability, particle aggregation, or lack of sufficient capping due to lower plant extract concentration.
CuNp (1:2): Incomplete reduction, minimal stabilization.
CuNp (1:2): Good balance, effective nanoparticle synthesis.
CuNp (1:2): Optimal conditions for rich capping and nanoparticle formation.

4.3 UV-Visible Results

Table 4.6: UV-Vis Absorbance Data

Wavelength (nm)	**Sample 1 (1:2)**	**Sample 2 (2:1)**	**Sample 3 (3:1)**	**Sample 4 (4:1)**
500	1.309	0.929	0.590	0.244
525	1.320	0.941	0.594	0.244
550	1.313	0.946	0.599	0.245
575	**1.327**	0.958	0.604	**0.247**
600	1.264	**0.962**	**0.611**	0.241

Table 4.7 Peak Absorbance Summary

Sample	**Peak Absorbance**	**Wavelength at Peak (nm)**
Sample 1 (1:2)	1.327	575
Sample 2 (2:1)	0.962	600
Sample 3 (3:1)	0.611	600
Sample 4 (4:1)	0.247	575

4.3.1 UV-Visible Spectrum of CuNp (1:2)

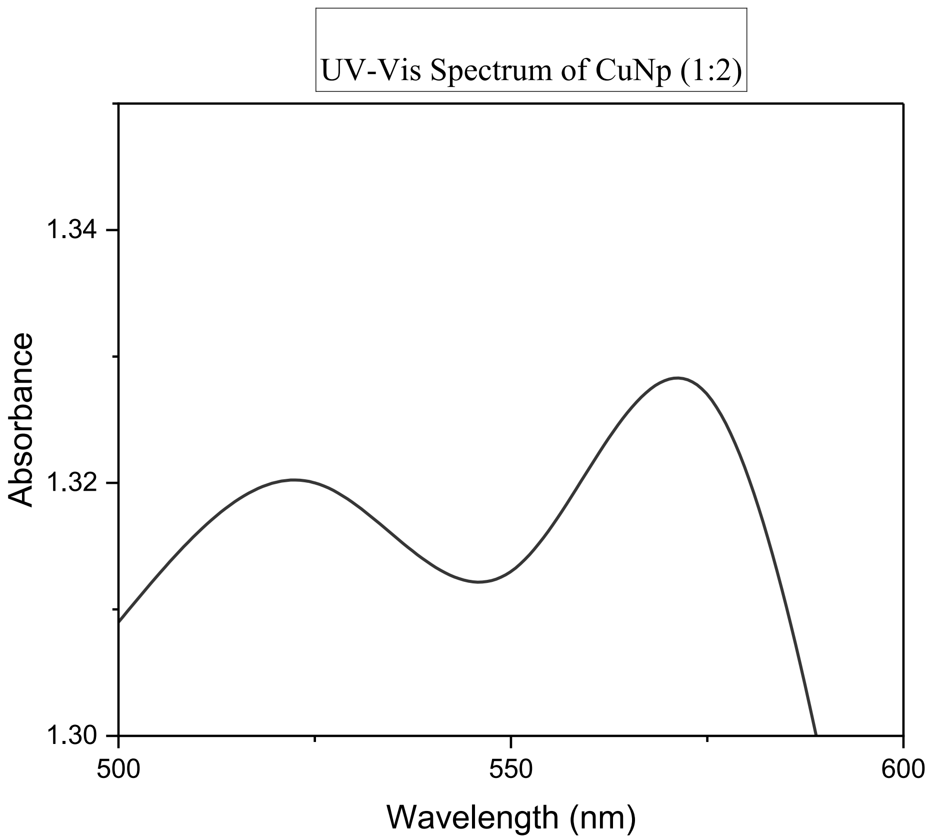

Figure 4.9: UV-Visible Spectrum of CuNp (1:2)

UV-Visible Spectroscopy by Ratio Interpretation

Sample 1 (1:2)

- Showed maximum peak absorbance (1.327 at 575 nm).
- Exhibits strong surface plasmon resonance, indicating nanoparticle formation.
- The very high absorbance may, however, indicate aggregation due to excess metal ions and inefficient capping agents.

4.3.2 UV-Visible Spectrum of CuNp (2:1)

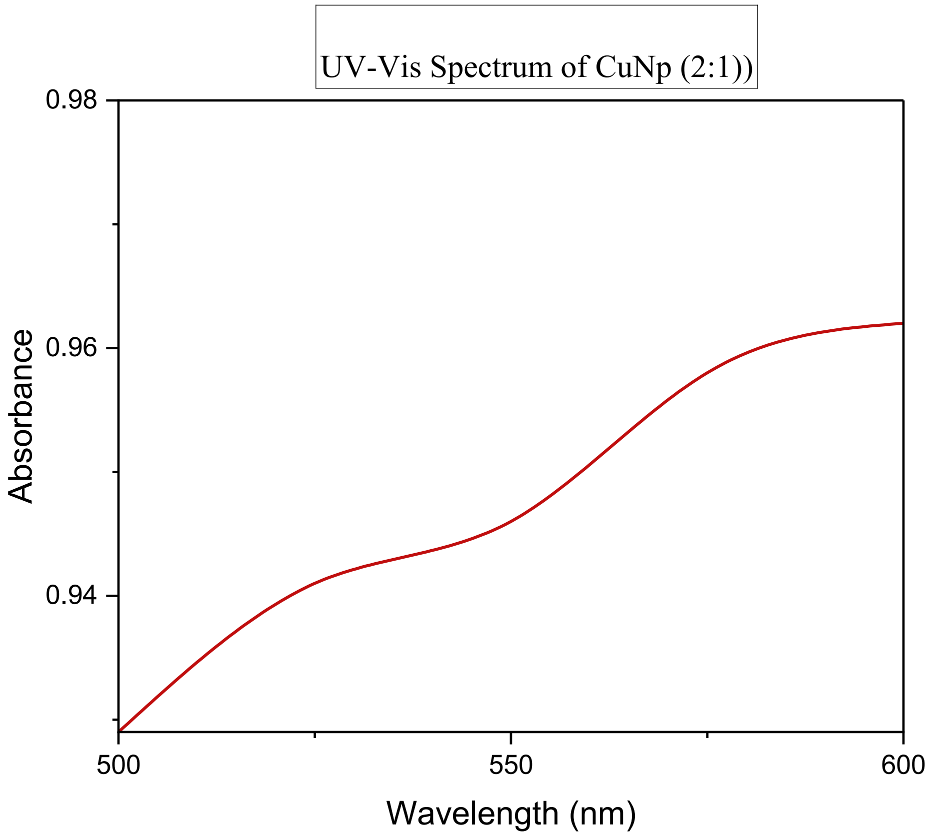

Figure 4.10: UV-Visible Spectrum of CuNp (2:1)

Sample 2 (2:1)

- Showed medium peak absorbance (0.962 at 600 nm).
- Indicates better control synthesis with enhanced particle dispersion.
- Red-shift of peak by a narrow margin indicates that the particles are efficiently stabilized relative to Sample 1.

4.3.3 UV-Visible Spectrum of CuNp (3:1)

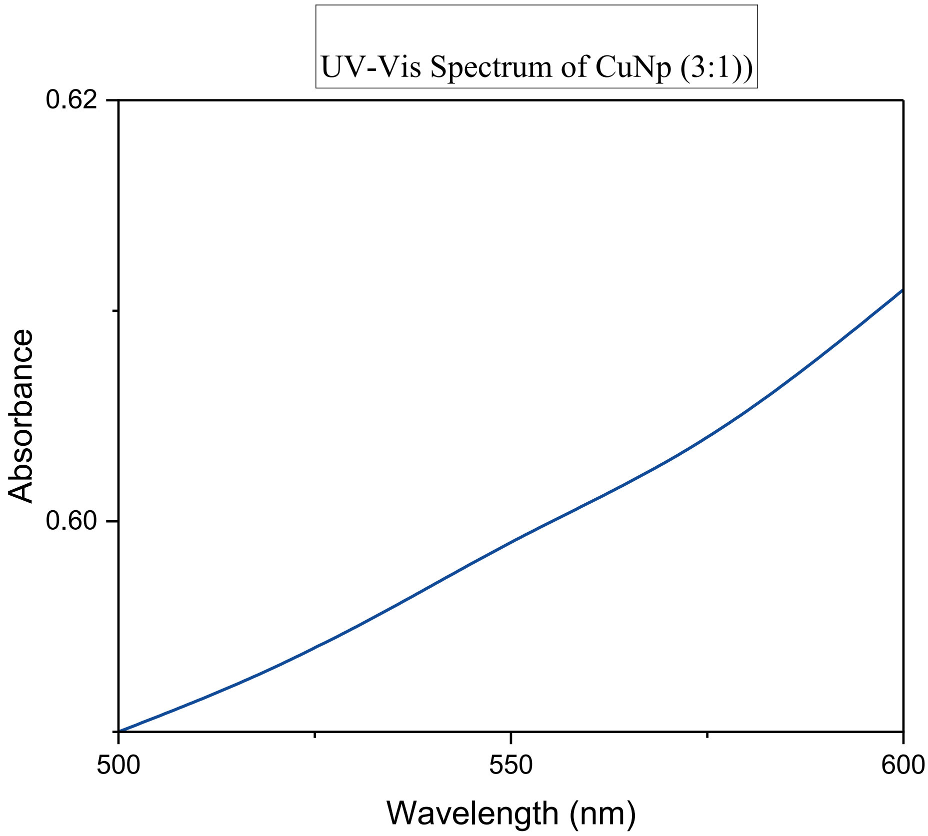

Figure 4.11: UV-Visible Spectrum of CuNp (3:1)

Sample 3 (3:1)

- Absorbance (0.611 at 600 nm) was also down, reflecting smaller or fewer nanoparticles.
- Reflects satisfactory balance of reduction and capping, with relatively homogenous particles.

4.3.4 UV-Visible Spectrum of CuNp (4:1)

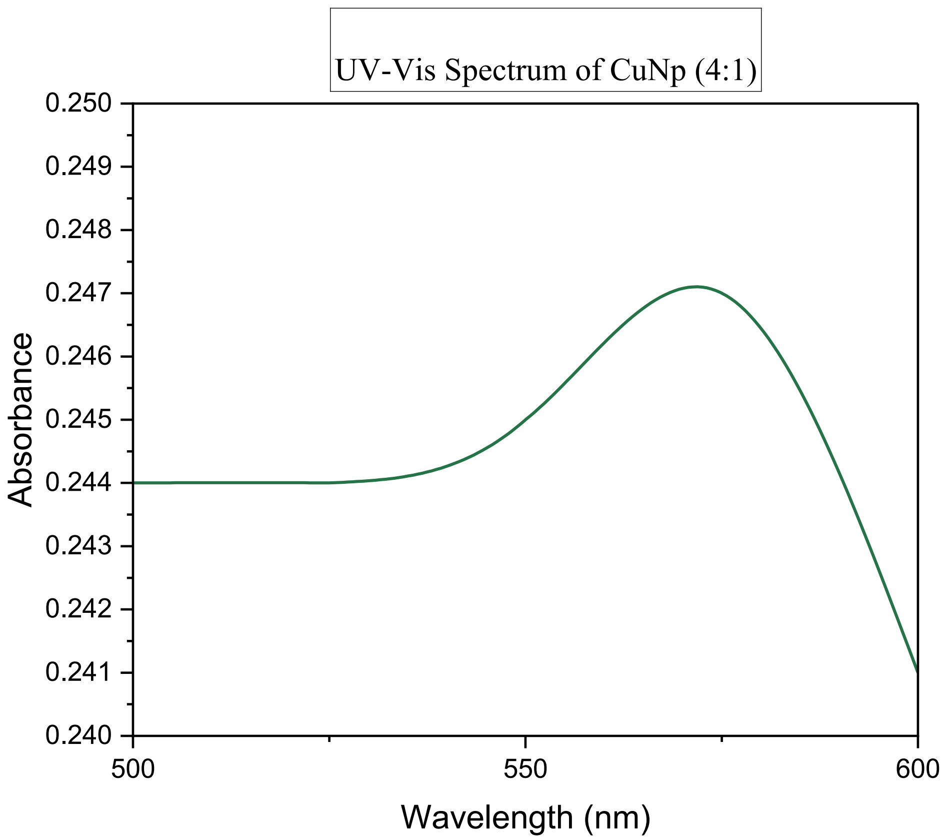

Figure 4.12: UV-Visible Spectrum of CuNp (4:1)

Sample 4 (4:1)

- Lowest absorbance (0.247 at 575 nm), reflecting lowest nanoparticle concentration.
- Excessive plant extract content may have resulted in overcapping, preventing nanoparticle completion.

4.4 Treatment of Wastewater using Effective Microorganisms and *Justicia gendarussa* Copper Nanoparticles (EM-CuNPs)

Table 4.8: Comparative values of all the treatments

Parameter	Synthetic Wastewater	After EM Treatment	After EM-CuNp Treatment
BOD_1 (mg/L)	7.24	6.60	5.59
Estimated COD (mg/L)	11.58	10.56	8.94
TDS/Conductivity	3.98 mS/cm	3.09 mS/cm	1266 µS/cm (1.266 mS/cm)
Nitrate (NO_3^-) / SA	54.8 mg/L	25.5 mg/L	24.3 mg/L
Chloride (Cl^-)	3.71 mg/L	2.64 mg/L	2.61 mg/L

Summary:

BOD_1: EM-CuNp gave highest reduction of nitrate, which indicates better breakdown of organic load.

Conductivity (TDS): EM-CuNp reduced the ionic content by a large margin (from 3.98 to 1.266 mS/cm), more than EM alone.

Nitrate: Both treatments lowered nitrate remarkably, with slightly better effectiveness in EM-CuNp.

Chloride: Minor increase in removal of chloride in EM-CuNp over EM alone.

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List of Publications with Acceptance letters and certificates

Acceptance Letter – AFMD-2025

Dear Prof./Dr./Mr./Ms. **Bidishna Basumatary**
Delhi Technological University

Greetings from the organizing committee of the International Conference on "Advanced Functional Materials & Devices for Sustainable Development (AFMD-2025)." The conference will be held in hybrid mode* (online & offline) at Atma Ram Sanatan Dharma College, University of Delhi, from March 03-05, 2025. It is being organized by the Department of Physics and Internal Quality Assurance cell (IQAC) of ARSD College.

We are pleased to inform you that the technical committee has accepted your participation, **titled**:

PP-81:-Bionanotechnology-Driven Systems for Environmental Studies: Innovations in Monitoring and Remediation
Type of Presentation: Poster Presentation:-Online via Google Meet/Zoom

*Program will take place as per following manner:

- ***Day-1 Offline Mode at ARSD College***
- ***Day 2&3 Online Mode through Zoom of Google Meet***

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Certificate of Participation

This is to certify that Prof./Dr./Mr./Ms.
BIDISHNA BASUMATARY
Delhi Technological University

has participated in the 3rd International Conference on "Advanced Functional Materials & Devices for Sustainable Development" (AFMD-2025) organised by Department of Physics under the aegis of IQAC ARSD College, University of Delhi, India during March 03-05, 2025 in hybrid mode.

He/She has presented **Poster** entitled:
PP-81:-Bionanotechnology-Driven Systems for Environmental Studies: Innovations in Monitoring and Remediation

Dr. Shankar Subramanian	Dr. Anjali Sharma	Prof. Vinita Tuli	Prof. Gyantosh Kumar Jha
Convener, AFMD-2025	Convener, AFMD-2025	Coordinator, IQAC	Principal/Patron AFMD-2025

Certificate No: ARSD/AFMD25/PP/084

Dear Researcher,

Greetings and Best wishes for the Day,

The Research paper entitled "**Toward Smart PGPR Systems for Millets: Bioinoculant Technology for Climate-Smart Agriculture**" is selected for the upcoming conference "**International Conference on Renewable, Environment and Agriculture(ICREA)**" on date & place: **01st Jun 2025 at Ajmer, India.**

Organized by: **SA (South Asian Research Center)**

Paper id: **SA-CREA-AJMR-010625-7707**

Place and Date: **01st Jun 2025 at Ajmer, India.**

Last date of Registration: **23RD May, 2025**

Authors Details: ***Bidishna Basumatary, Megha Shah & Jai Gopal Sharma***

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for presenting a paper entitled "Toward Smart PGPR Systems for Millets: Bioinoculant Technology for Climate-Smart Agriculture" at the International Conference on Renewable, Environment and Agriculture(ICREA) held in Ajmer, India on 01st June, 2025.

Conference Coordinator
South Asian Research Center

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