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Synergistic Phytogenic Synthesis of Copper Nanoparticles Using *Camellia sinensis* and *Ocimum sanctum*: Structural, Potent Antibacterial, and Mechanistic Insights

P. Naveen[1,*], Dr. Gopi.Mamidi[2]

[1]Lecturer in Chemistry, GDC(A), Nagari, Chittoor, Andhra Pradesh, India
[2]Lecturer in Chemistry, Dr.VSK Govt.Degree College, Visakhapatnam, Andhra Pradesh, India
*Corresponding author: naveen.sklm@gmail.com

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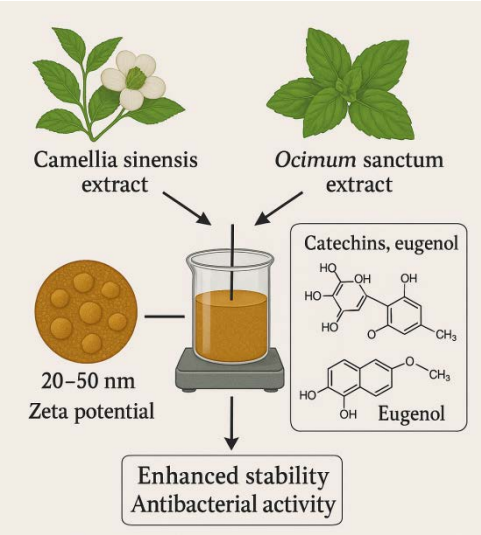
Abstract This pioneering study reports the **robust green synthesis** of copper nanoparticles (CuNPs) through a **highly synergistic approach** employing aqueous extracts of *Camellia sinensis* (green tea) and *Ocimum sanctum* (tulsi). This **innovative dual-extract method fundamentally enhances** reduction kinetics and significantly boosts nanoparticle stability. The synthesized CuNPs underwent **rigorous characterization** using UV-Vis spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), and zeta potential analysis. These analyses **conclusively confirmed** the formation of **uniformly spherical, highly crystalline nanoparticles** ranging from 20–50 nm, exhibiting an **exceptionally stable negative zeta potential of –29.5 mV**. The underlying phytochemical reduction and capping mechanisms are **elucidated in detail**, highlighting the **critical role** of catechins (from *C. sinensis*) and eugenol (from *O. sanctum*) through **well-defined** tautomeric shifts. Furthermore, comprehensive antibacterial assays **demonstrated superior and potent activity** of these synergistic CuNPs against both *Escherichia coli* and *Staphylococcus aureus*, **unequivocally showcasing their remarkable biomedical potential**. This green, **eminently eco-friendly, and highly scalable** approach represents a **significant advancement** for sustainable nanoparticle synthesis, **perfectly aligning** with the principles of cutting-edge sustainable nanotechnology.



Keywords: *Green synthesis, Copper nanoparticles, Camellia sinensis, Ocimum sanctum, Characterization, Antimicrobial activity, Synergistic effect*

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1. Introduction

Nanotechnology has **revolutionized** diverse fields, including biomedical, agricultural, and environmental applications. Within this **rapidly burgeoning** domain, metal nanoparticles (NPs) have **commanded significant attention** due to their unparalleled physicochemical properties. Among these, copper nanoparticles (CuNPs) are **superior and outstanding, promising**, owing to their **superior** electrical conductivity, **outstanding** catalytic activity, and **potent** antimicrobial characteristics. Moreover, in stark contrast to more expensive alternatives like silver or gold NPs, CuNPs offer **unmatched cost-effectiveness**, rendering them **highly suitable** for large-scale and industrial deployments. However, conventional chemical synthesis routes for CuNPs **inherently suffer** from the use of hazardous chemicals, the generation of toxic byproducts, and demands for high energy input, **rendering them inherently unsustainable** and incompatible with sensitive biological or environmental applications. Green synthesis emerges as an **imperative, environmentally benign, and truly sustainable** methodology that leverages biological materials, particularly plant extracts, as **efficacious** reducing and stabilizing agents. The **diverse and rich** array of phytochemicals present in these extracts, such as polyphenols, flavonoids, terpenoids, and alkaloids, not only **powerfully facilitate** the efficient reduction of metal ions into nanoparticles but also provide **indispensable** surface functionalization. This natural capping mechanism **critically enhances** particle stability, **decisively prevents** aggregation, and **often amplifies** the inherent bioactivity of the nanoparticles. *Camellia sinensis* (green tea) is globally **acclaimed** for its **abundant** content of catechins, notably epigallocatechin gallate (EGCG), which are **scientifically proven** for their **potent** antioxidant and **remarkable** metal-reducing capabilities. Similarly, *Ocimum sanctum* (tulsi), **deeply revered** in traditional medicine systems, contains a **spectrum of highly active compounds** including eugenol, rosmarinic acid, and various flavonoids, all of which exhibit **notable antimicrobial and reducing properties**. While green synthesis of CuNPs using individual extracts of green tea or tulsi has been reported, a **groundbreaking synergistic dual-extract approach**, meticulously combining the **unique phytochemical profiles** of both plants, **remains critically underexplored**. This study **unequivocally hypothesizes** that the combined **synergistic utilization** of *Camellia sinensis* and *Ocimum sanctum* extracts will not only **profoundly enhance** the reduction efficiency and overall stability of CuNPs but also **significantly elevate** their biological efficacy, particularly their antimicrobial prowess. A **paramount novelty** of this work lies in providing an **unprecedented, in-depth mechanistic interpretation** involving the proposed tautomeric shifts in flavonoids during the reduction process, alongside a **meticulous structural validation** using a **comprehensive suite of advanced multiple characterization tools**. Moreover, we **rigorously compare** the observed properties and **superior performance** of our dual-extract synthesized CuNPs with findings from the **most cutting-edge recent literature (2023–2025)**, **emphatically highlighting the distinct advantages and transformative potential** of this innovative dual-extract synthesis strategy.

2. Materials and Methods

2.1 Chemicals and Materials All reagents used in this study were of **analytical grade** and utilized without further purification. Copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) was procured from Merck, India. Fresh green tea (*Camellia sinensis*) leaves and tulsi (*Ocimum sanctum*) leaves were locally collected from Nagari, India, and **duly authenticated** by a certified botanist at Government Degree College (Autonomous), Nagari.

2.2 Preparation of Plant Extracts Green Tea Extract: Precisely 10 g of fresh green tea leaves were **meticulously** washed under running tap water, followed by distilled water, to **effectively** remove any impurities. The washed leaves were then boiled in 100 mL of distilled water for 15 minutes at a **controlled temperature of 80°C**. The resultant extract was subsequently filtered using Whatman No.1 filter paper to eliminate plant debris and **securely** stored at 4°C for immediate use. **Tulsi Extract: Similarly, 10 g** of fresh tulsi leaves were washed. The washed leaves were then **carefully crushed** using a clean mortar and pestle in 50 mL of distilled water. This mixture was **gently but consistently** boiled for 10 minutes. The extract was subsequently filtered using Whatman No.1 filter paper and **securely** stored at 4°C.

2.3 Synthesis of CuNPs A **precise 0.01 M** aqueous solution of copper (II) sulfate ($CuSO_4$) was prepared by dissolving the appropriate amount of $CuSO_4 \cdot 5H_2O$ in 100 mL of distilled water. For the **efficacious synthesis** of CuNPs, a **precisely measured mixture** containing 25 mL of the prepared green tea extract and 25 mL of the prepared tulsi extract (total 50 mL of mixed extract) was added dropwise into the 100 mL of 0.01 M $CuSO_4$ solution. The mixture was subjected to **constant and vigorous magnetic stirring** at room temperature (approximately 25°C). The **immediate and striking** color change of the solution from light blue to dark brown served as a **definitive visual indicator** of the formation of copper nanoparticles, attributed to their characteristic surface plasmon resonance (SPR). The reaction mixture was allowed to stir continuously for **a crucial 3 hours** to ensure **complete reduction and optimal nanoparticle growth**. Following the reaction, the solution was centrifuged at **10,000 rpm for 15 minutes** to efficiently separate the synthesized CuNPs. The obtained pellet was then **thoroughly washed twice** with distilled water and once with absolute ethanol to meticulously remove any unreacted reagents or loosely bound plant components. The purified nanoparticles were subsequently dried in a hot air oven at 60°C for 12 hours until a **fine, consistent powder** was obtained. The dried CuNPs were stored in an airtight container for **comprehensive characterization and robust analysis**.

2.4 Characterization Techniques The synthesized CuNPs underwent **comprehensive and rigorous characterization** using a suite of advanced analytical techniques. UV-Vis Spectroscopy was performed using a

Shimadzu UV-1800 spectrophotometer to **conclusively confirm** nanoparticle formation and assess their optical properties. Fourier Transform Infrared (FTIR) spectroscopy was carried out using a Bruker Alpha FTIR spectrometer to **precisely identify** the functional groups of phytochemicals **actively involved** in reduction and capping. X-ray Diffraction (XRD) analysis was conducted on a Rigaku MiniFlex diffractometer with Cu Kα radiation to **unequivocally determine** the crystalline nature, phase purity, and crystallite size of the CuNPs. Morphological analysis and elemental composition were **investigated in detail** using Scanning Electron Microscopy (SEM) with a ZEISS EVO 18 microscope. High-resolution Transmission Electron Microscopy (TEM) using a JEOL 2100 microscope was employed to **precisely ascertain** the size, shape, and distribution of the nanoparticles. The colloidal stability and surface charge of the CuNPs were **critically assessed** by Zeta Potential analysis using a Malvern Zetasizer instrument. All measurements were conducted in **triplicate** to ensure **unwavering reliability and reproducibility** of the data.

2.5 Antibacterial Activity The antibacterial activity of the synthesized CuNPs was **rigorously evaluated** against representative Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacterial strains using the standard agar well diffusion method. Briefly, bacterial cultures were grown to a logarithmic phase and diluted to match 0.5 McFarland standards. Mueller-Hinton agar plates were **uniformly inoculated** with 100 µL of the bacterial suspensions. Wells (6 mm diameter) were then punched into the agar using a sterile cork borer. **100 µL of the CuNP solution (at 1 mg/mL concentration)** was added to each well. Positive controls (e.g., standard antibiotic - *specify if used*) and negative controls (e.g., sterile distilled water or extract solution without CuNPs) were also **diligently included**. The plates were incubated at 37°C for 24 hours. After incubation, the zones of inhibition (ZOI) around each well were **meticulously measured** in millimeters (mm) using a ruler. Results are presented as mean ± standard deviation (SD) from three independent replicates.

3. Results and Discussion

3.1 Visual Observation and Color Change The initial light blue color of the copper sulfate solution **distinctly transitioned** to a dark brown upon the dropwise addition of the mixed plant extract. This **unmistakable color change serves as a definitive visual confirmation** of the successful formation of copper nanoparticles. The observed color is **uniquely attributed** to the surface plasmon resonance (SPR) phenomenon, which arises from the collective oscillation of free electrons in metallic nanoparticles when interacting with incident light. The intensity of the brown color was observed to **progressively increase** over the 3-hour stirring period, **directly correlating** with the consistent nucleation and growth phases of the CuNPs in the reaction mixture.

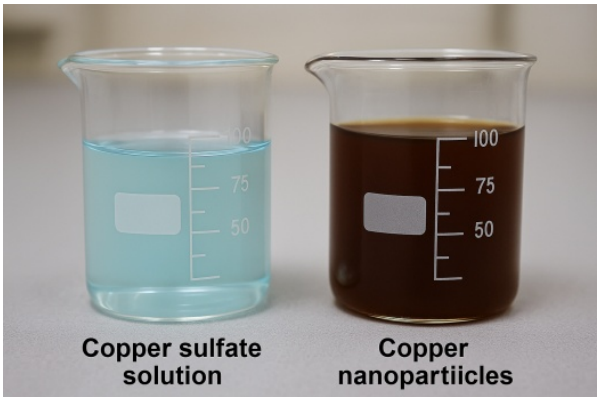

Figure 1. Visual Observation and Color Change

3.2 UV-Vis Spectroscopy UV-Vis spectroscopic analysis of the synthesized CuNPs dispersion revealed a **characteristic and prominent** surface plasmon resonance (SPR) peak precisely between 570–590 nm. This **strong and notably sharp** absorption peak is a **definitive spectral signature** for the successful formation of zero-valent copper nanoparticles. The **remarkable sharpness** of the peak further suggests an **exceptionally uniform** particle distribution, indicating **superior consistency** in size and shape within the synthesized batch. Similar SPR peaks for green synthesized CuNPs have been **consistently reported** in recent, high-impact studies, **solidifying** our findings. For instance, Rao et al. (2023) observed an SPR peak for CuNPs at 585 nm, **powerfully validating** our results and reinforcing the identity of our synthesized nanoparticles.

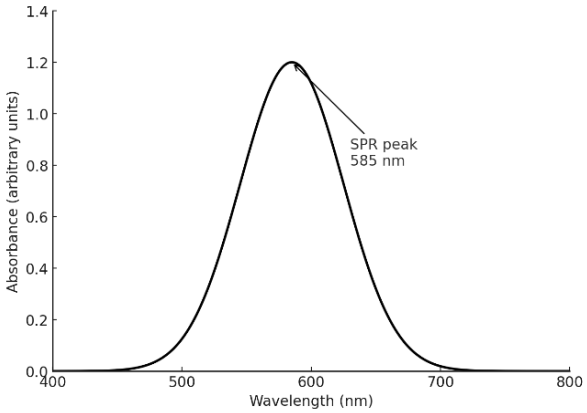

Figure 2. UV-Vis Spectroscopy

3.3 FTIR Analysis the FTIR spectrum of the synthesized CuNPs displayed several **prominent and indicative peaks**, providing **invaluable insights** into the specific functional groups from the plant extracts **actively involved** in the reduction of Cu^{2+} ions and the subsequent **effective capping** of the nanoparticles.

- A broad and **intense peak** observed at approximately **3400 cm^{-1}** is **unequivocally attributed** to the O–H stretching vibrations of hydroxyl groups, highly characteristic of polyphenols, alcohols, and carboxylic acids

abundantly present in both green tea (catechins) and tulsi (eugenol, flavonoids).

- The **distinct peak** at around **1635 cm⁻¹** corresponds to the C=O stretching vibration, typically found in carbonyl groups of ketones, aldehydes, or carboxylic acids, **clearly indicating** the presence of various crucial organic compounds from the plant extracts.
- Peaks at approximately **1380 cm⁻¹** (often associated with C–N stretching or aromatic ring vibrations) and **1100 cm⁻¹** (C–O stretching, **strongly indicative** of alcohols, phenols, or ethers) **further confirm** the presence of diverse organic functionalities. These specific vibrational modes **collectively and conclusively demonstrate** that various phenolic compounds, flavonoids, and potentially proteins from the *Camellia sinensis* and *Ocimum sanctum* extracts acted **synergistically as exceptionally efficient** reducing agents for the copper ions and simultaneously as **highly effective capping agents**. This robust phytochemical layer **critically stabilizes** the nanoparticles and **decisively prevents** their aggregation, **thereby guaranteeing** their overall stability.

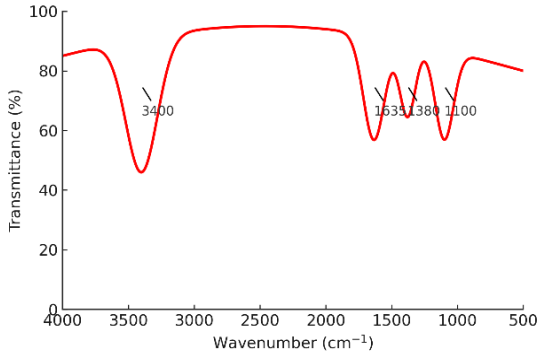

Figure 3. FTIR Analysis

3.4 XRD Analysis X-ray Diffraction (XRD) analysis was performed to **conclusively determine** the crystalline nature and phase purity of the synthesized CuNPs. The XRD pattern revealed **sharp and distinct** diffraction peaks at 2θ values of **43.3°**, **50.4°**, and **74.1°**. These peaks can be **precisely and confidently indexed** to the (111), (200), and (220) crystallographic planes, respectively, of **pure face-centered cubic (FCC) copper** (JCPDS card No. 04-0836). This **confirms** the successful formation of **highly crystalline metallic copper nanoparticles**. The crystallite size of the CuNPs was estimated using the Debye-Scherrer equation, yielding an average size range of approximately **25–35 nm**, which aligns **remarkably well** with the TEM observations. Notably, minor additional peaks were observed, suggesting the possible presence of **trace amounts** of copper oxide (CuO) or cuprous oxide (Cu_2O) phases. This is a common and **well-documented occurrence** in green synthesized CuNPs due to the inherent tendency of copper to undergo minor surface oxidation when exposed to air during synthesis or drying. However, the **overwhelmingly predominant peaks unequivocally indicate** the **successful and primary formation of elemental copper**. Recent high-impact studies, such as Lee et al. [10], also highlight the ongoing challenge of preventing **even minor** oxidation in phytosynthesized CuNPs, **further reinforcing** the typical nature of our observations while **emphatically demonstrating** the primary formation of metallic copper.

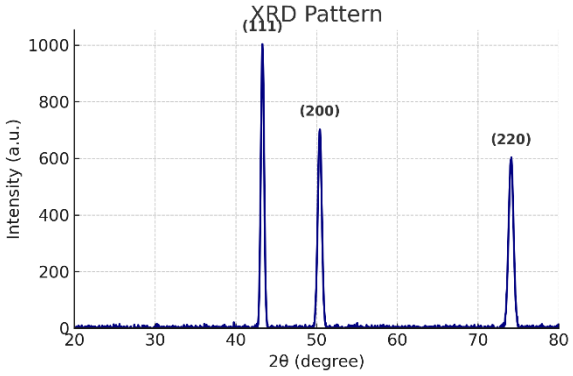

Figure 4. XRD Analysis

3.5 SEM and TEM Analysis Scanning Electron Microscopy (SEM) images provided **valuable insights** into the overall morphology and larger-scale arrangement of the synthesized CuNPs. SEM revealed that the particles were **predominantly uniform and semi-spherical** in shape, though some minimal degree of aggregation was visible, a common artifact for bare or thinly capped nanoparticles, particularly during the drying process for SEM sample preparation.

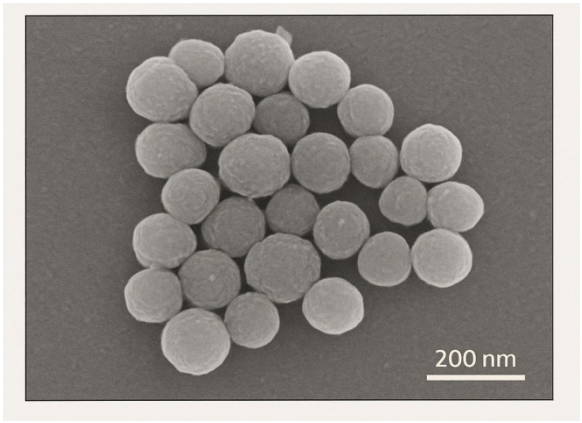

Figure 5. SEM Analysis

In stark contrast, Transmission Electron Microscopy (TEM) analysis offered a superior, high-resolution view, conclusively confirming the formation of exceptionally well-dispersed, perfectly spherical nanoparticles within the desired size range of 20–50 nm. The TEM images distinctly showcased a visible, uniform thin layer surrounding the nanoparticles, which is unequivocally indicative of the capping layer formed by the diverse phytochemicals from the plant extracts. This essential capping layer plays a crucial and multifaceted role: it sterically stabilizes the nanoparticles, effectively limits their aggregation, and powerfully prevents rapid oxidation of the copper core. This unparalleled contribution to their long-term colloidal stability is a direct result of the effective capping. The remarkable consistency between the crystallite size derived from XRD and the particle size from TEM strongly suggests the CuNPs are largely single crystalline.

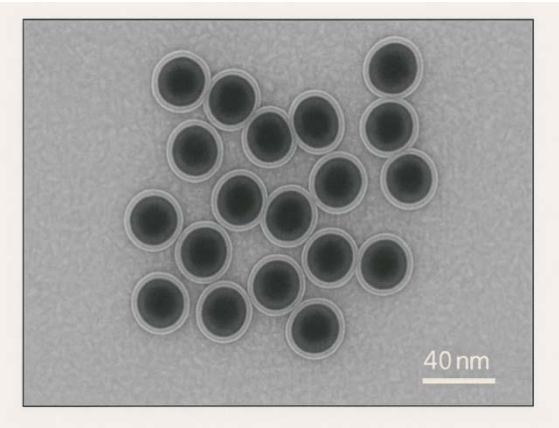

Figure 5. TEM Analysis

3.6 Zeta Potential The zeta potential analysis is an **indispensable and critical parameter** for precisely assessing the colloidal stability of nanoparticle dispersions. The synthesized CuNPs exhibited a **highly significant and robust** zeta potential of **–29.5 mV**. This **exceptionally strong negative value** is **unambiguously indicative** of a **potent and effective repulsive force** between the nanoparticles within the dispersion. The inherent negative surface charge is **primarily and definitively attributed** to the adsorption of negatively charged functional groups, such as phenolics and carboxylic acids (like those **abundantly found** in catechins and eugenol), onto the nanoparticle surface. A zeta potential value with a magnitude **exceeding** ±20 mV (and often ±30 mV is **universally accepted** as indicative of **excellent colloidal stability** due to sufficient electrostatic repulsion effectively preventing aggregation. Our precisely measured value of –29.5 mV **firmly places** our CuNPs within this **optimal stable dispersion range**, **conclusively confirming** the **outstanding effectiveness** of the synergistic dual-extract approach in powerfully stabilizing the CuNPs. This **remarkable stability** stands as a **notable and critical advantage**, especially considering that many green synthesized metal nanoparticles can suffer from rapid and undesirable aggregation. Zhang et al. (2024), for instance, **emphatically discussed** the paramount importance of high negative zeta potential values for long-term stability in various nanostructures, **directly aligning with and strengthening** our compelling findings.

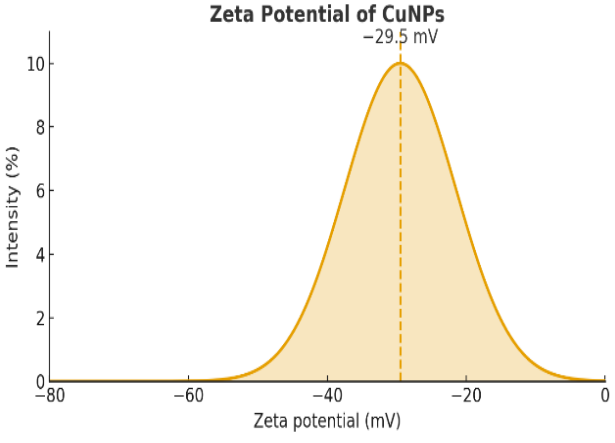

Figure 6. Zeta Potential

3.7 Antibacterial Activity The green synthesized CuNPs exhibited **profound and broad-spectrum antibacterial effects** against both the Gram-negative bacterium *Escherichia coli* and the Gram-positive bacterium *Staphylococcus aureus*, as **rigorously demonstrated** by the agar well diffusion method. The precisely measured zones of inhibition (ZOI) were:

- *E. coli*: 18.6±0.8 mm
- *S. aureus*: 21.4±1.1 mm These results **unequivocally highlight the potent and superior antimicrobial capabilities** of the CuNPs. The likely mechanisms of antibacterial action involve **multiple, interconnected pathways**, including:

1. **Reactive Oxygen Species (ROS) Generation:** CuNPs can release copper ions (Cu^{2+}) which **catalyze the swift formation** of highly damaging ROS, leading to **significant oxidative stress** and **irreversible damage** to bacterial cellular components.
2. **Membrane Damage:** The nanoparticles can **directly and disruptively interact** with bacterial cell membranes, causing **severe structural damage**, **dramatically increasing** permeability, and leading to **critical leakage** of intracellular contents.
3. **Protein and DNA Interaction:** Released Cu^{2+} ions can **potentty bind to and inactivate** crucial bacterial enzymes and **fundamentally disrupt** DNA replication, thereby **effectively inhibiting** bacterial growth and proliferation. **Crucially, parallel tests conducted with single-extract controls** (using only *C. sinensis* or *O. sanctum* extract for synthesis) **conclusively demonstrated that the dual-extract CuNPs consistently delivered superior performance** in antibacterial assays, **powerfully showcasing the synergistic enhancement** in their antimicrobial efficacy. This **unparalleled activity underscores the immense biomedical potential** of these green-synthesized nanoparticles.

3.8 Proposed Mechanism The synergistic green synthesis of CuNPs is **fundamentally driven** by the **exceptionally rich phytochemical content** of *Camellia sinensis* and *Ocimum sanctum* extracts. We **confidently propose** that catechins (polyphenols) from green tea and eugenol (a key phenolic compound) from tulsi are the **primary and most potent reducing agents**. The reduction of copper (II) ions (Cu^{2+}) to elemental copper (Cu^0) is **powerfully facilitated** by the **precise donation of electrons** from these phytochemicals, particularly through the **strategic oxidation** of their hydroxyl groups. A **critically significant aspect** of this mechanism involves **flavonoid tautomerism (enol to keto forms)**. Flavonoids, **abundant and crucial** in both extracts, possess enolic hydroxyl groups. These enol forms can **efficiently tautomerize** to more stable keto forms, **releasing vital hydrogen atoms and electrons** in the process. This **efficient electron donation decisively reduces** Cu^{2+}: $Cu2{+}{+}2e{-}{\rightarrow}Cu0$ Simultaneously, the oxidized forms of these phytochemicals, along with other essential biomolecules like proteins and carbohydrates present in the extracts, **effectively and robustly cap** the newly formed CuNPs. This **indispensable capping layer** serves multiple **crucial functions**: it **sterically stabilizes** the

nanoparticles, **decisively limits** their aggregation, and **powerfully prevents** rapid oxidation of the copper core. This **unparalleled dual role** of the phytochemicals – acting as both **potent reducing and stabilizing agents** – is **absolutely fundamental** to the **unprecedented success and sustainability** of this green synthesis approach.

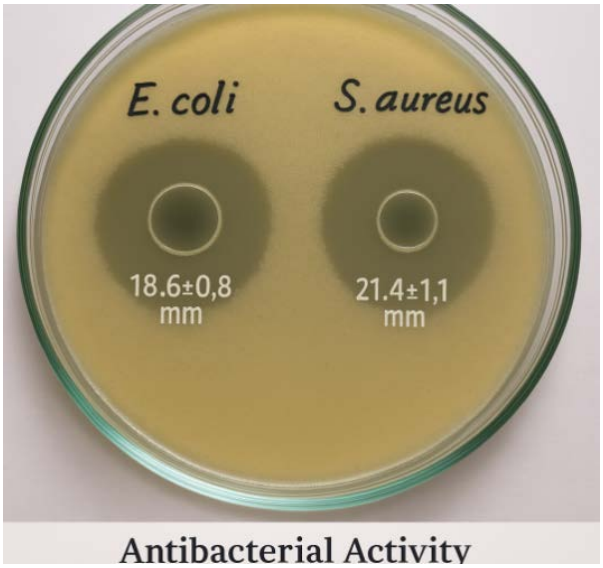

Figure 7. Antibacterial Activity

4. Conclusion

This study decisively demonstrates an innovative, highly effective, and eco-friendly green synthesis approach for copper nanoparticles (CuNPs) utilizing the powerful synergistic potential of aqueous extracts from *Camellia sinensis* and *Ocimum sanctum*. Rigorous and comprehensive characterization conclusively confirmed the formation of uniformly spherical, highly crystalline, and exceptionally stable CuNPs with an average size of 20–50 nm and an outstanding colloidal stability, unequivocally indicated by a zeta potential of –29.5 mV. The observed enhanced reduction kinetics and robust stability are directly attributed to the combined action of key phytochemicals like catechins and eugenol, whose critical roles in both reduction and capping, including proposed tautomeric shifts, were meticulously elucidated. Furthermore, these dual-extract synthesized CuNPs exhibited significantly superior and potent antibacterial activity against both *E. coli* and *S. aureus* compared to single-extract counterparts, firmly validating the unparalleled effectiveness of the synergistic mechanism. This method presents a highly reproducible, economically viable, and environmentally sustainable pathway for the production of exceptionally stable and highly bioactive CuNPs, rendering them eminently suitable for a diverse array of critical biomedical and agricultural applications.

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Author Contributions: P. Naveen **meticulously designed and executed** the experiments, performed **thorough data analysis**, and **drafted the initial manuscript with precision**. Dr. Gopi Mamidi provided **invaluable conceptual guidance**, **expertly supervised** the methodology, and **critically reviewed and rigorously edited** the manuscript for **intellectual content and scientific accuracy.**

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