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BIOSYNTHESIS OF COPPER NANOPARTICLES USING *TINOSPORA CORDIFOLIA* LEAVES: A GREEN APPROACH TO ANTIMICROBIAL STUDIES

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ABSTRACT

The environmentally sustainable synthesis of nanoparticles is a promising alternative to conventional chemical methods, addressing concerns of environmental toxicity and resource depletion. This study reports the biosynthesis of copper nanoparticles (CuNPs) using *Tinospora cordifolia* leaf extract (TCLE) as a natural reducing and stabilizing agent. The synthesis process leverages the bioactive compounds in *T. cordifolia* for the reduction of copper ions, resulting in eco-friendly nanoparticle production. The TC-CuNPs were characterized through DLS, UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FT-IR), and scanning electron microscopy (SEM) to confirm their structural, optical, and morphological properties.

The antimicrobial potential of the synthesized TC-CuNPs was evaluated against antibiotic-resistant microorganisms *Mycobacterium tuberculosis*. Antimicrobial efficacy was assessed using the Zone of Inhibition (Kirby-Bauer method) assays. The results demonstrated significant antibacterial activity, attributed to the generation of reactive oxygen species (ROS) and the release of copper ions, which disrupt microbial cell membranes and genetic material. The TC-CuNPs exhibited strong inhibitory effects, with larger inhibition zones indicating their potential as effective antimicrobial agents. This study highlighted the use of *T. cordifolia* as a sustainable resource for nanoparticle synthesis, emphasizing its role in advancing green nanotechnology. The findings pave the way for the development of eco-friendly antimicrobial agents with potential applications in biomedical, pharmaceutical, and environmental sectors, aligning with global sustainability goals.

Keywords: Copper nanoparticles, *Tinospora cordifolia*, Green synthesis, Antimicrobial activity

INTRODUCTION

The emergence of nanotechnology has created new possibilities in fields such as medicine, agriculture, and environmental science. Today, integrating green chemistry into the research of nanoparticles is a crucial focus in material science and engineering **[1, 2]**. Nanoparticles play a vital role in nanotechnology, as their unique characteristics make them essential in an array of applications. These particles generally measure between 1 and 100 nanometers and demonstrate distinct attributes, resulting in high-surface-area materials that are beneficial across various domains, including medicine, electronics, and environmental studies **[3]**. Indeed, nanotechnology stands out as one of the most advanced and swiftly progressing areas in contemporary material sciences **[4]**. This technology has transformed multiple scientific and technological disciplines, providing significant advantages to humanity through its diverse applications **[5-7]**.

Noble metals such as gold and silver have been thoroughly researched for their potential uses in areas like antimicrobial properties **[8-10]**, catalysis **[11-13]**, and medicine **[14, 15]**. Lately, copper nanoparticles (CuNPs) have gained popularity in their synthesis due to their special antibacterial attributes and lower costs compared to precious metals such as gold and silver **[16]**. The promising potential of CuNPs in medical labs and healthcare is fueling research interest in these materials. These tiny metallic particles are changing the landscape of biomedical research, treatments, and diagnostics, unlocking new avenues for progress **[17-20]**. Traditional nanoparticle synthesis methods frequently involve toxic substances and high energy usage, which present environmental and health concerns. In this regard, using plant extracts for biosynthesis offers a green and sustainable option, utilizing natural reducing and capping agents. Literature has reported on green synthesis derived from plant extracts **[21–23]**. Plant-based methods are among the most cost-effective, safe, and environmentally friendly techniques for generating nanoparticles **[24]**. Nanoparticles produced through plant-mediated biosynthesis are often more stable and can be generated more swiftly than those created by other synthesis approaches **[25]**.

Tinospora cordifolia is an important plant in traditional Indian medicine, known for its benefits in boosting energy, aiding digestion, and treating urinary infections **[26-27]**. The *Tinospora cordifolia* plant is recognized by different names in various languages, including Gulancha (English), Giloy (Hindi), Galo (Gujarati), Guduchi (Sanskrit), Shindilakodi (Tamil), Madhupa (Kannada), Thippateega (Telugu), and Gulancha (Bengali) **[28]**. This study concentrated on the biosynthesis of copper nanoparticles (CuNPs) utilizing the leaf extract of T. cordifolia (TCLE), as well as their characterization and assessment of antimicrobial properties. The CuNPs synthesized from TCLE are referred to as TC-CuNPs.

Materials and Methods

Preparation of *Tinospora cordifolia* extract

Fresh T. cordifolia leaves were gathered, rinsed, and air-dried. A total of 10g of the dried leaves was grounded into a powder and extracted using distilled water at a temperature of 60°C for 2 hours. The resulting extract was filtered through Whatman (41) and stored at 4°C for future use in synthesizing TC-CuNPs.

Phytochemical analysis

The phytochemical assessment of TCLE focused on identifying various bioactive compounds including flavonoids, alkaloids, carbohydrates, and others. Standard methods for qualitative phytochemical analysis were employed to detect the main phytochemicals present in the TLCE. Tests such as the Mayer test, $FeCl_3$ test, dilute iodine test, and lead acetate test were conducted to identify alkaloids, phenolics, and flavonoids, respectively, in the methanolic TLCE.

Biosynthesis of Copper Nanoparticles

In an Erlenmeyer flask, a 1M $CuSO_4$ solution was prepared, heated, and then mixed with 20 mL of TCLE while constantly stirring for an additional 5 hours at room temperature. During this process, the color of the solution changed from dark green to reddish-brown, indicating the successful synthesis of TC-CuNPs, which was further confirmed using UV-Vis spectroscopy. **[29]** Subsequently, the final mixture was centrifuged at 10,000–11,000 rpm, allowing for the separation of TC-CuNPs from the solution. Finally, the supernatant was removed, and the reddish-brown TC-CuNPs were collected. The transition from dark green to reddish-brown signifies the formation of TC-CuNPs, as illustrated in **Figure 1**.

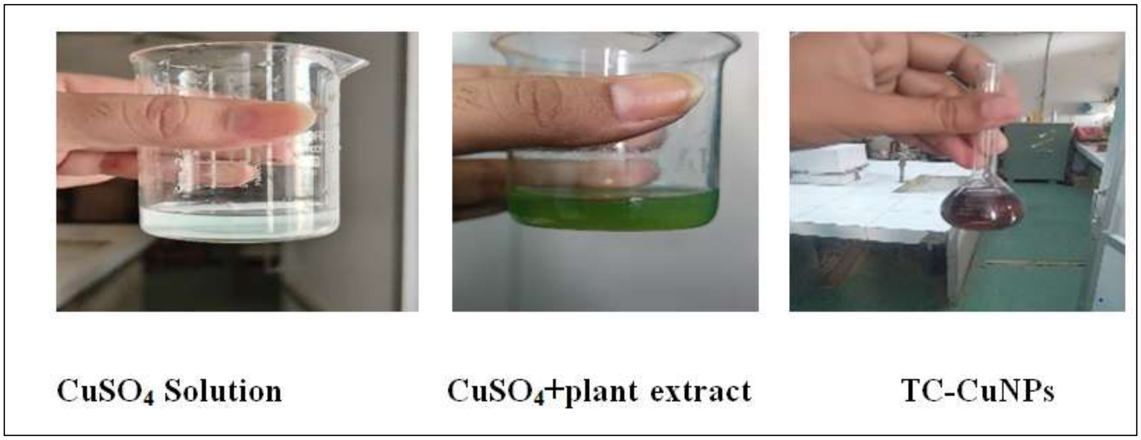


Figure 1: color change during synthesis of TC-CuNPs

Characterization of TC-CuNPs

• Dynamic Light Scattering (DLS): The instrument used for this purpose is Zetasizer Nano-ZSP (ZEN-5600). DLS analysis was conducted by dissolving 10 mg of TC-CuNPs powder in 20 mL of deionized water. The solution was mixed in a vortex mixer for 3-4 minutes to disaggregate any clusters, and then 0.5-1 mL was transferred into the zeta-disposable cell.

• UV-Vis Spectroscopy: The absorbance was recorded over the wavelength range of 200-800 nm to verify the formation of nanoparticles using a UV-3600 Plus from Shimadzu, Kyoto, Japan.

• FT-IR Analysis: The functional groups responsible for the reduction and stabilization of TC-CuNPs were identified using a Bruker ALPHA-E spectrometer.

• Scanning Electron Microscopy (SEM): The size and appearance of the TC-CuNPs were examined using the Thermo Scientific Apreo S model in conjunction with Energy Dispersive X-ray (EDX) spectrometry.

Antimicrobial Activity

The antibacterial efficacy of the synthesized TC-CuNPs was assessed using the Zone of Inhibition Method, specifically through the Kirby-Bauer technique. In this procedure, Mueller-Hinton Agar (MHA) plates were inoculated with 100 µL of Mycobacterium tuberculosis culture, which was adjusted to a 0.5 McFarland standard (approximately 1.5 × 10^8 CFU/mL). Discs containing 10 µL of varying concentrations of TC-CuNPs (from 0 to 100%) were placed on the inoculated plates. A disc that contained only the solvent served as the vehicle control, while a ciprofloxacin disc (10 µg) was utilized as the positive control. The inhibition zones surrounding each disc were measured to evaluate the antimicrobial effectiveness of the TC-CuNPs against *M. tuberculosis*. The dimensions of the inhibition zones indicated the degree of antibacterial activity. The inoculated plates were then incubated at 37°C for 24 hours in an incubator. After incubation, the plates were inspected for clear zones around the discs, which indicated bacterial inhibition. These areas, known as zones of inhibition, were measured and documented to assess the antimicrobial efficacy of the TC-CuNPs.

RESULTS AND DISCUSSION:

Phytochemical Analysis of TCLE:

The initial assessment of the phytochemical profile indicated that the methanolic TLCE is rich in a variety of essential phytochemicals including alkaloids, phenolics, tannins, flavonoids, etc., as shown in **Table 1**. The analysed phytoconstituents in different extracts of the formulation have been documented to have medicinal significance in various pathological conditions, like diabetes, cancer, and inflammation **[30]**. The presence of phenolics and flavonoids can disrupt the chain reaction of reactive oxygen species in cellular processes and safeguard the human body from the damages caused by reactive oxygen species. Due to the cardio-protective, anti-cancer, anti-diabetic, anti-aging, and neuroprotective properties of phenolic and flavonoid compounds, plants rich in these compounds may enhance the body's antioxidant capacity **[31]**.

Dynamic Light Scattering (DLS): DLS was utilized to examine both the Particle Size Distribution (PSD) and the Zeta Potential (ZP) of the synthesized copper nanoparticles (TC-CuNPs). The outcomes from the DLS analysis were illustrated as intensity-based Particle Size Distribution and Zeta Potential distribution graphs, presented in **Figure 2**, with an average particle size of approximately 82.29 nm. The PDI value of 0.457 indicated that the synthesized TC-CuNPs are moderately distributed. The measured particle size is within the range established by the American Society for Testing and Materials and the International Organization for Standardization for classifying a nanomaterial, which is defined as being between 1 nm and 100 nm. This indicates that the size and charge distribution of the produced nanoparticles may enhance the biological properties of CuNPs **[32]**.

UV-Vis spectroscopy: A change in color was noted after TCLE interacted with the TC-$CuSO_4$ solution under the specified conditions. The appearance of a reddish brown suggested that $CuSO_4$ had been reduced to nanoparticles. The color change was attributed to the interaction between the conduction electrons of the CuNPs and incoming photons **[33]**. The absorption spectrum of the resulting TC-CuNPs displayed a distinct peak around 346 nm, as shown in **Figure 3**, which is characteristic of copper nanoparticles (TC-CuNPs). This peak aligns with the surface plasma resonance (SPR) of TC-CuNPs, serving as an optical property that validates their successful synthesis. The presence of the surface plasma resonance (SPR) peak confirmed that TC-CuNPs had been successfully formed. With increased reaction time and additional biomolecules, the SPR signal intensified. This indicates that longer reaction durations and greater biomolecule concentrations contribute to the growth and stability of the nanoparticles **[34]**.

FT-IR Spectra Analysis

The FT-IR analysis of the produced TC-CuNPs has revealed several distinctive peaks **(Figure 4)**. A peak corresponding to the hydrogen bonded hydroxyl group (-OH) was detected at 3410.08 cm^{-1}. The peaks at 2750 cm^{-1} and 2895.08 cm^{-1} correspond to the C-H bond at varying hybridization states. A peak for the carbonyl group (>C=O) was identified at 1690.80 cm^{-1}. In addition to these characteristic peaks, there are other peaks observed at 1290.08 cm^{-1}, 1407.08 cm^{-1}, and 1510.08 cm^{-1}. The synthesized nanoparticles are encapsulated by phyto-constituents that are abundant in alcoholic and carbonyl compounds.

SEM of TC-CuNPs

The SEM analysis of the TC-CuNPs offered comprehensive insights into their morphology, indicating that the particles created granular aggregates at the nano-scale, as shown in **Figure 5**. SEM serves as an essential method for characterizing nanoparticles since it provides high-resolution images of the material's surface and structure. The particle size determined through SEM analysis was found to be in the range of 100 nm. The magnification employed for the SEM analysis was 400 K X **[35, 36]**.

EDX Analysis

The analysis using EDX was conducted in conjunction with the SEM analysis of the sample. EDX analysis serves as an excellent complementary method to SEM, offering significant insights into the elemental makeup of materials. The EDS analyses validated the composition and stability of the synthesized TC-CuNPs **(Figure-6b)**. Some faint signals for the C and S elements are observed **(Figure 6a)**. These faint signals might originate from the X-ray emission of macromolecules such as flavonoids, phenolic compounds, carbohydrates, glycosides, steroids, and tannins found in the TLCE **[37]**.

Antimicrobial Activity

In this research, the inhibition zones induced by TC-CuNPs against *M. tuberculosis* were noted **(Figure 7 and 8) and Table 2**. The antimicrobial mechanism of CuNPs involves the production of reactive oxygen species (ROS), leading to damage of cell walls and membranes, and interactions with proteins and DNA **[38]**. During this process, CuNPs can harm various components within microbial cells. Determining sensitivity involved measuring the inhibition zone, which is the area of the medium where bacterial growth is prevented due to sensitivity to the antibacterial agent. A larger inhibition zone indicates higher sensitivity of bacteria to the antibiotic. The synthesized TC-CuNPs showed effectiveness against *M. tuberculosis*. Chatterjee et al. concluded that the primary impact of CuNPs on microorganisms stems from the oxidation of metallic Cu ions, which leads to cell death through NP-mediated ROS production within the cells **[39]**. Consequently, this results in lipid peroxidation of cellular components, protein oxidation, and degradation of DNA. The experimental findings indicated a significant inhibition zone for *M. tuberculosis* caused by TC-CuNPs, confirming their antimicrobial properties.

The **Table 2** represented the antibacterial zone of inhibition data for TC-CuNPs are as follows:

- At 0% concentration and below 25%, there was no observed antibacterial activity.
- At 25%, a small zone of inhibition (average 6.9 mm) was noted, with minor variability (S.D. ± 0.36 mm).
- Higher concentrations (50% and 100%) showed progressively larger inhibition zones, indicating stronger antibacterial effects.
- The positive control (PC) showed the largest zone of inhibition (average 24.67 mm).

The results indicated that the tested *M. tuberculosis* strains were susceptible to all the synthesized TC-CuNPs, with complete inhibition of growth. The clear zones' sizes were directly related to the effectiveness of the CuNPs in suppressing *M. tuberculosis* growth, offering quantitative evidence of their antibacterial effectiveness **[40]**.

Table 1: Phytochemical Constituents of TCLE

S. No	Phytochemicals in TCLE	*Results*
(1)	Alkaloids	Positive
(2)	Saponins	Negative
(3)	Flavonoids	Positive
(4)	Phenol	Positive
(5)	Resins	Negative
(6)	Steroids	Positive
(7)	Tannins	Positive
(8)	Protein	Positive

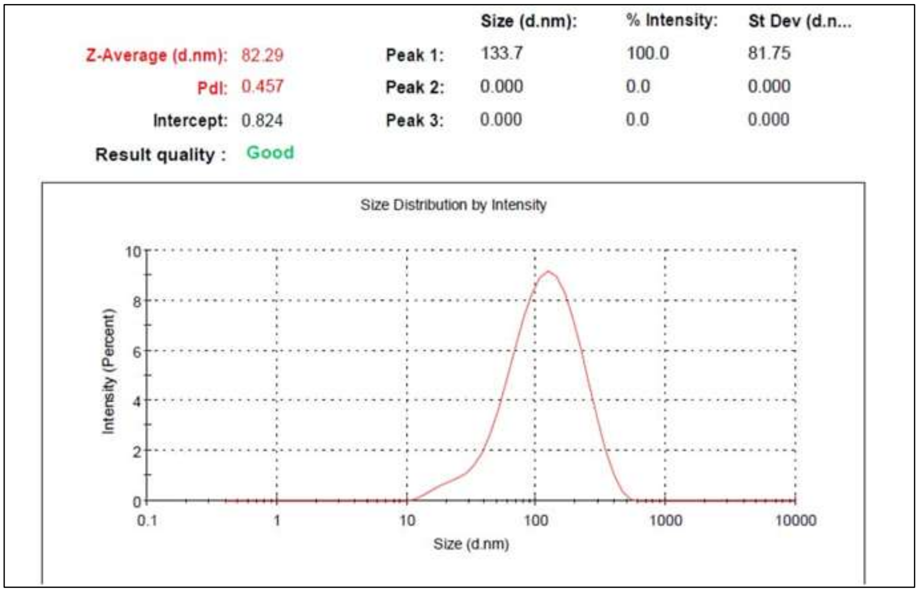


Figure 2: DLS results for the TC-CuNPs

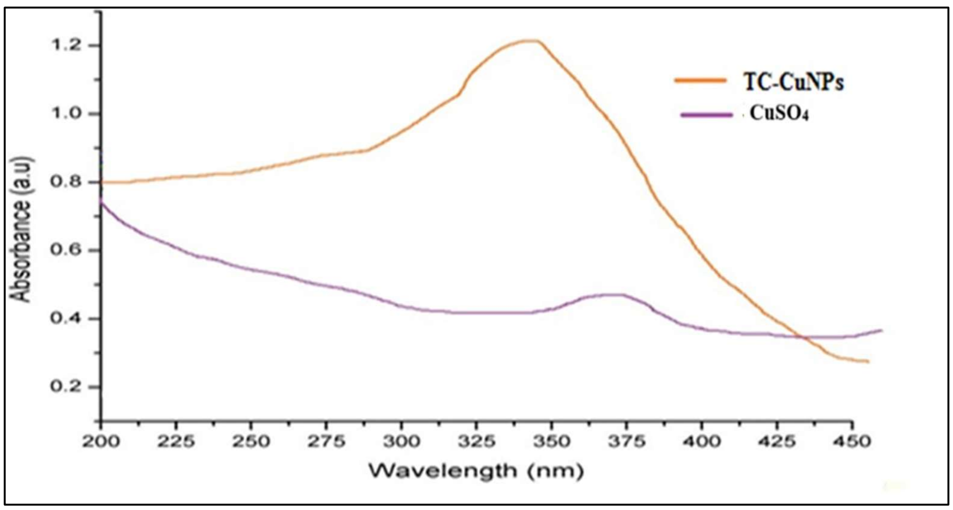


Figure 3: UV-Vis spectrum for the TC-CuNPs

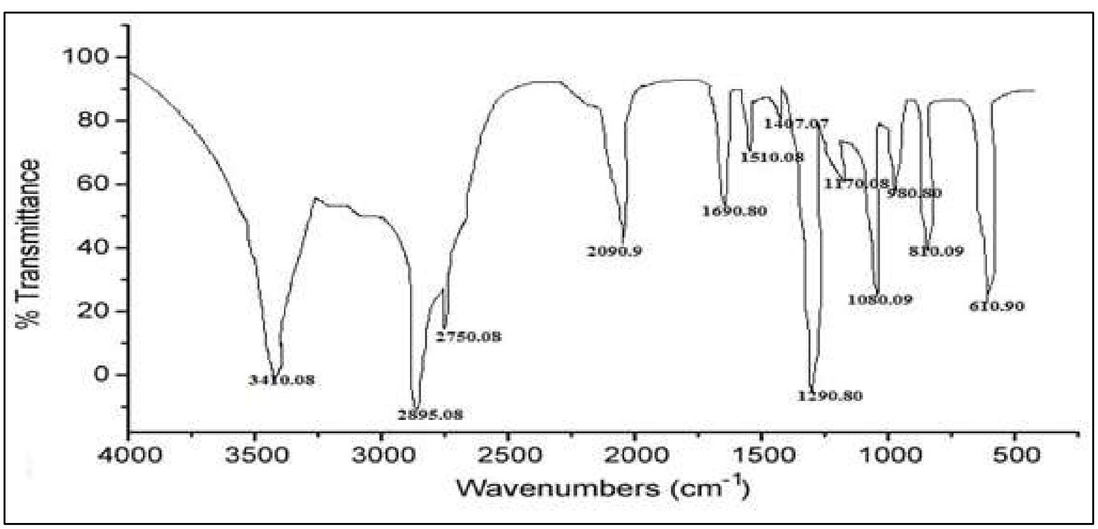


Figure 4: FT-IR spectrum of TC-CuNPs

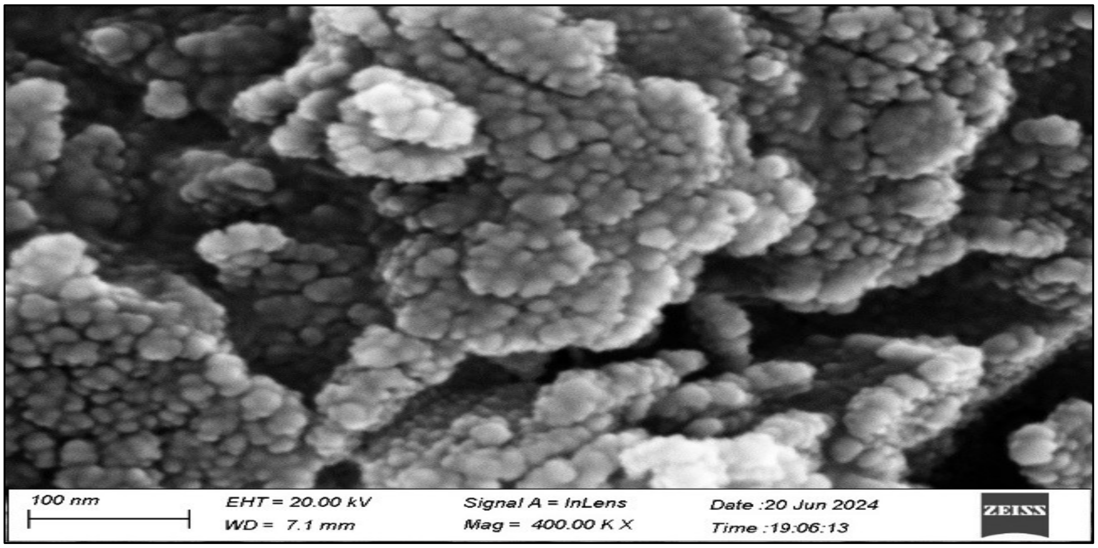
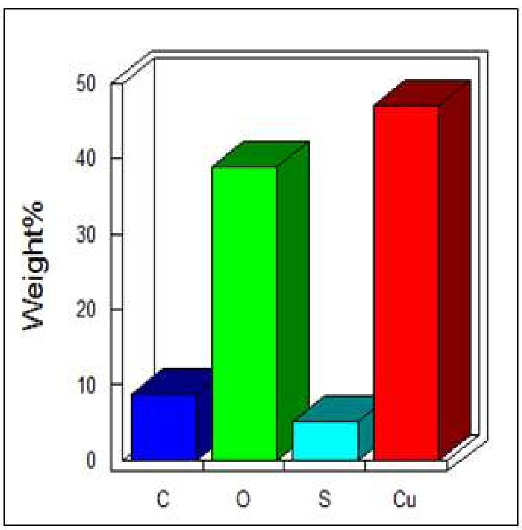
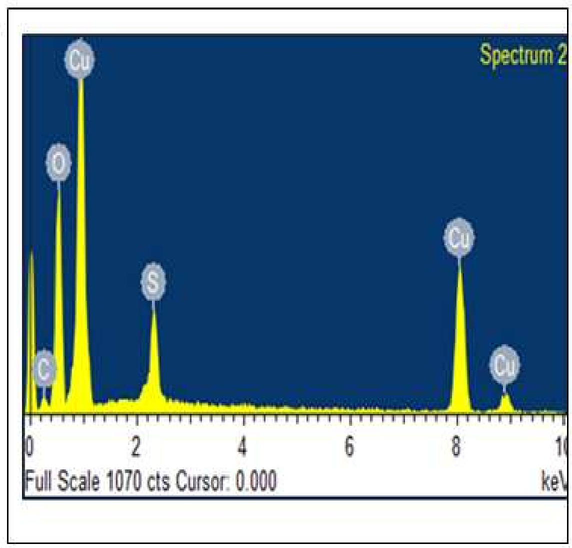


Figure 5: SEM Image of TC-CuNPs





(a) (b)

Figure 6:(a) %weight distribution histogram of TC-CuNPs, 6(b): EDX spectrum of TC-CuNPs

Table 2: antibacterial zone inhibition data for the TC-CuNPs

Conc. (%disk)	Plate A Zone size (mm)	Plate B Zone size (mm)	Plate C Zone size (mm)	Average Zone size (mm)	S.D. (±mm)
PC	26	24	24	24.66667	1.154701
0	0	0	0	0	0
6.25	0	0	0	0	0
12.5	0	0	0	0	0
25	7	6.5	7.2	6.9	0.360555
50	12.1	11.9	11.2	11.73333	0.472582
100	16	16.1	15.7	15.93333	0.208167

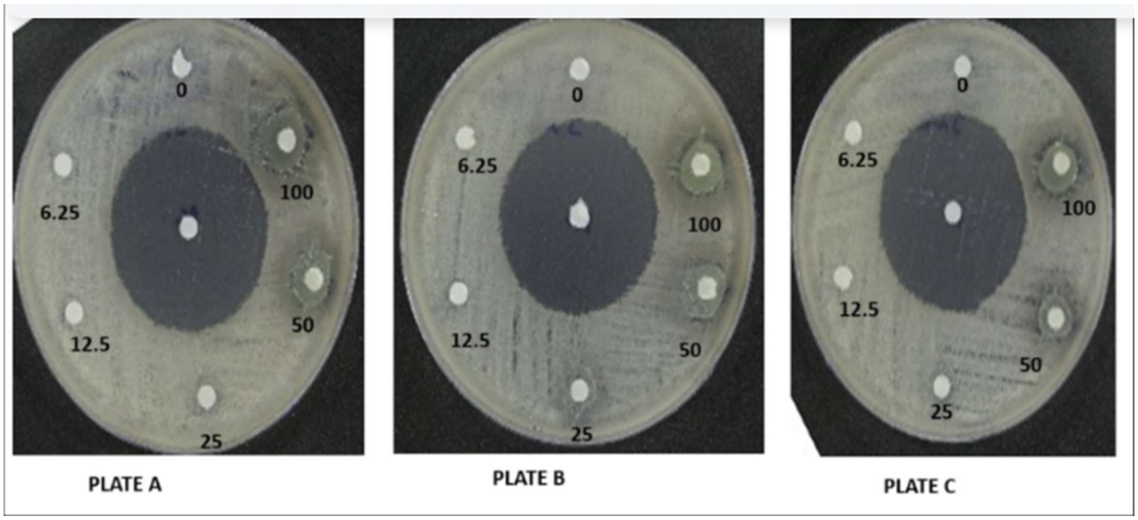


Figure 7: Zones of inhibition caused by TC-CuNPs against *M. tuberculosis*

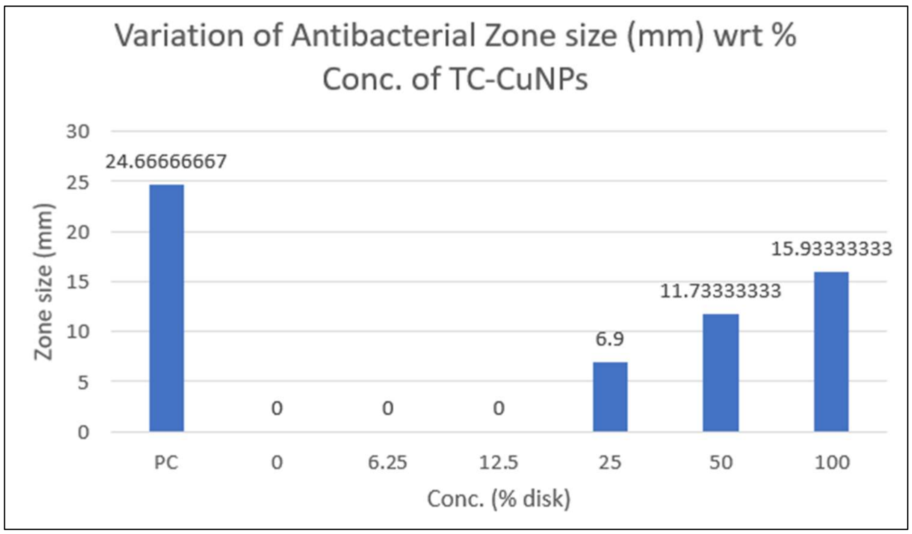


Figure 8: Antibacterial study of the synthesized TC-CuNPs

CONCLUSION

This research successfully illustrated the environmentally friendly production of copper nanoparticles (CuNPs) using leaf extract from *Tinospora cordifolia*, offering a sustainable substitute to traditional chemical methods. The characterization of the TC-CuNPs was conducted through DLS, UV-Vis spectroscopy, FT-IR and SEM to confirm their structural, optical, and morphological properties. The produced TC-CuNPs demonstrated significant antimicrobial activity against antibiotic-resistant pathogen Mycobacterium tuberculosis. These results emphasize the potential of green-synthesized TC-CuNPs for use in biomedical and environmental applications, while also highlighting the importance of plant-derived materials in promoting sustainable nanotechnology and addressing the increasing issue of antimicrobial resistance.

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