International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Green Synthesis, Antimicrobial and Dye Degradation Activity of Copper Sulphate Nanoparticles Derived from *Acalypha Indica* Linn.

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ABSTRACT

Nanotechnology is an embryonic field that grips countless impacts on the drug delivery system. Nanoparticles as haulers increase the capability of target-specific drug delivery to many folds hence are used in the treatment of dreadful diseases such as cancer, diabetes, etc. This boom has aimed at, to synthesize Copper sulfate nanoparticles using *Acalypha Indica* leaf extract. The synthesis of $CuSO_4$ NPs is confirmed through UV-Vis spectroscopy, showing an absorption peak at 295nm. The photocatalytic studies revealed that synthesized nanoparticles have the efficiency to degrade methylene blue dye. The nanoparticles were most effective against *Bacillus subtilis* and *Enterococcus*, while showing moderate activity against *Klebsiella pneumoniae*. These findings highlight the potential of green-synthesized Cu NPs in developing eco-friendly antibacterial agents, particularly for combating Gram-negative bacterial infections. Synthesized $CuSO_4$ Nanoparticles using *Acalypha indica*, with *Candida albicans* showing slightly higher sensitivity compared to Candida tropicalis. The dose-dependent efficacy and superior performance at higher concentrations suggest that these nanoparticles can serve as effective antifungal agents. These results clearly demonstrated that $CuSO_4$ NPs have potent antimicrobial and photocatalytic activities suggesting their potential applications as microbial agent and photocatalysts.

Keywords: Nanotechnology, Nanoparticles, *Acalypha Indica*, UV-Vis spectroscopy, Methylene blue dye and Microbial agent.

INTRODUCTION:

A nanoparticle (NP) is a small particle with a diameter ranging from 1 to 100 nm. NPs are the most widely produced nanomaterial due to their extensive industrial applications, which include aerospace, automotive, agriculture, cosmetics, textiles, food packaging, and additives, among many others. In recent years, the rapid growth of the global market for nanomaterials has incentivized research on the potential use of NPs for diagnostics, biomarkers, drug development and delivery, cell labeling, cancer therapy, and antiviral and antimicrobial agents. The latter have gained more interest due to the growing threat of antibiotic resistance. NPs have been integrated as antibacterial agents in the development of different products, such as creams to treat burns and injuries, and skincare products. The need for more cost-effective and sustainable alternatives for NP synthesis has drawn interest in biological methods. Among these, green synthesis has emerged as a straightforward, rapid, and sustainable approach. In the synthesis process with plants, aqueous extracts are used.

Copper is an essential trace mineral naturally present in some foods. In various metabolic pathways, it functions as a cofactor for numerous enzymes involved in energy production, and biosynthesis of connective tissue, neurotransmitters, collagen, and red blood cells. Copper also plays a significant role in supporting normal brain development, immune functions, dismutation of superoxide radicals, skin regeneration, angiogenesis, and healing processes. However, both copper deficiency and excess can cause illnesses in humans. Moreover, the antiseptic properties of copper have been documented extensively throughout history, with records as early as 3000 BC. Copper-based NPs have been proven to be effective against a broad range of clinically relevant pathogenic microorganisms. Copper and copper oxide nanoparticles (CuNP) generally have a major role in agriculture for suppliment of minerals. Its usage on plants have both positive and negative impacts. Due to oxidative nature, copper oxide nanoparticle has toxic effects on plants. Whereas using lower concentration of CuNP gives an appreciable impacts in growth of plants.[2]

Among the alternatives reported so far, nanoparticle synthesis using plant extract offers advantages over the use of fungi, algae, and bacteria. Plants are the reservoirs of naturally occurring chemical compounds and structurally diverse bioactive molecules. [1]

Dye-contaminated water has become a major pollutant from industries, posing a huge threat to aquatic/marine life. As a result, reducing the primary effluent colors before the harmful water enters water bodies is critical. Methyl red, one of the commonly used dyes, is released into the water reservoirs and causes water pollution. Copper NPs, can be used as an amazingly effective photocatalyst for methyl red dye degradation. These metal oxide NPs are also employed in a variety of applications, including photovoltaic devices such as solar cells, sensing, and photocatalysis, due to their important properties, such as strong adsorption ability, extra sites for functional moieties, high porosity, etc.,[3] In recent times, there has been a growing utilisation of Copper Nanoparticles (CuNPs) as agents with antibacterial properties. The techniques employed for synthesising CuNPs encompass both chemical and physical methods, including sol-gel processes, physical vapor deposition, chemical reduction, microwave irradiation, and thermal decomposition. However, these conventional approaches are associated with significant drawbacks such as high expenses, substantial energy requirements, and the generation of hazardous and toxic byproducts. The shift from physical and chemical methods to green synthesis methods is gaining momentum due to concerns associated with high energy consumption, the release of toxic substances, and the complexity of equipment and synthesis conditions.

Presently, green synthesis predominantly employs microorganisms such as fungi, bacteria, and algae, or extracts derived from leaves, flowers, roots, peelings, fruits, and seeds of various plants. Previous studies have underscored the advantages of green synthesis over chemical methods and highlighted the potential and promise of green synthesis. Among these approaches, the utilisation of plant extracts has garnered greater attention, largely due to its advantages, such as the absence of constraints related to microorganism culture and the ease of sourcing plant materials. Furthermore, employing plants for nanoparticle synthesis offers additional benefits, including the utilisation of safer solvents, more moderate reaction conditions, increased feasibility, and diverse applications in fields like surgery and pharmaceuticals.[4]

Thus, biosynthesized nanoparticles are free from toxic substances and they contain biochemical constituents which help in curing the diseases and also act as an alternative medicine for most of the life killing disorders.

The present study aimed to synthesise and characterise copper sulfate nanoparticles utilising *Acalypha indica* leaf extract as a mediator, and subsequently, assess their antibacteriall effectiveness against Gram positive and Gram Negative organism and its photocatalytic efficiency in the decomposition of organic dye.

METHODS AND MATERIALS

Collection, Processing, and Preparation of *Acalypha indica* Leaf Extract

Fresh Acalypha indica leaves were collected from Vellore District, Tamil Nadu, India, and kept in polyethylene zipper bags to minimize loss of freshness. The leaves were first thoroughly washed with running tap water and then washed twice with distilled water for the removal of impurities. After the washing cycle, the fresh weights of the leaves were taken. The leaves were oven-dried at controlled 60°C temperature for 24 hours to completely remove all moisture in the leaves. Afterward, the samples were powdered finely using an electric blender for further analysis, then stored in airtight polyethylene bottles to maintain their stability and prevent contamination.

Synthesis of Copper Nanoparticles (CuNPs) Using Chemical Reduction Methods

To prepare the aqueous extract, 4.8 g of powdered leaves were accurately weighed and mixed with 50 mL distilled water in a 100 mL beaker. The mixture was then heated and boiled while maintaining the boiling process for 15 minutes to adequately extract the bioactive compounds. After boiling, the extract was cooled to room temperature. The solid residues were separated from the mixture using Whatman grade 1 filter paper, after which it was transferred to a clean container and stored in a refrigerator at 5°C within 24 hours to preserve its chemical balance before subsequent application.

CuNPs were synthesized using a modified chemical reduction technique. The following aqueous solutions were prepared: the metal precursor solution, copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, 0.0398 mM, 25 mL) the reducing agent, L-ascorbic acid (0.1547 mM, 25 mL); and the stabilizing agent solution (RAFE or GJLE, 25 mL) . This copper solution was added to a 100 mL round-bottom flask and placed in the heating mantle set at 80°C with magnetic stirring running at 700 rpm. The ascorbic acid and stabilizing agent solutions were added dropwise to the copper solution with vigorous stirring. The reaction was monitored visually, from light blue (Cu^{2+}) to light green (Cu^{+}), then to dark yellow before forming a brown precipitate of Cu^{0} after 5 minutes. The reaction continued for 6 hours, after which the precipitate was separated by centrifugation at 4000 rpm for 10 minutes. The precipitate was washed with hot distilled water and anhydrous ethanol to remove unreacted reagents, dried in a vacuum desiccator, and stored at room temperature in an amber flask.

Characterization of Copper Nanoparticles

A UV-Vis spectrophotometer (Shimadzu UV-Vis 1601 series) was used to analyze the optical absorption of copper sulfate and copper nanoparticle suspension at room temperature in the wavelength range of 200–800 nm.

Catalytic Activity of *Acalypha indica*-Derived Nanoparticles in Dye Degradation

Catalytic degradation of the synthesized copper nanoparticles, CuNPs, was evaluated for dyes like methylene blue.

All the dyes solution mixed with 1 mL of 10 mM sodium borohydride, $NaBH_4$, and 3 mL of CuNPs. The reaction mixture was stirred and aliquots collected at scheduled intervals, centrifuged at 10,000 rpm for 10 minutes, and the supernatant analyzed via UV–Vis spectroscopy to monitor dye degradation. Photocatalytic experiments was carried out on methylene blue dye. For those, a 1 mL 1 mM dye solution was mixed with 1 mL of nanoparticles (CuNPs, 1 mg/mL) synthesized using concentration (1mM) cupric sulfate for CuNPs). The solutions were further diluted to 5 mL volume, then stirred in the dark for 2 hours, and thereafter, exposed to sunlight for 0–24 hours to get a degradation rate measurement. Blanks were prepared as dye + water solutions and the degradation was quantified using UV–Vis spectroscopy. Further experiments involved mixing CuNPs with sodium borohydride and dye solutions with volume being adjusted to 5 mL using distilled water. The degradation shown as decolorization of the solution was recorded for 10 mins and 30 mins time interval after which nothing significant was observed. All the experiments were repeated thrice, and mean percentages values of degradation are calculated to obtain accuracy and reproducibility. (Raina and Roy *et al*., 2024).

Antimicrobial activity

Test organisms

The antimicrobial properties of the produced copper nanoparticles were examined against both pathogenic bacterial species - Gram-positive (*E.coli*, *Pseudomonas, Salmonella typhi*, *Streptococcus*) and Gram-negative (*Bacillus subtilis*, *Klebsiella pneumonia*, *Enterococcus*, *S.aureus*) bacteria and fungal strains (*Candida albicans*, *Candida trophicalis*) were used.

Antimicrobial properties

Muller Hilton Agar (MHA) was used in the petri dish for the agar well diffusion method of antibacterial assay (Tripathi *et al*., 2013; Aziz *et al*., 2015). The relevant sterile bacteria and fungi, CuNP's and positive control (Ofloxacin 50 µg/mL) were added to each of the three MHA agar plates at the corresponding well. In every well, different concentrations of CuNPs, viz 10, 20, 30 and 40 µL were added and plates were incubated at 37 °C for 24 hours at room temperature. Each experiment was repeated in triplicates. The clear zone that developed around the well after incubation was measured.

RESULT AND DISCUSSION:

Synthesis of g-Cu NPs

The g-Cu NPs were synthesized by using copper sulphate as a precursor and plant leaf extract as a reducing and capping agent. The change in color from blue to light brownish visually indicates the formation of copper NPs. The g-Cu NPs were washed by deionized water and ethanol to remove any unwanted particles. Thereafter, g-Cu NPs were dried and ground (Figure 1) and later subjected to various characterization methods.

Figure 1: Schematic Diagram showing the synthesis of Copper Sulphate Nanoparticles from Acalypha Indica L aqueous extract.

Characterization of copper sulphate and *Acalypha indica* mediated copper sulphate Nanoparticles

UV – Vis Spectrophotometer

The UV-Vis spectra of copper sulphate and *Acalypha indica* plant extract-capped copper nanoparticles are different from each other with specific absorption characteristics reflecting their special chemical structures and interaction. In the spectrum of copper sulfate, a single major peak at 808 nm appears that is associated with the d-d electronic transitions of Cu^{2+} ions and affected by the aqueous surroundings (Figure 2). The production of the coordination complex $[Cu(H_2O)_6]$ 2+ gives copper sulfate in solution its blue colour. To the human sight, this complex ion appears blue because it absorbs a lot of red visible light. Figure 1 shows the significant absorbance of the initial solution in the red part of the visible spectral area about 800 nm, which is represented by the weakest absorbing spectrum (red). However, the spectrum of *Acalypha indica* extract-copper nanoparticle shows two prominent absorption peaks at 295 nm and 971 nm (Figure 3). The absorption peak at 295 nm corresponds to the electronic transitions associated with organic biomolecules present in the plant extract, whereas the broader absorption peak at 971 nm is characteristic of surface plasmon resonance (SPR), thus proving the nanoscale dimensions of copper particles.

Photocatalytic degradation of methylene blue by CuNPs

UV-Vis spectra of methylene blue degradation catalyzed by *Acalypha indica* copper nanoparticles (Cu NPs) after 10 minutes and 30 minutes illustrate a decrease in absorbance with time, indicating progressive degradation of the dye.

After 10 minutes, a characteristic peak was observed at a wavelength of 590 nm; the absorbance intensity is nearly 4.5 arbitrary units, indicating that it falls under the initial stage of degradation as active species released by Cu NPs start decomposing the dye molecules (figure 4)

At 30 minutes, the absorbance at the same wavelength is greatly reduced to about 3.5 (arbitrary units), which indicates a marked decrease in the concentration of dye due to enhanced photocatalytic activity (figure 5)

Absorbance intensity decreases from 10 to 30 min, showing the efficient role of copper nanoparticles synthesized from kuppaimeni (*Acalypha indica*) in facilitating the rate of degradation of dye, which may be attributed to higher surface area and generation of reactive oxygen species.

Antimicrobial activity of Copper Nanoparticles.

Evaluation of Copper Nanoparticles Against Gram-Positive Bacterial Pathogens

Copper nanoparticles (CuNPs) synthesized using *Acalypha indica* demonstrated notable antibacterial activity against both Gram-positive and Gram-negative bacteria, with a clear dose-dependent increase in inhibition zones. Among the tested strains, *Streptococcus* (Gram-positive) exhibited the highest susceptibility, with inhibition zones ranging from 6 mm at 10 µL to 12 mm at 40 µL, indicating robust antibacterial activity. Gram-negative bacteria were less sensitive, but the *Pseudomonas* species had an inhibition zone of 4 mm at 10 µL to 7 mm at 40 µL, while *Salmonella typhi* had inhibition zones ranging from 4 mm to 8 mm. *E. coli* had a moderate level of sensitivity, with the inhibition zones increasing from 5 mm at 10 µL to 11 mm at 40 µL (Table1; Figure 6). These results validate the increased susceptibility of Gram-positive bacteria to CuNPs. This is consistent with the observation of Ahmad [8] *et al*. (2020), in which Gram-positive bacteria were stated to be more susceptible to cell wall disruption due to nanoparticle action than Gram-negative bacteria. The standard antibacterial agent used in the study for gram-positive organisms, such as Bacillus subtilis, Enterococcus, and Staphylococcus aureus, showed 5 mm for all tested organisms. This constant activity suggests the baseline effectiveness for controlling gram-positive bacterial growth; it is always a reliable source for comparison among similar studies that evaluate antimicrobial agents.

The antibacterial activity of copper nanoparticles (CuNPs) synthesized from *Acalypha indica* against gram-positive bacteria (*Bacillus subtilis*, *Enterococcus*, and *Staphylococcus aureus*) demonstrated superior results compared to the standard (5 mm inhibition zone). For *Bacillus subtilis*, the CuNPs produced inhibition zones ranging from 6 mm at 10 µL to 11 mm at 40 µL, showing more than twice the standard's efficacy at the highest concentration. In the case of *Enterococcus*, inhibition zones ranged from 6 mm at 10 µL to 10 mm at 40 µL, consistently outperforming the standard at all concentrations. For *Staphylococcus aureus*, CuNPs showed equivalent activity to the standard at 10 µL (5 mm) but exceeded it significantly at higher concentrations, reaching 10 mm at 40 µL. These results clearly indicate that copper nanoparticles exhibited better antibacterial activity than the standard, particularly at higher concentrations, highlighting their potential as an effective alternative for managing gram-positive bacterial infections.

Effectiveness of Copper Nanoparticles on Gram-Negative Bacteria

Copper nanoparticles (CuNPs) synthesized from *Acalypha indica* were tested for their antibacterial activity against Gram-negative bacteria, including *Bacillus subtilis*, *Klebsiella pneumoniae*, *Enterococcus*, and *Staphylococcus aureus* (Table 2; Figure 7). The results, summarized in the table, indicate a concentration-dependent antibacterial effect, with inhibition zones increasing as the concentration of CuNPs increases

- ***Bacillus subtilis:***

The inhibition zone increased from 6 mm at 10 µL to 11 mm at 40 µL, demonstrating strong antibacterial activity. This trend indicates significant effectiveness of CuNPs in disrupting this Gram-negative bacterium, potentially due to their ability to penetrate bacterial membranes and generate reactive oxygen species (ROS).

- ***Klebsiella pneumoniae:***

This strain showed moderate sensitivity to the CuNPs, with inhibition zones ranging from 4 mm at 10 µL to 10 mm at 40 µL. The smaller inhibition zones compared to *Bacillus subtilis* suggest higher resistance, likely due to the thicker outer membrane characteristic of Gram-negative bacteria.

- ***Enterococcus:***

The inhibition zones ranged from 6 mm at 10 µL to 10 mm at 40 µL, similar to the trend observed for *Bacillus subtilis*. This indicates that CuNPs were effective against *Enterococcus*, with inhibition zones consistently outperforming the standard antibiotic control (5 mm).

- **Staphylococcus aureus**:

As a Gram-positive reference organism, *S. aureus* displayed significant sensitivity, with inhibition zones increasing from 5 mm at 10 µL to 10 mm at 40 µL. This result is consistent with the general observation that Gram-positive bacteria are more susceptible to nanoparticle-based antimicrobials than Gram-negative bacteria.

The concentration-dependent antibacterial activity observed in this study is a characteristic feature of nanoparticles, as higher concentrations increase the likelihood of nanoparticle interaction with bacterial cells. The strong activity against *Bacillus subtilis* and *Enterococcus* can be attributed to the ability of CuNPs to generate ROS, which cause oxidative damage to cellular components such as lipids, proteins, and DNA. Additionally, the nanoparticles may disrupt the bacterial cell wall and membrane, leading to cell death.

Antifungal Activity of Copper Nanoparticles Synthesized from *Acalypha indica*

Copper nanoparticles (CuNPs) synthesized using *Acalypha indica* were assessed for their antifungal activity against *Candida albicans* and *Candida tropicalis*. The agar well diffusion method was used to assess the antifungal activity, and the inhibition zones were measured at different concentrations of 10 µL, 20 µL, 30 µL, and 40 µL. Results indicated a dose-dependent increase in the inhibition zones, showing that CuNPs can be considered as effective antifungal agents (Table 3; Figure 8).

Candida albicans:

The inhibition zone increased from 3 mm at 10 µL to 11 mm at 40 µL, with strong antifungal activity. At 20 µL, the inhibition zone was 6 mm, which is higher than the standard antifungal agent (5 mm). The highest activity was recorded at 40 µL, with an inhibition zone of 11 mm, indicating high fungicidal activity.

Candida tropicalis:

Similar to *C. albicans*, *C. tropicalis* also exhibited a dose-dependent response with inhibition zones of 3 mm at 10 µL to 10 mm at 40 µL. The inhibition zone was 8 mm at 30 µL, and the highest activity was 10 mm at 40 µL; this was only slightly less sensitive than *C. albicans*. Both the fungi exhibited measurable sensitivity to CuNPs at all concentrations tested and the activity level was highly enhanced at higher nanoparticle doses

The results of this study show the potent antifungal activity of CuNPs synthesized using *Acalypha indica*, with *Candida albicans* showing slightly higher sensitivity compared to Candida tropicalis. The dose-dependent efficacy and superior performance at higher concentrations suggest that these nanoparticles can serve as effective antifungal agents. The eco-friendly synthesis approach further adds to their appeal for potential applications in clinical and environmental antifungal treatments.

CONCLUSION

The copper nanoparticles (CuNPs) made from *Acalypha indica* showed great promise as environmentally benign and strong antimicrobials and photocatalysts, demonstrating their adaptability in environmental and biological applications. In comparison to conventional antimicrobial treatments, the CuNPs had potent antibacterial and antifungal action, demonstrating a dose-dependent response against both Gram-positive and Gram-negative bacteria and harmful fungi. Furthermore, the effective photodegradation of methylene blue dye was made possible by their high surface area, stability, and light-absorbing capacity, suggesting their potential as photocatalysts for the treatment of wastewater contaminated with dyes. A viable substitute for chemically produced nanoparticles, the green synthesis of CuNPs employing *Acalypha indica* improves their environmental sustainability and biocompatibility. In order to successfully address environmental pollution and microbial illnesses, future research could concentrate on scaling up these procedures and evaluating their effectiveness in complicated systems, including industrial wastewater treatment.

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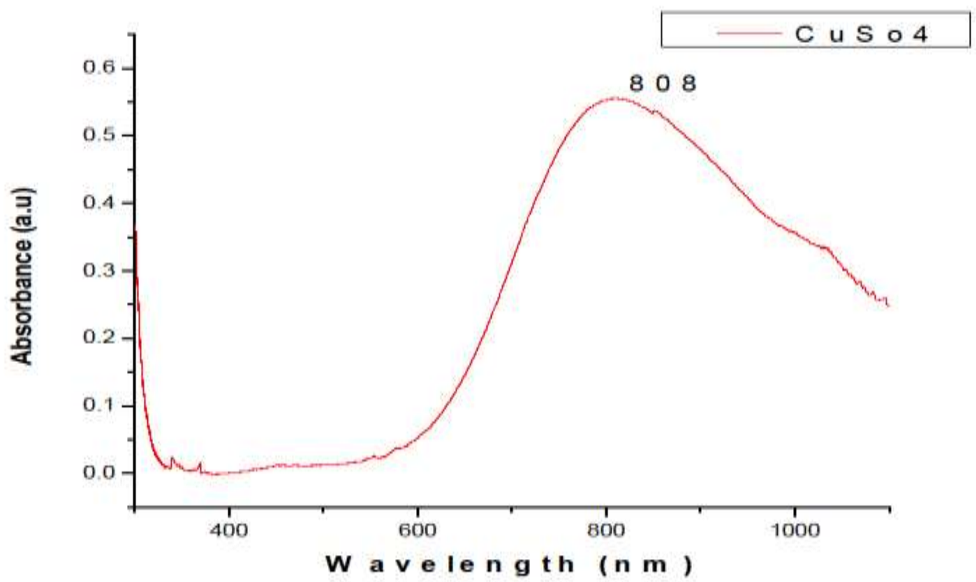


Figure 2: UV-visible absorption spectroscopy analyses of copper sulphate.

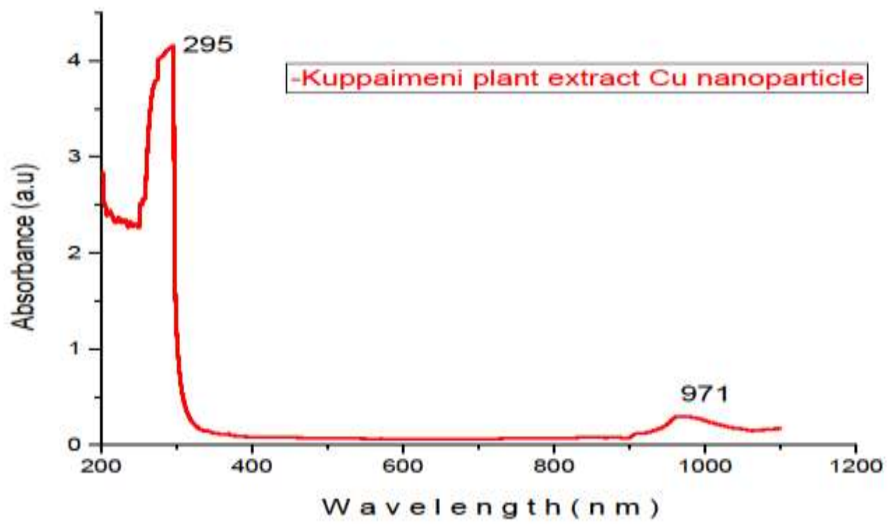
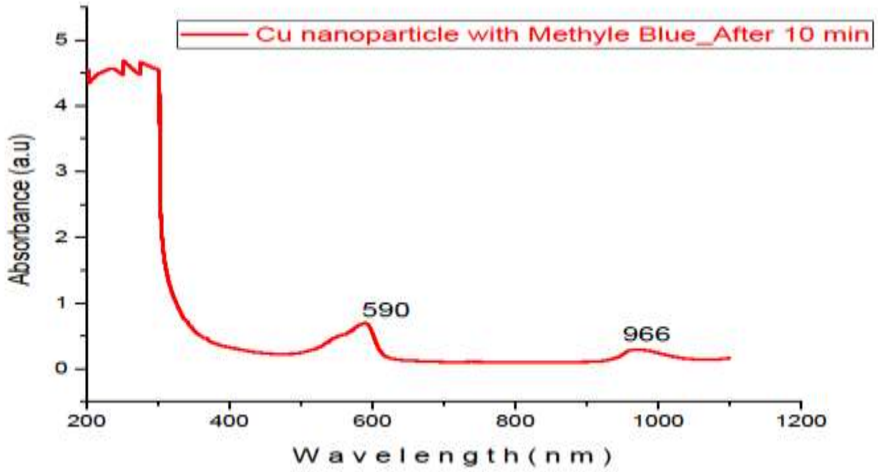


Figure 3: UV-visible absorption spectroscopy analyses of copper Nanoparticle



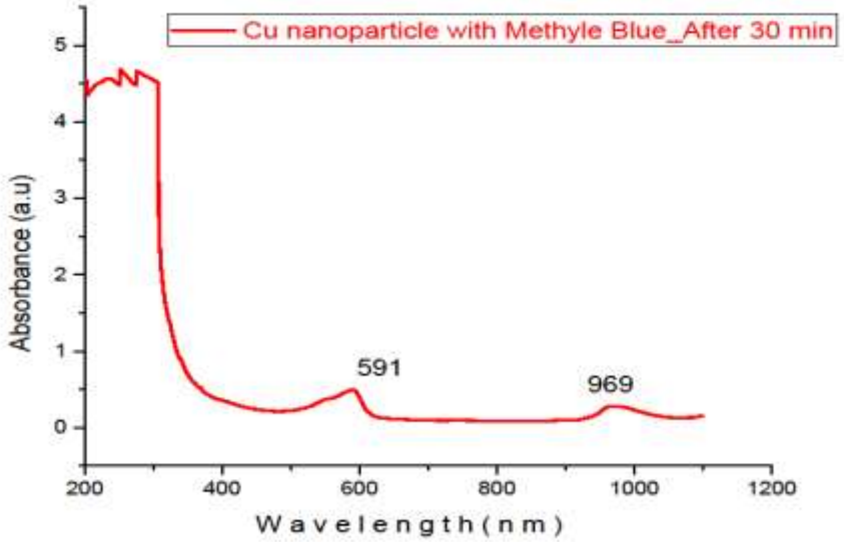


Figure 4 and 5: UV-Vis spectra showing the photocatalytic degradation of methylene blue under the influence of *Acalypha indica*-derived copper nanoparticles after 10 minutes and 30 minutes, highlighting a significant reduction in absorbance at 590 nm, indicative of dye degradation over time.

Table 1: Antibacterial Activity of Copper Nanoparticles Synthesized from *Acalypha indica* **Against Gram-Positive Bacteria**

Organisms **Concentration**	***Bacillus subtilis***	***Klebsiella pneumonia***	***Enterococcus***	***S.aureus***
10 µl	6 mm	4 mm	6 mm	5 mm
20 µl	8 mm	6 mm	7 mm	6 mm
30 µl	10 mm	7 mm	8 mm	8 mm
40 µl	11 mm	10 mm	10 mm	10 mm
Standard	5 mm	5 mm	5 mm	5 mm

Table 2: Antibacterial Activity of Copper Nanoparticles Synthesized from *Acalypha indica* Against Gram-Negative Bacteria

Organisms Concentration	***E.Coli***	***Pseudomonas***	***Salmonella typhi***	***Streptococcus***
10 µl	5 mm	4 mm	4 mm	6 mm
20 µl	7 mm	5 mm	6 mm	7 mm
30 µl	9 mm	6 mm	7 mm	10 mm
40 µl	11 mm	7 mm	8 mm	12 mm
Standard	5 mm	5 mm	5 mm	5 mm

Table 3: Antifungal Activity of Copper Nanoparticles Synthesized from *Acalypha indica* Against Fungal Pathogens

Organisms Concentration	***Candida albicans***	***Candida tropicalis***
10 µl	3 mm	3 mm
20 µl	6 mm	5 mm
30 µl	9 mm	8 mm
40 µl	11 mm	10 mm
Standard	5 mm	5 mm

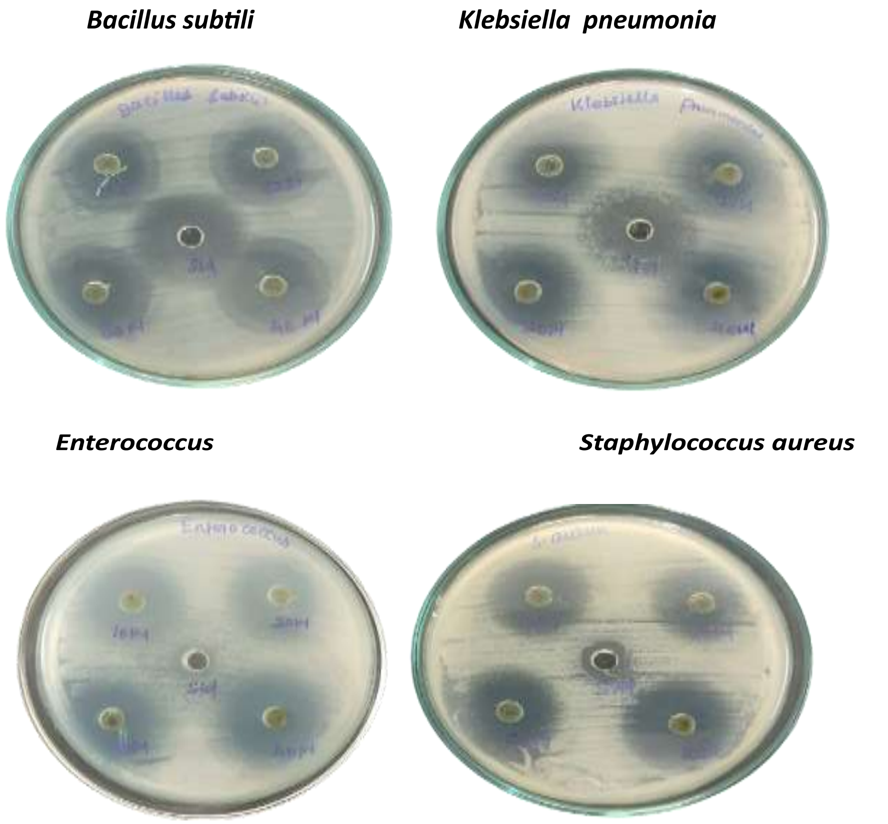
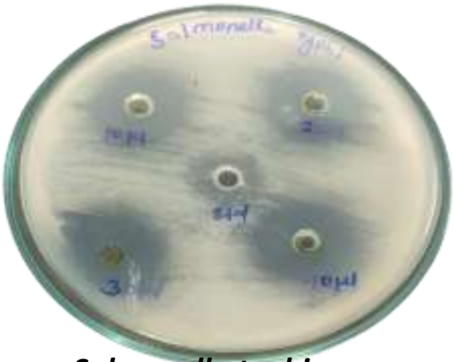


Figure 6: Plate showing the antibacterial activity of CuNPs against Gram positive organisms

E.Coli ***Pseudomonas***



Salmonella typhi ***Streptococcus***

Figure 7: Plate showing the antibacterial activity of CuNPs against Gram negative organisms

Candida albicans ***Candida tropicalis***

Figure 8: Plate showing the antifungal activity of CuNPs against Fungal Pathogens