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Leaf-Mediated Copper Nanoparticles Synthesis from Calotropis Gigantea and Their Characterization

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Abstract: *Nanoparticles are the tiny materials ranging from size 1 to 100 nm. Their higher surface-area-to-volume ratio is a very important and unique property. These nanoscale materials find use in a variety of more different areas, such as electronic, magnetic and optoelectronic, biomedical, pharmaceutical, cosmetic, energy, environmental, catalytic, and materials applications. Green-synthesis of copper nanoparticles (CuNPs) using Calotropis gigantea leaf extract is reported in this study. Among all methods of synthesis plant based biological method of synthesizing nanoparticles is one of the rich sources due to their nontoxic, clean, cost-effective and environmentally friendly approach. Calotropis gigantea is a widely used plant in the traditional medical system. The plant extract having the ecofriendly polyphenol which acts as a reducing and a capping agent. The aim is to provide information about the innovation regarding synthesis of metal nanoparticles through plant mediation and their characterization techniques concerned.*

Keywords: Synthesis; Calotropis gigantea; Copper Nanoparticles; and Characterization.

I. INTRODUCTION

Nowadays, nanoparticles are of scientific interest because they serve as a bridge between molecular structures and bulk volume materials. A bulk volume material should have constant physical property regardless of its size, but at the nanoscale, this is often not the case. [1] The nanoparticles have unexpected properties because of their small size, which confines their electrons and produces quantum effects. [2,3] The bulk material is compressed into tiny fragments by the top-down approach. There are various methods to synthesize metal nanoparticles, including sputtering, ball milling, radiation-induced synthesis, chemical synthesis, electrochemical synthesis, and green synthesis. [4] These metals have been known for their strong inhibitory effects on many biological and medicinal activities. Slightly acidic to neutral pH, in the range 5.5-7.0, yields the best productivity with smaller particle sizes. Increasing the reaction temperature to 80°C often speeds up nucleation and results in more uniform particles. [5] The chemical synthesis of metal nanoparticles using volatile and hazardous reagents requires high temperature and pressures, posing a risk to human exposure and environmental contamination. Heavy metals, including mercury, lead, and benzene, exposure can cause neurological damage, respiratory illness, cancer and other health issues. [6]

The development of the "green" method of synthesizing metal particles by using plant extract has become a prime focus of research. This method of synthesis of nanoparticles is more preferable as it is a sustainable, productive, and environmental-friendly approach that bypasses toxicity. Some of the assured examples of metals showing potential activities for green method synthesizing nanoparticles are gold, selenium, platinum, and copper. [7,8] The plant extract involves leaves, stem, flowers, and root, having the eco-friendly polyphenol which acts as a reducing and capping agent. [9] The *Calotropis gigantea* which is usually known as crown flower, which are commonly occurs on wasteland. The

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plant belongs to the family *Apocynaceae* which is grown widely throughout the tropical region of Asia and Africa. They are mostly found in wasteland. It has been used as a folk medicine in India for many years, and has been reported to have a variety of uses as effective in treating skin, circulatory, and neurological disorders. The Phyto active compounds present in the plant, such as Tannins and glycoside shows the potential abilities to fight against the toxic pathogen produce nanoparticles of multiple shapes and sizes by reducing metal ions, enabling nanoparticles synthesis. [10-12]

The green synthesis of copper nanoparticles by treating with *Calotropis gigantea* leaf extract could have a significant consequence in nanotechnology and the medicine industry. [13] The phytochemicals present in leaf extract, which are authoritative for reducing the $CuSO^4$, were confirmed using the colour change indication from bluish-green to brownish-black after incubation. [14] The study focuses on the detailed characterization techniques, including UV-Vis Spectroscopy, X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM) to confirm the formation of nanoparticles and their structural and morphological appearance. However, the study aims to investigate the synthesis of CuNPs by *Calotropis gigantea* leaf extract, which may result in the adsorption of fatal chemicals of the synthesized materials. [15]

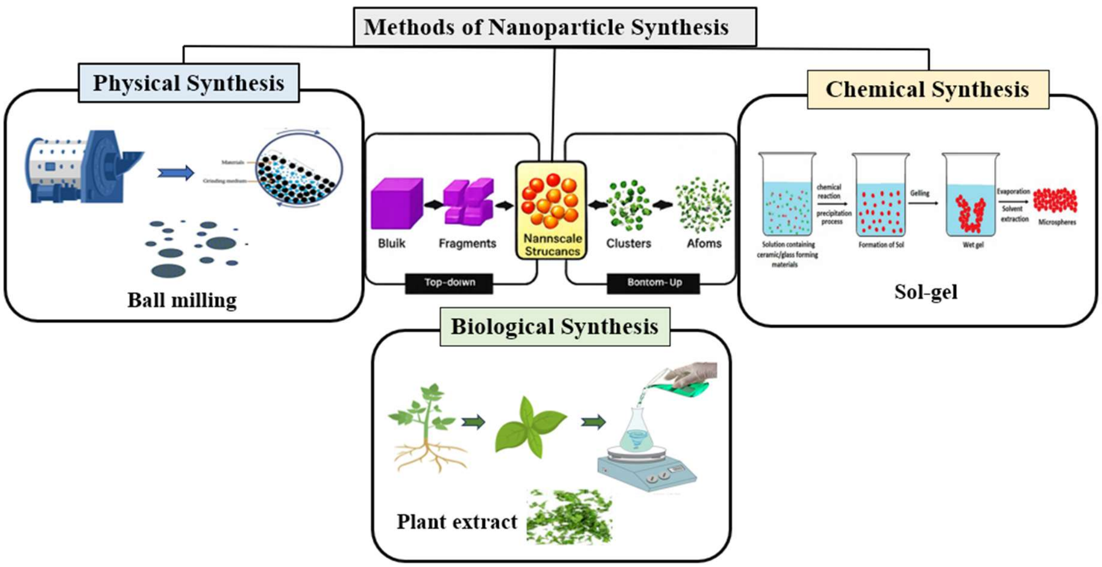

Fig 1: Conventional methods to synthesis nanoparticles.

II. METHODOLOGY

2.1Chemicals and reagents

The chemical, including Copper (II) sulphate pentahydrate ($CuSO_4.5H_2O$), was used to synthesize copper nanoparticles. Develop a comprehensive protocol based on all the suitable conditions and focusing on both preventive and curative strategies for overcoming challenges posed by various factors (temperature, time of incubation, and contamination).

2.2 Plant extract preparation

The green leaves of the plant *Calotropis gigantea* were collected form the local region of Nagpur, Maharashtra, India. The leaves were properly washed multiple times under running tap water. After complete washing, the leaves were allowed to dry under shade conditions. After 15-20 days, the leaves were completely dried. The dried leaves were collected and crushed into small pieces, and the resulting material was further processed using an electric mixture grinder to obtain a uniform fine powder. The freshly prepared powder of the plant *Calotropis gigantea* leaf was stored in an air-tight condition for further use.

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To prepare the leaf extract, the freshly prepared *Calotropis gigantea* leaf powder was weighed exactly 20 g. 200 ml of distilled water was poured into a conical flask, and the leaf powder was added and stirred vigorously. By using an Electrical Magnetic Stirrer, the mixture was heated at 80˚C for 30-35 minutes under continuous stirring conditions. After heating, the mixture was allowed to cool down at room temperature and was filtered using Whatman no. 1 filter paper. For better and clearer results, the solution was centrifuged at 1500 rpm for 12-15 minutes. The resultant *Calotropis gigantea* leaf extract was stored in the refrigerator at 4˚C for further synthesis of CuNPs as a precursor solution. [4,16]

Table 2.2: Summary of plant *Calotropis gigantea* leaf extraction

Plant Used (Common name)	Part of plant	Plant metabolites	Incubation	Temp (°C)	Formation of plant material
Calotropis gigantea (crown flower)	Leaves	Tannins, flavonoids, steroids, Proteins, and alkaloids	15-20 days	Room temperature	Powder form

2.3 Copper nanoparticles (CuNPs) synthesis preparation

The process of synthesizing copper nanoparticles follows: 0.05M of Copper (II)sulphate pentahydrate ($CuSO_4.5H_2O$) solution was prepared by dissolving copper sulphate pentahydrate in 100 ml distilled water. The freshly prepared *Calotropis gigantea* leaves extract was added dropwise in a 1:1 ratio. The reaction solution was incubated for 24 hours at room temperature. After 24 hours, the change in colour of the reaction from dark green to brownish-black was observed, which indicates the formation of CuNPs. The mixture solution was further centrifuged at 9,000 rpm for 10-15 minutes. The obtained palette was repeatedly washed with distilled water and 70% ethanol. The obtained precipitate was carefully collected and allowed to dry using an electrical Hot Air Oven at 80˚C overnight. The dried CuNPs powder was collected and scraped off. The CuNPs were stored in an air-tight condition. [17,18]

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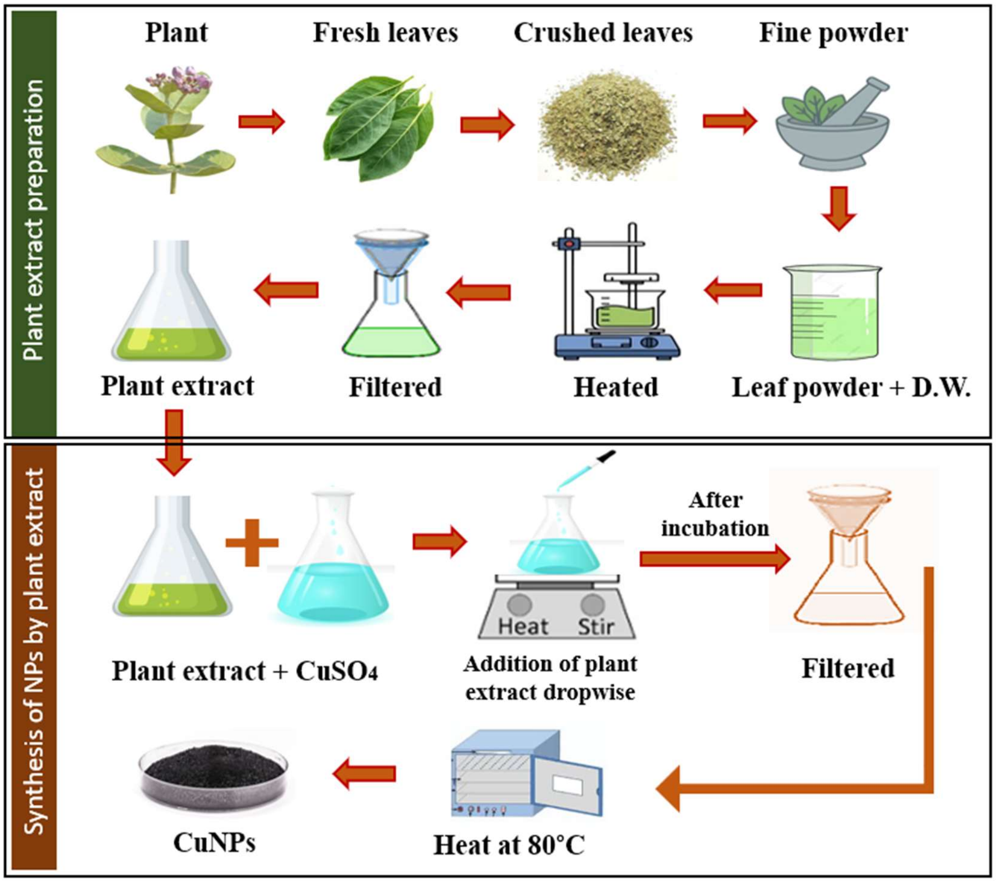

Fig 2.3: Plant-based synthesis of CuNPs using *Calotropis gigantea* leaf extract.

2.4 Characterization of copper nanoparticles (CuNPs)

To confirm the formation of copper nanoparticles (CuNPs), the formation was characterized by discolouration from dark green to brownish-black that occurred at the time of incubation. [19] The mixture of CuNPs reaction with *Calotropis gigantea* leaf extract was analyse by UV-Vis spectroscopy at 200-400 nm wavelength, and XRD was measured at 2-Theta degree. The FT-IR was observed from 4000-500 cm-1 Wavenumber, SEM and TEM were used to analyse a detailed high-resolution image of the sample to understand their shape, size, morphological, and topological structure of the CuNPs. [20,21]

III. RESULT AND DISCUSSION

Several approaches have been attempted to obtain a better synthesis of CuNPs using a biological method. Synthesis of CuNPs by *Calotropis gigantea* leaf extract has provided a clear, nontoxic, and environmentally friendly approach. [22] Aqueous extract of *Calotropis gigantea* leaf extract as a reducing and capping agent with $CuSO_4$ stock solution showed the colour reaction from dark green to brownish-black mixture solution after 24 hours of incubation. The change in colour indicates the formation of CuNPs. The control stock solution of $CuSO_4$ showed no colour change as compared to $CuSO_4$ with *Calotropis gigantea* leaf extract. [23]

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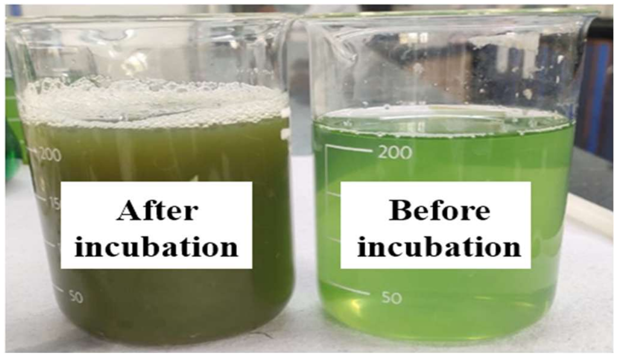

Fig 3: Comparative image of CuNPs synthesized using *Calotropis gigantea* leaf extract. The color change from green to brownish-black after incubation indicates the successful formation of CuNPs.

Factors affecting green synthesis of CuNPs:
Various limitations play a key role in identifying the characteristics of CuNPs synthesis, including temperature, incubation time, pH, and concentration of extract. It can affect the desired structure and morphology of CuNPs. Therefore, maintaining proper parameters could obtain unique properties of CuNPs synthesized by *Calotropis gigantea* leaf extract. [24]

Temperature: The solution temperature was maintained to impact the property of CuNPs synthesized by *Calotropis gigantea* leaf extract. Generally, the plant-based synthesis is performed at ambient temperature below 100°C. [25] The study suggested that mixture solution be maintained at room temperature for 24 hours, with increase in the temperature, the reaction mixture rate increases, reducing metal ions to form the nanoparticles, may form small size of copper nanoparticles. The size of CuNPs formed is an average size of 35 nm. With a change in temperature, the size of particles may vary. The report indicates the effect of temperature in the size of CuNPs synthesized by *Calotropis gigantea* leaf extract. [26]

Incubation time: The green synthesis of CuNPs with respect to the time of incubation may undergo aggregation and affect the properties of CuNPs synthesized by *Calotropis gigantea* leaf extract. [27] By comparing fresh and aged CuNPs, a change in shape, size, chemical bonding of the CuNPs was observed. The size of fresh CuNPs increased with aging under moderate conditions. It was observed that the aggregated CuNPs were oxidized highly compared to fresh prepared CuNPs synthesized by *Calotropis gigantea* leaf extract. However, incubation time does not highly influence the size of the nanoparticles, considering the other factors such as concentration of plant extract, pH, and temperature effect more on the formation of CuNPs. [28,29]

pH: The pH of the green synthesized copper nanoparticles mixture solution by plant extract is one of the factors affecting the shape and size of CuNPs. The ideal pH of the reaction mixture solution ranges from 7 to 9. The particles synthesized in an acidic condition of $pH < 7$ were relatively larger. An alkaline condition is favourable for synthesizing CuNPs, as evident from the synthesis of CuNPs occurring at the alkaline condition of $pH > 8$, which produces small-sized nanoparticles. It produces loosely agglomerated particles with sharp copper nanoparticles CuNPs with a small particle size.[30]

Concentration of plant extract: Plants containing metabolites and biomolecules as reducing agents and capping agents are the better choice for green synthesis of metal nanoparticles. The metabolite compositions depend on the part of the plants and their types. [31] Therefore, the amount of plant extract and the variety of biomolecules present in the plant

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extract plays a crucial role that can affect the synthesis rate of molecules for the instant reduction of metal ions and the stabilization of nanoparticles. [32] An increase in concentration of plant extract speeds up the reaction rate of metal ions, which increases the formation of nanoparticles of CuNPs. In this study, the plant *Calotropis gigantea* leaf extract was used. The plant leaf contains phytochemical properties contain in the leaf extract, which are reliable for the stabilization and reduction of the CuNPs. The leaf extract of the plant *Calotropis gigantea* was prepared by grinding the leaf into fine powder. The plant extract was used in a ratio of 1:1 with $CuSO_4$ stock solution dropwise. The addition of plant extract dropwise can lead to speeding up the reaction. The mixture solution was incubated at room temperature for 24 hours. The study provides information about the interaction of metal ions and concentration of plant extract used. [33-35]

3.1 Characterization of CuNPs by UV-Visible Spectroscopy

The UV-Visible spectroscopy confirms the formation of CuNPs synthesized by *Calotropis gigantea* leaf extract, the mixture solution changed from bright blue to greenish-brown by the addition of $CuSO_4$ to *Calotropis gigantea* leaf extract. [36,37] The change in colour indicated successful synthesis, whereas UV-Visible spectra analysis determines the particle size, ranging from 300 to 700 nm. In the study, UV-Visible spectroscopy analysis to confirm the CuNPs formation, monitoring the reduction of copper ions within the plant extract. The successful validation of CuNPs via UV-Visible spectroscopy, disclosing the highest peak observed at 430 nm absorbance, corresponds to the evidence by the appearance of the surface plasmon resonance band of copper nanoparticles. [38]

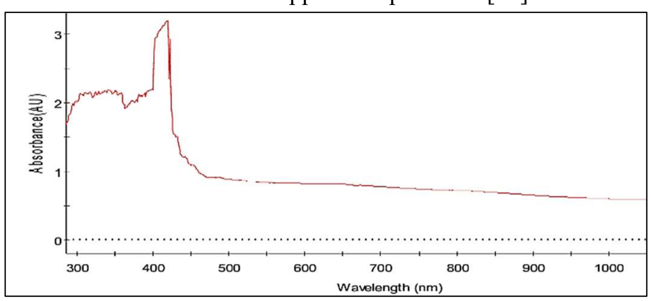

Fig 3.1: UV-Visible spectra of CuNPs

3.2 XRD analysis of CuNPs

The crystalline nature of CuNPs synthesized by using *Calotropis gigantea* leaf extract was confirmed through XRD analysis, with characteristic patterns indicating a face-centred cubic (FCC) crystal structure. The Bragg reflections were observed in the XRD pattern at different 2theta values. The main peaks observed at approximately 31.85° (2 theta) plane - (110), 38.18° (2 theta) plane - (111), 47.26°(2theta) plane - (200), and 74.45° (2theta) plane – (311) sets of lattice planes. In case the dominant CuNPs crystalline plane, the 38.18° peak is unusually high, suggesting it may arise from bio-organic residues/amorphous copper nanoparticles phases from the plant extract used for synthesis. The 47.26° peak at plane - (200) confirms the FCC copper nanoparticles. The study indicates the presence of crystalline CuNPs with an approximate particle size of 35 nm. Hence, the XRD patten confirms the formation of copper nanoparticles. [39]

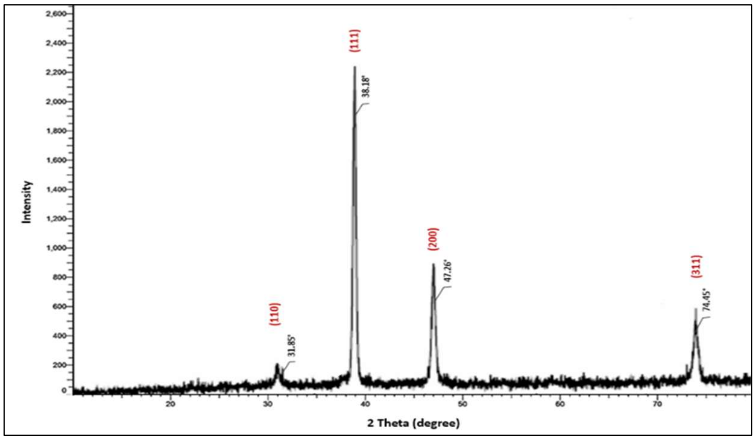

Fig 3.2: XRD analysis of CuNPs

Functional group analysis of CuNPs by FT-IR

CuNPs synthesised by *Calotropis gigantea* leaf extract undergo FT-IR spectroscopy, which is widely utilized to identify surface functional groups in CuNPs ranging a wavelength range of 4000-500 cm^{-1}, confirming absorption peaks at (3288, 2926, 1385, 1065, and 518 cm^{-1}) respectively. The spectral profiles of all samples indicated the existence of O—H stretching Alcohols groups, C—H stretching Alkane groups, C—N bending amine group from protein and amine, C—O stretching Alcohol and phenolic group, Cu—O/ Cu—N vibration metal-oxygen/nitrogen bond, confirming the CuNPs formation. The *Calotropis gigantea* leaf extract exhibited characteristic absorption peaks at 3320 and 1635 cm^{-1}, confirming the characteristic of O—H stretching from Phenol and Alcohol form plant metabolites and C=O stretching from Amine group from proteins or aromatic compounds. [40]

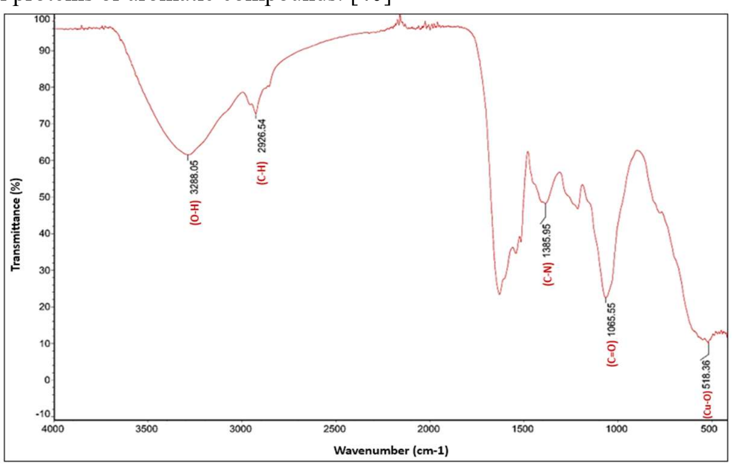

Fig 3.3: FT-IR spectra of CuNPs

3.3 Structural analysis of CuNPs by SEM

CuNPs synthesized with *Calotropis gigantea* leaf extract determine changing shape and size depending on the $CuSO_4$ concentration. The Scanning Electron Microscopy (SEM) technique is used to analyse the characterization to visualize the most advantageous results for size, shape, and stability of CuNPs. The surface morphological images were obtained with 100 % form of CuNPs by reducing metal ion with *Calotropis gigantea* leaf extract. The study proves the formation of the CuNPs and their morphological dimensions demonstrating the average size of particles ranging from 50 to 100

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nm. The structure was observed at 2 µm, showing that the size and shapes of the CuNPs proved to be spherical. The CuNPs synthesized by *Calotropis gigantea* leaf extract showed size and shape, which were confirmed by Scanning Electron Microscopy (SEM) analysis. [1,41]

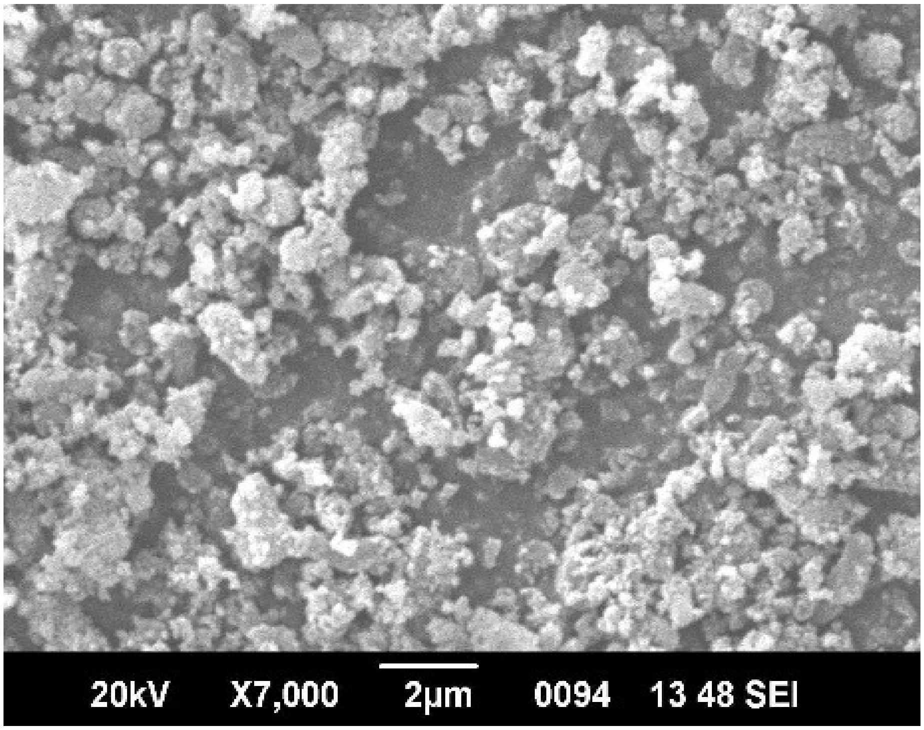

Fig 3.4: Structural analysis of by SEM. The image shows the surface morphological structure and spherical shape of the CuNPs.

Detailed analysis of CuNPs by TEM

Transmission Electron Microscopy (TEM) analysis confirms spherical shape of copper nanoparticles synthesized by *Calotropis gigantea* leaf extract. TEM analytical technique for characterization of nanoparticles is commonly used to obtain quality measures of particles such as size, shape, and detailed morphology. TEM analysis provides better resolution and additional analytical measurements. The shape and size of the CuNPs at 50 µm and 5 µm proved the spherical shape and approximately the size of particles up to 35.16 nm. The study confirms the formation of copper nanoparticles synthesized by *Calotropis gigantea* leaf extract with detailed analysis of particles morphology by using characterization techniques. [1,42]

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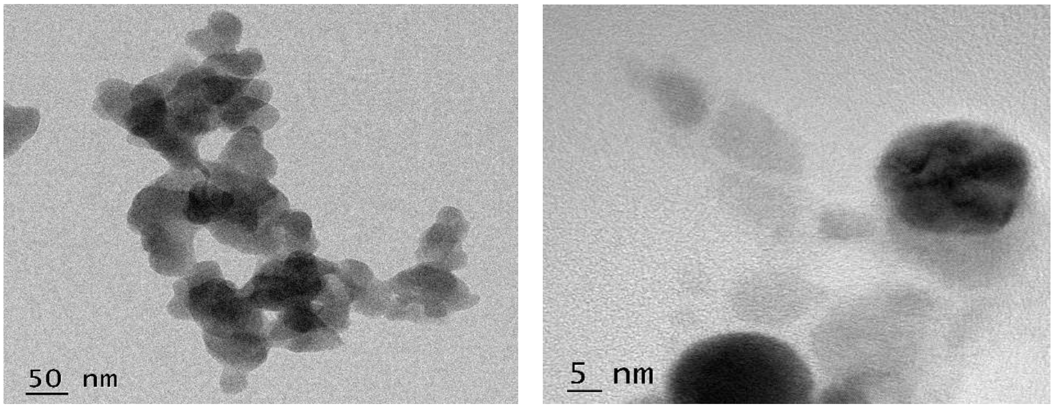

Fig 3.5: Detailed morphological analysis of CuNPs by TEM. The image analyses the average size 35.16 nm of CuNPs ranging at 50 µm and 5 µm.

IV. CONCLUSION

The sustainable synthesis of copper nanoparticles (CuNPs) using Calotropis gigantea leaves extract demonstrates promising attributes. The green synthesized copper nanoparticles are uniform in their shape and size, and the reaction was observed by a colour change from bright blue to greenish-brown after 24 hours of incubation. Characterization techniques, including UV-Visible spectroscopy, XRD, FT-IR, SEM and TEM confirm the purity, chemical bonding, and structural demonstration of CuNPs. The green synthesized CuNPs focused on healthcare aspects in the field. The study concludes that the CuNPs synthesized by *Calotropis gigantea* leaf extract are promising and biologically significant. [43-45]

Declaration

The authors declare that they have no known competing financial interests.

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