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Green synthesis of CuO nanoparticle using *Justicia Adhatoda* leaf extract and it's antibacterial and thermal asset

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Preparation and analysis of copper oxide nanoparticles (CuO Nps) are under investigation due to their electronic and wide clinical applications in nanoscience. Preparation was made to CuO Nps by using a medical plant *Justicia Adhatoda* and copper sulfate ($CuSo_4$). The crystal structure of prepared sample was confirmed by X-ray powder diffraction (XRD) technique, it is an orthorhombic crystal structure. The Fourier transform infrared spectroscopy (FTIR) affirms Cu-O stretching vibration. The zeta potential properties are utilized to search out the surface charge and long term stability of CuO Nps. The scanning electron microscope (SEM) pictures explain the surface morphological analysis of CuO Nps. EDAX is utilized to affirm the presence of copper and oxygen in NPs. The optical properties were researched by UV–DRS and PL. Thermal analysis of CuO Nps examined exploitation thermo gravimetric analysis (TGA) and differential thermal analysis (DTA). It was furthermore, it had been that CuO Nps have well anti bactericidal potential. The prepared Nps will be utilized for various applications in light of their eco-accommodating, nontoxic, and similarity for drug and diverse electronic applications.

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Keywords: Antibacterial activity, CuO, Justicia Adhatoda, Zeta potential

1. Introduction

Nanotechnology has covered a colossal space of analysis, which involves material scientists, molecular biologists, physicists, chemists, engineers, etc. Nanomaterial was an excellent invention for environmental advantages as a result of their use, saving raw materials, the consumption of natural resources, and reduced environmental pollution [1,2]. Besides, nanomaterial was of nice interest in biomedical applications. Various studies have targeted metal oxide nanoparticles (Nps) because of their electronic, optical, and catalytic properties [3]. Green nanotechnology is an excellent resolution to decrease the negative effects and increase the application of nano size materials. Plant extracts are treated in an eco-friendly queous medium. Because of the biomass abundance of many plants, the scientists have priority to the plant to execute the preparation of metal oxides Nps. It is attributable to their biomass profuseness and the stabilization of the surface of the metal oxide Nps [4]. The primary compounds of plants like amino acids, phenolic compounds, terpenoids, enzymes, peptides, polysaccharides, and tannins are accountable for the metal oxide particles reduction. Alongside this plant conjointly offers an improvement for Nps preparation as they are free from violent chemicals similarly as they offer natural reducing agents. The leaf extract is the most Significant for the assembly of metal oxide Nps because it is that the major resource for metabolites as a result of them is rejuvenated and non-devastating compared with different plant tissues. The leaf extract

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composition incorporates a nice impact on Nps synthesis. Since it might have the flexibility to arrange and stabilize metal oxide NPs. During this study, we synthesized metal oxide Nps by exploitation of *Justicia Adhatoda* leaf extract. During this analysis work, the *Justicia Adhatoda* plant is an Ayurved medicative plant obtainable in Tamil Nadu, and it's conjointly referred to as adhatoda. The plant leaves significantly contain vaccinating specialist alkaloid. The leaf extracts of the plant are used as a medication for coughs, colds, asthma, skin infections, fever, and inflammation. The green technique exploitation using greeny liquid leaf extract of *Justicia Adhatoda* has been used for the synthesis of oxide Nps[5]. The current technique is speedy, environmentally benign, and efficient in comparison to the chemical/physical technique of CuO NPs synthesis. Also, biosynthesized copper oxide Nps (CuO NPs) are the foremost predominant for biomedical applications within the rising field of nanoengineering science [6]. CuO NPs need to be proven they have effective health additives in medicine and also to show sturdy effectiveness against bacteria, fungi, viruses, and alternative microorganisms. Experimental information like XRD, FTIR, UV DRS, PL, SEM, and EDAX were explained like crystalline phase, particle size, elemental analysis, and surface morphology. The prepared Nps were analyzed by TG/DTA, Zeta Potential to survey thermal and electro kinetic properties and furthermore tried for their antibacterial action. Consequently, inside the current examination, the green amalgamation approach abuse leaf concentrate of *Justicia Adhatoda* was decreasing specialist for the blend of copper oxide Nps.

2. Materials and Methods

2.1. Biosynthesis process of CuO NPs using *Justicia Adhatoda* aqueous leaf extract

The *Justicia Adhatoda* leafs were washed to make them free from dust particles and surface contamination and dried well. Afterward, 15ml of leaves extract prepared by grinding the leaves and the extract was filtered and used for the synthesis process AR grade copper sulphate chemical utilized in the experiments was purchased from Madras Scientific Suppliers, India. Deionized water was used all over experimentation for making solutions and washing purposes. CuO Nps was synthesized using by 0.2 M of copper sulphate ($CuSO_4.5H_2O$) and *Justicia Adhatoda* leaf extract by coprecipitation technique. The mixture was then stirred for about four hours. A color change was observed to confirm the formation of Nps shown in (Fig 1). The obtained color change indicates the reduction of cu ions and the formation of CuO Nps with a pH of 7. The precipitate is collected and dried in an oven for 24 hours at 100°C, followed by a calcination process at the temperature of 500 °C for four hour, with the resulting yield of about 3.2 g, respectively. The dried samples were made into a fine powder and used for studies.

Fig. 1. Reaction process of CuONPS using leaf extract of Justicia Adhatoda.

2.2. CuO Nanoparticles (NPs) Characterization

The structural characteristics of synthesized CuO Nps using leaf extract of *Justicia Adhatoda* were determined through the X-Ray Diffractometer X'PERT PRO diffractometer. Functional groups were explored from Fourier Transform Infrared spectroscopy. The morphology was analyzed by scanning electron microscopes (SEM; JEOL – model JFC1600). The Zeta Potential was recorded by Malvern Zetasizer Nanosizer. Thermal behavior was studied by TG/DTA analysis (EXSTRAR 6300). The photoluminescence (PL) emission and excitation spectra were recorded by use of a Shimadzu RF-5301PC spectrofluorophotometer. The

determination of the antibacterial activity is determined by using Muller Hinton Agar medium (HIMEDIA- M173).

3. Results and discussion

3.1. XRD Analysis

From figure 2, CuO Nps structure was confirmed from the characteristic peaks of XRD. XRD studies shows a diffraction peaks at 2θ of 13.52°, 18.24°, 22.89°, 27.52°, 31.66°, 33.23°, 35.60°, 39.01°, and 46.96° which are assigned to the (111), (200), (212), (032), (133), (313), (042), (143), and (501) planes of orthorhombic CuO (JCPDS – 77-1898) respectively. All diffraction peaks can be indexed as orthorhombic structures. The average crystalline size of CuO Nps was calculated using the Scherrer formula [7] as 42 nm. The results of the present study indicated the successful preparation of CuO Nps from *Justicia Adhatoda* leaf extract.

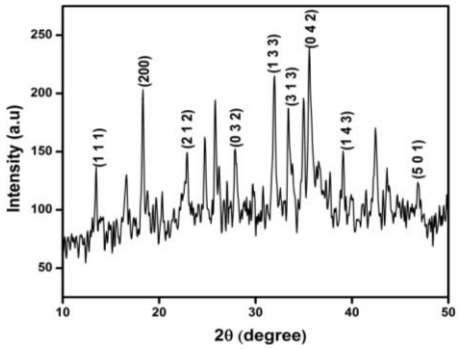


Fig. 2. XRD Analysis of CuO NPs.

3.2. Surface Morphology & Elemental Analysis

To study the morphology of prepared CuO Nps, SEM was used. SEM images of the CuO Nps using *Justicia Adhatoda* are shown in Figure 3. The green synthesized CuO-Nps reveals the formation of dense cuboids containing amorphous agglomerates [8]. The morphology and shape of the Nps are dependent on the reducing agent, which in this case, is the plant extract, these data are in full agreement with recent prestigious studies of the green preparing technique. EDAX spectrum confirmed the presence of Cu and Oxygen in the Nps [9]. The presence of S may be a residual contribution from the organic compound that emanated from the aqueous leaf extract. Thus, Justice Adathoda is found to be a strong eco-friendly reducing agent and the sample CuO NPs is free from impurity.

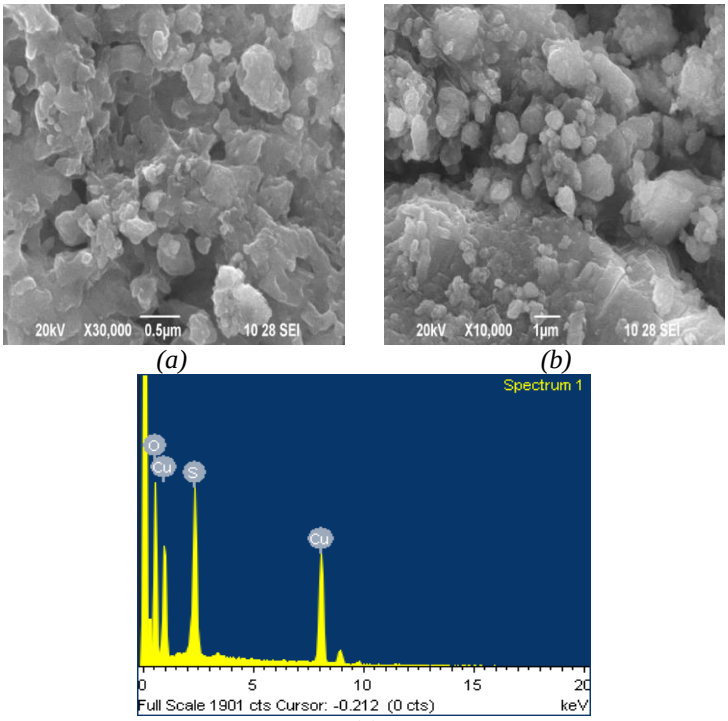


Fig. 3: SEM (a, b) & EDAX (c) Spectroscopy spectrum of biosynthesized CuO NPs

3.3. FT-IR Analysis

FT-IR spectrum shows the functional group of CuO NPs illustrated peaks in the range of 500 –4000 cm^{-1}. Intense broadband range of 3240 cm^{-1} is a O–H stretching vibration of the surface hydroxyl group of adsorbed water molecules [10]. This peak seems because of the nanocrystalline structure of CuO Nps, which exhibit a high surface – to - volume magnitude relation. The little band 2244 cm^{-1} is a O=C=O stretching vibration. The sharp absorption band 1623 cm^{-1} is a C=C bending vibration. The bond 1101 cm^{-1} is a C-O stretching vibration. The IR band 586 cm^{-1} confirms the formation of CuO Nps. [11,12].

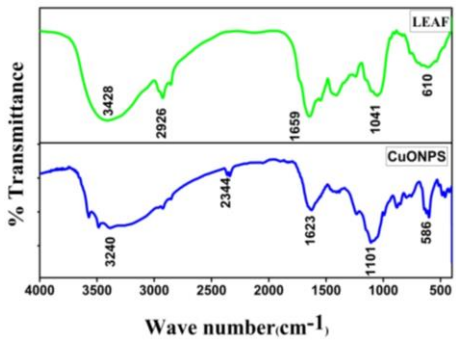


Fig. 4. FTIR Spectra of CuO NPs.

3.4. UV-DRS Analysis

The absorption spectrum is used to analyze the energy band and also the form of electronic transitions. Absorption spectrum of CuO Nps is shown in Figure 5. The optical band gap of the biosynthesized CuO Nps is calculated using the victimization of the Kubelka - Munk relation. To convert the reflectance information into a Kubelka - Munk function [13]. The bandgap energy of the prepared sample was calculable from the variation of the Kubelka-Munk function with photon energy. The figure shows the Kubelka-Munk plots for the CuO NPs which are accustomed to

confirm the bandgap energy related to their direct transitions. The worth of the band gap is decided from the intercept is found to be 2.01eV. This value is much more than the bulk CuO (1.2 eV). Band gap energy will increase with decreasing particle size because of quantum confinement effects.[14] The prepared Nps are extremely confined and also the absorption spectrum of them become more structured as a result of its electronic band structure changes to molecular level with nonvanishing energy spacing. Therefore the material desires more energy for an electronic transition from the valence band to the conduction band. Thus the CuO Nps are often employed in semiconductor devices like photo amplifiers, photovoltaic cells, and photo detectors.

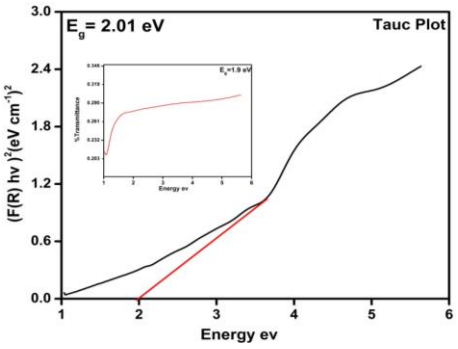


Fig. 5. UV-DRS Spectra of CuO NPs.

3.5. Photoluminescence Spectral Analysis

The prepared CuO Nps were studied for photoluminescence at normal temperature are shown in Figure 6. The photoluminescence spectra help understand the electron transition energies inside CuO Nps through finding the emission peaks and exploiting them to evaluate the corresponding electronic energy level. The broad emission maximum was observed at 392 nm. The comparatively low fluorescence emission maximum obtained may be due to the luminescence arising from the recombination of excitons and / or shallowly trapped electron-hole pairs of CuO-free excitons [15]. Thus, it declared that prepared Nps are fluorescent but not intensely fluorescent. The smaller the Nps, the shorter wavelength emission is due to the reduction in Nps size and increases in QC effect.

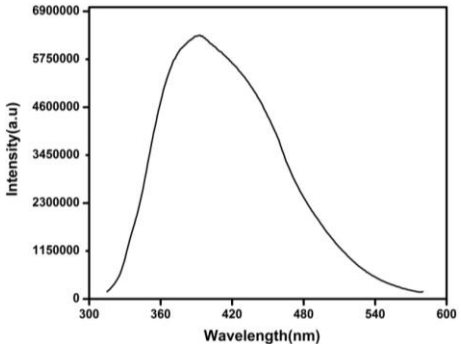


Fig. 6. PL spectra of CuO NPs.

3.6. TG-DTA Analysis

TGA is a method that measures the physical and chemical properties of a prepared sample as a performance of temperature with time. It is mainly used for determining a material that expelled either mass loss or gain due to oxidation, decomposition, or loss of volatiles. DTA is a technique that is on to obtain the chemical composition of a sample underneath heating conditions. Figure 7 shows in TGA, the CuO Nps was analyzed by heating and air atmosphere at the rate of 5°C/min, underneath the N2 atmosphere over the temperature range. The initial weight loss below

96°C was occurred by the water evaporation agrees with two resolved endothermic peaks on the DTA curve (105°C, 254°C) in this region. Second decomposition of the TGA curves at 166–227°C is the reason for the resolved exothermic within the DTA curves at about 336°C [16]. No change in the TGA curve is obtained above 550°C, which confirms the formation of CuO Nps. Therefore, physical and chemical changes of the prepared CuO Nps using *Justicia Adhatoda* leaf were studied by TG- DTA graph.

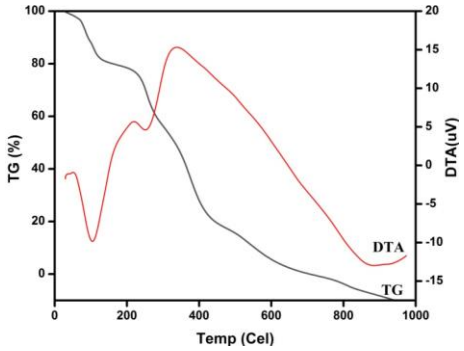


Fig. 7. TG-DTA of CuO NPs.

3.7. Zeta Potential

A zeta potential technique has been used to predict the long stability of the Nps in solution and also to understand the state of the Nps surface. The extent of zeta potential gives an implication of the potential strength of the biosynthesis CuO Nps using *Justicia Adhatoda* leaf extract. Electro-kinetic potentials of each Nps in colloidal scattering were indicated by zeta potential result, which shows the magnitude as 0.134 mV and 3.17 mS/cm for zeta potential and conductivity separately is shown in Figure 8. Positive zeta potential is significant for drug conveyance. Since it will work with effective adhesion to the membrane epithelial surface, prolonging the drug unleash and enhancing the drug bio-availability among the internal tissue of the skin owing, it is also charged at the skin surface. [17]. Zeta-potential measurements will give valuable information regarding the nature and behavior of Cuo Nps in environmental and biological systems.

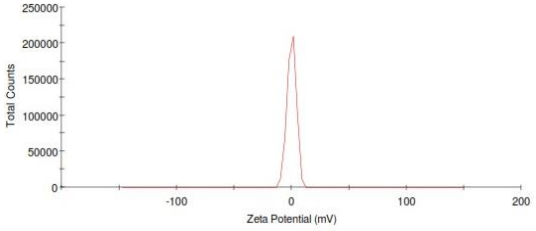


Fig. 8. Zeta Potential distribution of CuO NPs.

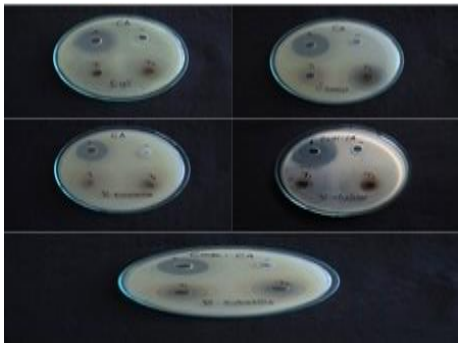
3.8. Antibacterial Activity

Agar well diffusion methodology is employed to evaluate the antimicrobial activity of the CuO NPs against microorganisms like *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Vibrio cholera, Bacillus subtilis* (Table. 1.). The sterilized 15- 20 ml of Mueller-Hinton agar was poured on glass Petri plates of the same size and allowed to solidify. once the solidified, the wells (4 wells/ plate) were created with a sterile cork borer of diameter 8 mm (20 mm apart from one another) were punched aseptically an each plate.[18] The standardized inoculants of the test organism were uniformly unfolded on the surface of these solidified media victimization sterile cotton swabs. The test volumes (40 µL from 10mg/mL) of the sample at desired concentrations were added to the primary 2 wells, one well with 80mcg of Gentamycin as positive control and the other one with DMSO as negative management. Then, the agar plates were

incubated in an incubator underneath 37°C for 24 hr. Once incubation, a clear zone was observed as shown in Figure 9. The Biosynthesis Nps show that CuO Nps would possibly act as an efficient antibacterial drug agent against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Bacillus subtilis* bacteria [19], it's known that copper Nps act on the cell membrane attributable to their affinity to amines and carboxyl teams on the cell surface therefore the sample will be utilized in skin infection treatment. The discharge of a drug through the skin can depend upon the physicochemical properties of the drug itself combined with the influence of the vehicle to change the drug penetration profile [20]. The positively charged NPs were found to be simpler in terms of skin diffusion than the charged ones. As mentioned, the interaction of CuO NPs with skin depends upon factors of things including factors of the electrical charge of the droplets. The zeta Potential results obtained suggested that positively charged CuO Nps are ready to carry with efficiency the nano drugs into the skin and after promoting the penetration of the drugs through the skin. The degree of skin binding is probably more necessary with the positively charged particles than with the negative one because it is understood that the skin is negatively charged at neutral ph [21].

Table 1. Various Antibacterial strains. Zone of Inhibition of CuO NPs.

S. No.	Organism	Standard Gentamycin (80 mcg)	Zone of Inhibition (mm)
1.	S. aureus	26	25
2.	E. Coli	25	-
3.	K. pneumoniae	24	21
4.	V. cholerae	23	-
5.	B. subtilis	24	22



Fig, 9. Various Antibacterial strains. Zone of Inhibition of CuO NPs.

4. Conclusion

In this work, we tend to successfully synthesize CuO Nps that are biocompatible, low toxicity, and eco-friendly using *Justicia Adhatoda* as a reducing agent. Synthesized Cuo NPs conditions were characterized using UV- DRS, PL, XRD, TG-DTA, Zeta Potential, and SEM. The XRD patterns showed the orthorhombic phase particle size range of 42 nm and also the UV–DRS spectrum confirms the bandgap energy to be 2.01eV. TG-DTA studies confirm the steadiness of the CuO NPs. The plant mediates CuO Nps have a positive zeta potential and good antibacterial activity. Therefore the bio-synthesized CuO Nps using *Justicia Adhatoda* leaf extract has a diversity of potential applications in the field of photo-voltaic devices, biomedicine and spintronics.

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