Rauvolfia serpentina-Mediated Green Synthesis of CuO Nanoparticles and Its Multidisciplinary Studies

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Abstract Copper oxide nanoparticles (CuO Nps) were successfully synthesized by solution combustion method using aqueous leaf extract of *Rauvolfia serpentina* as a fuel. The structure and morphology of the CuO nanoparticles (Nps) were characterized by powder X-ray diffraction (PXRD), UV–visible spectroscopy (UV–visible), scanning electron microscopy (SEM), transmission electron microscopy (TEM), etc. The PXRD patterns reveal the formation of monoclinic phase with crystallite structure. SEM images indicate that the particles have sponge-like structure being highly porous and agglomerated with large surface area. The average crystallite sizes were found to be in the range of 10–20 nm by Scherrer's method. The CuO Nps size was further confirmed by TEM. Further, CuO Nps exhibit good photocatalytic activity for the photodegradation of trypan blue dye, indicating that it acts as a promising semiconducting material. The antibacterial properties of CuO nanoparticles were investigated against pathogenic bacterial strains, namely Gram –ve *Escherichia coli* (NCIM-5051) and *Pseudomonas desmolyticum* (NCIM-2028) and Gram +ve bacteria *Staphylococcus aureus* (NCIM-5022) using the agar well diffusion method.

KEY WORDS: CuO; Nanoparticles; *Rauvolfia serpentina*; Photocatalytic activity; Antibacterial activity

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1 Introduction

In recent years, nanotechnology was well-developed, interdisciplinary field of research in science and technology. Metal nanoparticles have widespread popularity because of their application of in variety of fields including biomedical sciences, chemical industry, electronics, drug–gene delivery, and biosensor [1]. CuO nanoparticles were one of the most important catalysts used to eliminate industrial effluents in the environment. Earlier studies indicate that the catalytic reaction was apparently a structure-sensitive process, due to the involvement of the oxygen surface lattice of CuO nanoparticles [2]. CuO nanoparticles play an important role in the field of medicine in which they act as antioxidant [3], antimicrobial agents [4], and pesticide formulations [5]. A part from, it

has numerous applications like gas sensors [6], solar energy conversion [7], and catalyst [8]. Due to the versatile properties and diverse applications, various kinds of CuO nanostructures, namely nanorods, nanosheets, and nanodendrites, honeycomb, urchin, and dumbbell-like structures have been synthesized and studied [9–12].

The wide varieties of CuO nanostructures were prepared in either physical or chemical routes, namely sol–gel [13], microwave irradiations [14], thermal decomposition [15], and solid-state reaction method [16]. Chemical synthesis process lead to the presence of some highly toxic chemicals adsorbed on the surface that may have adverse effect in the medical applications [17].

Presently, material scientists and researchers focused on the development of novel routes to synthesized nanoparticles through green route as which is an eco-friendly, inexpensive, very cheap, without use of harsh chemicals [18]. Recently, green approached synthesis of metal oxide nanoparticles by plants, namely *Gloriosa superba* [19], *Carica papaya* [20], *Aloe barbadensis* Miller [21], *Tabernaemontana divaricate* [22], *Embllica officinalis* [23], and *Cassia fistula* [24].

In the present work are presented synthesis, characterization, and multidisciplinary properties of CuO nanoparticles prepared via greener route at low-temperature solution combustion using aqueous leaf extract of *Rauvolfia serpentina* as a fuel.

Rauvolfia serpentina was one of the medicinal species of flowering plant, which belongs to family Apocynaceae. It was also called ‘Indian snakeroot’ or ‘sarpagandha’ [25]. *R. serpentina* extract has been used to treat infections for thousands of years in Indian traditional system of medicines. Further, it was used for the treatment of fever, anxiety, epilepsy, snake bite, rheumatism, insanity, eczema, intestinal disorders, psychiatric disorders, nervous disorders, cardiovascular disorder, and bacterial infections and in the management of hypertension schizophrenia [26–28]. It mainly contains alkaloid, glycosides, steroids, reducing sugar, flavonoid, and tannins [29]. In the present work, an attempt has been made to synthesize CuO nanoparticles using aqueous leaf extract of *Rauvolfia serpentina* as a fuel, and photocatalytic, antibacterial activities were discussed.

2 Materials and Methods

2.1 Materials

Fresh leaves of *R. serpentina* were collected at Devarayanadurga forest, in Tumkur District, Karnataka, India. The leaves of *R. serpentina* were identified and authenticated by Taxonomist, Department of Botany, Tumkur University, Karnataka, India. All the chemicals were

purchased from Sigma-Aldrich Chemicals, India. The clinical isolations of pathogenic bacterial strains were purchased from National Chemical Laboratory (NCL), Pune. These strains were maintained on nutrient agar slant at 4 °C.

2.2 Preparation of Extract

Fresh leaves of *R. serpentina* (250 g) were thoroughly washed with running tap water, followed by distilled water to remove the dust particles. Further, shaded dried at room temperature, later leaves were powdered mechanically using mixer grinder and then sieved. Powdered plant material was subjected to successive solvent extraction using double-distilled water in Soxhlet apparatus at 40–60 °C (Merck, Mumbai, India) for 72 h. Extraction of plant material was filtered and concentrated in vacuum using rotary flash evaporator (Büchi, Flawil, Switzerland). Left over solvent was completely removed on water bath and finally dried in the desiccator. The crude plant extract material obtained from the aqueous solvents was labeled, and the formed product was stored in air-tight glass vials in the refrigerator.

2.3 Synthesis of CuO Nanoparticles

CuO nanoparticles were prepared by greener route at low-temperature solution combustion method [30] using aqueous leaf extract of *R. serpentina* as a fuel. In a typical experiment, 0.1 g of aqueous leaf extract of *R. serpentina* and stoichiometric amount of copper nitrate trihydrate was dissolved in 10 mL of distilled water in a petri dish under constant stirring for 10 min to get a homogenous mixture. The homogenized mixture was kept in a preheated muffle furnace maintained at (400 ± 10) °C for 5 min, and the reaction was completed. Then fine black-colored material was formed. The product was stored in airtight container until further use.

2.4 Material Characterization

The phase purity and crystallite size were determined by using X-ray diffraction analysis using Shimadzu-7000 with mono-chromatized CuK_α radiation ($\lambda = 1.541$ Å) with a nickel filter. The UV–Vis absorption of the sample was measured using a Shimadzu (UV-1800) spectrophotometer. The morphological features of nanoparticles were assessed by scanning electron microscopy (Hitachi—7000 Table top) and TEM (TECNAIF-30).

2.5 Experimental Procedure for Photocatalytic Degradation of Dye

The photocatalytic experiments [31, 32] were carried out with the help of batch reactor, having dimensions of

150 mm × 75 mm and using 125 W/m² medium pressure mercury vapor lamp as the UV light source. Solar light (750 W/m²) experiments were performed under sunlight between 11 am to 2 pm, when the solar intensity fluctuations were minimal, and the sun rays were not oblique, and catalytic load of 100 mg nanoparticles was used in 100 mL trypan blue dye solution at appropriate concentrations. Known volume of the slurry was drawn at specific intervals (30 min), centrifuged to remove the intervention of the catalyst, and assessed using a spectrophotometer for rate of degradation. The photodegradation efficiency (R) of trypan blue by CuO nanoparticles was calculated using the following equation:

$$R = (C_i - C_f)/C_i \times 100\%, \quad (1)$$

where C_i and C_f are the initial and final dye concentrations, respectively. The experiment was carried out by varying parameters such as concentration of dye, catalytic load, irradiation time and pH and nature of light (UV and sunlight).

2.6 Antibacterial Activity Studies

Antibacterial activity was screened by agar well diffusion method [33, 34] against pathogenic bacterial strains, namely Gram -ve bacteria *Escherichia coli* [NCIM-5051] and *Pseudomonas desmolyticum* [NCIM-2028] and Gram +ve bacteria *Staphylococcus aureus* [NCIM-5022]. Nutrient agar plates were prepared and swabbed using sterile L-shaped glass rod with 100 µL of 24-h mature broth culture of individual bacterial strains. The well was made by using sterile cork borer 6-mm wells was created into each petri plate. Various concentrations of CuO nanoparticles (500 and 1000 µg per well) were used to assess the activity of the compound. The compound prepared in sterile water was added into the wells by using sterile micropipettes. Simultaneously, the standard antibiotics (as positive control) were tested against the pathogens. Ciprofloxacin (Hi Media, Mumbai, India) as positive control. Then the plates were incubated at 37 °C for 36 h. After the incubation period, the zone of inhibition of each well was measured and the values were noted. Triplicates were maintained in each compound, and the average values were calculated for the ultimate antibacterial activity.

3 Results and Discussion

Figure 1 shows the PXRD pattern of CuO nanoparticles via greener route at low-temperature solution combustion method. All the diffraction patterns were indexed to be monoclinic crystalline structure (JCPDS 80–1916) of CuO Nps with lattice constant $a = 4.6965$ Å, $b = 3.4324$ Å,

Fig. 1 PXRD pattern of the synthesized CuO Nps

and $c = 5.1329$ Å and $\beta = 99.5287^\circ$. Further, no indications of a secondary phase or impurity peaks were obtained. The average crystallite size (D) was estimated by using Debye–Scherrer’s formula [35]:

$$D = K\lambda/(\beta\cos\theta) \quad (2)$$

where K is the shape factor (0.9), λ is the wavelength of X-ray (1.5406 Å) CuK_α radiation, θ is the Bragg angle, and β is the full width at half maximum (FWHM). The average crystallite size of CuO Nps was found to be in the range of 10–20 nm.

Figure 2 shows the room-temperature UV–visible spectrum of CuO nanoparticles synthesized using *R. serpentina* leaf extract. A broad spectrum reveals a characteristic absorption peak of CuO at 340–370 nm [36].

SEM images reveal that green synthesis of CuO nanoparticles shows sponge-like structure and highly porous, agglomerated, and irregularly shaped primary particles with large voids and pores. The pores and voids can be

Fig. 2 UV–Vis spectrum of the synthesized CuO Nps

attributed to the amount of gas escaped from the reaction mixture during low-temperature solution combustion method as shown in Fig. 3a, b. Through the pores of various sizes and shapes, the crystallites were interlinked to one another, and it is further confirmed by the TEM image as shown in Fig. 3c.

Figure 4a shows the effect of dye concentration on the photocatalytic activity of CuO Nps; its concentration range of trypan blue was 5–25 µg/L, catalytic load was maintained at 0.5 g, and pH value was 10. As the concentration of dye increases, time taken for complete degradation increases. At increased concentrations, more dye molecules were adsorbed onto the surface of catalyst resulting in decreased active sites on the catalyst (lesser number of hydroxyl and superoxide radicals) and then by reduced light penetration. Figure 4b shows the effect of catalyst load on the photocatalytic degradation of dye. In order to determine the optimal dosage of the catalyst, the catalytic load was varied from 1.0 to 7.0 g/L. The optimal load was found to be 5.0 g/L. Below this concentration, active sites on the surface of the catalyst increased resulting in rapid degradation. Above the optimal load, turbidity of the slurry increased, light penetration decreased, and thus availability of hydroxides and super oxides becomes minimal. Figure 4c shows the effect of pH value on photocatalytic degradation of trypan blue. It was clear that photocatalytic process strongly depends on pH value of the dye solution [37]. When pH value of the solution is adjusted by the addition of either 0.5 mol NaOH solution or 0.5 mol H₂SO₄ solution, the degradation efficiency is more in basic condition than in acidic condition. The variation of pH value alters the surface properties of CuO Nps, in turn, which leads to dissociation of the dye molecules. At the

condition of pH value of 10, per-hydroxyl radicals were formed, leading to the formation of hydrogen peroxide which gave rise to a number of hydroxyl radicals. When pH value is 10, the highest photocatalytic degradation is observed.

Mechanism of formation of possible radicals can be described as:

Figure 4d shows the effect of different light sources on photocatalytic degradation of dye using both the light sources such as sunlight and UV light, and it was clear that photocatalytic degradation of the an azo dye was more in UV light than in sunlight. This was due to the fact that UV light has higher intensity (lower wavelength or higher energy). So light can easily penetrate and form more number of radicals, which increases the rate of photocatalytic degradation of an azo dye.

Hydroxyl radicals were proved to be the major active species during the photocatalytic activity. The amount of

Fig. 3 SEM images a, b & TEM image c of the synthesized CuO Nps

Fig. 4 Photodegradation of trypan blue as a function of irradiation time under different experimental conditions: **a** Trypan blue concentration varies from 5 to 25 $\mu\text{g/L}$, 125 W/m^2 UV light, catalyst CuO Nps load 0.5 g, pH value 10; **b** catalyst CuO Nps load varies from 0.1 to 0.7 g, trypan blue concentration 5 $\mu\text{g/L}$, 125 W/m^2 UV light, pH value 10; **c** pH value varies from 2 to 12, trypan blue concentration 5 $\mu\text{g/L}$, 125 W/m^2 UV light, catalyst CuO Nps load 0.5 g; **d** under 125 W/m^2 UV and 750 W/m^2 sunlight irradiation, 100 mL trypan blue, catalyst CuO Nps load is 0.1 g, and pH value is 10

OH can be detected by using photoluminescence (PL) technique. The rate of formation of OH during the photocatalytic degradation reaction could be detected by using a simple, rapid, sensitive, PL technique using coumarin as probe molecule. Coumarin reacts with OH to produce highly fluorescent product 7-hydroxyl coumarin at a signal of ~ 495 nm in Fig. 5. In this method, 0.5 g of catalyst was dissolved in 100 mL of 0.001 mol/L aqueous solution in a borosil trough. The solution was allowed to undergo adsorption-desorption equilibrium with the photocatalyst, coumarin, and water before the irradiation. This reaction was irradiated under UV light of intensity 125 W/m^2 as a source of light. For every 15 min, aliquots were withdrawn, and the PL intensity at 456 nm increased linearly with the irradiation time, and it indicated that the formation of OH

at the surface of CuO was proportional to the irradiation time [38].

Antibacterial activity of CuO NPs was studied against Gram +ve *S. aureus* and Gram -ve *E. coli* and *P. desmolyticum* bacterial strains using agar well diffusion method. The highly significant antibacterial activity in *E. coli* (12.00 ± 0.00 mm) was less significant in *S. aureus* (6.33 ± 0.33 mm) and *P. desmolyticum* (4.33 ± 0.33 mm). Mechanism of the bactericidal effect of nanoparticles was generally attributed to the decomposition of bacterial outer membranes by ROS, primarily $\cdot\text{HO}$, which leads to phospholipid peroxidation and ultimately cell death. The pathogenic bacterial strains of Gram +ve bacteria *S. aureus* and Gram -ve bacteria, *E. coli* and *P. desmolyticum* with 500 and 1000 μg CuO Nps per well showed the zone of

Fig. 5 Detection of hydroxyl radicals with different irradiation time

inhibition shown in Fig. 6, and graphical representation of antibacterial activity of CuO nanoparticles is shown in Fig. 7. The data are shown in Table 1.

4 Conclusions

In this study, the synthesis of green approached CuO nanoparticles using aqueous leaf extract of *Rauvolfia serpentina* as a fuel by solution combustion method has been successfully carried out. The PXRD pattern revealed that monoclinic crystallite structures of CuO nanoparticles were formed. Morphological features of CuO nanoparticles were sponge-like structures being highly porous and agglomerated. TEM images revealed that crystallite size of CuO Nps was found to be in the range of ~10–20 nm. The

Fig. 7 Graphical representation of antibacterial activity

Table 1 Antibacterial activity of CuO Nps on pathogenic bacterial strains

Treatment (µg/µL)	<i>E. coli</i> (mm)	<i>S. aureus</i> (mm)	<i>P. desmolyticum</i> (mm)
Standard 5	15.83 ± 0.03*	13.03 ± 0.16	14.13 ± 0.09**
CuO Nps 500	10.10 ± 0.06**	3.17 ± 0.12	2.17 ± 0.03*
CuO Nps 1000	12.13 ± 0.09**	5.90 ± 0.01**	4.13 ± 0.09**

* Represents statistical significance $P < 0.05$; ** represents $P < 0.01$

carcinogenic dye such as trypan blue dye was found to degrade effectively by CuO nanoparticles in the presence of UV and sunlight. CuO nanoparticles showed significant bactericidal activity against Gram +ve and Gram –ve pathogenic bacterial strains in agar well diffusion method. The methodology employed suggests that natural occurring extract from plants can be used as both a reducing and stabilizing agent for the preparation of nanoparticles.

Fig. 6 Zone of inhibition of antibacterial activity of CuO Nps against: **a** *E. coli*; **b** *S. aureus*; **c** *P. desmolyticum*

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