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Predicting the Characteristics of Copper Oxide Nanoparticles using Nyctanthes Arbor-tristis Flower Extract via Antibacterial Study

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Abstract

Copper oxide (CnO) is an inorganic compound with monoclinic crystal structure. CuO nano particles attracted considerable attention due to its numerous applications in the field of optical, catalytic, mechanical, organic dye degeneration, biomedicine, pharmaceutics, cosmetics and different medical purposes. In the present study, copper oxide nanoparticles have been prepared by biological method using the flower extracts of Nyctanthes arbor- tristis as a reducing agent. The resulting samples were characterized using X-ray diffraction (XRD). Scanning Electron Microscopy (SEM). Fourier Transform Infrared Spectroscopy (FTIR), Energy Dispersive analysis of X-rays (EDX), UV-Visible Spectroscopy (UV) and Antibacterial activity. From XRD studies, the average crystalline size of the obtained sample was calculated by Debye-Scherrer formula and it was found to be 33.13 mn. The band gap energy of the synthesized nano panicle was estimated from UV studies and its value is 1.19 eV. The morphological characteristics were absorbed by SEM studies. The EDX and FTIR studies confirm the presence of Copper Oxide nano particles. The antibacterial activity of CuO-nano panicles on selected bacteria was done using agar diffusion method.

Keywords: XRD. FTIR. EDX, FTIR, Antibacterial, UV, CuO nanoparticles.

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1. Introduction

Nanotechnology is the manipulation of matter to create useful functional material devices by controlling the shape and size at the nanometer scale.[1] Nanotechnology includes the synthesis, characterization, exploration and utilization of nano structured materials which encompasses the production and application of physical, chemical and biological systems .[2] Nanoscience culminates in the development of safe ecofriendly nanoparticles.[3] Green synthesis methods are attractive as they are less toxic and environment friendly.[4] In Green synthesis, the biological organisms such as bacteria, yeasts, moles, algae, plant products and molecules in plants and micro organisms such as proteins, enzymes, phenolic compounds, amines, alkaloids and pigments are used as the reducing agent to perform the nanoparticle synthesis[5] with respect to the reaction parameters such solvent, temperature, pressure and pH conditions (acidic, basic and neutral).[6]

Copper Oxide nanoparticles have the high tendency of oxidization, thermodynamical stability, large surface area, penetration contamination power, catalytic activity and optical properties and so on.[7] Copper oxide nano particles found applications in solar energy transformations, high-tech superconductors, efficient antimicrobial agent,[8] organic catalyst,[9] steam reforming,[10], gas sensors[11]] etc.,. So far, bio synthesis of CuO NPs has been reported by plants extract such as solanum hycopersicum,[12] abutilon indicum,[13] Ocimum tenuiflorum,[14] Ocimum scantum,[15] Eichhornia crassipes,[16] etc,.

In my study, Nyctanthes arbor-tristis (night jasmine) flower has been used as it is one of the medicinal turbo in Indian origin like antihelminthie, antimicrobial, antiviral, antiallergic, anti diabatic and anti cancerous.[17] Nyctanthes arbor-tristis flowers are used in the treatement of cancer, scabis, mouth ulcers, gout etc,.[18, 19] The flower extract of Nyctanthes arbor-tristis have been used for the synthesis of zinc, gold, silver and titanium oxide nanoparticles.[20] Hence, Nyctanthes arbor-tristis flower extract is used as a biological reducting agent for the synthesis of copper oxide nanoparticles.[21]

2. Experimental Procedure

2.1 Preparation of Nyctanthes Arbor-tristis Flower Extract

Fresh flowers of Nyctanthes arbor-tristis are collected and washed several times with the distilled water. The flowers were dried without moisture and then finely powered. 5 grams of the powder was mixed with 50 ml of distilled water and boiled at 60°C for half an hour with simultaneous stirring. Then, the extract was cooled and filtered using whatmann.Nol filter paper and then stored in the refrigerator for further uses.

2.2 Preparation of copper nano particles

Aqueous copper (II) nitrate solution was prepared by dissolving 1M of copper (II) nitrate salt in 100 ml of distilled water and stirred for 30minutes. To prepare copper nano particles, the flower extract was added drop by drop to the aqueous solution of copper (II) nitrate hydrate [$Cu(No_3)_2.H_2O$] by stirring at room temperature. The colour change from blue to green indicates the presence of Copper nano particles. Then, NaOH pellets were added by maintaining the pH value of 8 and left for 2 days for ageing. The solution was centrifuged at 10,000 rpm for 5 minutes. The obtained precipitate was dried in the microwave oven for 12 hours at 100°C and a green colour powder was obtained by grinding with mortal pester.

The prepared samples were characterized by X-ray Diffraction (XRD), Scanning Electron Microscope (SEM), Energy Dispersive X-ray Spectroscopy (EDX), Fourier Transform Infrared (FTIR), UV-Vis Absorption and antibacterial studies.

3. Results and Discussion

3.1 X-ray diffraction

The X-ray diffraction is to measure the average spacing between layers or rows of atoms and to determine the orientation of a single crystal, size, shape and internal stress of small crystalline regions. The XR pattern of synthesized Cu nanoparticles using a leaf extract of Nyctanthes arbor-tristis is shown in Fig. 1. The XRD pattern shows a high crystalline-size of Cu sample level with diffraction angles of 12.8°, 25.8° and 33.8° which correspond to the characteristic face centered cubic (FCC) of copper lines indexed at (111), (200) and (222) respectively.[22,23] The size of the NPs obtained were estimated to be 33.13 nm using Debye-Scherrer Equation,

$$D = k\lambda/\beta \cos\theta$$

where, D = Thickness of the nano crystal, K = Constant, λ = Wavelength of X-ray, p = Width at half maxima of reflection at Bragg's angle 20, where θ = Bragg angle. The Table 1 shows the structural parameters of CuO nanoparticles.

3.2 Scanning Electron Microscope

The structure and morphology of the prepared sample were observed using SEM. The SEM images of CuO nanoparticles stabilized by extract prepared using copper nitrate are shown in Fig. 2. The morphology of copper nano particles was found to be irregular rock like structure. Thus, these SEM results confirmed the nanostructure behaviour of the synthesised particles.

3.3 Energy Dispersive X-ray spectroscopy

EDX analytical technique is used for the elemental analysis or chemical characterization of the sample. The presence of copper can be observed in the graph which is obtained from EDX analysis. Fig. 3 shows the peaks of Cu and oxygen. The percentage of composition of Cu and 0 are 29.48% and 70.52% respectively.

2.4 Fourier Transform Infra Red Spectroscopy

The FTIR spectrum results are shown in the Fig. .4. This helps to know the bio-molecules attached to the surface of CuO nanoparticles. The spectrum displays absorption spectra in the range between 3932 cm 1and 428 cm^{-1}. The band obtained at 3932 cm^{-1} specifies O-H stretching and the band at 1350 cm^{-1} and 883 cm^{-1} corresponds to O-H bending and indicates the formation of the CuO

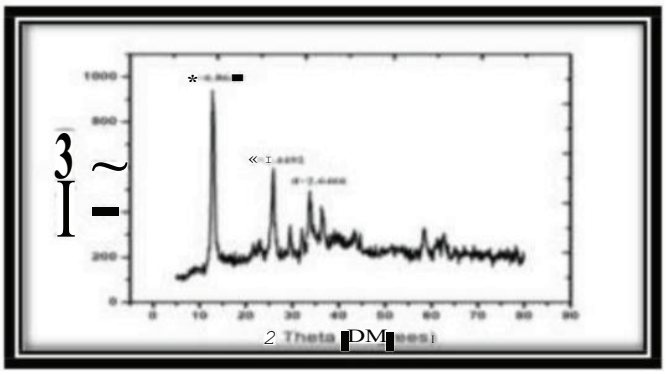

Fig. 1: XRD pattern for CuO nanoparticles

Table 1: Structural parameters of CuO nanoparticles

2θ (deg)	Interplanar distance(d)	Crystalline size ($Dx10^{-8}m$)	Dislocation density ($\delta \times 10^{15}$)	Micro-strain ($\Sigma \times 10-3$)
12.8859	6.8645	1.0952	8.3364	3.3055
25.8085	3.4492	1.0809	8.5585	3.3493
33.4820	2.6466	1.1375	7.7275	3.1825

nanostructure. The appearance, group and compound class of the corresponding absorption bands are indicated in the Table 4.

2.5 Ultra Violet-Visible Spectral Analysis

The optical properties of the prepared copper oxide nanoparticles were characterized by UV-Visible spectral analysis. The CuO nanoparticles were analyzed by UV-Visible spectrophotometer from the absorption range of 200-1200 nm with the maximum absorption at 237nm is shown in the Fig. 5. Using the lambda maxima of synthesized copper oxide, the band gap energy of copper oxide nanoparticles sample was calculated and found to be 1.19 eV.

2.6 Antibacterial Activity

The antibacterial activity of CuO nanoparticles synthesised using Nyctanthes arbor tristis flower extract were tested by well diffusion method through the zone of inhibition. Liquid Mueller Hinton agar media and the Petri plates were sterilized by autoclaving at 121°C for about 30 minutes at 15 lbs pressure. Under aseptic conditions in the laminar airflow chamber, about 20 ml of the agar medium was dispensed into each Petri plate to yield a uniform depth of 4 mm. After solidification of the media, 18 hrs culture of Gram positive microorganisms such as *Bacillus cereus (MTCC 430), Staphylococcus aureus (MTCC 3160),* Gram negative microorganisms such as *E.coli (MTCC 1698)* and *Klebsiella pneumoniae (MTCC10309)* obtained from IMTECH, Chandigarh were swabbed on the surface of the agar plates. Well was prepared by using cork borer followed with loading of 50 pi and 100 pi of each sample to the distinct well with sterile distilled water as negative control and gentamycin (30mcg/disc) as positive control. The sample loaded plates were then incubated at 37°Cc for 24 hours to observe the zone of inhibition. The CuO nanoparticles are easily enter the nucleus of the bacteria and provides an surface area for interactions that hinders growth mechanism.

4. Conclusion

The present synthesis is capable of producing copper oxide nanoparticles via Green method and to demonstrate

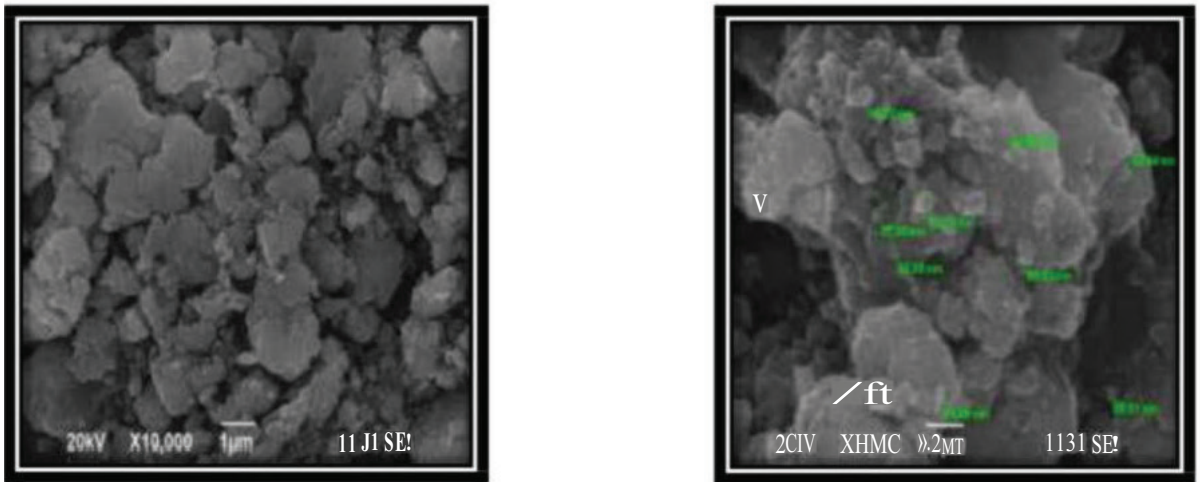

Fig. 2: SEM images of CuO nanoparticles

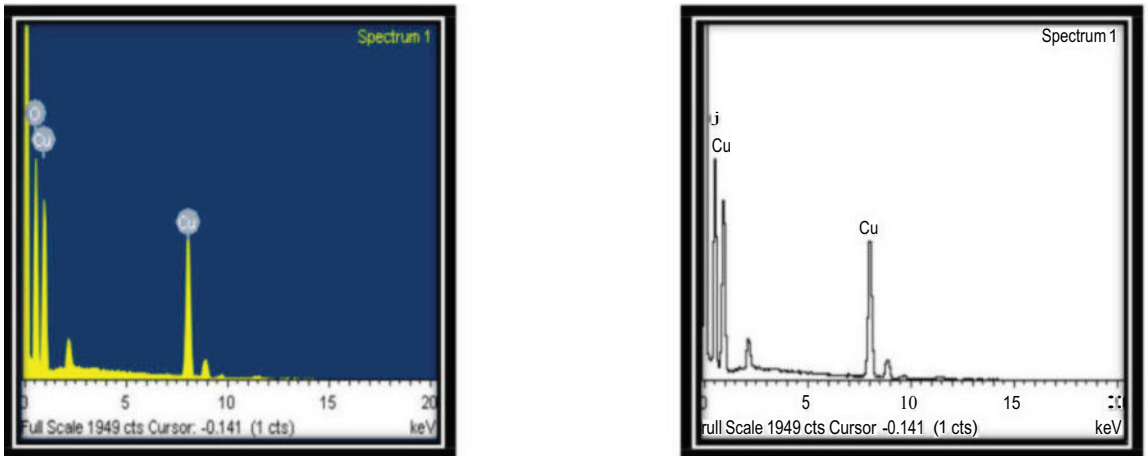

Fig. 3: EDX of CuO nanoparticles

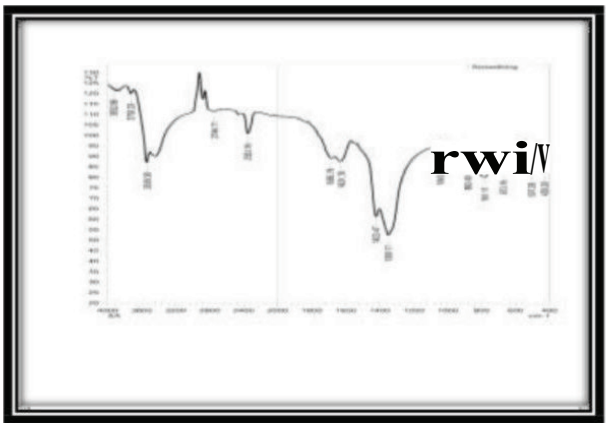

Fig. 4: FTIR of CuO nanoparticle

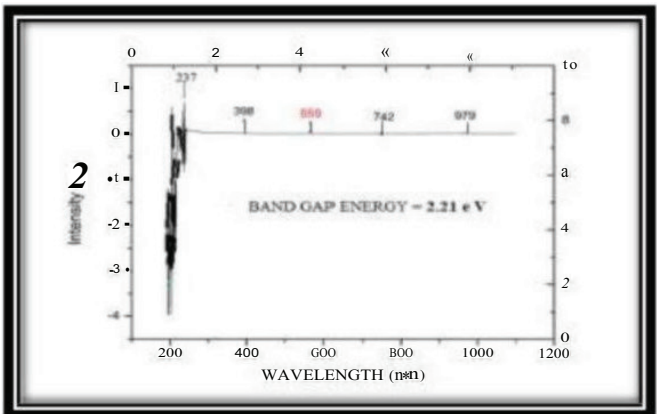

Fig. 5: UV-Visible spectrum of CuO nanoparticles

Table 4: FTIR of CuO nanoparticles

Absorption cm^{-1}	Appearance	Group	Compound class
3932.86	Medium, sharp	O-H stretching	Alcohol
2744.71	Medium	C-H stretching	Aldehyde
1685.79	Weak	C-H stretching	Aromatic compound
1350.17	Medium	O-H bending	Alcohol
883.40	Strong	O- H bending	1, 3 disubstituted

Fig. 6: Zone of inhibition against (A) Eschericia coli (B) Bacillius cereus (C) Staphylococcus aureus (D) Klebisella pneumoniae.

the importance of environmental friendly preparation of nanoparticles. Thus, the study confirms that the copper oxide nanoparticles could be prepared by using Nyctanthes arbor-tristis flower extract. The XRD pattern indicates nano crystalline size of the particles that was found to be 33.13 nm. The SEM analysis shows the irregular rock structure of the prepared CuO nanoparticles. The EDX indicates the compositions of CuO nanoparticles i.e., 29.48% of Cu-content and 70.52% of O-content. The FTIR shows the corresponding functional groups of synthesized CuO nanoparticles. The optical properties of the prepared CuO nanoparticles were characterized by UV-Visible spectral analysis and the band

Table 6: Antibacterial activity of CuO nanoparticle

S. No	Micro organisms	Zone of inhibition in Diameter (mm)			Std. Antibiotic (Gentamycin) 30mcg/disc
		Control 100 pi	50pi	100 pi	
1	Bacillus cereus	Nil	24	28	30
2	Staphylococcus aureus	Nil	27	31	36
3	Escherichia coli	Nil	28	29	29
4	Klebisella pneumoniae	Nil	21	30	32

gap energy was calculated as 1.19 eV. The antibacterial activity of CuO nanoparticles against pathogenic bacteria such as Escherichia coli, Klebsiella pneumonia, Bacillus cereus and Staphylococcus aureus shows the highest inhibition at lowest concentration against pathogenic bacteria. The studies concluded that the synthesis of copper oxide nanoparticles using plant extracts is more beneficial as it is an economical, energy efficient, low cost and environment-friendly process than the bio hazardous chemical synthesis.

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