

Green synthesis with antibacterial investigation of Copper nanoparticles using *Azadirachta indica* (neem) leaf extract

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ABSTRACT

In the present work, green mediated synthesis of copper nanoparticles has wide interest due to its inherent features such as eco-friendliness and low costs. The resulting copper nanoparticles were characterized by X-ray diffraction (XRD) to study the crystalline nature of the synthesised copper nanoparticles. The functional group of the copper nanoparticles was analysed by Fourier transform infrared spectroscopy (FTIR) and energy dispersive spectroscopy (EDAX) was used to detect the presence of elemental copper. The antibacterial activity against Gram positive microorganisms such as *Bacillus cereus* and *Staphylococcus aureus*, Gram negative microorganisms such as *E.coli* and *Klebsiella pneumonia* were studied. The zone of inhibition reveals the good antibacterial activity against these human pathogenic bacteria.

Keywords: Azadirachta indica, Copper Nanoparticles, XRD, FTIR, EDAX, Antibacterial activity

I. INTRODUCTION

In the current research, the green synthesis of nanomaterials using various parts of plant extracts is a growing field in Nanoscience, with accent to avoid the usage of toxic chemicals. It is an ecofriendly technique for synthesis of nanomaterials because of their unique properties compared to the bulk materials. In the green synthesis method, the plant extract used in the synthesis of nanoparticles act as both reducing and capping agents[1].The plant mediated green synthesis of nanoparticles is relatively fast and cost effective technique.

Copper is an essential element for human nutrition. Copper sulphate is a commonly used germicide and antiseptic agent [2]. Copper sulphate also inhibits

bacterial growth such as Escherichia coli. Neem, being a purifying agent, clears toxins from the blood. The amazing health benefit of neem cures acne and skin irritations. It controls the formation of free radicals[3]. It heals the skin infected by strong diseases like chicken pox and small pox when treated with neem tree paste due to antibacterial properties.

II. METHODS AND MATERIAL

PREPARATION OF NEEM EXTRACT

The Azadirachta indica (neem) leaves were collected from in and around Erode District. About 100g of the collected leaves are thoroughly washed with water and cut into pieces. Then it was boiled with 100 ml of deionized water for 15 minutes at 80°C. The resulting product was filtered using Whatmann's No.1 filter

paper. The solution was stored in cleaned container and kept in refrigerator for further use.

SYNTHESIS OF COPPER NANOPARTICLES

The Copper nanoparticles are synthesized by addition of, 20 ml of Azadirachta indica leaf extract with 100 ml of 1.2M aqueous $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution in a 250 ml beaker. The solution colour changes from sea blue to green this colour changes indicates reduction of copper nanoparticles. The beaker was then kept overnight at room temperature for aging. The Cu nanoparticles solution thus obtained was purified by repeated centrifugation at 12,000 RPM for 15 min. Then the Cu nanoparticles were dried in oven at 80°C . The dried powder was further calcinated in muffle furnace at 400°C . The synthesis flowchart is shown in Fig. 1.

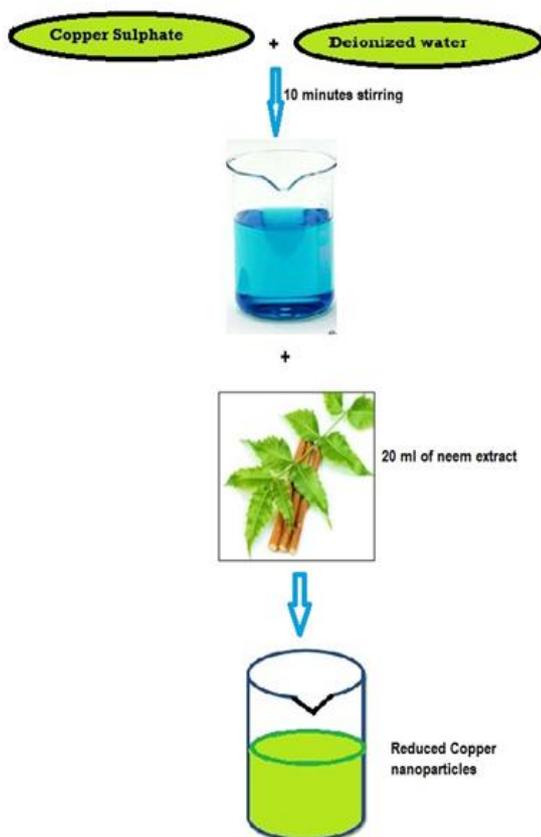


Fig. 1 Synthesis flowchart

III. RESULTS AND DISCUSSION

A) XRD ANALYSIS

The crystallography analysis was carried out by X-ray diffraction method. X-ray diffraction pattern of the prepared Cu nanoparticles is shown in the Fig. 2. The straight line and sharp peaks shows that the synthesized powder containing crystalline nature[4-6]. The average crystalline size is determined using Scherer formula,

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

Where,

D- Crystallite size of nanometer,

λ - Wavelength of the monochromatic X-ray beam (0.1540nm)

β - Full width at the half maximum for the diffraction,

θ - Diffraction angle (deg).

The characteristics peaks of apatite can be identified in the range of 2θ , using the Scherer formula. The micro strain can be calculated from the following equation

$$E_{\text{Strian}} = \beta / 4 \tan\theta$$

Where,

β - Width half maximum of the peak in radians,

θ - Diffracted angle of X-ray pattern.

The dislocation density can be calculated

$$\delta = 1/D^2$$

Where,

D- Particle size of the sample.

The average crystalline size of Cu nanoparticles synthesised using neem is 21.655 nm.

2θ (deg)	d (Å)	FWHM β (deg)	Crystalline Size D (nm)	Micror Strain (m)	Dislocation Density (δ) ×10 ¹⁵ m
35.26	2.52	0.6919	24.098	0.1162	1.722
31.91	2.8	0.7375	22.4098	0.3225	1.991
18.34	4.8	0.8733	18.4566	0.6754	2.935

Tabulation: 1

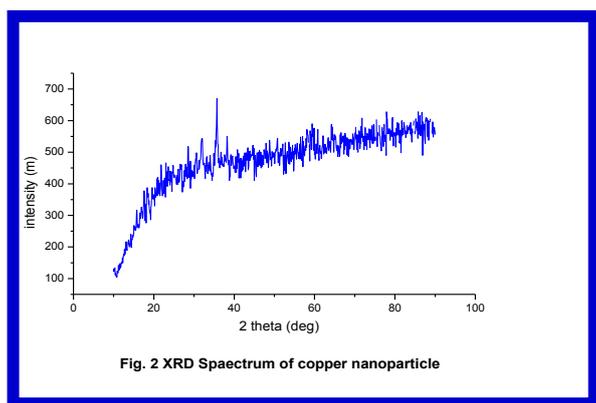


Fig. 2 XRD Spaectrum of copper nanoparticle

B) FTIR ANALYSIS

The FTIR spectra of copper nanoparticles synthesized by green synthesis method using Azadirachta indica leaf extract shown in the Fig. 3. The IR spectrum of synthesized copper nanoparticles shows band at 1618 cm⁻¹, 787.93cm⁻¹ corresponds to presence of alkene, aromatic ring phenyl group[7]. The peak at 1618 cm⁻¹ indicates the presence of copper nanoparticles[8] and is listed in tabulation: 2.

WAVELENGTH (Cm ⁻¹)	STRETCHING
1618	Presence of Carbonyl group
655.28	Presence of C-Cl group
596.49	Presence of C-Br group

Tabulation: 2

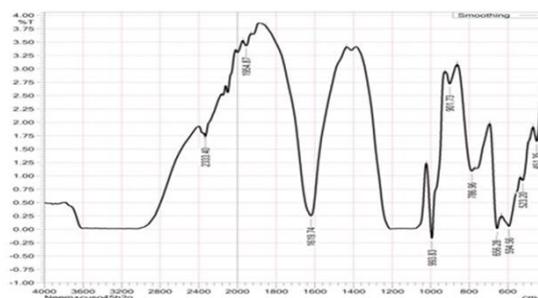


Fig. 3 FTIR Spectrum of Copper nanoparticles

C) EDAX ANALYSIS

The elemental composition analysis of copper nanoparticles reveals the presence of Cu, O, S. EDAX spectrum of copper sulphate+neem extract is shown in Fig. 4 confirms the presence of copper nanomaterial.

D) ANTIBACTERIAL ACTIVITY

The copper nanoparticles synthesized using neem extract is tested for antimicrobial activity by well diffusion method[9-12]. Liquid nutrient 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 20ml of the agar medium was dispensed into each Petri plate to yield a uniform depth of 4mm[13]. After solidification of the media, 24 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 pneumonia* (MTCC10309) obtained from IMTECH, Chandigarh were swabbed on the surface of the agar plates[14-15]. Well was prepared by using cork borer followed with loading of 100 µl of each sample to the distinct well with sterile distilled water as negative control and Tetracycline (30mcg/disc) as positive control[16-17]. The sample loaded plates were then incubated at 37°C for 24 hours to observe the zone of inhibition shown in Fig. 5(a, b, c, d). The zone of inhibition of synthesised copper nanoparticles against *Bacillus cereus*, *Staphylococcus aureus*, *E.coli* and *Klebsiella pneumonia* is listed in tabulation: 3.

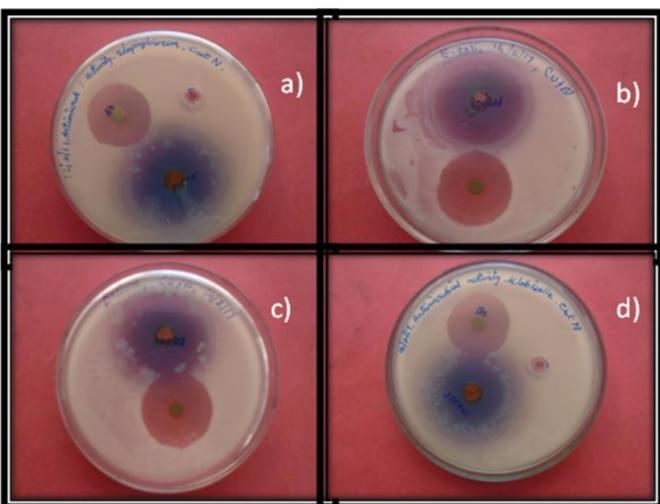
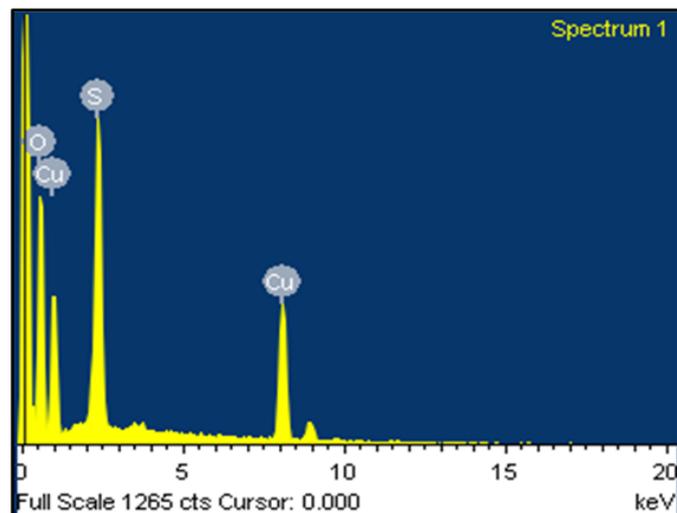


Fig.5 Zone of inhibition against a) *Staphylococcus aureus* b) *E.coli* c) *Bacillus cereus* & d) *Klebsiella pneumoniae* microorganisms

Fig.

4 EDAX Spectrum of Copper Nanoparticles

S. No.	Micro organisms	Zone of Inhibition in Diameter (mm)		Std. Antibiotic (Tetracycline) 30mcg/disc
		Contro l	Copper nano particles	
		100 µl		
1	<i>Bacillus cereus</i>	Nil	35	32
2	<i>Staphylococcus aureus</i>	Nil	35	28
3	<i>Escherichia coli</i>	Nil	39	29
4	<i>Klebsiella pneumoniae</i>	Nil	30	28

Tabulation : 3

IV. CONCLUSION

Copper nanoparticles were successfully prepared by green synthesis method using neem extract. The XRD spectrum reveals that the synthesised copper nanoparticles using neem extract have an average crystalline size of 21.6548 nm. The FTIR spectrum confirms the presence of copper nanoparticles at the peak 1619 cm⁻¹ and presence of C-Cl stretching, C-Br stretching, phenyl, aromatic bending groups respectively. The EDAX exhibits the elemental composition of CuNPs. The spectrum reveals the presence of copper nanomaterial. The antibacterial activity study confirms that CuNPs synthesised using neem have stronger inhibition against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. This green synthesis method is carried

out by easily available starting materials, inexpensive to carry out in any laboratory, use of toxic reagent is avoided and pollution free.

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