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Antimicrobial Activity Of Copper Oxide Nanoparticles Synthesised Using Artocarpus Heterophyllus Leaf Extract

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Copper-based nanomaterials can be used in conductive films, nanofluids, catalysis, gas sensors, magnetic storage media, batteries and semiconductors and also as persuasive antimicrobial agents. The present study outlines the development of a method to synthesize copper oxide nanoparticles (CuONPs) using Artocarpus Heterophyllus leaf extract and calcinated at 400°C which is eco-friendly and non-toxic. The XRD graph showed the good crystalline nature of CuO nanoparticles. The physical interaction of macromolecules with Copper Oxide nanoparticles and the formation of nanoparticles if confirmed by UV-Vis Spectroscopy. The average crystalline size around 43.69 nm was observed by the Debye–Scherrer formula. The antibacterial activity of CuONPs was determined by Kirby-Bauer disk diffusion method against E.coli. It was reported that the synthesized CuONPs demonstrated a significant inhibitory activity against Escherichia coli.

Keywords: Green synthesis, leaf extract, XRD, UV-Vis, Antibacterial

INTRODUCTION

Nanostructure materials have attracted a great deal of attention because of their physical, chemical, electronic and magnetic properties show dramatic change from higher dimensional counterparts and depends on their shape and size. Many techniques have been developed to synthesize and fabricate nanostructure materials with controlled shape, size, dimensionality and structure. The performance of materials depends on their properties. The properties in tern depend on the atomic structure, composition, microstructure, defects and interfaces which are controlled by thermodynamics and kinetics of the synthesis. In general, the synthesis of nanomaterials can be carried out by bottom up and top-down method of integration in which the components spontaneously assemble typically by bouncing around in a solution or gas phase until a stable structure of minimum energy is reached. Self-assembly is important to nanotechnology and is thus a promising method for assembling automatically precise devices.[1-6]

Green synthesis aims to promote innovative chemical technologies to reduce or eliminate the use and production of hazardous substances in the design, manufacture, and use of chemical

products. This involves minimizing or, if possible, eliminating the pollution produced in the synthesis processes, avoiding the consumption and wastage of nonrenewable raw materials, using hazardous or polluting materials in product manufacturing, and reducing the synthesis time. Paul J. Anastas, considered the father of green chemistry, defined it as "a work philosophy that involves the use of alternative tools and pathways to prevent pollution," referring to both the design of the synthetic strategy and the treatment of possible secondary products originating from that route. The use of plant species, algae, or microorganisms such as bacteria or fungi is one of the most commonly used resources for this procedure. Various compounds from plants or microorganisms, including terpenes, polyphenols, alkaloids, carbohydrates, proteins, and genetic materials, play an important role in the synthesis of nanoparticles by acting together [7-10].

In the present studies, an attempt has been made to prepare copper oxide nanoparticles using Artocarpus herterophyllus leaves extract by green synthesis method. The prepared copper oxide nanoparticles were subjected to the structural, optical and antibacterial analysis.

MATERIALS AND METHOD

Copper (II) nitrate trihydrate, deionized water, ethanol was used in the present work for the synthesis of nanoparticles

The extract was prepared by collecting the selected Artocarpus Heterophyllus and washed in running tap water and then rinsed with distilled water for the removal of impurities. 500g of leaves were chopped into small pieces with a sterile knife and then boiled for 10 min at 600 ºC filtered using Whatman's no. 1 filter paper. The filtrate was collected in clean, dry 250ml conical flask and stored in refrigerator for further use [11].

Add approximate amount of Copper II nitrate trihydrate in 100 ml of extract and then stirrer for 2.30 hours for the temperature of 40-600 ºC. Colour change to dark brown and the solution was left one day for aging process. Then the solution is certifuge and the precipitate is filter out using A-filter paper. It was dried out and precipitate of copper nanoparticles was collected. Removal of residual organics and the stabilization of the materials were carried out by calcinations for 2hrs, at 400^0C. Then compound was grounded and ready for analysis [12].

RESULTS AND DISCUSSION

XRD Studies

X-ray diffraction is a technique mainly used to establish orientation and crystalline nature of the CuO nanoparticles which gives information on translational symmetry size and shape of the unit cell from peak positions. Figure 1 represents the powder x-ray diffraction pattern for the synthesized CuO NPs. The peaks standing state for 2Θ values of 35.37, 38.55, 48.63,61.34 and 67.91 are recorded as (1 0 -1), (1 1 1), (2 0 2), (1 1 -3) and (1 1 3) levels which are in good arrangement of CuO particles which affirmed the fabrication of crystalline structure. It was observed that all the corresponding peak agreed with the database of the Joint Committee on Powder Diffraction Standards (JCPDS CardNo. 48-1548) [13].

The average crystallite size calculated by Scherrer formula,

$$D = \frac{0.9\lambda}{\beta \mathrm{Cos}\theta}$$

Where

λ = wavelength of X-ray radiation

β = full width at half maximum (FWHM) of the peaks at the diffracting angles Θ

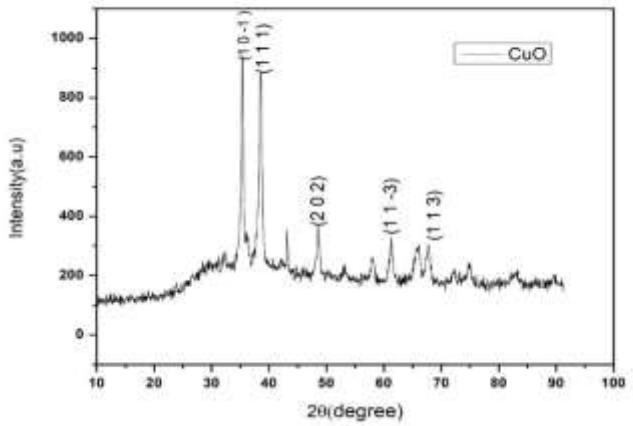
Θ = Angle of Diffraction

The Average crystalline size of CuO nanoparticles are calculated from different peaks using Scherrer Formula is 43.69 nm.

Table 1 Crystalline size of the prepared sample for the prominent peaks

Sample	2Θ	FWHM	D (nm)	Average size (nm)
CuO	35.3743	0.167	49.949	43.69
	38.5515	0.205	41.068	
	48.6340	0.19	45.904	
	61.3438	0.193	47.898	
	67.9094	0.285	33.619	

Fig 1 X-Ray diffraction pattern of synthesized CuO nanoparticles



By applying Braggs Law Inter-planar distance (d-spacing) have been calculated. The values of d-spacing calculated for the synthesis of CuO nanoparticles shown in Table. These values agrees with the standard values form JCPDS file.

Table 2 The Calculated values of d-spacing for the synthesis CuO Nanoparticles

Sample	d spacing(A°)		Miller Indices (h k l)
	Observed	Standard	
CuO		2.530	(1 0 -1)

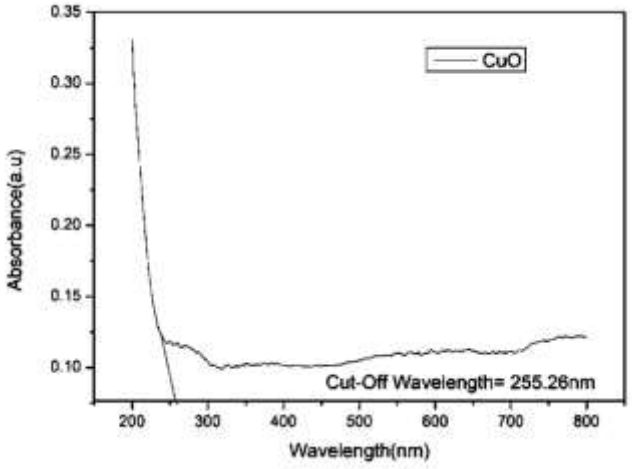
	2.535 2.333 1.418 1.870 1.510 2.093 1.379	2.324 1.410 1.867 1.505 2.324 1.379	(1 1 1) (2 0 0) (2 0 2) (1 1 -3) (0 2 2) (1 1 3)

UV Vis SPECTRAL STUDIES

The absorption edge is found so that the type of transition and bandgap value can be determined. The absorption spectra were used to study the energy band and the type of electronic transition. Absorption spectra of Cuo nanoparticle can be shown in the fig 2 which shows a strong fundamental absorption edge approximately due to direct transition of electrons. Optical absorption shows that the direct bandgap compared to indirect band gap permits us to determine the crystallinity of a material. The functional relationship αhυ and photon energy for CuO nanoparticles is presented in fig 3. The E_g can be obtained by extrapolating the linear portion to the photon axis. If the direct bandgap is higher that the indirect bandgap, the material will be crystalline. The calculated direct band gap value is 4.86eV which is higher than the bulk band gap value (3.51eV). Hence only the direct transition relation absorption as observed and there was no indirect transition absorption peak (fig 3).

The observed increasing band gap could be described to the presence of intragap states and quantum confinement effect.

Fig 2 Absorption of CuO Nanoparticles



The spectra indicates that the CuO nanomaterials have minimum absorption in the entire region. The intensity of absorption completely disappears at particular critical wavelength. That critical point is called Cut-off wavelength. The Cut-Off wavelength of CuO nanomaterial is 255.26nm and it indicates π-π^* transition shown in fig 2.

Optical Band Gap Energy Spectra

The graph is plotted between photon energy hυ and $(\alpha h\upsilon)^2$. By extra pointing the linear portion of a curve to absorption coeffiecient (α) becomes zero as shown in fig 3.

According to planck's equation, the optical band gap energy of CuO nanoparticle was related as, Eg = hc/eλ, (eV)

Where, h – plancks constant (6.626×10^{-34} J s)

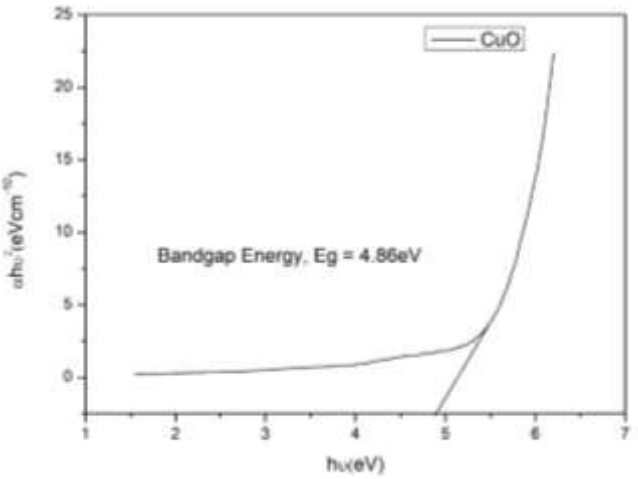
C – velocity of light in the vacuum (3×10^8 m/s)

λ – Low Cut-Off Wavelength (255.25nm)

e – Charge of electron (1.602×10^{-19} C)

The calculated band gap energy of CuO nanoparticle energy was 4.86eV.

Fig 3 Tauc Plot for CuO Nanoparticle



The optical band gap energy of the synthesized material was estimated by using Tauc's relation by plotting $(\alpha h\upsilon)^2$ vs hυ as shown in fig.3. From the graph optical energy band gap of CuO is found to be 4.86eV. The material having lower band gap energy and lower absorbance and is useful in the field of optoelectronic and biomedical applications.

ANTIMICROBIAL ACTIVITY

The application of nanoparticles as antimicrobial agents is gaining importance in the field of biology. The copper oxide nanoparticles have been synthesized and tested for various applications in medicine. Nanoparticles are increasingly used to target bacteria as an alternative to antibiotics. Nanotechnology may be particularly advantageous in treating bacterial infections. Examples include the utilization of nanoparticles in antibacterial coatings for implantable devices and medicinal materials to prevent infection and promote wound healing, in antibiotic delivery systems to treat disease, in bacterial detection systems to generate microbial diagnostics, and in antibacterial vaccines to control bacterial infections.

Microbial sensitivity to nanostructures has been found to be extremely different, depending on the microbial species and on the experimental set-up. Different methods that have been used

to test the antimicrobial activity of copper nanomaterials are itemized as follows: disc diffusion test also defined zone of inhibition (ZoI), minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and counting the number of colony-forming units.
MIC is defined as the lowest concentration of a material that inhibits the growth of an organism; while MBC is defined as the lowest concentration of a material that inhibits the growth of an organism in batch cultures, this can be determined from broth dilution MIC tests by subculturing to agar media without antibiotics.
Hence, a quantitative comparison of the bioactivity effects of Cu-nano-antimicrobials is not possible in all cases, since the antimicrobial effectiveness was studied using different experimental parameters, such as methods, time of contact, and microorganism strain, as well as its initial concentration.
The antimicrobial tests were conducted according to NCCLS, 1993 (National Committee for Clinical Laboratory Standards. (1993a). Performance standards for Antimicrobial Disk Susceptibility Tests. The antimicrobial activity was determined by agar well diffusion method. The principle of this method is that the antimicrobials existing in the sample are permitted to spread out into the medium and these antimicrobials interact in the plate sowed with the test organisms. The resultant zones of inhibition against the organisms will be fairly circular since there will be a convergent lawn of growth. The diameter of the resulting zone of inhibition in this method can be measured in millimeters [14-16].

Antibacterial Test

Muller Hinton Agar Medium (MHI Agar Media) was used for bacterial culture. The medium for the antibacterial test was prepared by liquifying 33.8 g of the commercially available Muller Hinton agar medium in 1000 ml distilled water. The dissolved medium thus obtained was steamed at 15 lbs pressure at 121° C for 15 min. Then the steamed medium was blended well and drained onto the 100 mm Petri plates (20–30 ml/plate) while molten and these Petri plates consisting of 20 ml MHA medium were sowed with bacterial culture of E. coli, P. aeruginosa, K. pneumonia, S. mutans and S. aureus (growth of culture adjusted according to McFarland Standard, 0.5%). Then wells of 10 mm were drilled and varied concentrations of the sample such as 1.0 mg/ml were added. DMSO (10%) was used as the solvent for sample preparation. Then the plates were set at 37°C for 24 h. The antibacterial capacity was determined by estimating the diameter of the inhibition zone created around the well. In the antibacterial test, streptomycin was taken as a positive control.
Bactericidal Concentration (MBC) tests were done using the CLSI protocol (CLSI Methods for determining bactericidal activity of antimicrobial agents. Approved guideline, NCCLS document M26-A, CLSI, 950, Wayne, USA). The materials required for the MBC test were the nutrient broth and Muller Hinton Agar (MHA) plates. The medium was made by liquifying 38 g of the MHA medium in 1000 ml distilled water. The liquified medium was steamed at 15 lbs at 121 C for 15 min. After steaming, the media (20 ml) was allowed to cool to 60 C and was poured to pre-sterilized Petri plates. Then the plates were permitted to solidify in a laminar airflow chamber. The nutrient broth of 23 g was dissolved in 1000 ml of distilled water and was steamed at 121 C, 15 lbs for 15 min. The minimum bactericidal activity was determined against K. pneumonia. The initial steps were done as in the MIC protocol. The sample concentration for the MBC test were 0.0625 mg, 0.125 mg, 0.250 mg, 0.5 mg, 1.0 mg, 1.5 mg

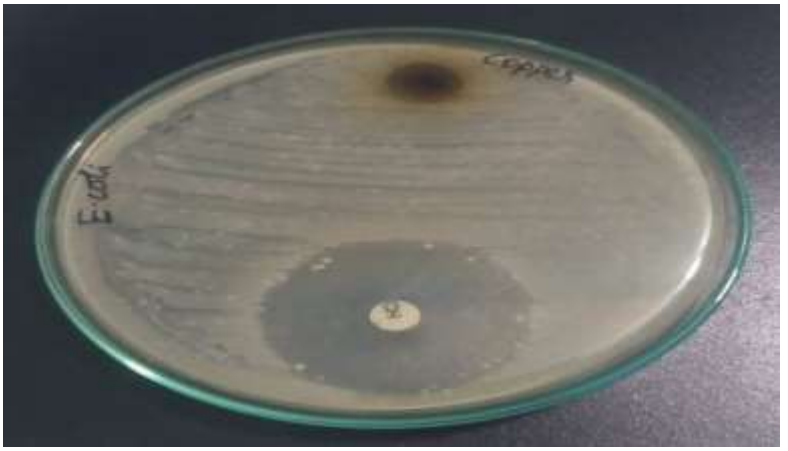
and 2.0 mg. After 24 h of incubation, 20 µl from each well was swabbed onto MHA plates; the constituents of the well were not shaken before the removal of the definite volumes. The test plates were then incubated at 37 C for 48 h. After incubation, the test plates were observed for the survival of colony-forming units (CFU).

Bacteria are a large group of single-celled prokaryote microorganisms. There are two different types of cell wall in bacteria, called Gram-positive and Gram-negative. The names originate from the reaction of cells to the Gram stain, a test long-employed for the classification of bacterial species [17-18].

Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids, while Gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins.

Antibacterial activity

Fig 4 The photograph showing the zone of inhibition by the plant extract against the bacteria E.Coli



The antibacterial activity was examined by standard disk diffusion method against Gram-negative(E.coli) bacteria for CuO NPs synthesized using extract of Artocarpus Heterophyllus leaf. The diameter of inhibition zones (mm)of each well with CuO NPs and Gram negative bacteria by a paper strip is shown in fig 4. Antibacterial results revealed that the synthesized copperoxinanoparticles might be used as excellent antimicrobial activity against a range of bacteria.

Tested Microorganism

Escherichia coli

Escherichia coli are normal flora in the body of human beings and they can be non-pathogenic, commensal or pathogenic. When pathogenic they usually cause urinary tract infections, systematic infections and enteric infections. The development of resistance by Escherichia coli

due to increasing in the use of antimicrobial agents has led to the use of medicinal plants extracts against it. Medicinal plant extracts have shown to have antimicrobial activity against enteropathogenic Escherichia coli found in food material. Traditional products used in food preserving (spices) have antimicrobial activity against multiple antibiotic resistant Escherichia coli isolated from water. Other studies carried out on plants with a medicinal value such as Allium sativum has shown antimicrobial activity against Escherichia coli.

Table 3 Zone of inhibition (in diameter) shown by E.Coli

Name of Microbial Strains	Bacteria	Zone of inhibition	
		CuO	Standard
Gram Negative Bacteria	E.Coli	12	40

CONCLUSION

In the present work, we have successfully synthesized CuO nanoparticle using Artocarpus heterophyllus leaves extract by green synthesis method. Structural, optical properties AMD antibacterial activity of prepared CuO nanoparticles was studied. It is concluded that the synthesis of copper oxide nanoparticles by a green approach using Artocarpus heterophyllus extract is inexpensive, very easy to carry out in any laboratory and also nontoxic. XRD analysis confirmed the crystalline structure of CuO nanoparticles and investigated the size in the range of 43.69nm. Optical properties of CuO nanoparticles were analysed/classified through UV absorption. The direct band gap of CuO nanoparticles was found to be large. The antibacterial activity of CuNPs was determined by Kirby-Bauer disk diffusion method against E.coli. It was reported that the synthesized CuNPs demonstrated a significant inhibitory activity against Escherichia coli.

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