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Characterization And Antimicrobial Activity of Copper Oxide Nanoparticles Synthesized by Crocus Sativus Extract

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ABSTRACT

This study aimed to evaluate the antimicrobial activity of green synthesized copper-oxide nanoparticles (CuO-NPs) using Crocus sativus. The microorganisms were supplied from Baghdad Teaching Hospital. Using C. sativus, CuO nanoparticles were synthesized in a green manner, and their properties were assessed using UV-visible spectroscopy at the peak of 260 nm. Atomic force microscopy (AFM) was used to measure the particles' size and form, and x-ray technology was used to more precisely quantify their dimensions. diffraction (XRD). The biomolecule and functional group were determined by Fourier transform infrared spectroscopy (FTIR). Different concentrations of nanoparticles (50, 75, and 100 µg/ml) were subjected to the well diffusion method for antibacterial activity testing. The findings indicated that the CuO-NPs had a spherical form and a 0.154 nm size. The average diameters of the AFM pictures were found to be 11.89 nm, 2.62 nm, and 24.55 nm. The following growth zone inhibition values for the various bacteria species used were revealed by the nanoparticles' antimicrobial activity results: Staphylococcus aureus 18 mm, Staphylococcus epidermidis 18 mm, Klebsiella ssp 15 mm, Candida albican 18 mm, and Escherichia coli 17mm.

Keywords: CuO NPs, Saffron, Antimicrobial, Nanoparticles

I. INTRODUCTION

The widespread description of nanotechnology is manipulation of matter on, molecular, super-molecular and atomic scale. Most nanoparticles are made composed of a few hundred atoms with at least one dimension sized from 1 to 100 nm [1]. Nanomaterials' study interest has increased rapidly due to their distinct physical and chemical properties, which differ from those of raw materials, such as but not limited to diffusion, conductivity, electrical resistivity, hardness and strength,

chemical reactive properties, and useful antimicrobial activity [2,3]. Metal oxide nanoparticles have caused the most interest because they are commonly utilized as industrial catalysts, chemical-sensing devices, in medical field, disinfection, as antimicrobial agents, filler substances, opacifiers, catalysts, semiconductor-related industries, and in the creation of microelectronics, sun care and cosmetic [3, 4, 5,6].

Copper oxide (CuO) nanoparticles attracted more study attention due to their simple synthesis process. In a low-cost technique, they can be obtained by employing copper salts such as sulfate, chloride, and others. These nanoparticles can be used in a variety of biomedical fields based on their properties, particularly their anti-tumor and antimicrobial properties [7]. A recent study revealed a sensitive and selective method for detecting a virus that is based on the principle of labeling the antibody (Ab) with the construction of a sandwich compound made of CuO-NPs that binds to the antigen (Ag) of the H1N1 virus [8].

CuO nanoparticles have the ability to inhibit both gram-positive and gram-negative bacteria through a variety of ways. The first mechanism involves copper ions binding to DNA molecules. This connection disrupts the spiral form of DNA by crosslinking the nucleic acid strands. The second mechanism is disrupts the cell membrane by copper and enters the cell that led to kill bacterial cell, or disturbing the key enzymes that are important to the most cellular activities. Third mechanism, the nanoparticles work on the cell membrane directly through the negatively charged molecules' (e.g DNA) attaching to the positively charged copper nanoparticles in cell membrane, leading to disrupt membrane and cause cell lysis [9]. The aim of this investigation was to synthesize copper oxide nanoparticles from Crocus sativus plant extract and evaluate their activity on microorganisms obtained from clinical samples.

II. MATERIALS AND METHODS

Test microorganisms

Pathogenic microorganisms (E. coli, S. aureus, S. epidermidis, and Klebsiella ssp and Candida albicans), supplied from Baghdad Teaching Hospital.

Collection of Crocus sativus petals

Petals of Crocus sativus plant were obtained from Baghdad local markets in October 2022. and confirmed by the Ministry of Health's herbarium.

Preparation of aqueous extract of Crocus sativus

The Crocus plant were collected, cleaned to remove the particles dust, then the dry petals grinded to fine powder by using electrical grinder. 2 g of Crocus petals powder was unsettled with 100 ml of distal water. The mixture was boiled at 80 °C for 10- 15 min using a hot plate stirrer. Then filtration by using filter paper and centrifuged to eliminate any remaining contaminants and produce a clear crimson solution. The extract was cooled and stored in refrigerator at 4°C for further used [10].

Preparation and synthesis of CuO nanoparticles

Copper nitrate Cu (NO3)2 (1.8 g, molar mass = 187.56 g/mol) was mixed with 100 ml of distilled water to generate 1M of copper oxide. The mixture was heated until copper nitrate dissolved, then 10 ml of Crocus extract was added drop by drop to 100 ml of copper nitrate with continuous mixing by a magnetic stirrer at 120 °C for 24 hours. When the color was changed from blue to green. The mixture was allowed to cool to room temperature, then centrifuged for 20 minutes at 3500 rpm. The procedure was done twice, after which the deposit was eliminated and dried at 130°C for 6 hours. The black precipitate that had formed was kept in test tubes to be used in the next experiments [11, 12].

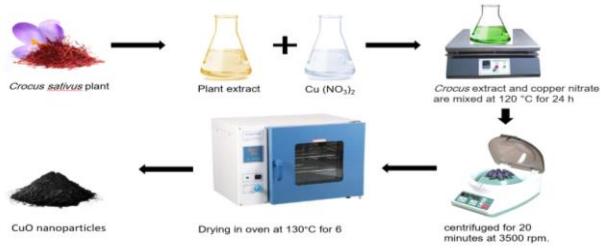

Figure 1. Scheme of Green Synthesis of CuO Nanoparticles from Crocus Plant Extract.

Identification and Characterization of Copper Oxide Nanoparticles:

UV–visible Spectroscopy

A UV-Visible spectrometer of type UV-2610 made in the UK by Biotech Engineering Management Company was used to confirm the synthesis of CuO NPs, a change in color and UV-visible absorption spectrum are used as indicators for synthesis. The absorbance of the solution combination was measured at various time periods, and the maximum absorption was detected by scanning at 200-1100 nm.

Atomic force microscopy (AFM): Microscopy is a cheap tool and a transition that came about when the scanning microscope was created. In this process, camber strengths are mechanically calculated by means of a versatile cantilever between the atomic point of the metal tip and the wall. An electronic activity along the surface can be examined by a scanning tunneling microscope, and the individual atoms can be manipulated. This approach can also be used in the analysis of chemical interactions with other forces, such as magnetic and electrostatic forces. [13]. In order to measure CuO particles' size and form, atomic force microscopy was performed at the University of Technology.

X-ray Diffraction (XRD):

X-ray spectroscopy is an extremely valuable technique for characterizing many different types of materials. XRD peaks are produced by the constructive interference of a monochromatic beam of X-rays spread at particular angles from each set of lattice planes in a sample. The distribution of atoms within the lattice determines the density of peak. As a result, the XRD pattern represents the fingerprint of periodic atomic configurations in a given material [14]. The XRD spectrum of the produced CuO nanoparticles was performed at the University of Technology utilizing XRD (XRD 6000/Shimadzu/Japan) using CuK radiation at = 1.5406 for two values ranging from 20 to 80°. The average particle size (D) of the produced nanoparticles was determined using the Debye-Scherrer equation [15]

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) is commonly used for the chemical analysis of bio-medical samples [16] and the detection of functional groups (active group) present in the sample [17]. For detection of the functional groups on CuO-NPs and identification of their function in the synthesis of copper oxide nanoparticles, FT-IR analysis was performed (FTIR 8400S/Shimadzu, Japan) in the Technology University.

Photoluminescence (PL):

Photoluminescence was applied to analyse the fluorescence characteristics of a material. Using fluorescence spectra, one may ascertain the molecular structure of a material.

III. RESULTS AND DISCUSSION

In the current investigation, biosynthesis of CuO NPs by using Crocus extract as a reducing agent causes change in color from white to blue to green color within a few minutes after mixing figure (2).

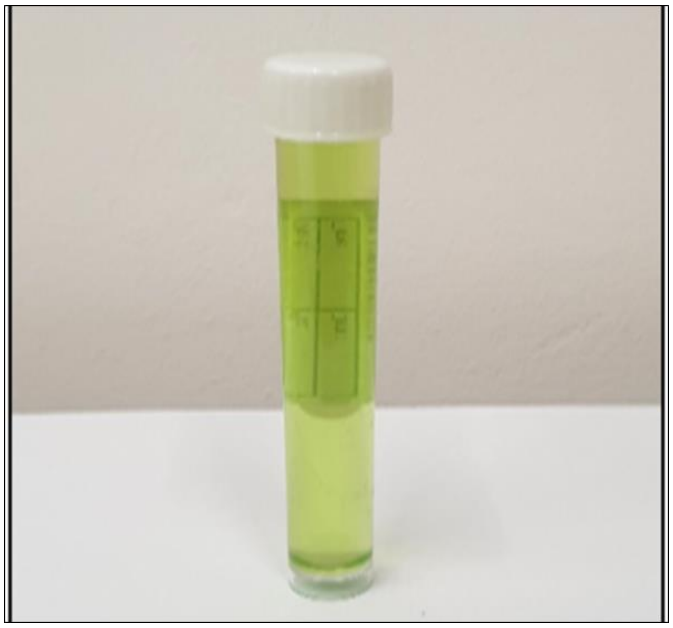

Figure 2. Shows CuO NPs after mixing with saffron extract and the color becomes pale green.

UV–visible Spectroscopy

The presence of nanoparticles of copper oxide was verified using UV-visible spectroscopy. Figure 5 (A) showed the wavelength-dependent absorbance of CuO following its production by green synthesis. The results show that translucence qualities rapidly increase with increasing absorbed particle size width, and are sufficient in the spectral area between 200 and 1100 nm. Furthermore, it has a noticeable peak at 240. The optical absorption properties of Cu-oxide nanoparticles are showed in Figure 5(A); as wavelength increases, the absorption spectra get narrower. A peak with a wavelength of 308 nm was found. This is caused by the formation of copper oxide in solution and is referred to as a Plasmon resonance.

CuO nanoparticles exhibited a high level of transparency in the visible and infrared range, with a maximum absorption of around 1.17 at 200 nm [18]. The graph of (αhv)2 vs photon energy (hv) in Figure 5(B) on the right shows the band gap of copper oxide, which was determined by Tauc's relation to be 3.8 eV. The band gap increases to 4.2, a value that could be related. to the effect of quantum size. The optical spectra of nanostructured objects exhibit a blue shift due to the quantum size effect. The band gap of a bulk semiconductor shifts to a higher energy level due to the quantum size effect [19]. The two energy gaps could be caused by the presence of copper oxide's mono-, binary-, and tri-elements.

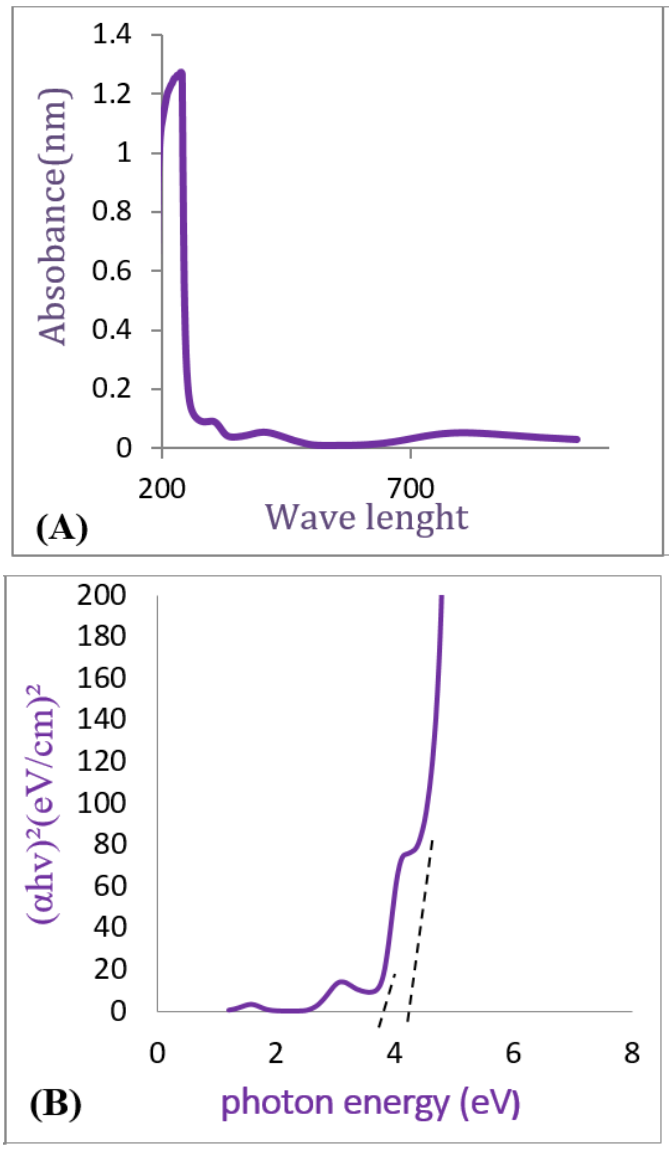

Figure 5. UV–Vis spectra of CuO NPs sample prepared by Crocus plant extract and (B) (αhv)2 versus hv For CuO NPs

Atomic Force Microscope (AFM) Results

A three-dimensional image of a CuO thin film grown at substrate temperature is presented in Figure 3. The grains were uniformly distributed, with each columnar grain reaching upward. According to AFM investigation, the pore's average

grain size was roughly 11.89 nm, with an RMS roughness of 2.62 nm and an average roughness of 24.55 nm, as shown in Table 2.

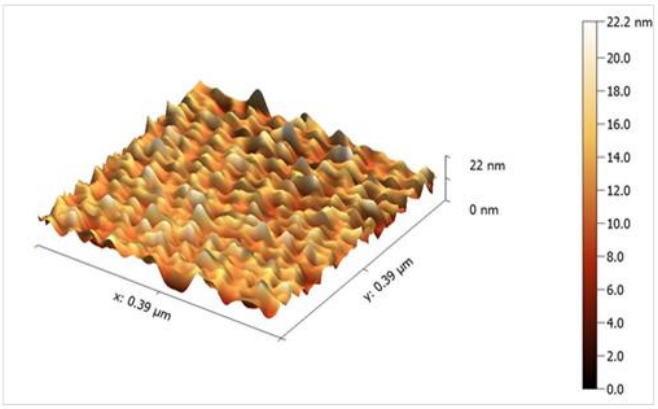

Figure 3 : 3D Image of AFM (Green synthesis)

Table 1. The parameter of copper oxide surface

Average value	11.89 nm
Minimum	0.00 nm
Maximum	24.55nm
Median	11.89 nm
Ra	2.06 nm
Rms	2.62 nm
Rms (grain-wise)	2.62 nm

Structural analysis of CuO and nanoparticles by XRD

XRD analysis can be used to identify metallic Nano-powders. The peak positions of the diffraction pattern (XRD) were used to determine the crystallinity of CuONPs. Copper nanoparticles have incredibly strong diffraction lines, with four distinct peaks detected at 2θ values of 32.49,31.9, 39.18, and 52.95o, respectively, and the values (110), (311), (111), and (220) are Miller indices in Figure 2. The size of the crystallites of the biosynthesized CuO nanostructure was determined using the Debye-Scherrer formula to:

$$D = k\lambda / \beta \cos\theta \quad \ldots\ldots\ldots (1)$$

where D= average crystallite size, K= A constant equal to 0.94, λ = the wavelength of X-ray radiation (0.154 nm).

The kind of the created nanoparticles must be determined by carefully examining the film using X-rays. Table 1 illustrates the different diffraction angles that were observed. The biggest crystal size of Cu was found to be 85.13, while the smallest crystal size of Cu2O was 13.69. Because the Diffraction Peaks are indexed to a hexagonal structure (JCPDS-05-0661 and JCPDS Card Number 45-0937), these matched the regular Peaks Card Number quite well.

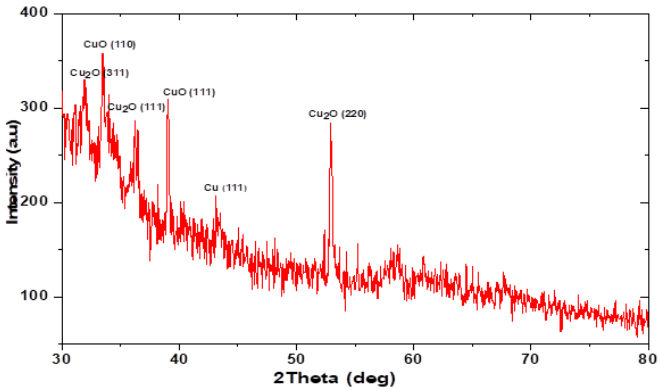

Figure 2: XRD diffraction pattern of CuO nanoparticles prepared from *Crocus Sativus* (Saffron) flower extract

FTIR Spectrum

The FTIR analysis was used to identify the biomolecules and functional groups in the produced CuO NPs.

Figure 4 displays the FTIR spectra of CuO nanoparticles that were biosynthesized. Wide absorption regions between 2750 and 3500 cm-1 can be seen in the FTIR spectrum of CuO NP, which are mostly caused by O-H and C-O groups. It was discovered that the O-H stretching vibration of the surface hydroxyl groups of adsorbed water molecules produced a strong broad band in the 3200–3550 cm^{-1} region [20]. This occurs as a result of materials composed of highly surface-to-volume-ratio nanocrystal lines absorbing a lot of moisture. CuO nanoparticles have a high surface-to-volume ratio nanocrystal line structure, which is responsible for this peak. Because to the stretching vibration of O=C=O, there is a small band at about 2100 cm1. The strong

absorption band at about 1640 cm-1 is caused by the C=C bending vibration and possibly by C-O and Cu(II) of CuO's bidentate ligand coordination [21] The sharp rise at 1350 cm-1 is mostly caused by the C-H bond of an alkane. The peak rises to 667 due to the aromatic bending vibration of the CuO bond. The infrared bands in the locations mentioned above confirm that.

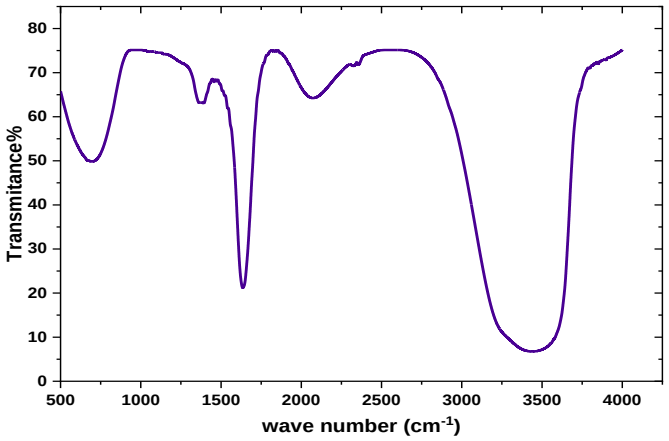

Figure 4. The FTIR spectra of CuO produced using flower extract from Crocus sativus (saffron)

Photoluminescence (PL):

Photoluminescence used to analyse the fluorescence characteristics of material. Using fluorescence spectra, one may ascertain the molecular makeup of a material. An emission spectrum at 320 nm wavelength is detected. Figure (6) illustrates how biosynthesized CuO nanomaterials glow when an energy gap of 3.8 eV is present.

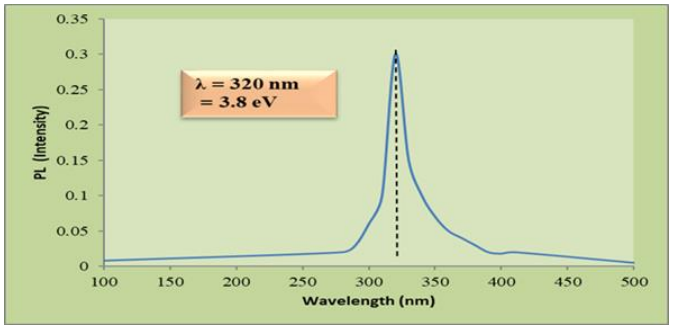

Figure. Photoluminescence spectra of CuO

The presence of phytochemicals (Crocus plant extract) linked to the CuO nanoparticles explains this. Muthuvel *et al.* [22] report that biologically produced. Compared to CuO nanoparticles made chemically, copper oxide nanoparticles have a higher PL intensity.

Antimicrobial activity of (CuO) NPS

In order to determine the antimicrobial activity of biosynthesized CuO nanoparticles against different pathogenic microorganisms, the diffusion method using agar-well was used. This method was applied to four bacteria, including *E. coli*, *S. aureus*, *S. epidermidis*, and *Klebsiella ssp* and one fungus is *Candida albicans*. The results demonstrated in figure (7) shows that the CuO nanoparticles have antibacterial activity against different bacterial species such as *S. aureus* (18 mm), *E. coli* (17 mm), *S. epidermidis* (18 mm), *Klebsiella ssp* (15 mm) and *C. albicans* (18 mm). Also, observed in his research *Pseudomonas fluorescens, Candida albicans*, and *Escherichia coli* are effectively inhibited by copper oxide nanoparticles. On the other hand, [23, 24].

CuO NPs' antibacterial activity is primarily due to their ability to create reactive oxygen species (ROS). ROS is mostly produced by electron transfer by nanoparticle surfaces, which is influenced by the locations of the energy bands. Because of their increased reactivity, ROS damaged the bacterial cell membrane by decomposing its contents (lipids and proteins) into simple byproducts. The ruptured bacterial cell membrane not only failed to sustain the rising K+ ion gradient within the cell but also failed to hold the cellular matrix. A lack of K+ ions in the bacterial cell inhibited vital activities such as respiration and cell division [25].

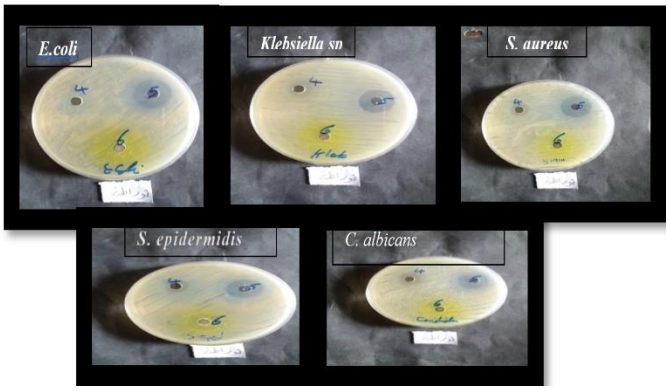

Figure. The antibacterial activity of CuO nanoparticles prepared by Green Synthesis method using C. sativus extract

Table. Antibacterial activity of CuO nanoparticles prepared by Green Synthesis method using *C. sativus* extract

microorganisms	*S. epidermidis*	*S. aureus*	*E.coli*	*Klebsiella sp*	*C. albicans*
Inhibition zone (mm)	18	18	17	15	18

IV.ACKNOWLEDGMENT

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