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Biogenic Synthesis of Copper Oxide Nanoparticles Using *Pimpinella anisum* Seed Extract Characterization and Antibacterial activity

NUHA Y. ELAMIN[1,2]* and AMEL TAHA[3,4]

[1] Department of Chemistry, College of Science, IMSIU (Imam Mohammad Ibn Saud Islamic University), P.O. Box 5701, Riyadh 11432, Kingdom of Saudi Arabia.
[2]Department of Chemistry, Sudan University of Science and Technology, P.O. Box 407, Khartoum 11111, Sudan.
[3]Department of Chemistry, College of Science, King Faisal University Al-Hassa P.O. Box 400 Hufof, 31982, Saudi Arabia.
[4]Department of Chemistry, Faculty of Science and Technology, Al-Neelain University, Khartoum 11121, Sudan.
*Corresponding author E-mail: NYElamin@imamu.edu.sa

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ABSTRACT

Recent attention has been drawn to green methods for preparing metal oxide nanoparticles due to their ease of preparation and environmental friendliness in comparison with chemical and physical methods. By using an Eco-friendly reducing agent, *Pimpinella anisum* seed extract, this study describes a method for synthesizing CuO nanoparticles that are based on biological principles. Copper oxide nanoparticles were produced utilizing a precipitation technique involving *Pimpinella anisum* extract, ($CuCl_2.2H_2O$), and (NaOH). To determine the morphology of CuONPs and their elemental composition, shape, size, and type of bonds present in the sample, UV–Vis spectrophotometry, XRD, SEM, and EDS analysis have all been performed. Spectra of the UV-Visible spectrum showed an absorption peak between 250–300nm, which confirms the formation of CuO nanoparticles. FT-IR spectral showed bands related to CuO vibration at 529 cm^{-1} are, suggesting the production of CuO nanoparticles. Analysis by X-ray diffraction revealed that the CuO nanoparticles produced were monoclinic in phase and had mean size of 22nm. Based on SEM analysis, the obtained nanoparticles have an aspherical shape. EDX analysis was used to confirm copper and oxygen presence. The weight percentage of the latter was (71.2% Cu and 28.8% O), respectively. Four pathogenic microorganisms were used to evaluate the antibacterial efficacy of green-synthesized CuO nanoparticles. As a result, the CuONPs prepared to have the potential for application as antimicrobial agents, suggesting that they may be useful for therapeutic applications in e future.

Keywords: *Pimpinella anisum*, CuO, Biogenic synthesis, Antibacterial.

INTRODUCTION

Nanoparticles metal and metal oxides have gotten considerable consideration from researchers owing to their special visual, conductor, and catalytic characteristics. Due to their prospective uses in various disciplines, such as industrial, medical, pharmaceutical drug delivery, and catalytic applications, scientists have recently placed a significant emphasis on metal nanoparticle synthesis[1,2]. Numerous chemical and physical processes are utilized to produce varied types of metal nanoparticles. However, these techniques are costly, need high pressure and radiation, grand negate hazardous and dangerous by products that are environmentally risky[3]. Hence, there is a need for the development of new, mild, harmless, and a natural product for metal/metal oxides production in an aqueous environment[4–6]. Using herbs attract the of researchers consideration the as most appropriate biological synthesis methods in a comparison with other synthesis methods[7-9]. Due to the wide abundant of the medicinal plants and its costly effective, there is a considerable growth in the synthesis of copper oxide nanoparticles utilizing green chemicals.[10–12]

Copper oxide nanoparticles have attracted interest among numerous metal nanoparticles because they exhibit mechanical and biological features using modern technologies[13]. Copper oxide nanoparticles have been discovered to be particularly effective in gas sensors, photocatalytic applications, and the biomedical sciences for the treatment of numerous disorders[14].

Pimpinanella anisum L. (Apiaceae), is an annual medicinal plant commonly known as anise, it is widely distributed in Europe, Turkey Central Asia, and Egypt. *Pimpinella anisum* is a well-known culinary plant, utilized in the manufacture of renowned liqueurs and also valued for its therapeutic qualities[15]. Anise seed extract is a rich source of flavonoids, phenols, proteins, and numerous B-complex vitamins, such as pyridoxine, niacin, riboflavin, and thiamin, in addition to possessing antioxidant properties[16]. Aqueous and ethanolic extracts of seeds have also been reported to have antibacterial action.[17] In this study, we synthesized and characterized CuO NPs using *Pimpinella anisum* seed extract as a stabilizer and reducing agents.

EXPERIMENTAL

Chemicals

All chemicals ($CuCl_2$ and NaOH) were obtained from Sigma-Aldrich in St. Louis. Agar powder, yeast extract, and Peptone were Purchased from Merk KGaA, Darmstadt, Germany. Pathogenic microorganisms were supplied by the medicine faculty, King Faisal University, Saudi Arabia. The remaining reagents utilized in this study are laboratory-grade. To prepare all sample solutions, deionized water was used.

Preparation of *Pimpinella anisum* extract

In order to create *Pimpinella anisum* extract, 5 g of dried fine seeds were combined with volume of (100 mL) distilled water. The mixture was then brought to a boil for 10 min, a color change of solution was observed from transparent to brown yellow. The mixture was subsequently cooled and filtered using Whatman No. 1 filter paper. The extract was kept at room temperature for future use in. experiments.

Synthesis of copper oxide nanoparticles

In a 250 mL beaker, 0.1 M $CuCl_2 2H_2O$ and 100 mL deionized water are mixed. Specific volume of the extract and copper chloride dihydrate solution were mixed and heated the to 80°C with steady stirring. In a burette, 40 mL of 2 M sodium hydroxide NaOH was drawn to be added drop by drop to the mixture. The mixture color changed rapidly from blue to dark brown. The quick formation of the black precipitate at the bottom of the beaker. Black precipitate was obtained after three washes with deionized water and filter paper Whatman No.1. The washed precipitate was then dried at 120°C for 3 hours. The product was calcined for four hours at 500°C.

Characterization

Fourier-transform infrared spectroscopy (FTIR) Cary (360) California, United States of America, was utilized to conduct FT-IR measurements on sample material. Morphological analysis was preformed using (FE-SEM) technique (FEI, QUANTA FEG, 250 high-resolution field emission scanning electron microscope) equipped with a high-angle, angular darkfield sensor and an X-ray energy dispersive spectroscopy system (EDX) to determine the particle crystallinity and size. To identify the particles crystallinity X-ray diffractometer was used (EMPYREAN via radiation of CuKα at wavelength of 1.54A), the crystalline phases were identified via XRD analysis. BET surface area (BET SSA), used to determine the surface area and porosity. The pore length and volume were calculated using an adsorption-desorption isotherm of nitrogen at 77 K boiling point.

Assessment of the antibacterial inhibitory potentials of CuO nanoparticles

Biosynthesized CuO nanoparticles' antibacterial efficacy was evaluated against Four species of dangerous germs, including two *Gram-negative* bacteria (*E. coli*) and *Salmonella*, and two *Gram-positive* bacteria (*Marsa, Staphylococcus aureus, Bacillus subtilis*). Various concentrations (0, 2, 6, 10, 14, 18, and 22ppm) of produced CuO nanoparticles were employed to investigate the antibacterial activity against harmful microbes. In addition, a comparative assessment was conducted using a commercial amoxicillin concentration of 22ppm. A suspension of nanoparticles was made using (5 mL of the broth of nutrient (NB) medium and CuO nanoparticles) and peptone, yeast extract (10 mL) and NaCl (5 mL)[18,19]. Pathogenic inoculum (100 mL) was introduced into the medium of nutrient broth supplemented with nanoparticles (5 mL) during the logarithmic phase. under sterile environments for microorganisms. The absorbance was carried out spectrophotometrically at 600nm after incubated for 24 h of at 3^0C for all samples.

RESULTS AND DISCUSSION

Recent research has focused heavily on the green production of metal nanoparticles due to their limitless possibilities[4]. The creation of copper oxide nanoparticles was confirmed by UV-Visible spectroscopy and visually noticed as a color shift. When the extract of *Pimpinella anisum* seeds is applied to a solution of copper chloride, a clear change in the color instantly was observed from light blue to a brown[20]. Strong absorbance between 250 and 300nm in Fig. 1 indicates the production of copper oxide nanoparticles. The occurrence of SPR absorption provides information on the nanoparticles' structure and size[13,21].

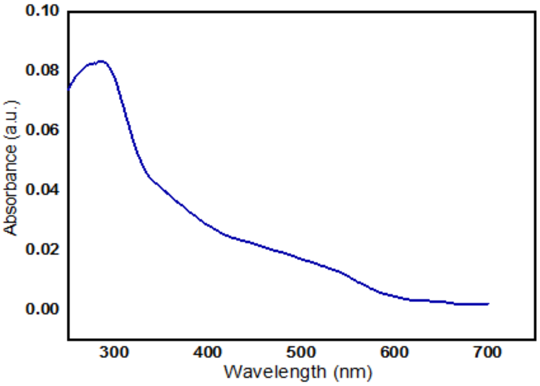

Fig. 1. UV-Vis spectra of green synthesized CuO NPs using extract of *Pimpinella anisum* seeds

To identify the biomolecules in Pimpinella anisum seeds extract used to reduce or synthesize CuO NPs, FTIR analysis was carried out. Fig. 2 shows the FTIR spectrum of green synthesized CuO nanoparticles. The presence of the OH group of absorbed moisture on the copper oxide NPs surface is characterized by the broadband at 3440 cm^{-1}. Stretching vibrations of carboxylic bonds had an absorbed band at 1670 cm^{-1}; additionally, at 1370 cm^{-1} a peak for O-H bending vibrations in combination with Cu atoms was observed, while peaks at 591 cm^{-1} are owing to stretching vibrations of Cu-O[22]. These results showed that the biomolecules of *Pimpinella anisum* seeds extract are responsible for the surface stabilization of CuO NPs.

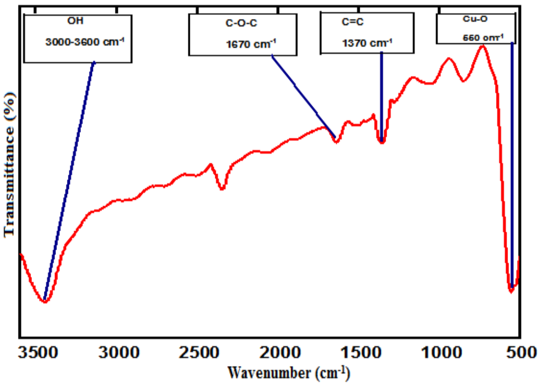

Fig. 2. FTIR spectra of synthesized CuO nanoparticles

As shown in Fig. 3, the morphology of biosynthesized CuO nanoparticles was evaluated using FE-SEM. The synthesized CuO NPs have a size in nanometers with a spherical morphology, consistent with previous studies.[9,12] A SEM image showed that the synthesized nanoparticles were monodispersed and spherical, with a diameter ranging from 46.9-72.8nm.

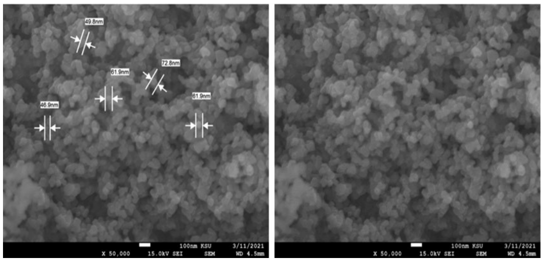

Fig. 3. SEM images of CuO NPs

CuO-NPs were subjected to a 15 keV EDX assay, which revealed the presence of copper (Cu) and oxygen (O). According to EDX analysis, the percentages mass of copper and oxygen were 28.80 and 71.20, respectively. The EDX spectrum did not reveal any further elemental impurities. EDX examination revealed that CuO had a uniform distribution of copper-to-oxygen with a 3:1 atomic ratio. An elemental analysis confirms the existence of copper oxide.

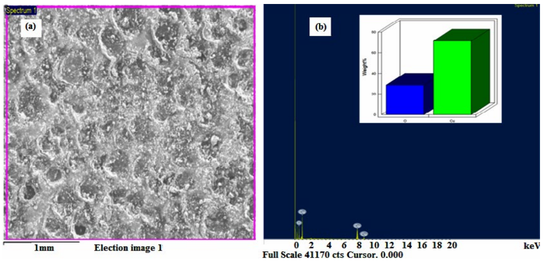

Fig. 4. EDX analysis of CuO nanoparticles

Nanomaterials can be identified by their specific surface area, which is one of the primary indicators of their nanoparticle content. CuO NPs were characterized by their surface area (BET). The approach is based on constant-temperature single-gas adsorption. As an adsorbate on the surface of CuO NPs, nitrogen gas was utilized. To assess the surface texture parameters of CuO, Brunauer Emmett Teller (BET) and Barrett Joyner Halenda (BJH) diagrams were utilized. Based on the N2 sorption isotherm, the isotherm is of Langmuir type IV (Fig. 5a), with a relative pressure (P/Po=0.0-0.99). In addition, the hysteresis loop is type H3 (Fig. 5b), confirming that the nanoparticles with slit-shaped pores are mesoporous. The results indicate that CuO NPs have a surface area of 14,034 m^2/g, which is a considerable value. In addition, the pore volume and radius are 0.02cc/g and 15.897A0, respectively. The antibacterial capabilities of CuO NPs were influenced by their high surface area. Due to its enhanced ability to absorb UV light[17] and generate radical species, the presence of nano CuO with a wide surface area can boost antibacterial activity. Owing to the enhanced production of Cu^{2+} ions and the reactivity of the oxygen, the CuO NPs were able to destroy bacterial cells (ROS).

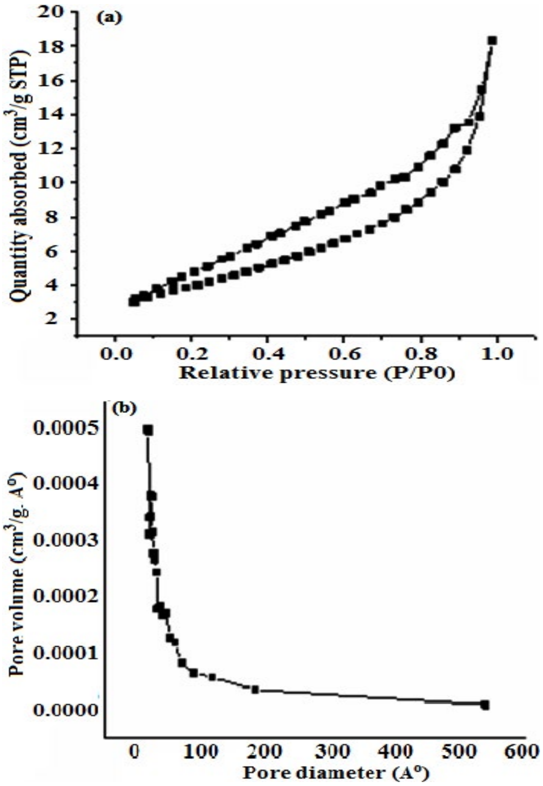

Fig. 5(a). (BET) and N2 adsorption–desorption isotherm (b) (BJH) pore size distribution of the CuO nanoparticles samples

To examine the crystallinity of CuO nanoparticles, XRD diffraction analysis was performed. Fig. 6 depicts the CuO nanoparticles' XRD pattern. The formation of tiny, well-crystallized copper oxide nanoparticles is permitted for this result. The obtained results demonstrated discrete diffraction peaks at 2 theta=32.816, 38.844, 66.08, and 68, which are assigned to 002, 111, 311 and 113 planes of a monoclinic phase of copper oxide nanoparticles. The XRD spectrum revealed the crystalline nature of the biosynthesized CuO NPs. In accordance with (ICSD087122), the peak positions indicated that CuO possesses a monoclinic structure[21]. The CuO nanoparticles average size was determined by the Scherer equation :

$$D=0.9\lambda/\beta \cos\theta \quad (1)$$

Where λ is X-ray radiation wavelength, β is the full width at half maximum (FWHM) for the peaks at the angle. It was determined and the it was around 22 nanometers in diameter.

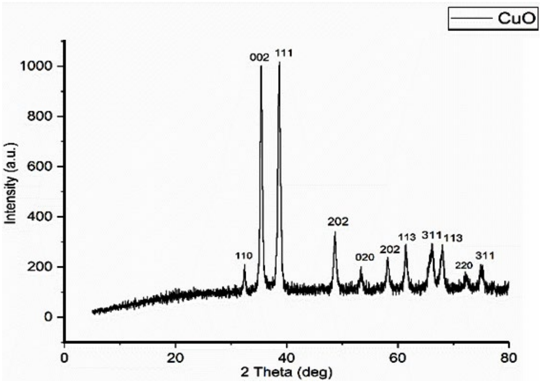

Fig. 6. XRD Pattern of CuO nanoparticles

Antibacterial activity of CuO nanoparticles

The results of the antibacterial assessment of CuO nanopowder in contrast to five species of human pathogenic bacteria; are *Escherichia coli, Marsa, Pseudomonas aeruginosa Klebsiella Pseudomonas*, and *Candiia*. Fig. 7. Our results showed that CuO nano powder are highly active in the tests of the pathogenic bacteria and fungi employed in this experiment. The presence of biomolecules capped the synthesized nanopowder the bacterial efficacy was high which can explained by the linking of CuO with the terpenoids from the extract during the process of capping.[23] The increasing of CuO nanopowder concentration inhibits the virulence of microbial pathogens.

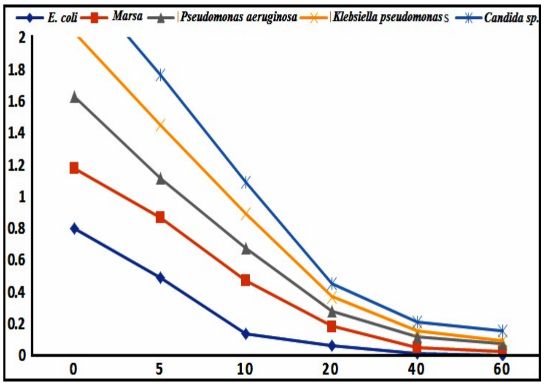

Fig. 7. Antibacterial activity of biosynthesized CuO nanoparticles

Table 1: XRD parameters of CuO NPs

No.	B obs. [°2Th]	B std. [°2Th]	Peak pos. [°2Th]	B struct. [°2Th]	Crystallite size [nm]
1	0.263	0.008	32.422	0.255	32.4
2	0.433	0.008	35.421	0.425	19.6
3	0.393	0.008	38.69	0.385	21.9
4	0.354	0.008	48.744	0.346	25.2
5	0.742	0.008	53.349	0.734	12.1
6	0.354	0.008	58.304	0.346	26.3
7	0.393	0.008	61.461	0.385	24.0
8	0.472	0.008	66.215	0.464	20.4
9	0.629	0.008	72.324	0.621	15.8
10	0.472	0.008	74.918	0.464	21.6
					Average= 21.93

CONCLUSION

Based on this study, we found that biogenic synthesis of CuO nanopowder from *Pimpinella anisum* seed extract is simple, economical, and environmentally friendly. Nanopowder synthesized was initially confirmed by the presence of SPR bands at 340nm in UV-Vis spectra. A spherical particle with a diameter of 22nm was detected. Copper nanoparticles with crystalline nanostructure are confirmed by the XRD spectrum. Finally, the green CuO NPs generated exhibited stronger antibacterial infections; this property makes the CuO NPs more relevant in domains such as medicine.

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Conflict of interest

The authors declare no conflict of interest.

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