Green Synthesis, Characterization and Antimicrobial activity of CuO nanoparticles (NPs) Derived from Hibiscus sabdariffa a plant and CuCl

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Abstract: This study was the synthesis of CuO NPs using Hibiscus sabdariffa plant extracts and CuCl. Copper Oxide CuO prepared by simple a chemical method. Analytical techniques such as Ultraviolet-Visible (UV-Vis), XRD and SEM (XRD analyses confirmed the characterization of the prepared CuO NPs. (XRD) measurements that the (CuO) thin film was poly-crystalline and there is no trace of the other material. Crystallite sizes 18.99nm calculated using the Scherrer's equation. SEM was used to estimate the average diameter CuO NPs was less than 100 nm. From the optical properties the energy gap was 5eV. This study focused on effect of CuO on the Antifungal and different type of bacterial. CuO had an inhibitory effect against fungal more than bacterial

Keywords: CuONPs, Hibiscus sabdariffa plant, Antibacterial Activity, Bacillus, E. coli and Enterobacter.

1-Introduction

The understanding of biological processes at nanoscale is a major driving force behind the development of nanotechnology. Nanoparticles are now available with a number of applications. Nanoparticles are about one billionth of a meter in size, about 100,000 times the width of human hair. There are new strains of bacterial fungus, showing resistant antibiotic characteristics. Antibiotics that can function effectively as an antibacterial agent must therefore be identified [1]. Cells usually 10μm in size consist of living organisms. The cell parts are however considerably smaller and are in the sub-micron domain. There are smaller proteins of just 5 Nm, the standard dimension of smallest man-made nanoparticles. There are even smaller proteins. The simple compare of this size gives us an idea to spy on cellular machines without much intervention, using small samples of nanoparticles. [2,4]. Shirley et al. (2010) reported synthesizing silver nanoparticles with substantial antibacterial potential for action against multiple-drug resistant bacterial gram positives and gram-negative strains. Application against dermatophytes was tested for the antifungal effect of silver
nanoparticles[3]. The aluminum oxide nanoparticles have antimicrobial activity. There has been a simple and robotic process for synthesizing nanoparticles of metallic copper with high antibacterial strength against E-coli. [4,5]. Copper oxides are one of metal oxides studied by nature and its good electrical and optical properties for a number of reasons. Copper oxide, a major semiconductor and have formed two well-known oxides CuO and CuO₂, each of them p-type with 2.1 to 2.6 eV band gap energy, respectively, which has increased interest in the development of copper oxides. The use of latte oxide is based on its cosmetic and antimicrobial properties for consumer goods such as pillowcases and sockets[6,7]. However, CuO with different nanoforms are summarized using different methods in many research papers [8,9]. Due to its antibacterial and antiviral properties, copper and its complexes have been used for decades as desinfectants. The increased antimicrobial activity of Cu-NPs was proposed as a result of their large volume-to-surface ratio and [10,11] compare to copper salts. This work seeks to prepare CuO NPs for antibacterial and antifungal products.

Experimental part

CuO NPs has been produced by Green synthesis of CuCl with Hibiscus sabdariffa plants is a highly cost-effective, safe, non-toxic, ecofriendly way of synthesis which can be done on a big scale. CuCl and Hibiscus sabdariffa plants as beginning material and twofold refined water as a dissolvable were utilized to obtain CuO NPs. in first, 9.89g took of CuCl was in 100 ml deionized water as a dissolvable to get a specific molar fixation at room temperature. It mixed for 60 min at 60 ± 10 °C temperature. Second, the Hibiscus sabdariffa was set up by dissolving 1.g in100 ml deionized water the solution was stirred for half an hour, then plant extract solution filtered with a filter paper to get rid of impurities. Finally, both solutions mix by magnetic stirrer at 60 °C for 1 h. The change in color was the indication for synthesis of solution NPs green. As shown in Fig1

![Photo image of CuCl, Hibiscus sabdariffa plant and deionized water](image)

**Fig (1):** Photo image of CuCl, Hibiscus sabdariffa plant and deionized water

**Microbial suspension preparation**

In regular saline, take the loop full of colony bacteria or Candida and their turbidity compared to standard McFarland solution 0.5 was around 1.5x10⁶CFU/ml for bacteria - 1.5x10⁶ CFU/ml for mold and yeast.

To test the antibacterial activity, the following steps used:

1- Take 250 μL of each microorganism's microbic suspension (Escherichia coli, Staphylococcus aureus, Bacillus subtilis, and Candida albicans) and spread them over the Muller Hinton agar plate using L-Glass rod Shape.
2-To dig well on the surface of the inoculated plate (6 mm), a sterilized cork borer was used and then 150 μl of CuO nanoparticles were applied to each pore and the plates were incubated at 37 °C (bacteria), 30 °C (Candida albicans) for 24 hrs.

3-The inhibitory effect of CuO nanoparticles on bacteria and Candida albicans was determined by inhibition zones measured in (mm) around each well.

**Fig (2):** Schematic diagram of cultivation of fungi and bacteria

**Result and dissection**

1- **X-ray diffraction analysis**

The XRD pattern of CuO NPs prepared by Green synthesis method as shown in Fig. 3. The spectrum of CuO is similar to that of pure CuO, suggesting that single-phase CuO with a monoclinic structure according to card number (JCPDS-05-0661 and JCPDS card number 45-0937). The average crystallite size (D) was 18.99 nm calculated using the Scherer formula as follows when, λ is wavelength for X-ray [12,13]

\[
D = \frac{0.9\lambda}{\beta \cos\theta} \quad ...... (1)
\]

**Fig (3):** XRD patterns of CuO thin film
CuO nanoparticles shown in SEM images 4. The sizes estimated to less than 100 nm, suggesting that the sample is nanostructured and has no certain morphology.

Fig (4): FE-SEM of an agglomerated of CuO NPs

Optical properties of CuO Thin film

After deposition on a glass substrate in the range of 200 to 900 the optical spectra of a CuO sample, recorded on UV-Vis absorption, as shown in Figure 4. The wavelength transmission is displayed in Fig.5. It has been found that the film's transmission increases as the wavelength increases. The transmission was reduced by the wide absorption of the particle size in the UV region and in the region visible in NIR[14].

Fig (5): Transmission for CuO thin film
The relationship between the absorption coefficient $\alpha$ and the incident photon energy $h\nu$ can be used to determine the value of the optical band gap. It can be written as:

$$(\alpha h\nu)^2 = A(h\nu - E_g)^n$$

where $A$ is a constant, $E_g$ is the band gap of the material and the exponent $n$ depends on the type of transition. $n$ may have values $1/2$, $2$, $3/2$ and $3$ corresponding to the allowable, allowed in direct, forbidden direct and forbidden indirect transitions respectively [15]. The sample's approximate band gap is $5eV$ as in fig.6, which is greater than for CuO bulk ($2.1eV$), it is more than the values recorded, due to decreased particle size. When the magnitude of Nano-crystals are smaller than the excited Bohr radius, Pair of electron hole, quantum confinement effect occurs and the band gap energy starts to increase with the decrease of particle size. This agreement with [5].

**Fig (6):** Tau's plot of CuO NPs thin films

**Antifungal and Antibacterial activity of CuO NPs**

Plant mediated synthesis of CuONPs exhibited a good the antifungal activity against and antibacterial activities that the Causing human diseases using agar well diffusion method. The results showed clearly those CuO NPs, which had efficient against these, isolates of antifungal as shows in fig (7) and Table 1. It has also found more in inhibitory area diameter of *Candida* isolates than isolates of bacteria when investigated in bacteria activity of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Klebsiella pneumonia* as shown in fig.8 and table 1. The literature also explains potential mechanisms for the activity of CuO NPs as an antifungal and antibacterial. CuO NPs can enter the cell by diffusion and endocytosis; they interfere with the functioning of mitochondria in cytoplasm contact, induce ROS (reactional oxygen species) and $\text{Cu}^{2+}$ to be released. These released ions can penetrate the membrane and reach the DNA, causing nuclear damage such as irreversible chromosome damage, causing cell death [16, 17], as shown in fig9.
**Fig. (7):** Antifungal Activity of CuO NPs

**Fig. (8):** Antibacterial Activity of CuO NPs
Fig. (9): Schematic presentation showing the activity of CuO NPs against bacteria strain

Table 1: antifungal and antibacterial effect of CuO NPs

| Compound (control 6mm) | Candidiasis | Escherichia Coli | Klebsiella pneumoniae | Staphylococcus aureus | Bacillus subtilis |
|------------------------|-------------|------------------|----------------------|-----------------------|------------------|
| CuO                    | 3.2 cm      | 2.0 cm           | 1.7 cm               | 1.5 cm                | 1.5 cm           |

Conclusion

CuO nanoparticles can be prepared by simple chemical method, use of CuO nanoparticles, the main application were antifungal and inhibitory influence against some pathogenic bacteria with suppression zone (control) about 6mm for CuO. It had more a inhibitory influence against antifungal subtilis (3.2 cm). we would thank Mustansiriyyah University for support in this work.

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