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Study Characterizations of CuO nanoparticles by using the chemical and green method and their effect on biofilm formation

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ABSTRACT

Nanomaterials have a wide range of applications due to their chemical and physical properties which depend on the size of the interesting particles compared to the size in the micrometer range. The importance of copper oxide nanomaterials increased due to their potential use in many technological fields. In this study CuO nanoparticles were synthesized via chemical and green method by using *Eucalyptus camaldulensis* leaves extract. The synthesized CuO nanoparticles were characterized by using SEM and UV – Vis analysis. CuO NPs are highly stable and have significant effect on both gram positive and negative bacteria. Using these methods nanoparticles can be synthesized without the use of organic solvents, expensive raw materials and complex equipment. Besides simplicity, the advantage of producing nanoparticles through this method is that it is convenient, flexible, fast, cost-effective and pollution-free.

Keywords: Copper oxide nanoparticles, green method, characterized, and antimicrobial activity.

1. INTRODUCTION

In recent years, nanotechnology and nanoparticles that based on the product and application are now increasing day after day due to the different fields of science such as biotechnology, physics, chemistry, materials science, engineering and medicine [1]. Nanoparticles differ from bulk materials [2] these molecules are isolated because of their unique optical, electronic and chemical properties [3]. They show fantastic properties and very useful, which can be exploited for a variety of structural and non-structural applications [4-5]. Several studies have been carried out to extract many natural products for screening antimicrobial activity but attention has not been focused intensively on the study of combinations of these products for their antimicrobial activity [6-7]. *E. camaldulensis* have the ability to synthesize a wide variety of chemical compounds. These compounds in addition to basic or primary metabolites include, phenolic compounds, terpenes, steroids, alkaloids, and other chemicals substances known as secondary metabolites [8-9]. These compounds that have prominent effect on the animal systems and some possess important therapeutic properties which can be and have been utilized in the treatment and cure of human and other animal diseases for themselves against the attack of predators such as insects, fungi and herbivorous mammals [10-11]. The objective of this study is to evaluate the effect of nanoparticles synthesized from plant and chemical extracts of bacteria and fungi.

2. MATERIALS AND METHODS

2.1 Chemical method of synthesis CuO NPs

CuO nanoparticles were prepared by chemical precipitation method in a typical synthesis of 1.2 M $Cu(NO_3)_2$ was dissolved in 100 ml distilled water then stirred for 10 min until it dissolved completely, then 1M of (NaOH) solution was added drop after drop to Cu

$(NO_3)_2$ solutions under constant stirring for 60 min. The reaction mixture forms bluish solution then changed into the dark brown completely after 1 h. The CuO precipitate was washed several times with distilled water to remove the native impurities in the product then dried in the hot air furnace at 200°C for 2 h until turned to black color completely, as shown in fig. (1).

Figure 1: Synthesis of Copper oxide nanoparticles by using chemical method.

2.2 The synthesis of CuO NPs by using the green method

2.2.1 The preparation of hot aqueous extract from *E. camaldulensis*

The fresh leaves of *Eucalyptus camaldulensis* were collected from the gardens of University of Baghdad then washed with distillated water several times, after drying in the oven at 50 C° the leaves were milled by using an electric mill. The leaves powder weighting (200 gm) were mixed thoroughly with (600 ml) of boiled distilled water then homogenized on the magnetic stirrer for 2h even a color of aqueous solution varies from watery to light yellow, then filtered and centrifuged at 8000 rpm for 15min, then kept at 4C° until use.

2.2.2 The synthesis of CuO NPs by using aqueous extract of *E. camaldulensis*

Aliquot of 100 ml aqueous extract from *E. camaldulensis* was heated at 80°C by using a magnetic stirrer then 15g of copper nitrate was added thoroughly to the extract and left for 10 min until changed the color of the solution to black green then centrifuged at 8000 rpm for 15min. The solution was placed in the oven at 200 °C for 2h; a black powder was obtained and collected carefully then stored for characterization purposes as shown in fig. (2).

Figure 2: The synthesis of Copper oxide nanoparticles by using green method

2.3 The effect of CuO NPs on biofilm formation

The determination of minimum inhibitory concentration (MIC) was determined by using tube macro dilution method while the MBC was calculated as the lowest concentration that kills 99.9% of the initial microbial population [12]. Biofilm formation assays were performed using 96- well microtiter plate based on the protocol by Goh, S. et al (2013) with minor modifications [13]. Microbial strains were cultured briefly in the TS broth overnight then resulting culture was diluted to 1:100 (TSB + 1% w/v glucose) each well of microtiter plate was loaded with 100 ml of medium and 100 µl of CuO NPs except the well of control without CuO NPs solutions and the plate was incubated at 37Cº for 24 h. The cultured microbes were removed using sterile distilled water, then 0.1% w/v crystal violet solution was added to the wells and left to stain for 10 min at room temperature then removed by submerging the plate in a water tray and left to air dry. The wells stained with 95% ethanol were treated for 10 min at room temperature and measured optical density (OD) in a small plate reader at 630 nm [14].

3. RESULTS AND DISCUSSION

3.1 The optical absorption analysis of CuO nanoparticles:

The optic absorption spectrum [15] was used to study the optical properties of the synthesized CuO nanoparticles, the optical energy band gap for CuO nanostructure has been calculated using absorption edge. Fig. (3) shows that UV-VIS absorption spectra of the chemical synthesized of CuO NPs have been

recorded to measure their band-gap. While noted that the CuO NPs manufactured via using aqueous extract of *E. camaldulensis* leaves will decrease the wavelengths and thus lead to increasing in the energy gap, as shown in fig. (4).

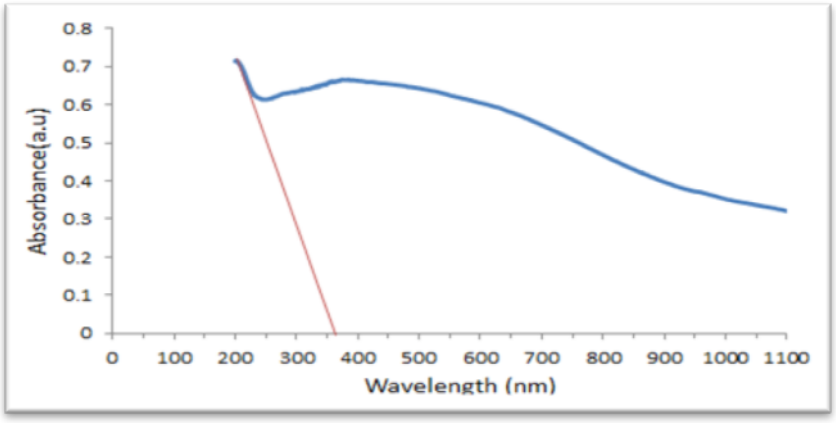

Figure 3: UV-vis absorbance spectra of CuO NPs via chemical method.

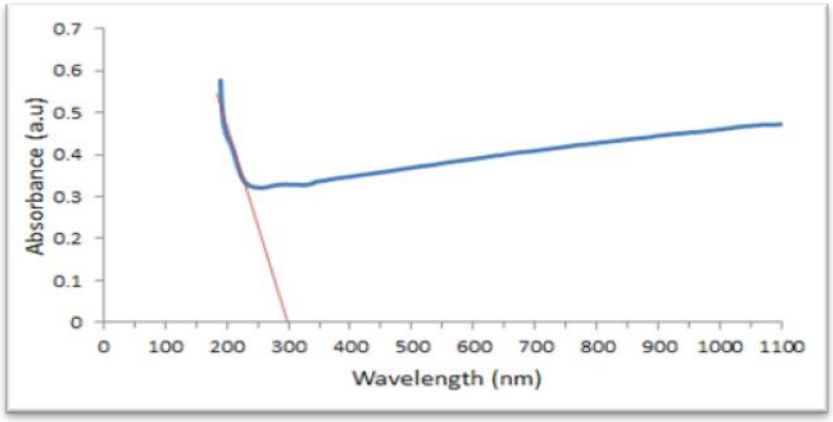

Figure 4: UV-vis absorbance spectra of CuO NPs synthesized by green method.

3.2 Scanning Electron Micrographs (SEM):

The surface morphology of the copper oxide nanoparticles synthesized by using chemical method was examined using scanning electron microscopy (SEM), as shown in fig. (5) which contains two types of structures, one of which is a cortical or cellulose structure and the other is a nano rod structure that is matrixed with each other. As the nano rod, the mass has a diameter (thickness) and a length; we observe that the thickness is approximately 40 nm and length 500 nm. Fig. (6) shows the scanning electron microscopy of copper oxide nanoparticles synthesized by using aqueous extract of *E. camaldulensis*, the SEM images shows that the copper oxide nanoparticles are crystalline solid blocks of different dimensions, as there are small masses of less than 50 nm that are united with each other. There are also large masses of more than 100 nm.

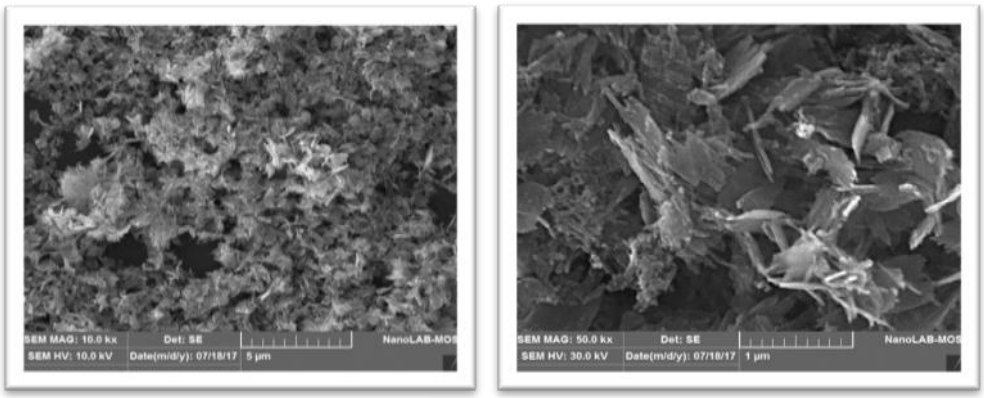

Figure 5: Scanning electron micrograph (SEM) of CuO NPs synthesized by using chemical method.

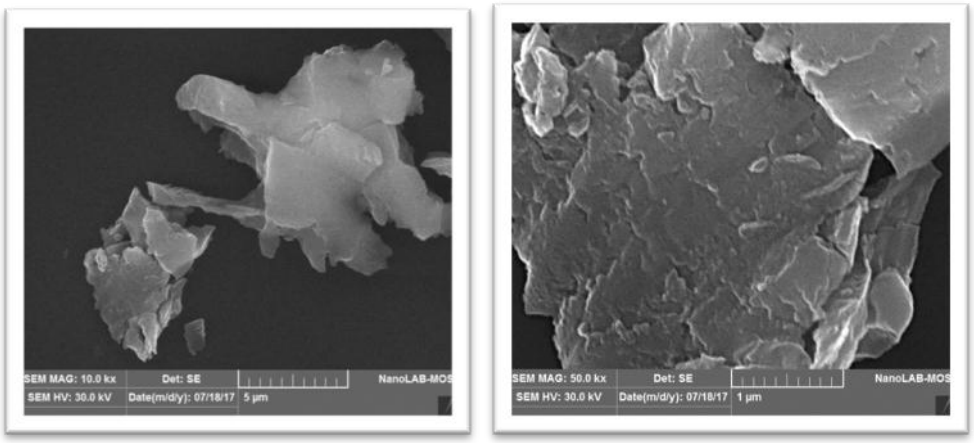

Figure 6: Scanning electron micrograph of CuO NPs synthesized by the aqueous extract of *E. camaldulensis*.

3.3 Biofilm effect of CuO nanoparticles

3.3.1 Determination of minimum inhibitory concentration (MIC) and determination of minimum bacterial concentration (MBC)

The results obtained that MIC values of CuO nanoparticles were found to be (0.1, 0.75, 0.05, 0.025, 0.21) µg/ml for *E. coli*, *S. aureus*, *S. epidermidis, K. pneumonia*, and *Candida albicans* respectively. These results of MIC values are confirmed by determining MBC that showed bacterial inhibitory in those concentrations for copper oxide nanoparticles.

3.3.2 The effect of CuO nanoparticles on biofilm formation

The results showed a difference in the biofilm growth depends on the type of nanoparticles and microbial pathogens as shown in table (1,2). The synthetic CuO nanoparticles (sample 1) displayed highest inhibition effect on gram negative bacteria (*K. pneumonia*, *E. coli*) respectively, as shown in figure (7) and then followed by gram positive bacteria (*S. aureus*, *S. epidermidis*) which have less effect on biofilm formation compared to the control. While the green synthetic CuO NPs (sample 2) had the same effect on biofilm formation of *E. coli*, *S. aureus* and *K. pneumonia* but less effect against *S. epidermidis* biofilm formation compared with the control. These results were similar with (Maqusood et al. 2014 and Azhar et al. 2017)[16-17], who used simple methods of manufacturing copper oxide nanoparticles and proved that the copper oxide nanoparticles have high effectiveness against bacteria. Fig (8) shows that the two types of nanoparticles offered the same effect against *C. albicans* biofilm formation which may be due to the differences in cell wall composition. The structure of the cell wall plays an important role in the tolerance or susceptibility of bacteria and fungi in the presence of nanoparticles and diffusion within biofilm matrixes by changing the surface from hydrophilic to a highly hydrophobic towards nanoparticles due to altering the expression of cell wall proteins [18]. These results were in the same line with the results of Amiri et al. 2017 and Ghasem et al. 2016 [19-20], CuO NPs showed a significant reduction in *C. albicans* biofilm.

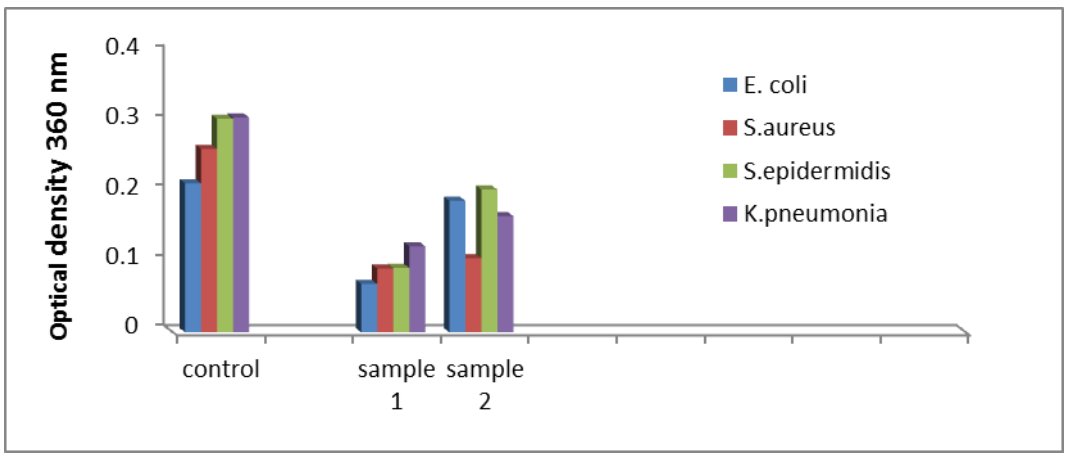

Figure 7: The differences in biofilm growth of bacteria after treatment with different CuO NPs.

Table 1: The effect of CuO NPs on biofilm growth in bacteria.

Bacterial isolates	Control	Treatment	
		CuO NPs Chemical method	CuO NPs Green method
Escherichia coli	0.213	0.069	0.188
Staphylococcus aureus	0.262	0.092	0.106
Staphylococcus epidermidis	0.305	0.092	0.204
Klebsiella pneumonia	0.307	0.065	0.166

Table 2: The effect of CuO NPs in biofilm growth on fungi (OD at 360nm)

Fungal isolates	Control	Treatment	
		CuO NPs Chemical method	CuO NPs Green method
C. albicans	0.207	0.166	0.172

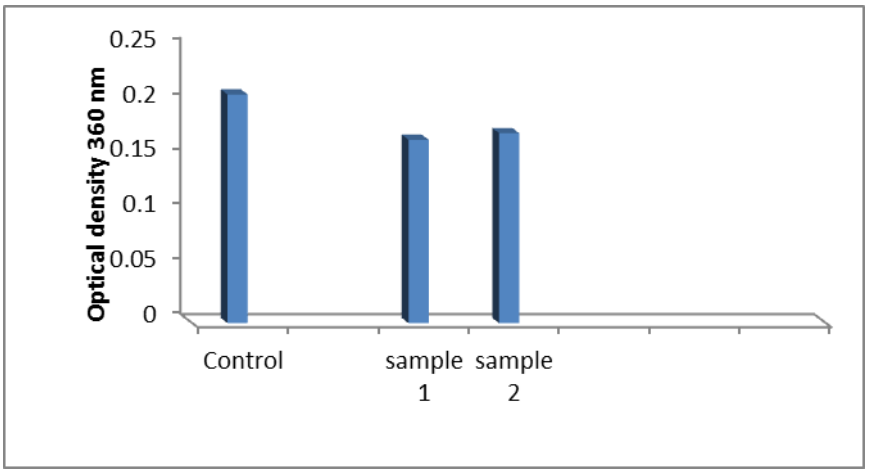

Figure 8: The differences in biofilm growth of *C. albicans* after treatment with different CuO NPs

4. CONCLUSION

A very versatile, nontoxic and environmental friendly approach for the synthesis CuO nanoparticles has been introduced in this paper. The optical characteristics of copper oxide nanoparticles were studied using UV-VIS analysis. The peak absorption confirmed the formation of copper oxide nanoparticles. The SEM photographs shows good agglomeration of CuO nano particles. Further antimicrobial activity of plant extract and synthesized copper nanoparticles were investigated in the biofilm method. From the results it is clear to know that the copper nanoparticles from chemical and green method also have the ability to inhibit the growth of various pathogenic microorganisms like *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumonia and C. albicans.*

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