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SYNTHESIS OF COPPER OXIDE NANOPARTICLES USING DIFFERENT LOCAL PLANTS AND STUDY THEIR BIOLOGICAL ACTIVITY

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Abstract
The inherent sophisticated structure of plants inspires researchers to use it as a natural template for synthesizing functional nanoparticles. In this study, pure copper oxide nanoparticles were synthesized using copper sulfate pentahydrate ($CuSO_4.5H_2O$) as a source of copper metal and plants leaves extracts (Pomegranate, Lettuce and Orange) as a reduction agent and natural inexpensive and renewable template. The crystal structure and morphologies of the copper nanoparticles were characterized by X-ray diffraction and field emission scanning electron microscopy. The CuO nanoparticles synthesized showed variant grain size and shapes in due to used different leaf extracts plant. This combination of CuO nanostructures with different plants leaves extracts exhibited remarkable antibacterial properties.

Keywords: green synthesis, copper oxide nanoparticles, local plants extracts, antimicrobial.

I. INTRODUCTION

In recent years, Nanotechnology has attracted many researchers from various fields like biotechnology, physics, chemistry, material sciences, engineering, medicine. Nanoparticles are synthesized by physical and chemical Methods, these are suffering from drawbacks like expensive reagent, hazardous reaction condition, longer time, tedious process to isolate nanoparticles [1,2]. Hence, there is scope to develop new methods for the synthesis of nanoparticles which should be required inexpensive reagent, less drastic reaction condition and eco-friendly. In recent time, Cu and Cd nanoparticles have attracted much attention of researchers due to its application in wound dressings and biocidal properties [3,4], potential industrial use such as gas sensors, catalytic process, high temperature superconductors and solar cells [5-7]. In the majority of cases, metal participates in its nitrate form, thus inducing a strong antimicrobial effect but when metal nanoparticles (MNPs) are used the surface area exposed to different types of microbes increases considerable [8]. MNPs are a very important part of nanotechnology mainly because they do not induce modification on living cells and, so, are unable to cause microbial resistance. Recent studies revealed that MNPs have the ability to attach to cell walls and alter cellular respiration. MNPs are widely used in biology and medicine especially because of their attractive and unique physiochemical properties. Many bacterial cultures were used for different kind

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of nanoparticles some are cadmium nanoparticles (Cd NPs) biosynthesis was done by Clostridium thermoaceticum [9]. To the best of our knowledge, the use of local plants leaf extracts at room temperature for greener synthesis of CuO nanoparticles has not been reported. Hence the present study was carried out to synthesize and characterize the copper oxide nanoparticles using local plants leafs extracts and study biological effectiveness.

II. MATERIALS AND METHODS

1. Chemical and reagents

Copper sulfate $CuSO_4.5H_2O$ (98%) was obtained from Department of Chemistry, College of Education for Pure Science, University of Diyala, Iraq. local plants were obtained from gardens in Iraq.

2. Preparation of the Extract

Preparation of green reducing and stabilizing agent. The plants (Pomegranate, Lettuce and Orange) were collected from the local market, Diyala, Iraq. Washed the leaves plants with deionized water to remove impurities. Cutting the leaves plants into small pieces. Take (25 g) cut leaves in100 mL of deionized water and set the temperature to 80°C after some time the water will tum greenish indicates the formation of leaf extract in water. Now filter out the extract and pour it into the burette, this extract would be used as reducing agent for copper oxide nanoparticles synthesis.

3. Synthesis of CuO nanoparticles

The salt solution was prepared by dissolving a 1.0 g copper sulfate pentahydrate ($CuSO_4.5H_2O$) in 100 mL of deionized water. The extracts solution (Pomegranate, Lettuce or Orange) was gradually added to the solution with stirring at (50 °C), the temperature of the solution was raised to (80 °C) under stirring and sodium hydroxide solution (1 M) was added dropwise to the mixture (salt solution and leaves extract), to obtain the number of pH at approximately 12, the mixture was left at constant stirring for 3 hours. Then, the mixture was filtered; the precipitate was collected and washed with deionized water and ethanol to obtain a pH of approximately 7 , CuO nanoparticles was formed (CuO –P, CuO –L or CuO –O by leaf extracts of Pomegranate, Lettuce or Orange respectively) in water after calcination process at 550 °C Scheme 1.

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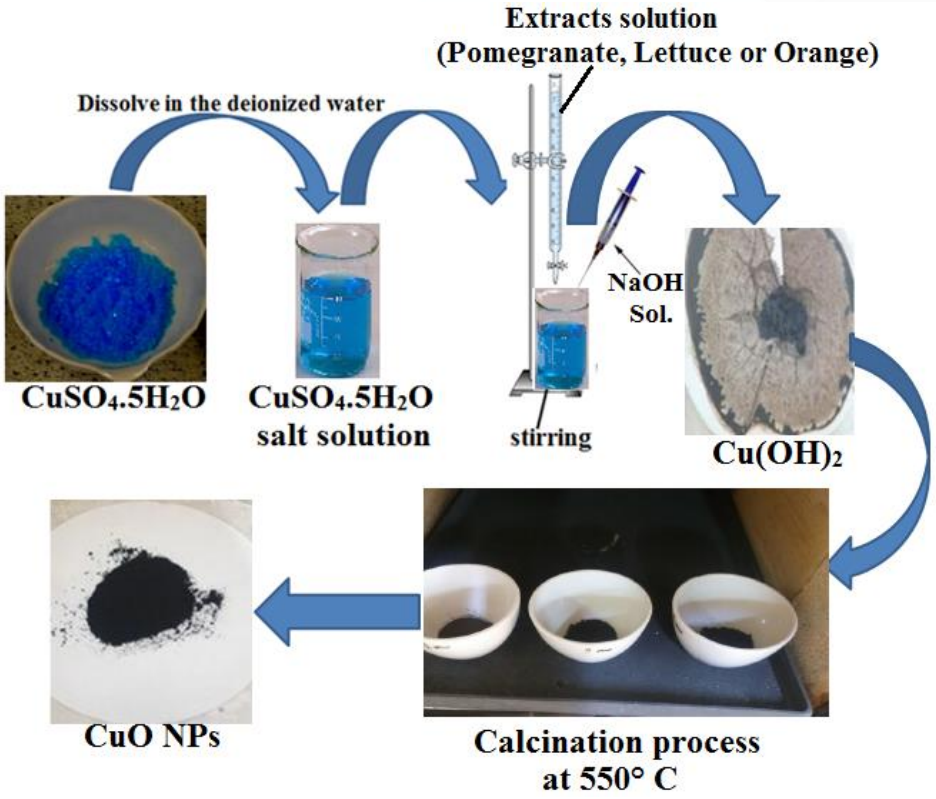

Scheme 1. Preparation of Copper oxide nanoparticles by Pomegranate, Lettuce or Orange leaves extract as a reduction factor with NaOH as precipitation agent

4. Bioactivity methodology

4.1. Material and Methods

Staphylococcus aureus and Escherichia coli isolates were cultured on Blood agar and MacCkonky agar. Candida albicans and C. tropicalis isolates were cultured on sabouraud dextrose agar and Candida Chromogenic agar.

4.1.1. MacFarland turbidity standard

The preparing solution from the company (Biomeriex) was used in calibrating the number of bacterial cells, as it gives an approximate number of cells 1.5 x 108 cells/mL .

4.1.2. Muller Hinton agar

This medium was prepared by dissolving 38 g in 1L of distillated water and sterilized by autoclave at 121°C and under pressure 15 pounds for 15 minutes cooled and poured into sterile dishes and kept in the refrigerator until use.

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ISSN: 2776-1010 Volume 4, Issue 11, November 2023

4.1.3. Determination the Antimicrobial activity of CuO nanoparticles by agar well diffusion method;

A number of bacteria colonies were transported by loop to prepare the suspended bacteria and put it in tubes contain brain heart infusion broth to activate the bacteria. The tubes were incubated for (18 - 24) h at 37 °C. The suspended bacteria were compared to the standard MacFarland solution (1.5 x 108) cells/mL. After that the bacteria suspended was spread by Sterile Swab, it was spread on the plates containing Muller Hinton agar and then left the plate for a while to dry. A holes were made with a diameter of 5 mm in the culture media by using sterilized a cork borer. 100 µl of the material were added to each hole individually by micropipette. After then, incubate the dishes at 37 °C for 24 h. The effectiveness of each concentration was determined by measuring the diameter of the inhibition zone around each hole.

4. Characterization

The purity of the synthesized copper oxide nanoparticles (CuO NPs) was confirmed XRD analysis (Bruker, German), using Cu Kα radiation in the range of 2θ = 20–80°c at a scanning rate of 5° min^{-1}.The structural morphology was examined by Field Emission Scanning Electron Microscopy (FESEM) (Hitachi SX-650, Tokyo, Japan). The UV–Vis absorption spectra were recorded on UV–Vis spectrophotometer (Perkin Elmer, USA).

III. Results and Discussion

1. X-ray diffraction analysis

The copper oxide nanoparticles CuO NPs that papered was synthesized by eco-friendly method using three leaf extract plants, and it was characterized using powder XRD in which to confirm the particles of copper oxide and to know the structural information for all products. Figure 2 shows the XRD pattern of CuO nanoparticles. The pattern of a clearly shows the main peaks for all CuO NPs (CuO-P, CuO-L and CuO-O) at (2θ) 35.83°, 38.60°, 40.48°, 50.39°, 56.80°, 61.98°, 66.17° and 69.48° corresponding to the (110), (111), (202), (220), (020), (113), (220) and (311) planes, respectively. By comparing JCPDS no: 48-1548 [10], the typical pattern of green synthesized of all CuO NPs is found to possess an cubic structures with average crystalline size 34.61, 32.29 , and 31.13 nm for CuO-P, CuO-L and CuO-O NPs respectively, was estimated using Debye–Scherrer's equation.[11] This different of nanoparticles size, may be due to the bioorganic compounds occurring on the surface of the CuO NPs and appearances of these peaks are due to the presence of phytochemical compounds in the different leaf extracts.[12] These unpredicted peaks were has high intensity in the case of CuO- P NPs sample compare as CuO-L and CuO-O NPs.

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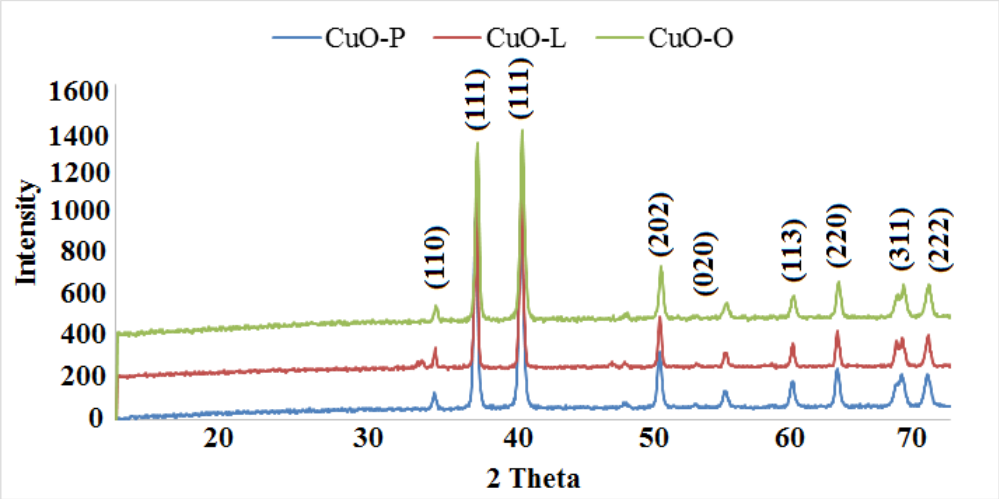
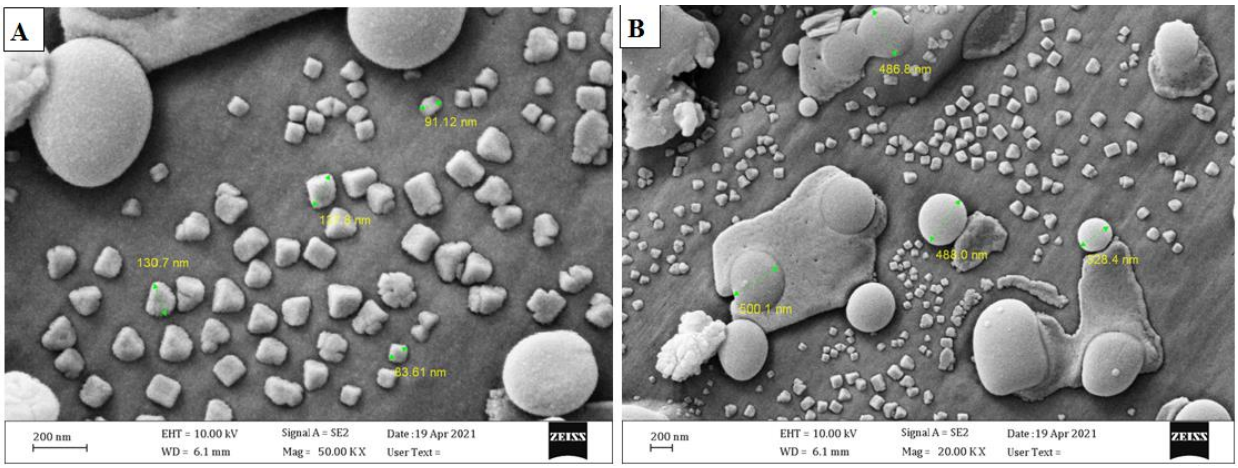


Fig. 2. X-ray diffraction pattern of CuO –P, CuO –L and CuO –O NPs.

2. FE-SEM analysis

The shape and composition of the all copper oxide nanoparticle were determined using FE-SEM. Figure 3 (A,B, and C) shows FE-SEM images of copper oxide nanoparticle were prepared using different plants leaves extracts, revealing cubic, spherically and irregular shaped particle with some agglomeration and aggregation. Figure 3 (A) return to CuO-P have been cubic nanoparticles with average size at 110 nm, while CuO-L gave irregular shape of nanoparticles with average size at 447 nm, Figure 3(B), but in the case of CuO-O shows spherically shape of nanoparticles with average size at 60 nm, Figure 3(B). Thus, FE-SEM analysis depicts the potentiality of plant extract solution type to effect on the aggregation and average size diameter of CuO NPs.



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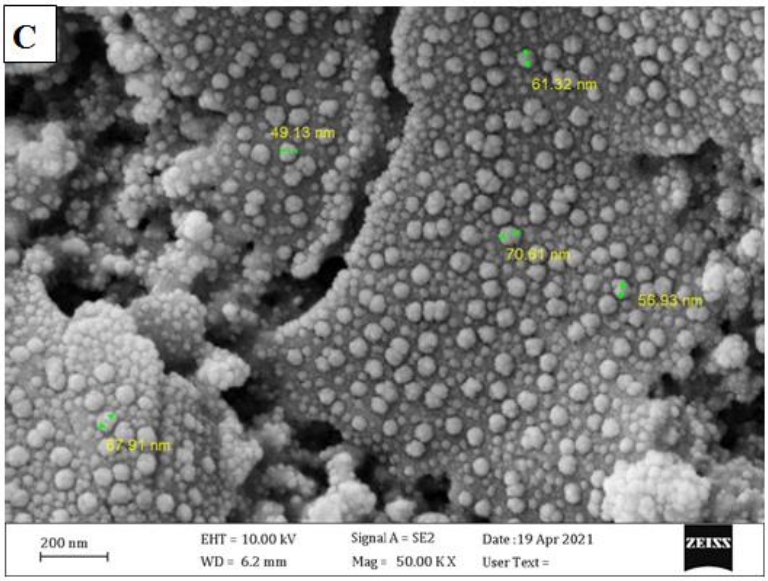

Fig.3. FE-SEM images of (A) CuO –P, (B) CuO –L, and (C) CuO –O NPs

3. Bioactivity

The biological activity of CuO nanoparticles;

The activity of CuO nanoparticles against two types of bacteria (E. coli) and (S. aureus) at two different concentrations (50 µg/ml, 100 µg/ml) were study and represented in table (1). The prepared materials proved high ability to inhibit the bacteria used in this study, as shown in figures (4) and (5). The effectiveness results of the CuO nanoparticles prepared were compared with standard antibacterials; for the (E. coli) type which were: Chloramphenicol Gentamycin and Imipenem, while for bacteria of the S. aureus type they are; Clindamycin, Levofloxacin and Ciprofloxacin

Table 1. Result for synthesized nanomaterials against Gr-ve (E. coli) and Gr+ve (S. aureus) bacteria

Sample	Concentration (µg / ml)	Zone of inhibition (in mm)	
		Bacteria	
		E. coli	S. aureus
CuO-P	100	37	50>
	50	31	40
CuO-L	100	37	50>
	50	40	44
CuO-O	100	43	50>
	50	45	34
Chloramphenicol	100	24	15
	50	21	24
Gentamycin	100	28	25
	50	21	24

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ISSN: 2776-1010 Volume 4, Issue 11, November 2023

Imipenem	100	24	26
	50	26	22
Ciprofloxacin	100	0	0
	50	24	28
Levofloxacin	100	21	32
	50	28	28
Clindamycin	100	28	24
	50	21	26
DMSO	100	0	0
	50	0	0

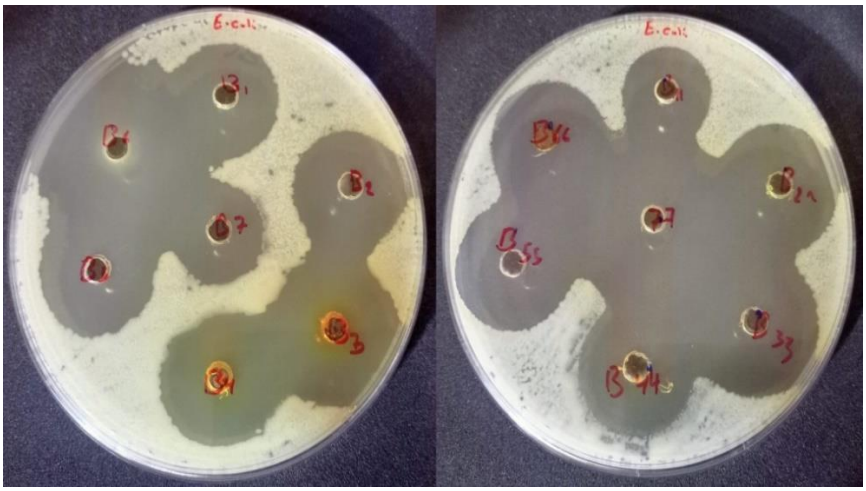

Figure (4): A comparison of E.coil bacteria in the presence of the two concentrations (50µg /mL) and (1000µg /mL).

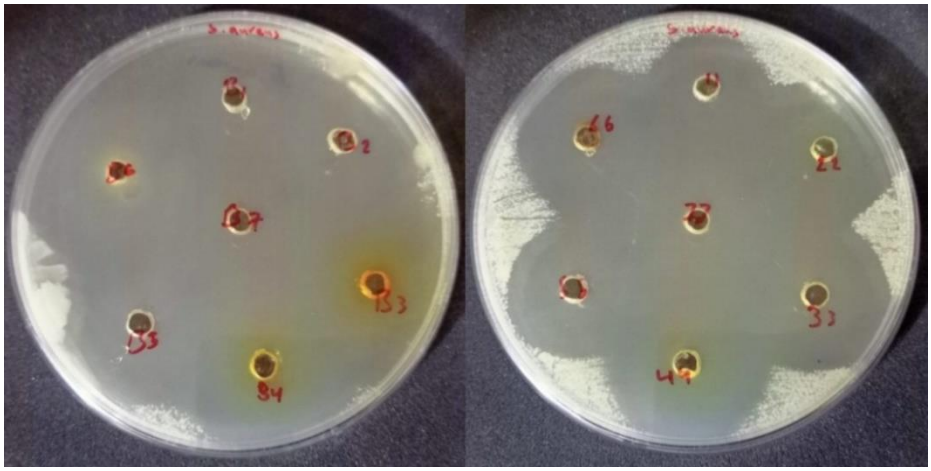

Figure (5): A comparison of S. aureus bacteria in the presence of the two concentrations (50µg /mL) and (1000µg /mL).

ISSN: 2776-1010 Volume 4, Issue 11, November 2023

V. Conclusions

In this work the green synthesis of stable CuO nanoparticles using aqueous extract of Pomegranate, Lettuce and Orange leaves extract as reducing and stabilizing agent was study. The XRD spectrum showed cubic crystal structure of CuO NPs with different average crystalline size about 34.61, 32.29 , and 31.13 nm for CuO-P, CuO-L and CuO-O NPs respectively. The FE-SEM image shows agglomeration of the NPs is present in the all CuO NPs. Hence, we observe that the leaves extract plant different has a clear effect on the shape and size of the copper oxide nanoparticles. On other hand, all CuO NPs synthesized were more effectiveness than standard antibacterials used

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