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REVIEW

A novel green synthesis of CuO nanoparticles in pear extract and their anticancer and antibacterial activities

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Abstract

Copper oxide nanoparticles (CuO NPs) were synthesised using a reduction agent from aqueous pear extract (*Pyrus communis* L.) CuO NPs were investigated by transmission electron microscopy, scanning electron microscopy, and X-ray diffraction (XRD) analysis. The XRD pattern of CuO NPs exhibited a face-centred cubic structure. Fourier transform infrared spectroscopy exhibited a stretching vibration of CuO at 408.91 cm^{-1}. The bactericidal activity of CuO NPs showed significant inhibitory activity against pathogenic bacterial strains, which were investigated against the Gram-negative bacterium *Acinetobacter baumannii* and the Gram-positive bacterium *Streptococcus pyogenes*. CuO NPs also showed significant cytotoxicity against human colon cancer (HT-29) cells. Overall, this study revealed that pear extract was an effective reducing agent for CuO NPs formation, exhibiting good biological properties.

Keywords: Green synthesis, CuONPs, Nanoparticles, *Streptococcus pyogenes*, Acinetopacter *baumannii*

1. Introduction

Nanotechnology is a field of study that spans several disciplines. Wide-ranging applications are possible with new nanowide substances (1–100 nm) [1]. Large area, thermal conductivity, size, load capacity, shape, crystal structure, and morphological surfaces are some of the novel characteristics of nanoparticles (NPs) that confer materials with into biomedical and biotechnological applications [2]. The production and self-assembly of NPs are considered the cornerstone of nanotechnology [3]. Green synthesis is an alternative way of using

plants to create NPs and has many benefits, such as reduced use of harmful chemicals, low toxicity, and ease of manufacture [4]. CuO NPs have been synthesised using pear extract. Pears (*Pyrus* spp.) are some of the most popular stone fruits in fresh and processed forms. Pears are a rich source of alkaloids, tannins, carbohydrates, steroids, amino acids, cardiac, and coumarin.

They are high in fibre, low in calories, and have antibacterial, antioxidant, and antifungal properties. In wound-healing models, *Pyrus communis* is found to be helpful by boosting wound contraction, decreasing epithelisation, and reducing scar area [5]. Regular consumption of pear lowers the risk of oesophageal, bladder, and lung cancer [6]. Pears contain ursolic acid, which prevents cancer by inhibiting aromatase activity [7].

Pears can help with mucous-membrane inflammation, intestinal problems, chronic gallbladder problems, arthritis, and gout. Pears are high in carotene, zeaxanthin, and vitamin C, all of which help reduce the levels of inflammation causing C-reactive proteins [8]. Pears are high in vitamin C, phenolic compounds, and copper, all of which help protect cells from free-radical damage [9]. Copper oxide (CuO) is a smart transition-metal oxide with a low band gap (2.0 eV) and unique nanoscale properties, such as strong electrochemical activity and high conductivity.

Surface area, redox potential, and solution stability are all important factors [10]. CuO is one of the most extensively used NPs, second only to noble-metal NPs, owing to its various potential applications, such as in catalysis [11]. CuO NPs can reportedly be applied in gas sensors, waste treatment, catalysis, batteries, food preservation, high-temperature superconductors, solar-energy conversion, photovoltaic devices, dye removal, field-emission emitters, and agriculture [12].

Apart from the previously described applications, CuO NPs have unique anticancer, antibacterial, and antioxidant properties, making them a promising biomedical tool [13]. Copper oxide NPs have an extended shelf life compared with other organic antimicrobials [14]. The synthesis of CuO NPs has three different methods that focus on the use of microorganisms, polysaccharides, plants, and enzymes: physical (i.e., thermal evaporation and pulsed-laser deposition), chemical (i.e., sol-gel, sonochemical, and spray pyrolysis), and biological techniques [15].

In synthesising NPs, these approaches require poisonous compounds or use a great deal of energy. Thus, green synthesis is an alternate method of utilising plant metabolites, microorganisms, and algae to create effective NPs. Green synthesis has various benefits, such as reduced use of harmful chemicals, biocompatibility, low toxicity, ease of manufacturing, cost effectiveness, and ability to control the synthesis process [16].

Colorectal cancer (CRC) is one of the most common cancer-related deaths in the world. It is the third leading cause of cancer death, following lung and prostate cancer tumours and lung and breast malignancies. Lack of physical activity, alcohol drinking, smoking, a low-fibre, high-fat diet, obesity, and insufficient fruit and vegetable consumption are all potential risk factors for the development of CRC [17].

The human digestive system and have a positive impact on their host is affected by the body's microbial flora [18]. The most often utilised human intestine cell lines are HT-29 cell line primarily comprises undifferentiated cells, with a small percentage of differentiated cells (3% to 5% of total cells) [19]. HT-29 is an epithelial cell line derived from a primary tumour collected from a 44-year-old Caucasian female

patient with colorectal adenocarcinoma in 1964 [20].

Streptococcus pyogenes is a Gram positive, aerotolerant bacteria belonging to the *Streptococcus genus*. These bacteria are extracellular and consist of nonmotile, nonsporing cocci (round cells) that cluster together. They are therapeutically significant for humans because they are a rare but pathogenic component of the skin microbiota that can cause Group A *streptococcal* infection. *S. pyogenes*, sometimes known as group A *Streptococcus*, is the most common species that carries the Lancefield group A antigen [21].

Acinetobacter baumannii is a Gram-negative bacterium that is often small, virtually spherical, and rod shaped (coccobacillus). In humans, it can be an opportunistic pathogen affecting persons with weakened immune systems, and it is becoming more common as a hospital-acquired (nosocomial) infection. Meanwhile, other *Acinetobacter* species are frequently detected in soil samples (leading to the common misconception that *A. baumannii* is a soil organism [22].

A. baumannii is most commonly associated with wound and burn infections, as well as ventilator-related pneumonia and urinary tract infections. It is also a common source of nosocomial bacteraemia. These infections frequently result in increased morbidity and death, particularly amongst immunocompromised individuals, owing to intrinsic and acquired antibiotic resistance [23].

The present study aimed to prepare CuO NPs through biological method by using pear extracts and investigate their efficiency against the HT-29 cancer cell line, the Gram-negative bacterium *A. baumannii*, and the Gram-positive bacterium *S. pyogenes*.

2. Material and methods

2.1 Materials

HT-29 cell lines were obtained from the Cancer Research Center Baghdad. RPMI-1640, Trypsin-EDTA, and fetal bovine serum were obtained from Capricorn (Germany). Dimethyl sulfoxide (DMSO) was obtained from Santa Cruz Biotech (USA), and MTT was procured from Bio World (USA).

Muller–Hinton (M-H) agar and copper (II) nitrate dehydrate Cu $(NO_3)_2 \cdot 3H_2O$ were procured from Hi-Media (India) and Riedel-de Haen (Germany), respectively. Deionised (DI) water was obtained from Chem-Lab (Belgium). Fresh pears were purchased in the month of November from a local market in Iraq.

2.2 Methods

2.2.1 Preparation of pear extract

To remove dust from fresh pears, they were separated from the branches and seeds and washed several times with DI water. The pears (250 g) were smashed well with 250 mL of DI water to form a homogeneous mixture in the mixer. The product was passed through Whatman No. 1 paper. A clear white solution was obtained, as shown in (Fig. 1).

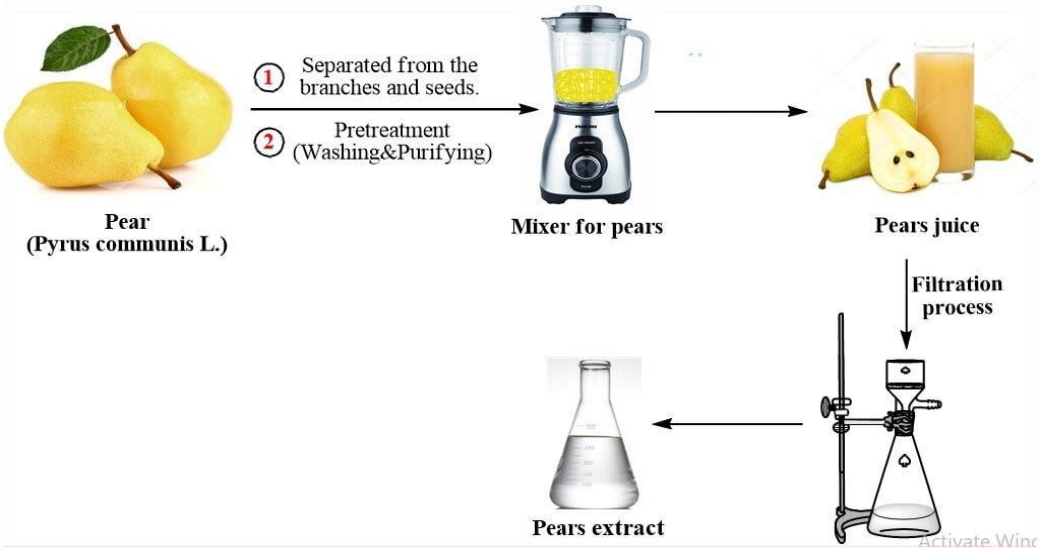


Fig. 1. Preparation of pears extract

2.2.2 Synthesis of CuO NPs

CuO NPs were synthesised by mixing 10 mL volumes of 0.02 M copper (II) nitrate trihydrate solution Cu $(NO_3)_2.3H_2O$ (MW = 241.60 g/moL) and 30 mL of the pear extracts while stirring the mixture. Then, 1M NaOH solution was added dropwise to adjust the mixture's pH to 9 (monitored using a pH meter) as required.

The mixture was stirred continuously for 2 h and heated at 70-80 °C. The color changed from bluish green to dark green, which confirmed the formation of CuO NPs. After cooling down to 25 °C, the solution was separated by centrifugation at 5000 rpm for 15 min. The steps are as shown in Fig. 2.

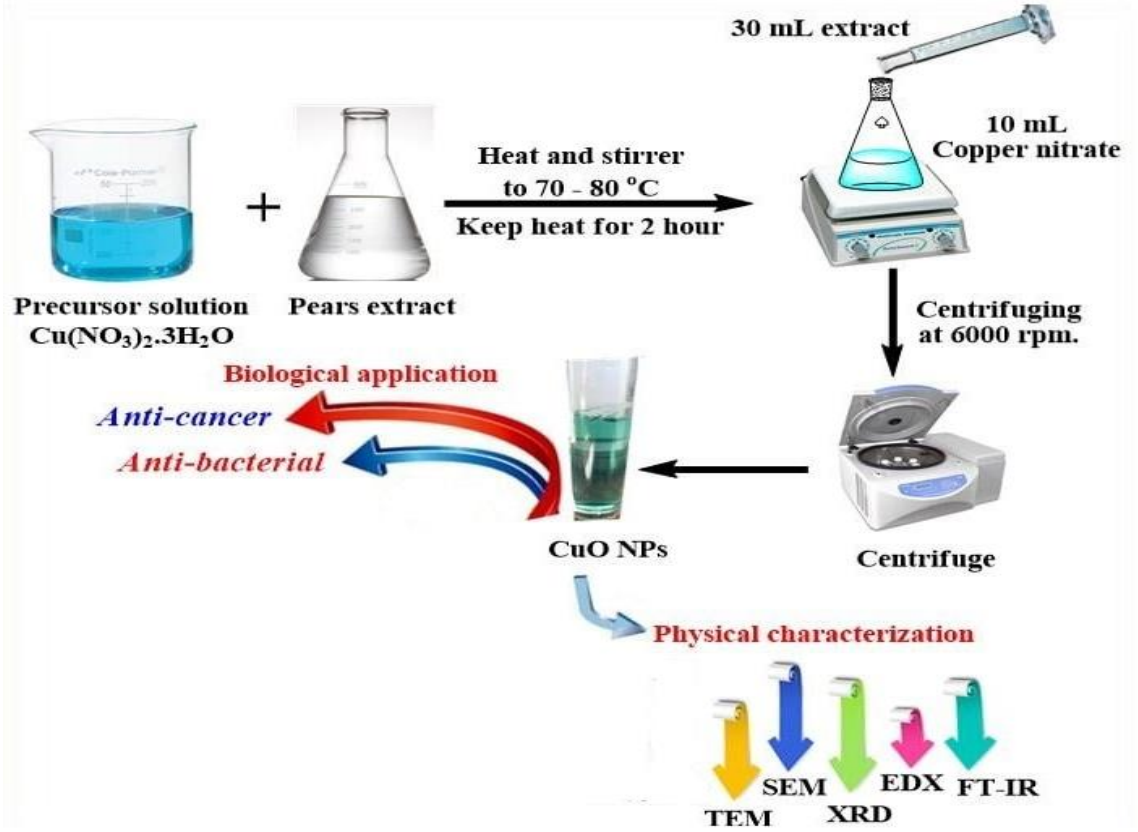


Fig. 2. Synthesis of CuO nanoparticles

2.2.3 CuO NP formation mechanism

In the initial activation step, Cu (II) cations were extracted from Cu $(NO_3)_2$ salt precursor dissolved in DI water. During mixing, the copper ions reacted with the pear extract-derived bio-organic compounds such as soluble saccharides through oxidation reduction. The abundant electron-rich natural biomolecules containing hydroxyl groups with considerable reduction capabilities reduced the copper cations from divalent oxidation state to metallic form, which was immediately converted into CuO NPs as result of the superior chemical reactivity of the bare nanoscale copper metal surface. Evidently, the segregated copper atoms gradually combined to produce CuO NPs.

Finally, in the termination step, stable CuO NPs were obtained. Pear-derived natural bio-macromolecules such as starch with linear and branched structures surrounded the nucleated NPs, thereby creating a protective shield and restricting the growth of CuO NPs [24]. Additionally, the steric forces resulting from the biological macromolecules maintained the separation of the capped NPs from one another, thereby preventing their agglomeration.

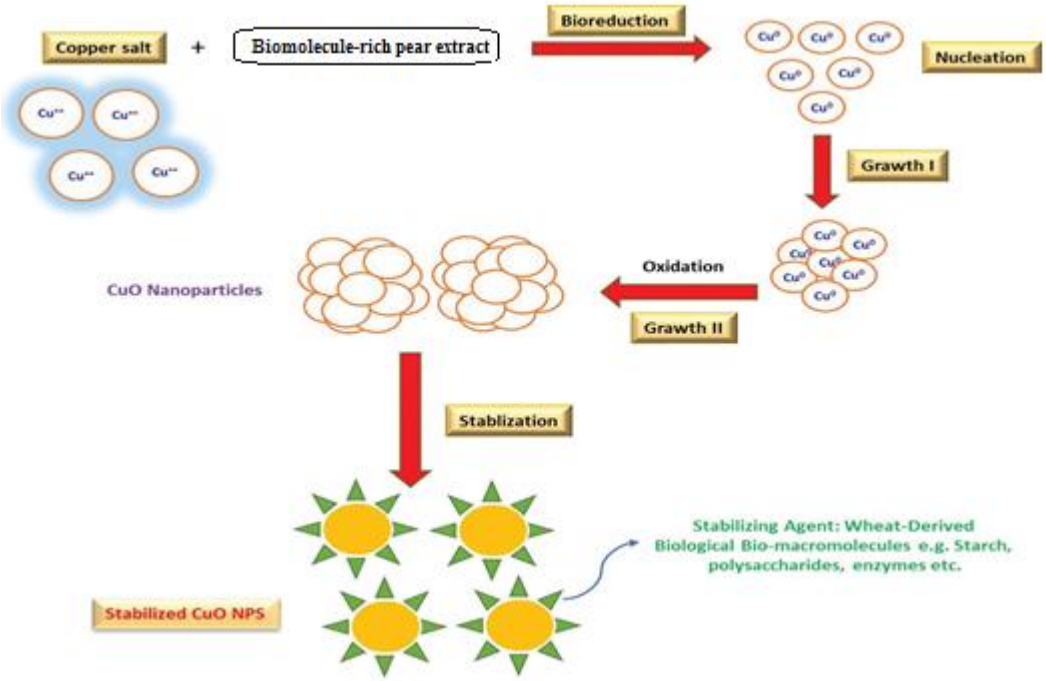


Fig. 3. Mechanism of CuO NPs formation [24]

2.2.4 Characterisation of CuO NPs

CuO NPs were subjected to transmission electron microscopy (TEM) and scanning electron microscopy (SEM) analyses. The crystalline size and structure of NPs were determined through X-ray diffraction (XRD) using an automated diffract meter (Shimadzu 6000 XRD) and energy-dispersive X-ray spectroscopy (EDX) instrument. The CuO NPs solution was scanned by Fourier transform infrared spectroscopy (FT-IR; Shimadzu 8400), to identify the functional groups in the sample.

2.3. Anti-cancer activity

2.3.1. Cell culture

HT-29 cells were maintained in RPMI-1640 supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 80% confluence twice a week and incubated at 37°C [25,26].

2.3.2 Cytotoxicity determination using MTT assay

To determine the cytotoxic effect of CuO NPs, MTT assay was conducted using 96-well plates [27,28]. Cell lines were seeded at 1×10^4 cells per well. After 24 h or when a confluent monolayer was achieved, cells were treated with CuO NPs at different concentrations (125, 250, 500, and 750 ppm).

Cell viability was measured after 72 h of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT and incubating the cells for 2.5 h at 37 °C. After removing the MTT solution, the crystals remaining in the wells were solubilised by adding 130 µL of DMSO followed by 37 °C incubation for 15 min with shaking [29].

Absorbency was determined on a microplate reader at 492 nm, and the assay was performed in triplicate. The inhibition rate of cell growth (cytotoxicity percentage) was calculated as follows [30,31]:

$$\textbf{Inhibition rate} = A - B / A \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where A is the optical density of the control, and B is the optical density of the samples [32].

To visualise the cell shape under an inverted microscope, cells were seeded onto 24-well micro titration plates at a density of 1×10^5 cells mL^{-1} and incubated for 24 h at 37 °C.

Then, the cells were exposed to CuO NPs for 24 h. After the exposure period, the plates were stained with crystal violet stain and incubated at 37 °C for 10-15 min [30]. The stain was washed off gently with tap water until the dye was completely removed. The cells were observed under an inverted microscope at 100× magnification, and the images were captured with a digital camera attached to the microscope [33-35].

2.3.3 Statistical analysis

The obtained data were statically analysed using an unpaired *t-test* with Graph Pad Prism 6 [36]. The values were presented as the mean ±SD of triplicate measurements [37].

2.4 Antibacterial activity

Using an agar well-diffusion experiment, the antibacterial activity of the produced CuO NPs was studied against the Gram-negative *A. baumannii* and the Gram-positive *S. pyogenes* bacterial strains [38,39]. In sterile Petri dishes, around 20 mL of MH agar was poured aseptically.

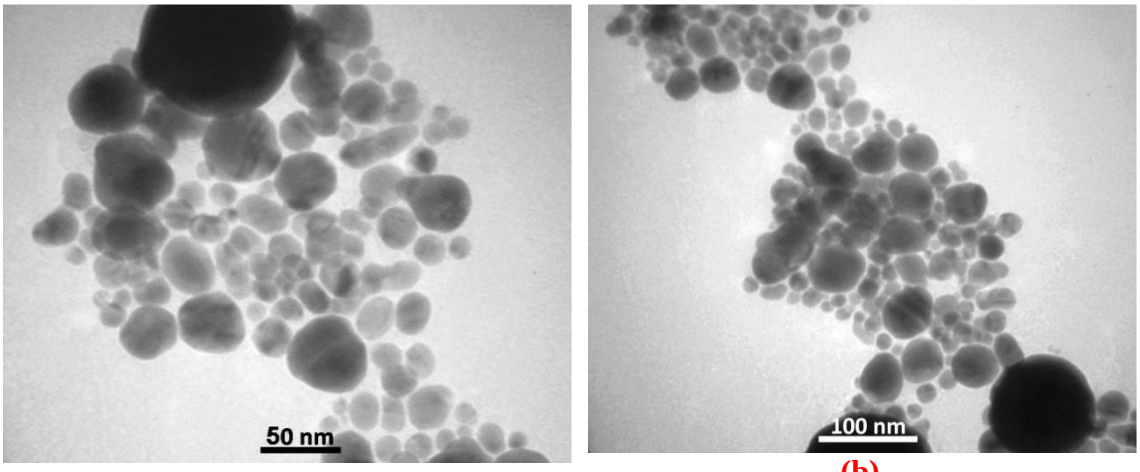
A sterile wire loop was used to capture the bacteria from their stock cultures [40]. After cultivating the organisms, a sterile point was used to bore 6 mm-diameter wells in the agar plates. CuO NPs in various concentrations were injected into the bored wells. The CuO NPs and the test organisms were cultivated in plates and incubated overnight at 37°C before measuring and recording the average diameter of the zones of inhibition [41,42].

3. Result and discussion

3.1 TEM

CuO NPs were studied morphologically, and their size and structure were determined by TEM analysis. CuO nanomaterials were mostly spherical (Fig. 4). The NPs were stacked roughly parallel to one another and had a wide size variety. Furthermore, the produced CuO comprised numerous NPs.

The diameter of the CuO NPs on display ranged within 30-50 nm, consistent with the X-ray result.

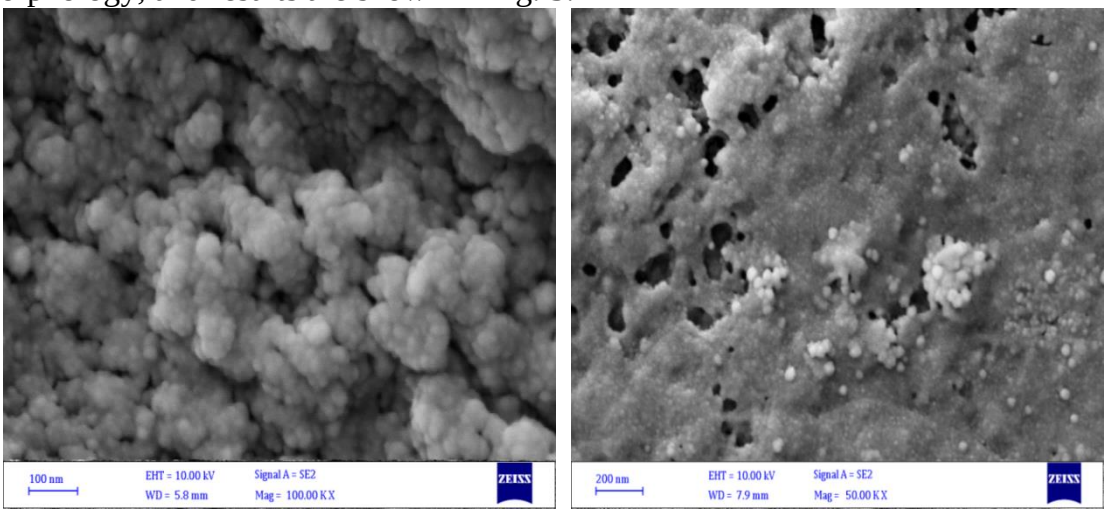


(a) **(b)**

Fig. 4. TEM micrograph CuO NPs **(a)** 50 nm and **(b)** 100 nm

3.2 SEM

CuO NPs produced from aqueous pear extract with diameters ranging from 30 nm to 50 nm were subjected to SEM analysis to assess their shape and surface morphology, and results are shown in Fig. 5.



(a) **(b)**

Fig. 5. SEM micrograph CuO NPs **(a)** 100 nm and **(b)** 200 nm

3.3 EDX

EDX analysis confirmed presence of the prepared copper oxide NPs, as shown in (Fig. 6).

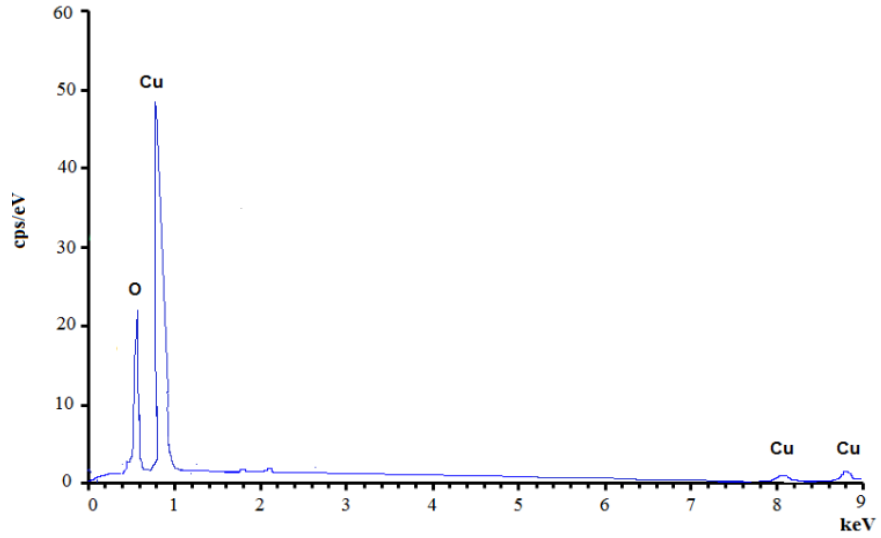


Fig. 6. X-ray energy-dispersive spectroscopy of the prepared CuO NPs

3.4 XRD analysis

Fig. 7 shows the XRD images of copper oxide NPs compared with the standard reference JCPDS No. (05-0661). CuO NP diffraction showed peaks at (110), (111), (002), (202), (202), (113), (113), and (222) which were attributed to the angles 27.56°, 35.53°, 38.62°, 48.75°, 61.62°, 66.22°, 68.24°, and 75.14°, respectively. This finding suggested that all these peaks belonged to the prepared nanostructured copper oxide with a face-centred cubic (fcc) structure. According to the standard reference and upon matching with the Joint Committee on Powder Diffraction Standards card, it was found to be identical and from its composition (fcc).

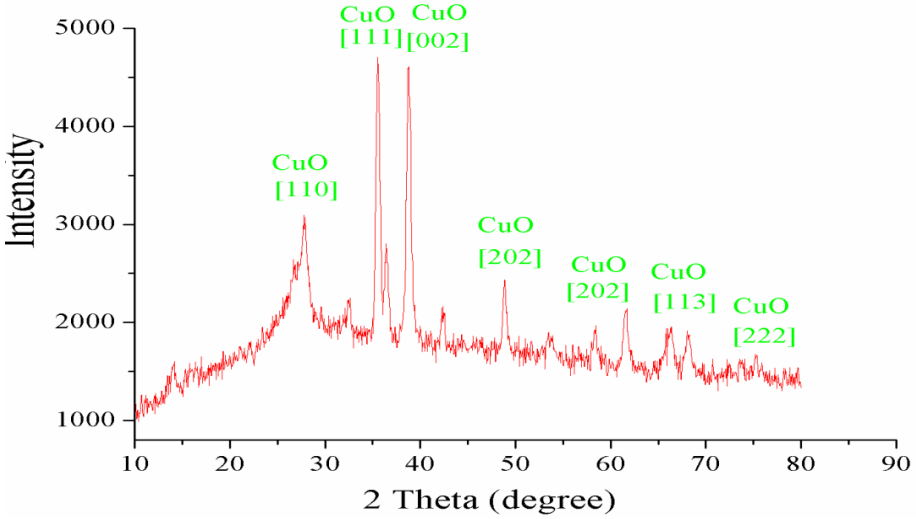


Fig. 7: Shows X-ray diffraction of CuO NPs compared to standard reference for copper oxide JCPDS (05-0661)

3.5 FT-IR

In the FT-IR range of CuO NPs, the band was approximately 3251.98 cm^{-1} because of O-H vibrations extending in water, alcohol, and phenols, as well as N-H extending in amines (Fig. 8). In the C–N stretch, the band was approximately 2133.27 cm^{-1}. The band at 1631.78 cm^{-1} was due to the C-C stretch in the fragrant ring and C=O stretch in phenols and N-H bond in amines. The sharp peak observed within 540-570 cm^{-1} was attributed to the vibrational phonon of CuO NPs. which was attributed to the vibration of CuO NPs. This result indicated the successful production of CuO NPs [43].

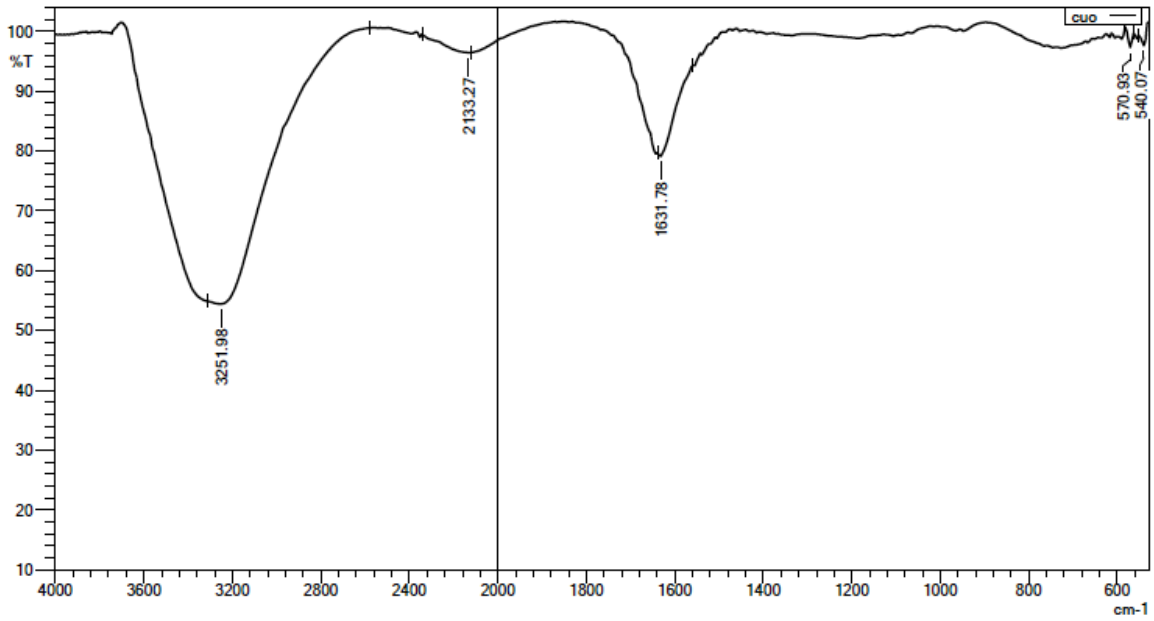


Fig. 8. Shows FT-IR spectrum of CuO nanoparticles

3.6 Anti-cancer activity

The electrostatic characteristics of CuO NPs are crucial to biomedical applications. In cancer and medication-delivery applications, CuO NPs have also been used as a convenient alternative material. Compared with macroscale CuO, several tests revealed that CuO NPs had significant cytotoxicity against several types of cancer cells. Under physiological settings, CuO NPs had a positive charge. Given that cancer cells contain high amounts of anionic phospholipids on their outer membrane, an electrostatic bond that promotes cellular uptake and cytotoxicity forms [44]. The effects of CuO on colon-cancer cells were investigated. The capacity of CuO to limit the proliferation of cancer cell lines was used to examine their anti-proliferative effect. Fig. 10 shows the findings of this investigation. Results showed that the prepared CuO had the power to degrade and kill colon-cancer cells. CuO had a concentration-dependent action against cancer cells, as shown in (Fig. 9).

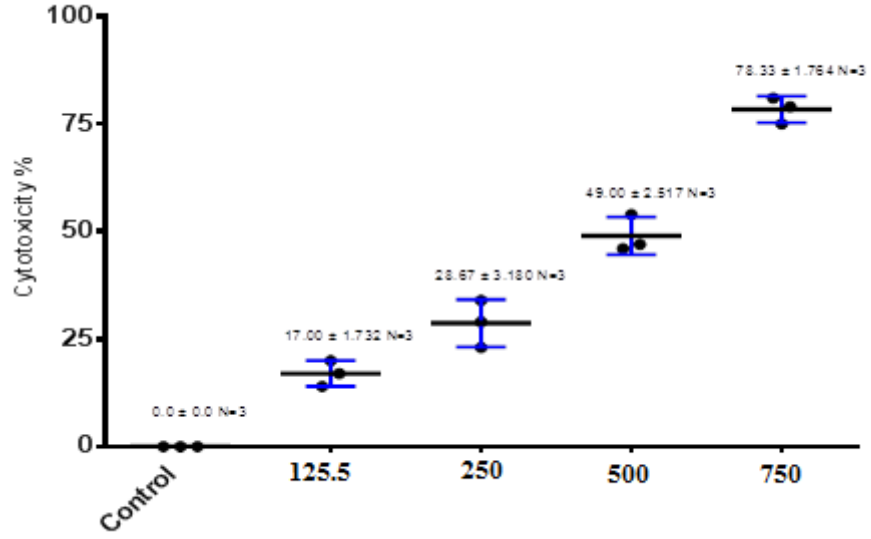
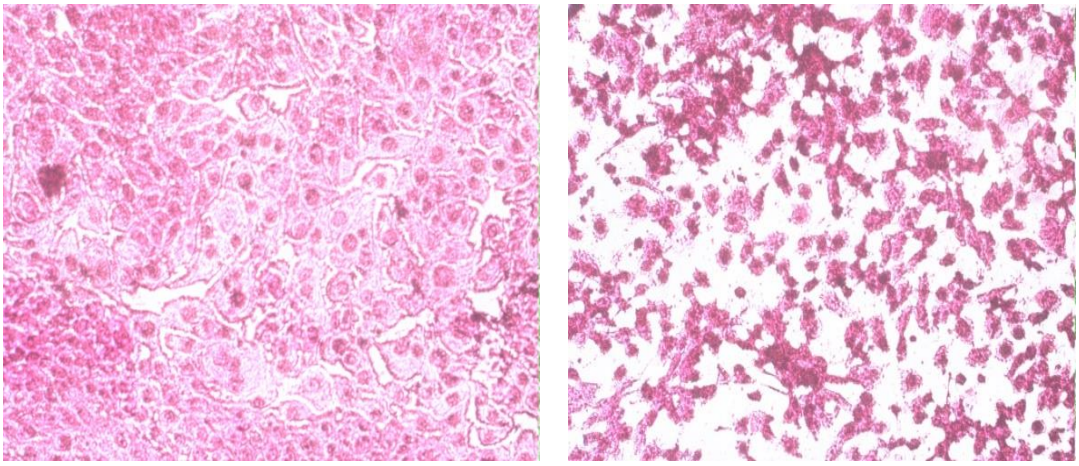


Fig.9. Cytotoxicity of CuO NPs in HT-29 cells. concentrations (125, 250, 500,750) ppm



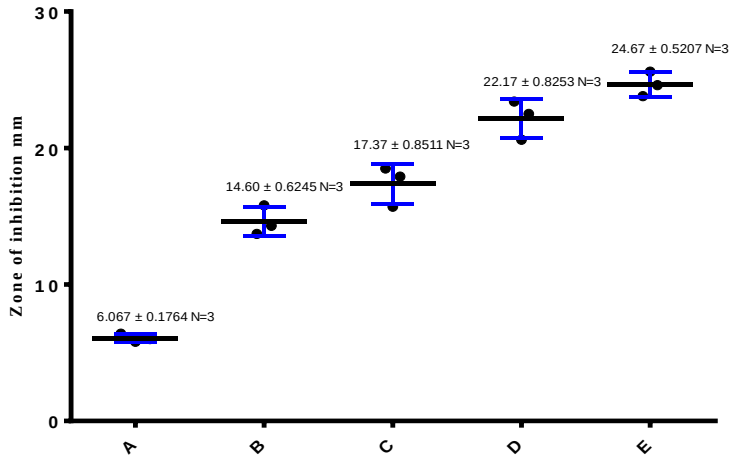
(a) **(b)**

Fig. 10. **(a)** Control untreated HT-29 cells, **(b)** Morphological changes in HT-29 cells after treated with CuO NPs

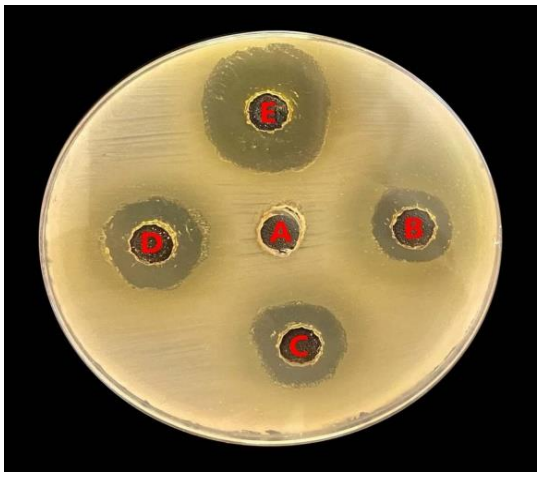
3.7. Anti-bacterial activity

Antibiotic resistance is a major contributor to hospital-acquired illnesses, and managing it necessitates the use of pharmaceutical formulations with various side effects. Consequently, researchers have concentrated on a combination of low-risk and effective bacterial resistance treatment. Using nanomaterials and assessing their antibacterial activities can help reduce healthcare-associated infection. CuO NPs show significant antibacterial activities against a broad spectrum of pathogenic microorganisms, including *S. pyogenes* and *A. baumannii*. CuO NPs' antibacterial activity was determined by serial-dilution techniques (Figs. 11 and 12) [45].

CuO NPs activity as an antibacterial at different levels in *S. pyogenes* was illustrated through inhibitory zones of various widths. As given in Table 1, copper oxide NPs had an inhibitory zone with diverse sizes against *A. baumannii*, as shown in Table 2. Significant growth inhibition was detected with increased concentration CuO NPs.



(a)



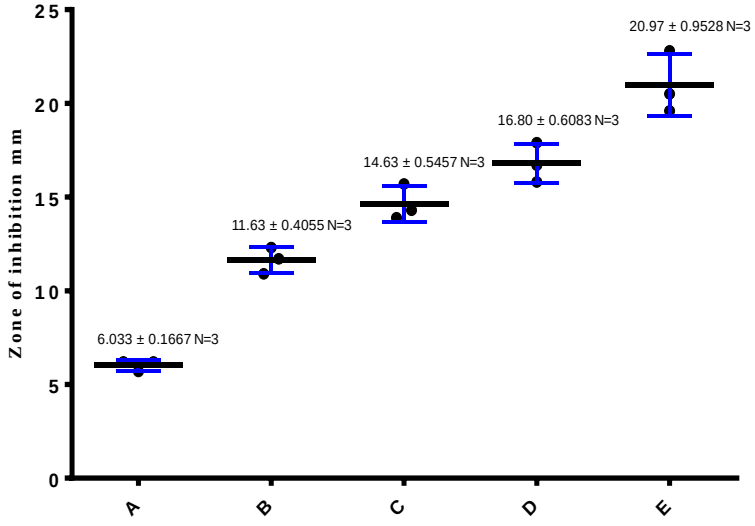
(b)

Fig.11: **(a)** Zone of inhibition for CuO NPs in mm

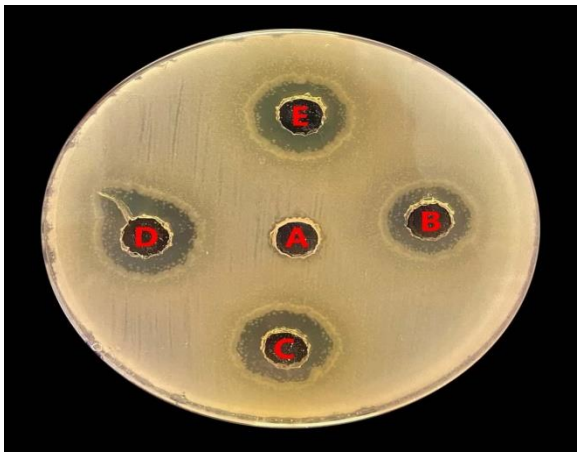
(b) Antibacterial activity of CuONPs against *Streptococcus pyogenes*. **A**. control untreated bacterial strains. **B**. bacterial strain treated with 62.5 ppm. **C**. bacterial strain treated with 125 ppm. **D**. bacterial strain treated with 250 ppm. **E**, bacterial strain treated with 500 ppm

Table (1): Growth inhibition of CuO NPs against *Streptococcus pyogenes*

Concentration	Inhibition zone mm
62.5 ppm	14.60±0.62 mm
125 ppm	17.37±0.85 mm
250 ppm	22.17±0.82 mm
500 ppm	24.67±0.52 mm



(a)



(b)

Fig.12. **(a)** Zone of inhibition for CuO NPs in mm

(b) Anti-bacterial activity of CuO NPs against *Acinetobacter bumannii*. **A**. control untreated bacterial strains. **B**. bacterial strain treated with 62.5 ppm. **C**. bacterial strain treated with 125 ppm. **D**. bacterial strain treated with 250 ppm. **E**. bacterial strain treated with 500 ppm

Table (2): Growth inhibition of CuO NPs against *Acinetobacter bumannii*

Concentration	Inhibition zone mm
62.5 ppm	11.63±0.40 mm
125 ppm	14.63±0.54 mm
250 ppm	16.80±0.60 mm
500 ppm	20.97±0.95 mm

4. Conclusion

CuO NPs can be used as anticancer and antibacterial medicines, offering considerable potential as an anticancer and antimicrobial delivery method. CuO NPs were synthesised using aqueous pear extract. This method of processing NPs was easy, environmentally friendly, cost effective, and energy efficient. Further research into the potential applications of CuO NPs as anticancer and antibacterial agents is needed based on our findings.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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