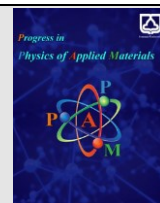




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Comparative Study of Cu and Fe-Doped ZnO Nanoparticles: Synthesis, Characterization, and Multifaceted Bioactivities

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ABSTRACT

Metal oxide nanoparticles display significant roles in antimicrobial and anticancer activities. In the present study, Cu, and Fe-doped ZnO nanoparticles have been synthesized and investigated for their antioxidant, antibacterial, and anticancer properties. The above-mentioned nanoparticles have been synthesized by the low-cost and simple sol-gel method. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay was conducted to assess the antioxidant activity. The antibacterial activity of NPs was tested against *E. coli* and *S. aureus* bacteria according to the broth microdilution method in Mueller Hinton Broth. The anticancer potency on cancerous AsPC-1 cell lines was examined. The structural and morphology of samples confirm that all NPs formed with different crystallite sizes in the hexagonal wurtzite system. The DPPH assay showed the $Zn_{1-x}Cu_xO$ to have more antioxidant properties than other samples. We observed that the *S. aureus* bacterium is more sensitive to NPs than the *E. coli* bacterium. The strongest and weakest substances used for these bacteria are $Zn_{1-x}Cu_xO$ and $Zn_{1-x}Fe_xO$ NPs, respectively. Our anticancer results showed that the loaded drug on the NPs surfaces has more anticancer properties than pure drugs. ZnO and $Zn_{1-x}Fe_xO$ NPs possess similar anticancer properties approximately, while sunitinib@ $Zn_{1-x}Cu_xO$ eliminates 92% of cancer cells at 200 $\mu\text{g/ml}$ concentration. We observed that ZnO, $Zn_{1-x}Fe_xO$, and $Zn_{1-x}Cu_xO$ NPs have antioxidant, antibacterial, and anticancer properties. Adding copper dopant to ZnO NP significantly increases its anticancer property.

1. Introduction

Currently, various cancers have been the leading cause of human death with metastasis and secondary infection treatments of cancers have general methods like surgery [1], chemotherapy [2], radiotherapy, and immunology [3]. Therefore, one should find a way to overcome the resistance of cancer cells to usual treatments with less harmful side effects. In cancer therapy like the analgesics used during and after surgery might have on long-term outcomes such as cancer recurrence [4]. In addition, chemotherapy has frequent toxic side effects such as hepatitis, cholestasis, and

steatosis [5]. Likewise, radiotherapy damages the DNA in surrounding healthy tissue of cancer cells and causes accelerated aging [6]. Microbial contamination is a pressing issue in health care, being responsible for nearly 40% of the 50 million global deaths annually due to diseases caused by bacteria like *Escherichia coli* and *Salmonella* [7, 8]. Different therapeutic applications have been paved using various nanomaterials with recent developments in nanotechnology [9], so they are of considerable interest. Multi-functional metals and metal oxide NPs have significant applications in antimicrobial activities, biomedicine, pharmaceuticals, food industry and healthcare

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[10-12]. Metal oxide NPs like: TiO_2 [13], ZnO [14], CuO [15], SiO_2 [16], SnO_2 [17], and MgO [18] exhibit interesting antibacterial properties and anticancer potential [19-22]. Among these, ZnO has been particularly noted for its anticancer and antibacterial activity [23, 24]. Also, it has been indicated by several researchers that ZnO nanostructures can be successfully used against various cancer cell lines [25-28], both gram-positive and gram-negative bacteria [29-32]. Zinc oxide is an affordable, non-toxic, and well-behaved metal oxide suitable for therapeutic applications compared to other metal oxides. Antioxidants contribute to enhancing the body's natural antioxidant defense mechanisms. They support the body's production of endogenous antioxidants and activate various enzymes

involved in antioxidant pathways, such as superoxide dismutase, catalase, and glutathione peroxidase. These protective systems collaborate to maintain balance and prevent the organism from experiencing excessive oxidative stress [33].

We synthesized ZnO NPs with Fe and Cu dopants using a simple and affordable sol-gel method and investigated their antioxidant, antibacterial, and anticancer activity. Additionally, the anticancer drug (sunitinib) was loaded on the ZnO NPs and compared with the NP's anticancer properties which will provide novel opportunities in cancer nanotechnology.

The experimental research procedure is shown as a schematic in Fig. 1.

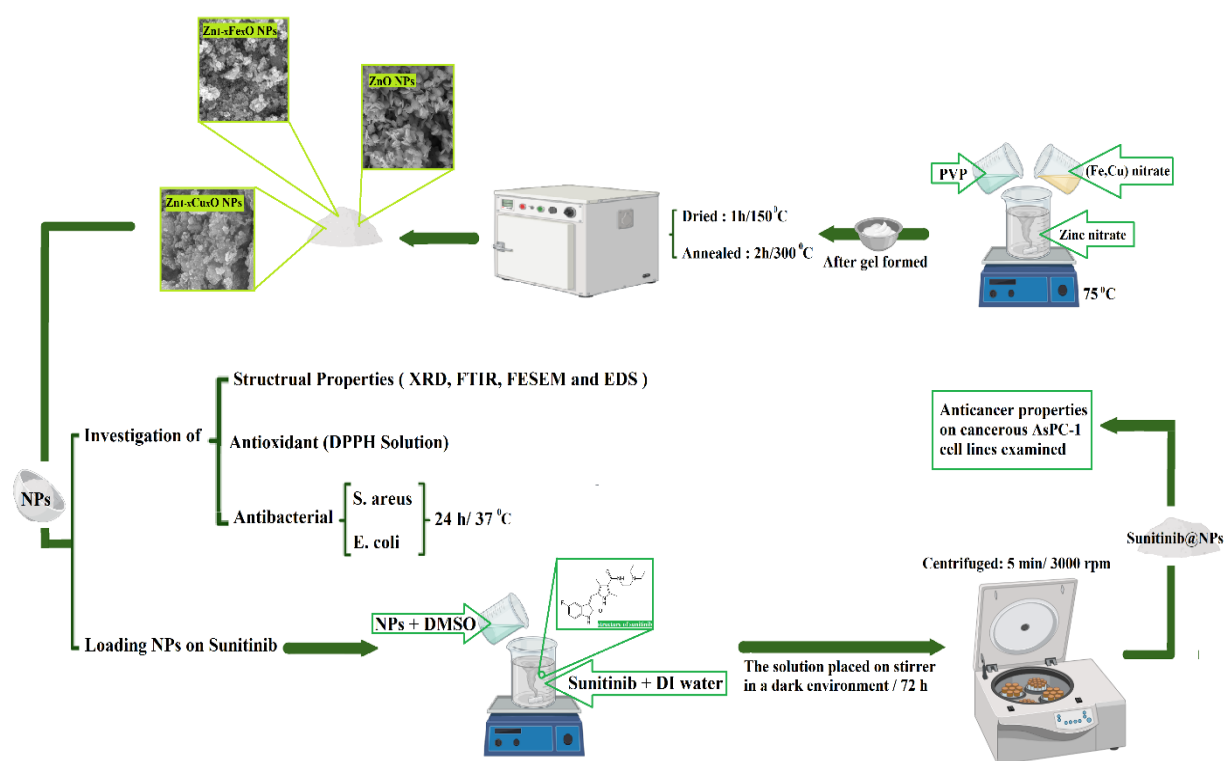


Fig. 1. Schematic of the experimental procedure

2. Materials and methods

2.1. Material

The chemicals used in this study were laboratory-grade and prepared by Merck, and chemical materials include Zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), Iron (II) nitrate nonahydrate ($\text{Fe}(\text{NO}_3)_2$), Copper (II) nitrate trihydrate ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$) and Polyvinylpyrrolidone (PVP) ($\text{C}_6\text{H}_9\text{NO}$)_n. Deionized water, dimethyl sulfoxide (DMSO, $\text{C}_2\text{H}_6\text{OS}$), DPPH (2,2-diphenyl-1-picrylhydrazyl), and methanol (CH_3OH) were also used. For the antibacterial test, *E. coli*¹ (ATCC 25922, negative gram) and *S. aureus*² (ATCC 29213, positive gram) bacteria were purchased from IROST³. To test for anticancer, sunitinib 25 mg capsule and AsPC-1 cell line belonging to human pancreatic cancer were supplied from the Pasteur Institute of Iran.

2.2. Method

The following describes the synthesis method of pure and doped ZnO NPs.

Undoped ZnO NP Synthesis:

At first, 1 g of zinc nitrate hexahydrate (a source of zinc) is dissolved in 10 ml of methanol to prepare the precursor solution. The precursor solution is placed on the stirrer and stabilized at 70 °C. Then, 0.1 g of PVP is dissolved in 5 ml of distilled water which acts as a stabilizer or surfactant, helping to control the size and shape of the NPs. This solution is then added to the precursor solution. The mixture solution is heated to 75 °C to induce gel formation. This means the liquid solution thickens and forms a semi-

1 Escherichia coli

2 Staphylococcus aureus

3 Iran Scientific and Industrial Research Organization

solid gel. After that, the gel is dried at 150 °C for 1 hour to leave behind a dry powder. Finally, the powder is annealed at 300 °C for 2 hours. This step helps to crystallize the material, improving its structural properties [34].

Doped ZnO NPs Synthesis:

The target concentration of the dopants (Fe or Cu) in the ZnO nanostructure is 10 weight percent. Dopant sources were iron and copper using iron (II) nitrate and copper (II) nitrate, respectively. The iron or copper nitrate is dissolved in 10 ml of methanol to prepare the doping resources. This solution is then added to the initial zinc nitrate solution before gel formation. In the final step, the PVP solution added them. The rest of the process (gel formation, drying, annealing) is similar to the undoped ZnO preparation.

Load Sunitinib Capsules on NPs:

To load the sunitinib drug on the ZnO NPs, two 25 mg sunitinib capsules were dissolved in 5 ml deionized water and stirred until completely resolved as the primary solution. 0.2 g of NPs were poured into 5 ml of DMSO until completely dissolved in the solution. The NP solution was added to the primary solution and stirred in a dark environment for 72 h. After the reaction time, the final solution was centrifuged at 3000 rpm for 5 minutes.

Antioxidant Activity:

To assess the antioxidant activity of ZnO NPs, the DPPH assay was conducted following the methodology described by Miliauskas et al., with minor adjustments [35]. In this experiment, a 0.1 mM ethanolic DPPH solution (4 mL) was prepared, and 3 mL of the NP solution was added to the DPPH solution. The mixture was thoroughly vortexed to ensure proper mixing. After an incubation period, the solution's absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Spectrum SP-UV500DB). The DPPH radical activity was determined using the following formula [36]:

$$\text{Antioxidant activity} = \frac{(z - x)}{z} \times 100\% \quad (1)$$

The absorbance value denoted as *z* represents the control parameter, while it is denoted as *x* for the sample.

Antibacterial Activity:

The antibacterial effectiveness of NPs was evaluated against two types of bacteria: the gram-negative *Escherichia coli* (*E. coli*, ATCC-25922) and the gram-positive *Staphylococcus aureus* (*S. aureus*, ATCC-25923). This assessment was carried out using the broth microdilution method in Mueller Hinton Broth (MHB), a widely used culture medium that supports the growth of these bacterial species and allows for precise determination of antimicrobial activity. The bacteria strains were cultured in trypticase Soy Broth (TSB) and incubated for 24 hours at 37°C. After incubation, the culture medium containing the grown bacteria was centrifuged at 5000 rpm for 20 minutes, the supernatant was discarded and the bacteria were washed twice with

normal saline. Finally, the precipitated contents were dissolved with physiological serum and reached a concentration of 0.5 McFarland standard (1.5×10^8 CFU/ml) [37]. The bacterial suspension was used as inoculum in the antibacterial assays.

Serial dilutions of the NPs were prepared to achieve initial concentrations in TSB, resulting in final concentrations of 1024, 512, 256, 128, 64, 32, 16, 8, 4, and 2 mg/l on a plate. After adding 100 µl of bacterial inoculum to each suspension, it was incubated at 37 °C for 24 hours. After the incubation period, the microplates were read using an ELISA reader at a wavelength of 600 nm. The minimum lethal concentration of NPs for bacteria was also investigated [38]. At the end of the experiment, the percentage of inhibition of microbes in the presence of NPs was calculated using the following formula [39]:

$$\text{Inhibition rate} = \frac{(D_{\text{control}} - 0.5) - (D_{\text{treatment}} - 0.5)}{(D_{\text{treatment}} - 0.5)} \times 100\% \quad (2)$$

where *D* means colony diameter, the higher the inhibition rate value shows stronger the inhibitory effect.

Anticancer Activity:

We tested the anticancer activity of samples using the AsPC-1 cell line of human pancreatic cancer. The cell line was cultured in culture medium RPMI (BIO-IDEA; Iran) containing 10% FBS (Gibco; USA) and 1% antibiotic (BIO-IDEA; Iran). To evaluate cytotoxicity, we conducted a detailed assay using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method, sourced from Sigma Aldrich. This assay measures cell viability based on the metabolic activity of living cells. After treatment, we quantitated the intensity of the resulting color, which correlates directly to the number of viable cells. This approach provides us with insight into the cytotoxic effects of the tested compounds, allowing for a thorough understanding of their potential impact on cellular health. At first, 3×10^3 cells per ml and ZnO NPs were seeded in each well of a 96-well plate. After 24 hours, 10 ml of MTT solution with 5 mg/ml concentration was added to the cell culture medium, and the plate was incubated for 3 hours. Then the cell supernatant was removed, and 80 µl of DMSO was added to each well. After 30 minutes, the contents of the wells were pipetted several times to flush out the sediment inside the cells. The optical density (OD) from the solution dye was assessed at 570 nm employing an ELISA reader. The cell viability rate (VR) an important measure used to assess the health and functionality of cells in various experimental contexts, was calculated by the following equation [40]:

$$\text{VR} = \frac{A}{A_0} \times 100\% \quad (3)$$

where *A* represents the absorbance of the cells treated with formulations and *A*₀ refers to the absorbance of the control group.

3. Structural Characterization

The crystal structure of the samples was characterized using X-ray diffraction (XRD) with a Philips instrument, Energy-dispersive X-ray spectroscopy (EDS) using a TESCAN VEGA 3, and Fourier transform infrared spectroscopy (FT-IR) with a Thermo AVATAR. The morphology of the NPs was investigated using field emission scanning electron microscopy (FESEM) with a TESCAN MIRA III.

4. Results and Discussion

The XRD patterns of nanostructures of ZnO, Zn_{1-x}Fe_xO, and Zn_{1-x}Cu_xO are presented in Fig. 2, that the diffraction peaks observed in all samples correlate with the standard diffraction pattern for ZnO crystals JCPDS (01-072-0627), which features a hexagonal wurtzite-type structure [41]. Since there are no additional peaks beyond those of undoped ZnO in the diffraction patterns of Zn_{1-x}Fe_xO and Zn_{1-x}Cu_xO, it can be concluded that the samples have a single-phase crystalline structure consistent with bulk ZnO. This indicates that some of the zinc sites in the ZnO nanostructures have been substituted with iron (Fe) and copper (Cu). The Miller indices for the planes corresponding to each peak are illustrated in Fig. 2.

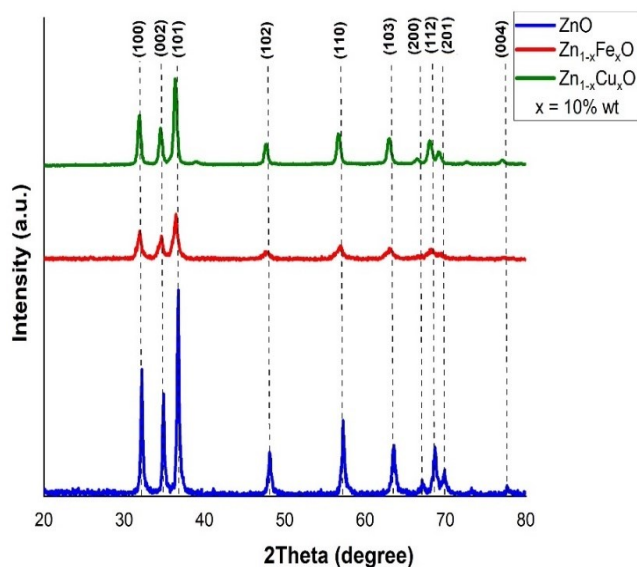


Fig. 2. XRD patterns of the ZnO, Zn_{1-x}Fe_xO, and Zn_{1-x}Cu_xO NPs

By analyzing the peak widths obtained from the X-ray diffractogram, we can accurately estimate the average diameter of the nanocrystals. The average size of the nanocrystals was determined using the well-known formula of Scherrer equation [42]:

$$D = \frac{0.9\lambda}{\beta \cos \theta} \quad (4)$$

In this context, D , λ , β , and θ represent the crystal size, the wavelength of the X-ray source (0.154 nm), the broadening of the diffraction line at full-width half maximum, and the position of the diffraction peaks,

respectively. The crystallite size is obtained by averaging over the size of all the peaks. They are given in Table 1.

Table 1. The crystallite size of NPs

Dopant source	D (nm)
Bare	31.98
Fe	32.09
Cu	58.82

The results presented in Table 1 indicate that the addition of Cu and Fe dopants leads to a slight variation in the crystallite size compared to the bare sample. This difference is likely due to a minor mismatch among the Zn, Fe, and Cu ions. The findings suggest that the Cu and Fe ions effectively replace the Zn ions in the samples without significantly altering the overall crystal structure [43], and the type of impurity causes a change in the crystallite size.

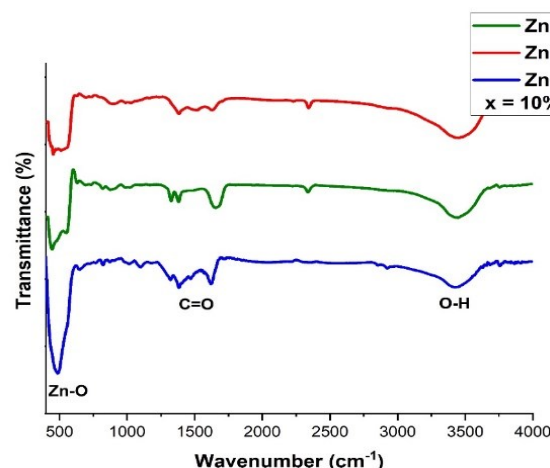


Fig. 3. FTIR spectra of ZnO prepared samples

FTIR spectra of the NPs were presented in Fig. 3, in the range of 400-4000 cm⁻¹. The peak observed between 450-500 cm⁻¹ is attributed to the stretching modes of Zn-O [44]. The reduction in the intensity of the Zn-O peak is due to the substitution of impurities in the Zn position by adding impurities. The broad peak between 3200-3600 cm⁻¹ is associated with the bands of bonded hydroxyl groups of the vibration of adsorbed water molecules [45]. In addition, two distinct peaks are observed at 1072 cm⁻¹ and 1356 cm⁻¹, which are characteristic of the carboxyl group [45].

The NP morphologies were analyzed using the FESEM technique, as shown in Fig. 4. The results indicate that the formation of hexagonal ZnO varies with different dopant elements, and impurities have notably altered the morphology.

The energy dispersive X-ray spectroscopy (EDS) analysis of the NPs presented in Fig. 5 indicates that the ZnO NPs exhibit peaks corresponding only to 'Zn' and 'O'. In contrast, the doped ZnO NPs also display peaks for 'Fe' and 'Cu', confirming the incorporation of iron and copper

ions into the ZnO lattice, is accompanied by a reduction in the percentage of zinc.

In Fig. 6, the scavenging percentages of DPPH were measured to assess the antioxidant activity. As we know, The DPPH assay is a relatively simple and widely used method for evaluating a substance's antioxidant capacity. DPPH is a stable free radical. In its radical form, it has a

deep violet color and absorbs strongly at a specific wavelength (usually around 515-520 nm). This strong absorbance allows us to track changes in its concentration using a spectrophotometer. When an antioxidant molecule is added to the DPPH radical solution, the antioxidant donates either a hydrogen atom or an electron to the DPPH radical. This neutralizes the DPPH radical, converting it to its non-radical form (DPPH-H).

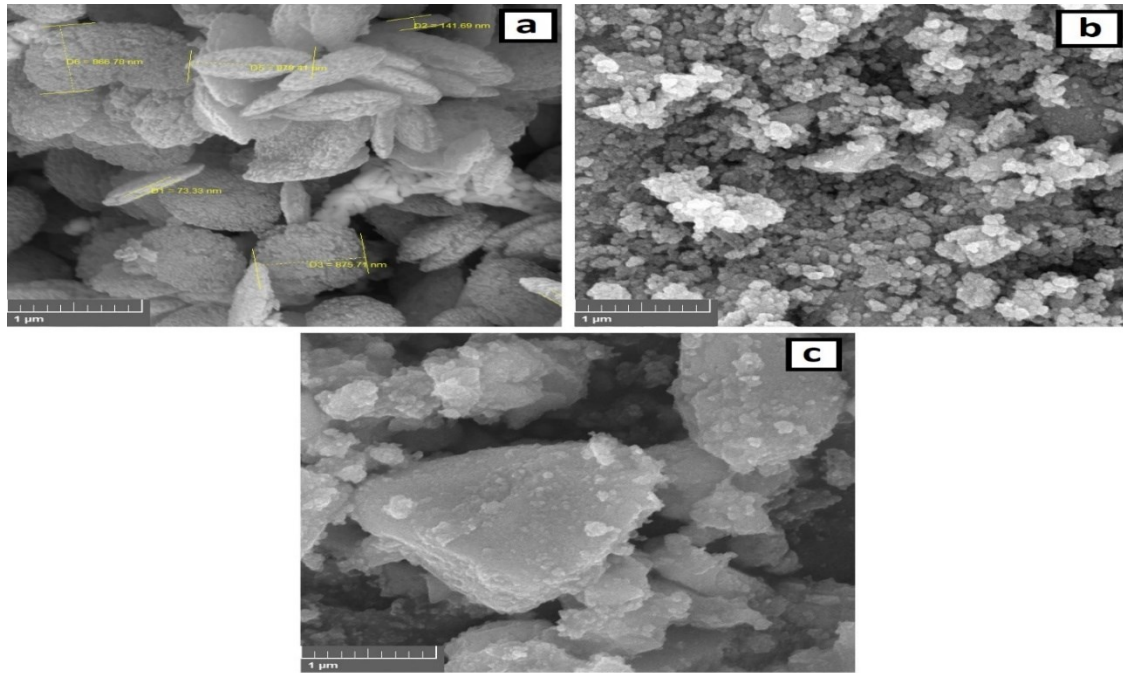


Fig. 4. FESEM images of (a) ZnO, (b) $\text{Zn}_{1-x}\text{Fe}_x\text{O}$ and (c) $\text{Zn}_{1-x}\text{Cu}_x\text{O}$ NPs

As the DPPH radical gets neutralized, the violet color of the solution fades. The extent of this color change is directly proportional to the antioxidant capacity of the tested substance. The more antioxidants present in the sample,

the more DPPH radicals will be reduced, leading to a greater decrease in absorbance (and lighter color). This color change is measured using a spectrophotometer where DPPH absorbs most strongly [46, 47].

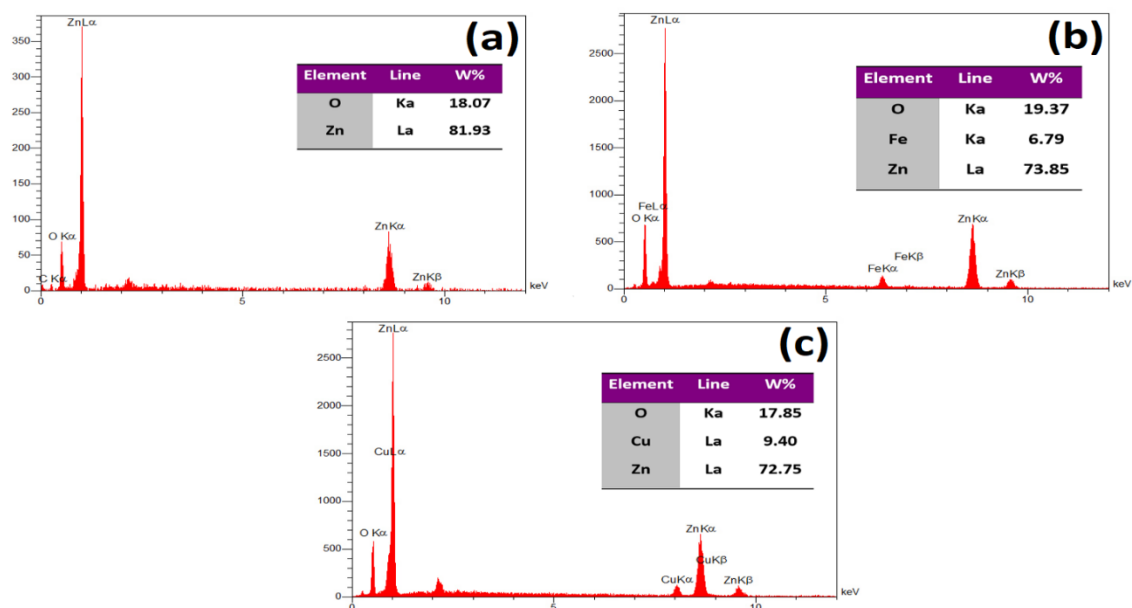


Fig. 5. EDS of the (a) ZnO, (b) $\text{Zn}_{1-x}\text{Fe}_x\text{O}$, and (c) $\text{Zn}_{1-x}\text{Cu}_x\text{O}$ NPs

The radical scavenging percentages for different samples were as follows: ZnO exhibited a scavenging percentage of 48.46 ± 2.23 , $\text{Zn}_{1-x}\text{Fe}_x\text{O}$ showed 69.12 ± 4.34 , $\text{Zn}_{1-x}\text{Cu}_x\text{O}$ demonstrated 88.98 ± 3.43 , and EU80PEG20Gin10% displayed $73.16 \pm 3\%$. Fig. 6, shows that adding impurity improved the antioxidant properties, and copper dopant was more suitable than iron.

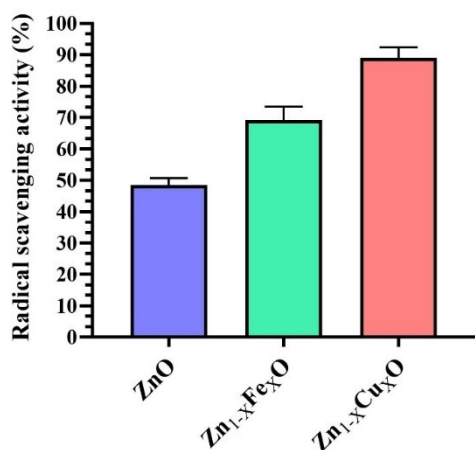


Fig. 6. Antioxidant properties of ZnO NPs

Fig. 7 shows the inhibition percentage of *S. aureus* and *E. coli* bacteria by NPs. Based on the results obtained from

this experiment, $\text{Zn}_{1-x}\text{Cu}_x\text{O}$ NPs showed more powerful antibacterial effects than the other two NPs, and $\text{Zn}_{1-x}\text{Fe}_x\text{O}$ had the least inhibitory effect on both bacteria. Among the bacterial samples tested, *S. aureus* bacterium (gram-positive) was more sensitive to NPs (Fig. 7a), and *E. coli* (gram-negative) one showed more resistance to NPs (Fig. 7b). Both bacterial types are affected by reactive oxygen species (ROS) generated by NPs. However, *S. aureus* bacterium is more vulnerable to oxidative damage due to its simpler cell wall and fewer protective mechanisms compared to *E. coli* one. This increases the effectiveness of NPs in disrupting *S. aureus* bacterium and also Copper ions released from $\text{Zn}_{1-x}\text{Cu}_x\text{O}$ NPs contribute to the antibacterial effect, and *S. aureus* bacterium is generally more sensitive to copper due to less efficient regulation of copper uptake compared to *E. coli* bacterium. Although both bacteria can form biofilms, *S. aureus* bacterium typically has a more robust biofilm structure, which may make it more susceptible to NP penetration if the NPs are highly reactive. In contrast, *E. coli* bacterium has more efficient mechanisms for expelling NPs, which may contribute to its higher resistance. *E. coli* bacterium strains often possess more mechanisms for resisting antimicrobial agents, such as efflux pumps, which might reduce the effectiveness of NPs against it compared to *S. aureus* one.

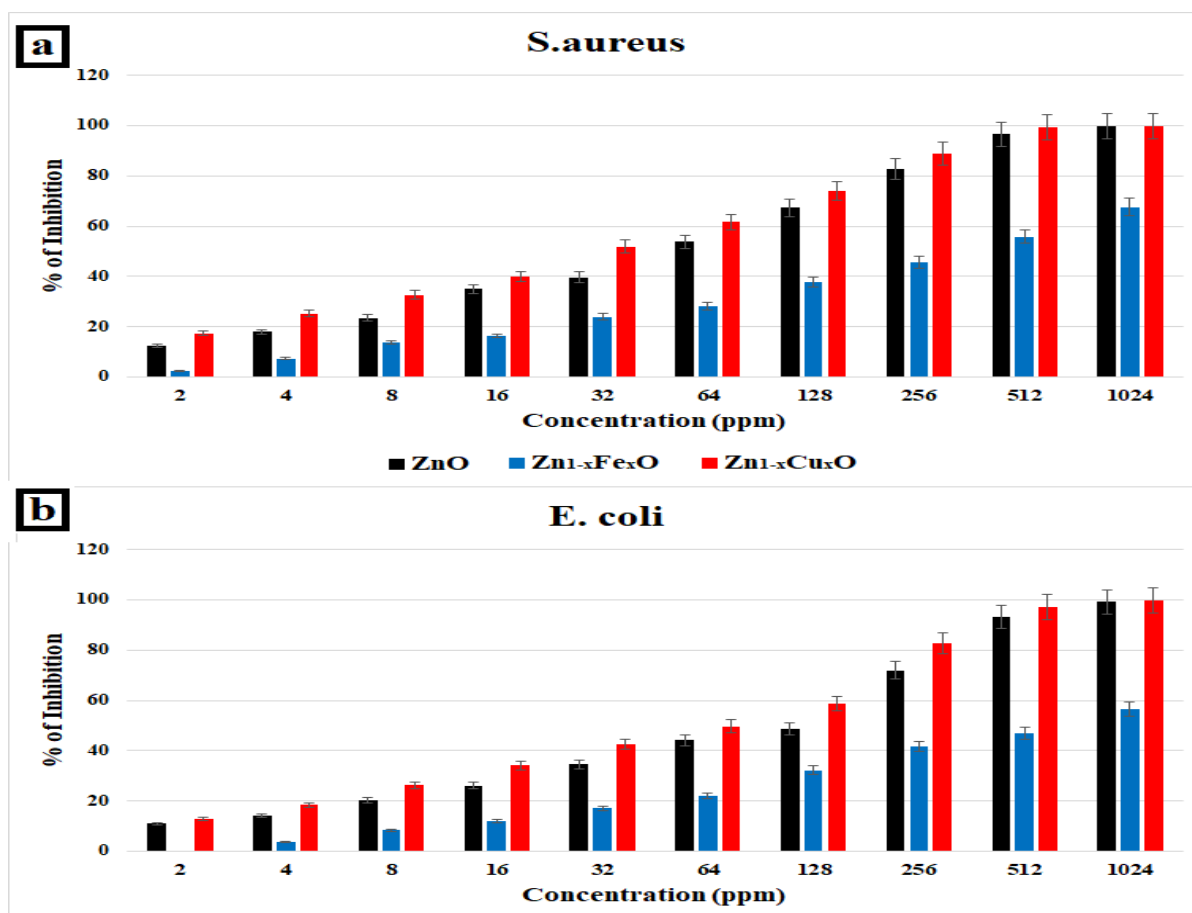


Fig. 7. Antibacterial effects of concentrations of 2 to 1024 ppm of NPs against (a) *S. aureus* and (b) *E. coli* bacteria

The 50% inhibitory concentration (IC-50) of the three synthesized NPs reported in Fig. 8, based on which the powerful substance used is $Zn_{1-x}Cu_xO$ NPs, whose IC-50 for *S. aureus* and *E. coli* bacteria was 80.77 and 176.44 ppm, respectively, and the weakest of them was $Zn_{1-x}Fe_xO$ NPs, whose IC-50 for *S. aureus* and *E. coli* bacteria was 555.31 and 715.57 ppm, respectively.

The difference in antibacterial activity between Gram-positive *S. aureus* and Gram-negative *E. coli* is primarily due to structural variations in their cell walls. Gram-negative bacteria possess an outer membrane rich in lipopolysaccharides, which serves as an extra protective barrier against the entry of NPs. This outer membrane limits the permeability of NPs and reduces their direct interaction with the bacterial cell. Additionally, the negative charge on the outer membrane's phospholipids can repel positively charged NPs, further hindering their

ability to penetrate the cell. Even when NPs breach this barrier, the periplasmic space—a region between the outer membrane and the thin peptidoglycan layer—contains enzymes capable of neutralizing reactive oxygen species (ROS) produced by NPs, thereby providing additional resistance. In opposition, Gram-positive bacteria have a thicker peptidoglycan layer but lack the outer membrane of Gram-negative bacteria. This structural simplicity allows NPs to access the cell more easily. The interaction of positively charged copper ions with the negatively charged teichoic acids within the Gram-positive cell wall may also lead to more effective bacterial membrane disruption and enhanced ROS generation, contributing to their increased susceptibility. The structural and biochemical differences between Gram-positive and Gram-negative bacteria highlight the strong antibacterial effects of copper-doped NPs specifically against Gram-positive species, as indicated by previous studies [48, 49].

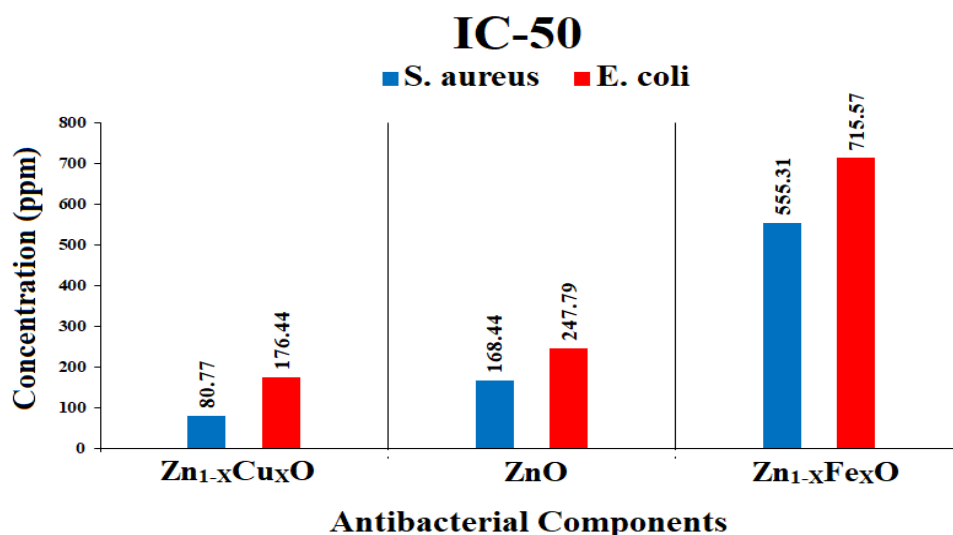


Fig. 8. The amount of IC-50 (Inhibitory Concentration 50%) related to NPs synthesized on *S. aureus* and *E. coli* bacteria

The results of Fig. 9a were analyzed by SPSS software. Fig. 9 shows that cell survival was alleviated by enhancing NP concentration. The percentage of cell death induced was determined using the MTT assay. The percentage of viable cells compared to the control illustrates that ZnO and $Zn_{1-x}Fe_xO$ at a concentration of 50 $\mu\text{g/ml}$ did not have a lethal on cancer cells, but at other concentrations, they have a lethal on cancer cells. Comparing ZnO and $Zn_{1-x}Fe_xO$ with each other on cancer cells, the results show no significant difference in the lethal on cancer cells, while $Zn_{1-x}Cu_xO$ in all concentrations has a lethal on cancer cells more meaningful than ZnO and $Zn_{1-x}Fe_xO$ in various concentrations. Fig. 9b, after loading the drug on the NPs, shows that NPs have significantly increased the anticancer properties of the sunitinib drug in different concentrations. Two NPs of ZnO and $Zn_{1-x}Fe_xO$ acted almost similarly to each other, while $Zn_{1-x}Cu_xO$ has significantly increased the anticancer effect of the drug compared to the other NPs, sunitinib@ $Zn_{1-x}Cu_xO$ destroyed 92% of cancer cells in 200 $\mu\text{g/ml}$ concentration. Copper participates in redox reactions through Fenton-like processes, cycling between

$Cu(I)$ and $Cu(II)$ states. This redox activity generates reactive oxygen species (ROS), such as hydroxyl radicals ($\cdot OH^*$) and superoxide ions ($\cdot O_2^-$), which can damage cellular macromolecules, including DNA, proteins, and lipids. Cancer cells, already under higher oxidative stress, are particularly susceptible to further ROS-induced damage. Zinc is redox-inactive and does not participate in similar ROS-generating reactions. While zinc ions can contribute to anticancer effects by stabilizing biomolecules and enzymes, its impact on oxidative stress is minimal compared to copper [50, 51]. Excess copper disrupts the delicate balance of intracellular metal ions. Copper overload interferes with the function of metalloproteins and enzymes, leading to cellular dysfunction and apoptosis. Copper targets mitochondria, leading to mitochondrial membrane potential collapse, ROS overproduction, and inhibition of oxidative phosphorylation. This can trigger apoptosis via mitochondrial pathways [50, 52]. Our results show that ZnO , $Zn_{1-x}Cu_xO$, and $Zn_{1-x}Fe_xO$ NPs demonstrated less cytotoxic effects on the cancer cell line at low concentrations. The anticancer result showed that the prepared NPs and the drug-loaded on them could be useful as targets against cancer cells.

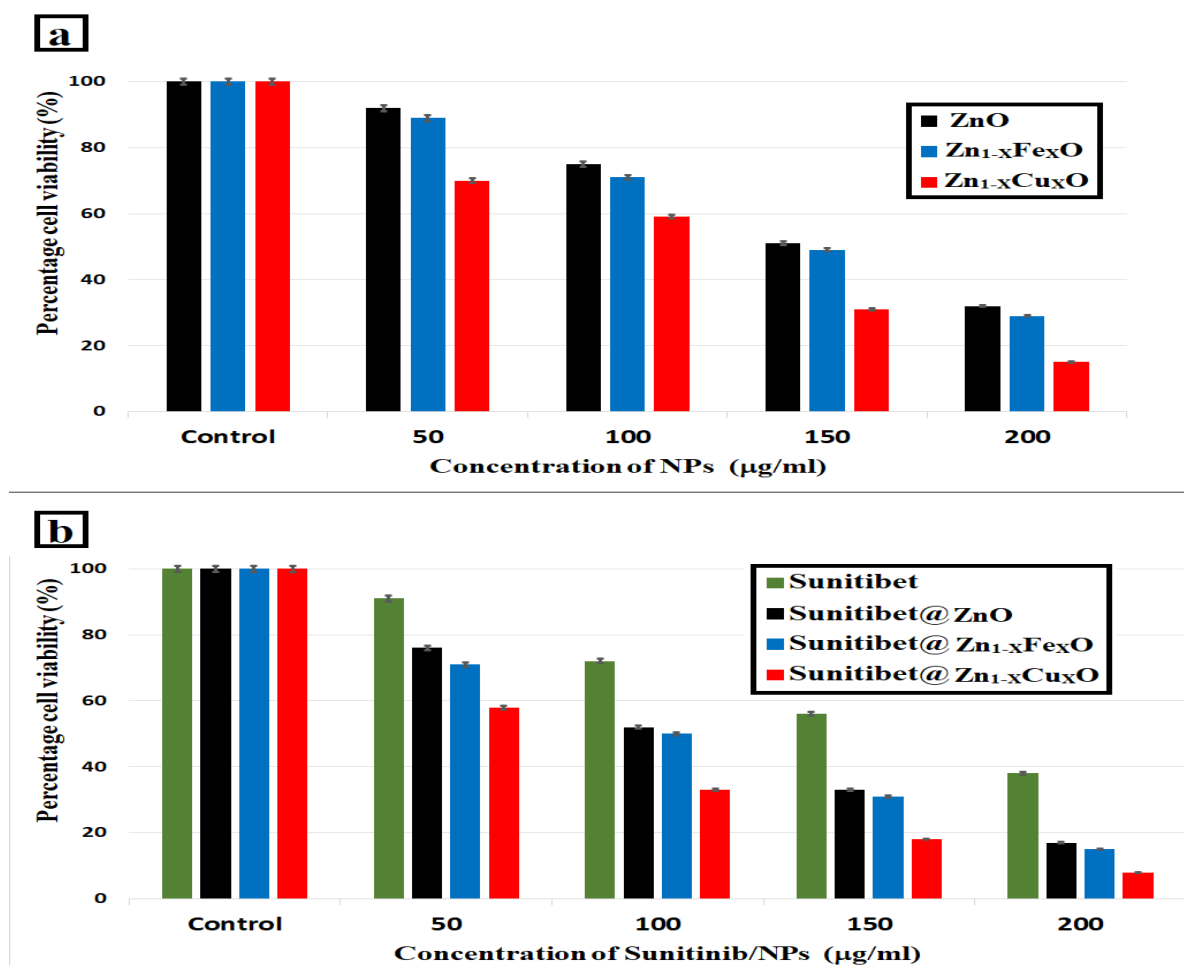


Fig. 9. Cytotoxic effect of (a) NPs and (b) Sunitinib@NPs in AsPC-1 cell lines at various concentrations (50, 100, 150, and 200 µg/mL) of NPs for 48h

5. Conclusions

This study has presented a low-cost and simple route with a short synthesis time for NPs using the sol-gel method. The structural characterizations confirmed the formation of the hexagonal wurtzite type ZnO nanostructure without extra peaks with different crystal sizes and morphologies. The antioxidant activity showed adding impurity improved the antioxidant properties, and copper dopant was more suitable than iron by 88.98. The inhibition percentage of *S. aureus* and *E. coli* bacteria by NPs was investigated, among the bacterial samples tested, *S. aureus* bacterium (gram-positive) was more sensitive to NPs, and *E. coli* (gram-negative) bacterium showed more resistance to NPs. The powerful substance used is Zn_{1-x}Cu_xO NPs for *S. aureus* and *E. coli* bacteria at 80.77 and 176.44 ppm, respectively. Also, the weakest is Zn_{1-x}Fe_xO NPs for *S. aureus* and *E. coli* bacteria at 555.31 and 715.57 ppm, respectively. The study explored the scavenging properties of DPPH free radicals, a well-known stable free radical frequently used in the assessment of antioxidant activity. Additionally, the anticancer effects of NPs were examined on the AsPC-1 cell line, which is derived from pancreatic cancer cells. The purpose of this research was to assess whether NPs have antioxidant properties and could restrain the proliferation of cancerous cells. The results

obtained from assays showed that drugs loaded on NPs were better than drugs in the studies. Two NPs of ZnO and Zn_{1-x}Fe_xO acted almost similar to each other. At the same time, Zn_{1-x}Cu_xO has significantly increased the anticancer effect of the drug compared to the other NPs, so sunitinib@Zn_{1-x}Cu_xO destroyed 92% of cancer cells in 200 µg/ml concentration.

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