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Unlock the potential of Magnetic Copper-Iron based Nanoparticles: Green Synthesis, Characterization, and Anti-Tumor Activity Comparison between ternary ($CuFeO_2$) and spinel ($CuFe_2O_4$) copper iron oxide nanoparticles

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Abstract:

Magnetic nanoparticles (MNPs) have become a crucial nanomedicine (NM) component for administering traditional chemotherapeutics. However, the NM delivery system restricts therapeutic efficacy, necessitating alternative strategies. This study designed nanoparticles with proper physicochemical structure characteristics to determine their application. The study synthesized ternary copper ferrite and spinel nanoparticles ($CuFeO_2$ and $CuFe_2O_4$) using *Boswellia carteri* resin extract. Powder-XRD, Z-potential, and SEM studies characterized the $CuFeO_2$ and $CuFe_2O_4$ nanoparticles with sizes ranging from 19.7-23.5 nm and zeta potentials of -18.3 to -16.5 mV. Furthermore, we compared the biological activity of copper ferrite nanoparticle types against various cancer cell lines and normal fibroblast cells to evaluate their potential as anticancer agents. Our findings demonstrate that $CuFe_2O_4$ nanoparticles exhibit significant cytotoxicity against K562 leukaemia cells, with IC_{50} values of 6.18 µg/ml and 5.87 µg/ml for $CuFeO_2$ and $CuFe_2O_4$ nanoparticles. However, MCF-7 and MDA-MB-231 breast cancer cells showed reduced sensitivity to $CuFeO_2$ nanoparticles, with higher IC_{50} values than leukaemia cells. Interestingly, $CuFe_2O_4$ nanoparticles displayed a promising safety profile towards normal fibroblast cells, indicating a favorable margin of safety. The results show differential sensitivity towards different cancer cell types based on the crystalline structure and the metallic ratio content. Understanding the correlation between the biological reactivity and the crystalline forms of copper ferrite nanoparticles and the Metal ratios could pave the way for designing novel, more potent selective anticancer agents with improved safety profiles.

Keywords: magnetic ferrites, copper, cytotoxicity evaluation, green chemistry, plant extracts, ABO_2, AB_2O_4, Spinel, ternary.

1. Introduction

Nanoparticles (NPs) have become transformative tools in cancer therapy due to their unique physicochemical properties at the nanometer scale. Their key attributes, such as high surface area-to-volume ratios, tunable surface chemistries, and controlled drug delivery capabilities, make NPs effective for overcoming challenges like multidrug resistance (MDR) and tumor heterogeneity [1]. Additionally, engineered nanoparticles are increasingly being explored as direct therapeutic agents. They can leverage their reactivity, magnetic, or redox properties to target cancer cells while minimizing systemic side effects specifically [2-3].

Copper oxide nanoparticles (CuO-NPs) and their derivatives are widely recognized for their versatile applications in industrial and biomedical fields. In the biomedical domain, their uses include wound healing, antimicrobial coatings, and anticancer therapies, primarily due to their ability to release cytotoxic copper ions and generate reactive oxygen species (ROS) [4-6]. However, the inherent toxicity of isolated CuO-NPs poses a significant limitation for their use in therapeutic settings. Recent research has focused on integrating copper with other metals, such as iron, to address this challenge of reducing toxicity while enhancing therapeutic efficacy [7,8]. These hybrid systems take advantage of the complementary properties of their constituent metals, striking a balance between therapeutic potential and biocompatibility. Copper-iron oxide nanoparticles exhibit remarkable synergistic effects, stemming from copper's ability to promote oxidative stress and iron's capacity for redox cycling [9]. These interactions enhance ROS production through the Fenton and Haber-Weiss reactions, selectively inducing apoptosis in cancer cells [10]. While iron oxide nanoparticles (FeO-NPs) possess inherent anticancer properties, their elevated cytotoxicity toward mammalian cells and associated systemic risks necessitate careful application [11-14]. Consequently, copper-iron bimetallic nanoparticles, including spinel-structured $CuFe_2O_4$ and delafossite ternary-structured $CuFeO_2$, have emerged as promising alternatives due to their multifunctional nature and tailored biological activities [15-18].

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The unique structural characteristics of copper-based ternary compounds significantly influence their chemical properties and potential biological uses. For instance, the crystal architecture of CuFeS has been associated with its effectiveness in drug delivery applications, highlighting the role of structure in biological interactions [19]. Furthermore, $CuFe_2O_4$, possessing a spinel structure with a sturdy three-dimensional lattice, demonstrates consistent magnetic behaviour, rendering it suitable for applications in targeted magnetic drug delivery, hyperthermia treatments, and as a contrast medium in medical imaging [20-222]. On the other hand, the layered configuration of ternary delafossite-type $CuFeO_2$ imparts distinctive redox properties, enhancing its utility in therapies that leverage reactive oxygen species (ROS) for therapeutic effects.

In a broader context, ternary nanomaterials such as CuFeS display notable plasmonic attributes that affect their bandgap energy and surface plasmon resonance. These properties are crucial for applications in photodynamic and photothermal therapies. Exposure to near-infrared (NIR) light triggers localized surface plasmon resonance in these ternary systems, facilitating ROS production through energy transfer to nearby oxygen molecules, thus generating singlet oxygen or other reactive intermediates [23-25]. The effectiveness of ROS generation is heavily dependent on the creation and interaction of electron-hole pairs with their surroundings, a factor that could significantly influence therapeutic outcomes.

It is hypothesized that the application of $CuFeO_2$ nanoparticles in biological systems may strongly correlate with increased ROS production, potentially enhancing their ability to induce apoptosis and address intricate cancer resistance mechanisms. This promising avenue merits deeper exploration [26]. Moreover, the combined therapeutic and diagnostic capabilities of compounds like $CuFe_2O_4$ and $CuFeO_2$ highlight their potential in theragnostic platforms. Beyond their role in cancer treatment, these nanoparticles also exhibit antimicrobial and antioxidant effects, broadening their scope for various biomedical applications [27].

In line with the growing emphasis on sustainability, recent advancements have enabled the green synthesis of $CuFe_2O_4$ NPs using biogenic methods. One such approach involves employing plant extract as a reducing agent [28]. This eco-friendly synthesis eliminates the need for harsh and hazardous chemical precursors, aligning with global efforts toward low-toxicity nanomaterial production. In this regard, *Boswellia Carteri* resin extract has a high content of polyphenolics, flavonoids, boswellic acid, and waxy materials that provide not only reducing agents but also stabilizing properties, facilitating controlled NP synthesis, thus resulting in enhanced morphology and biocompatibility [28]. This strategy presents a compelling and sustainable alternative to conventional methods, contributing to a broader movement toward environmentally conscious nanotechnology development.

This study investigated the preparation of both $CuFeO_2$ and $CuFe_2O_4$ nanoparticles using the Boswellia carteri plant. It explored further the crystal structure correlation to their anticancer potential against critical cancer models, including K562 leukemia cells and MCF-7 and MDA-MB-231 breast cancer cells. The results demonstrate significant anticancer activity, likely driven by elevated ROS production and specific interactions with tumor cell membranes. By addressing research gaps—particularly the sparse literature on Cu(I)-Fe(III)-based nanoparticles like $CuFeO_2$—this study aims to contribute valuable insights to the field.

2. Experimental

2.1. Materials and Methods

All solvents and anhydrous iron (III) acetate were bought from Aldrich and utilized exactly as supplied. Powder X-ray diffraction (P-XRD) and scanning electron microscopy were used to analyze the structure and composition of $CuFe_2O_4$ nano-ferrites. PANalytical's X'Pert PRO X-ray diffraction system was used to obtain the P-XRD at 40 kV and 30 mA with a step of 0.02o over the range of 4–60^o using Cu/Kα radiation with nickel filter. Using JEOL 6400 and FEI QUANTA 200, scanning electron microscopy micrographs of the samples' cracked surfaces were captured. DLS was used to measure the size and zeta potential of various NPs. A 0.45 m filter was used to filter the NPs suspensions, and three analyses were performed on each batch.

2.2. Preparation of the $CuFeO_4$ and $CuFe_2O_4$:

The preparation was done according to Al-hunaiti et al. [28]. After being bought from India, the *Boswellia Carteri* resin was extensively cleaned with distilled water. They were broken into little pieces, and roughly 100 g was poured into 1000 ml of water and heated at 65°C for around 300 min. After passing the extract through filter paper, the filtrate was mixed with a solution of $Fe_3O(OAC)_6.(H2O)$ in 0.1 M copper acetate, and the plant extract was added dropwise to the mixture. At room temperature, 5 mL of a 0.2 M NaOH solution was stirred for an hour. After centrifuging the resultant solution for 10 min at 14000 rpm, three milliliters of ethanol were added for washing. After that, it was calcinated for four hours at 800°C, yielding 90.0% $CuFe_2O_4$-NPs. We followed a procedure similar to the above mentioned one to synthesize a $CuFeO_2$ compound with a different metal ratio. We mixed 0.25 M of copper acetate with a 1.8 M iron acetate solution at room temperature and allowed it to stir for 1 hour. Powder-XRD, and SEM characterized the synthesized nanoparticles. Smaller NP particle sizes can be isolated with this preparation technique. Compared to other documented methods, it has the benefit of being smaller and requiring less temperature (Table 1). The sol-gel-prepared $CuFe_2O_4$ and the novel nanoparticles were also compared (Table 1).

Table 1. A comparison between biogenic and other methods for $CuFe_2O_4$-NP preparation:

Type	Temperature [°C]	Np Size [nm]	Reference
Sol-gel	90	40	69
Co-precipitation	90	30	70
$CuFe_2O_4$	Room temperature	19.7±0.35	This work
$CuFeO_2$	Room temperature	23.5±0.2	This work

2.3. MTT Assay

The MTT cytotoxicity assay was conducted to assess the anticancer potential of biosynthetic bimetallic nanoparticles against various cancer cell lines. The half-

maximal inhibitory concentration (IC50) of the nanoparticles was ascertained over the human triple negative MDA-MB-231 breast cancer cell line, MCF-7 breast cancer cells, the leukemic K562 cell line, and fibroblast cells (normal human epithelium). In a 96 well plate, the amount of $10X10^3$ were plated and incubated in a humidified incubator at 37°C with 5% CO_2 overnight to firmly attach to the bottom surface. Starting from the concentration of 200 µg/ml down to the concentration of 0.195 µg/ml, cells received treatments of the nanoparticles. The stock solution consisted of 20 mM of *Boswellia carteri* resin aqueous extract, resin-capped-$CuFeO_2$, and resin-capped-CuFe2O4 nanoparticles. The resin aqueous extract was prepared in dimethyl sulfoxide (DMSO). The percentage of DMSO remained below 2% for all treatments considered. Ultrasonication was utilized to dissolve the tested nanoparticles and the aqueous extract thoroughly. After a 72-hour treatment period, an MTT cytotoxicity assay was performed.

2.4. Statistical analysis

The IC50s of each tested NP were determined using the logarithmic trend line of cytotoxicity graphs (log(concentration versus inhibition)) in GraphPad Prism 8 program (GraphPad program, Inc.). The data, which are shown as mean ± SEM, were from three separate trials, each of which having three triplicates. The Student's t test was used to compare the differences. Statistical significance was defined as a p value of less than 0.05.

3. Results and Discussion

3.1. Characterization of the NP

Figure 1 illustrates the room temperature powder XRD diffractograms for $CuFe_2O_4$ (Fig. 1a) and $CuFeO_2$ NPs (Fig. 1 b). The P-XRD pattern is consistent with previously published reports, and the presence of sharp diffraction peaks indicates the existence of spinel-type CuFe2O4 nanoparticles [22]. The diffraction peaks observed at 28.5, 35.6, 38, 42, and 63 correspond to the (220), (311), (200), (400), and (440) crystallographic planes, respectively (JCPDF-card no.96-6901-2439). The diffractogram's absence of peaks corresponding to common contaminants such as CuO, Cu_2O, Fe_2O_3, and $CuFe_2O_4$ confirms the NPs' purity. Subsequently, the particle size of the $CuFe_2O_4$ NPs was determined using the Debye-Scherrer equation based on P-XRD data, revealing an average particle size of 19.7 nm. Meanwhile, the ternary $CuFeO_2$-NP adopts a delafossite structure (R3m), where FeO_6 octahedra form layers and Cu+ ions linearly bond to oxygen ions, creating a second layer. This structure was confirmed by distinct P-XRD peaks (15.40°, 31.19°, 34.46°) corresponding to specific crystallographic planes in XRD analysis, showing characteristic peaks corresponding to the (012), (104), (110), and (113) planes. The highest point corresponded to the standard information for bulk $CuFe_2O_4$, which possesses a rhombohedral structure, and has an average particle size of 23.5 nm. The morphology of the crystalline particles was confirmed via SEM images (Figure 2a, b). According to the SEM results, the synthesized $CuFe_2O_4$ NPs are uniformly distributed and almost spherical, while $CuFeO_2$ shows orthorhombic cubic morphology.

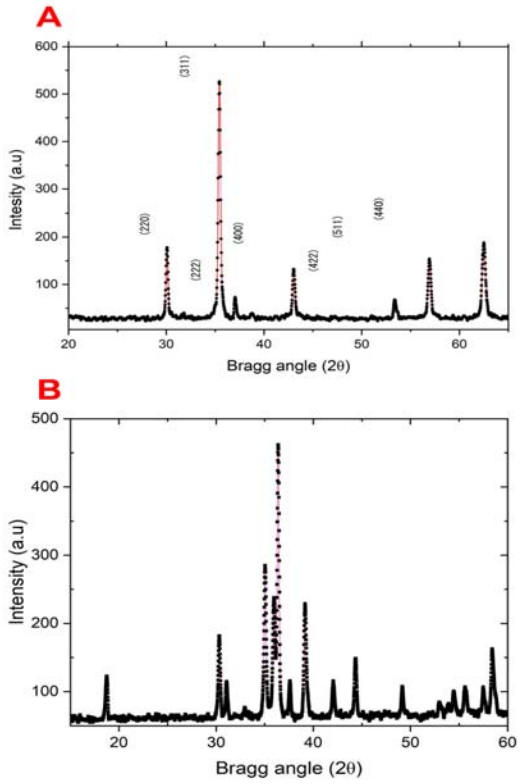

Figure 1. P-XRD pattern of the synthesized a) $CuFe_2O_4$-NPs, b) $CuFeO_2$-NPs.

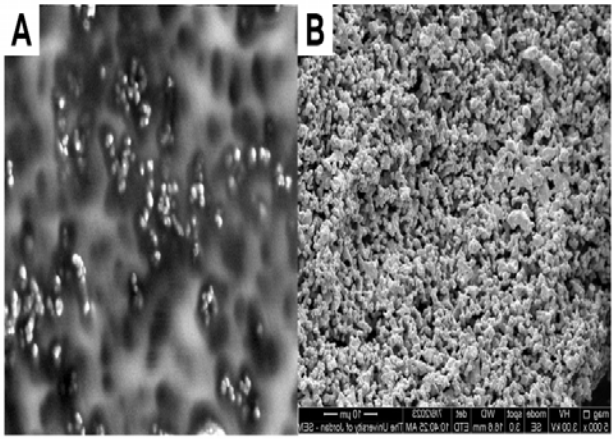

Figure 2. (a) SEM images of the pure spherical spinel $CuFe_2O_4$-NP and b) $CuFeO_2$-NP

3.2. Dls and ζ-potential

The fate and use of magnetic nanoparticles in biomedicine are considerably defined by their size and surface electric charge, among other relevant characteristics. The mean size (and PdI) of $CuFe_2O_4$ and $CuFeO_2$ are 20.8±3.5 nm and 26.0±4.2, respectively (Table 2). The zeta potential of the prepared NPs (zeta potential, in mV) of the two nanoparticle formulations: spinel $CuFe_2O_4$ with moderate negative charge and size (-18.3±0.15 mV), and the ternary nanoparticles are slightly less negatively charged with a ζ-potential of -16.5±0.8 mV; the negative ζ-potential values indicate moderate colloidal stability for both nanoparticles, with sufficient

electrostatic repulsion, which is consistent with the presence of surface hydroxyl groups that deprotonate in aqueous environments, leading to electrostatic repulsion and preventing aggregation. The smaller size of $CuFe_2O_4$ suggests a higher surface-to-volume ratio, potentially enhancing reactivity in catalysis or drug delivery applications. In contrast, the slightly larger size of $CuFeO_2$ may be advantageous for size-dependent magnetic or optical properties. Both nanoparticles are suitable for applications requiring stable dispersions. However, further optimization of ζ-potential and size control could improve their performance in specific use cases, such as environmental remediation or biomedical applications [26].

Table 2. Size and ζ-potential of the prepared particle.

NP type	Size (nm)	PDI (nm)	z-potential
Spinel $CuFe_2O_4$	20.8± 3.5	0.304±0.007	-18.3±0.15
Ternary $CuFeO_2$	26.0± 4.2	0.223±0.023	-16.5±0.8

3.3. The biological activity

With IC_{50} values of 6.18 µg/ml and 5.87 µg/ml, respectively, the present study shows the intense activity of CuFeO2 and CuFe2O4 NPs capped with Boswellia carteri extract against K562 leukemia cell lines (Table 3). Furthermore, the same nanoparticles had higher IC50 values (50.6 and 20.19 µg/ml, respectively) and decreased sensitivity in MCF-7 cancer cells. Another breast cancer cell line (MDA-MB-231) similarly shows this reduced sensitivity, with high IC_{50} values of 66.78 and 29.28 µg/ml, respectively. Remarkably, resin-capped $CuFe_2O_4$ nanoparticles are more specific and selective for a particular type of cancer because of the varying sensitivities of the evaluated cell lines. In our previous study, we synthesized a novel bimetallic Zinc-iron NPs using *Boswellia carteri* extract, where it exhibited a powerful anticancer against K562 and MDA-MB-231 cell lines with IC_{50} values 4.53 µM and 4.19 µM, respectively [16]. According to a study, $CuFe_2O_4$ nanoparticles target a specific area that the breast cancer MCF-7 cell line lacks. On the other hand, additional research is required to determine their targets and illustrate the molecular processes underlying the cytotoxicity of $CuFe_2O_4$ nanoparticles with resin caps. Interestingly, all our examined cells were safe and unaffected by the 1% DMSO, and our control cells treated with this concentration were healthy. Accordingly, the nanoparticles themselves, not their solvent (DMSO), were responsible for any anticancer action that the studied particles may have had (Table 3). Remarkably, the promising finding of our novel resin-capped $CuFeO_2$ and $CuFe_2O_4$ NPs is their safety toward normal fibroblast cell line, where the IC_{50} values are 21.5 and 26.52 µg/ml (Table 3), which is a good indication for a margin of safety. In other words, in comparison with K562 leukemia cells, resin-capped $CuFeO_2$ and $CuFe_2O_4$ nanoparticles need three and five times, respectively, more to inhibit the proliferation of the normal fibroblast cells, which is indeed a good indication of the safety of this compound. The potent and selective effect of the prepared nanoparticles, which is more pronounced in K562 cells and less evident in breast cancer cells while remaining safe for normal cells, is the strength point of the prepared system. This suggests their potential as a powerful anti-cancer therapy that selectively targets specific cancer types without harming normal tissues, addressing a significant challenge associated with conventional chemotherapy approaches. This selectivity of the biological reactivity of the NPs is related directly to the crystalline structure. Such a finding opens a new potential for metallic NPs to minimize off-target effects and adverse reactions in cancer therapy. The discovery of these effective biosynthesized NPs presents a promising approach to combating cancer. They offer cost-effectiveness and safety with reduced side effects on normal cells, potentially improving disease prognosis and overall patient health. Surprisingly, the results show that CuFe2O4 NPs capped with *Boswellia carteri* extract reduced the issue of multidrug resistance and showed anticancer efficacy against K562 leukemia cells. Thus, for enhanced therapeutic efficacy against cancer cells, the produced NPs may be a useful agent in a combination treatment with chemotherapy. Our findings have clinical significance since these synthetic NPs may help combat cancer cell resistance while lowering the issue of toxicity to healthy cells.

Table 3. IC_{50} values of the synthesized NPs and Boswellia carteri resin aqueous extract on different cancer cell lines

Compound	Fibroblast	K562	MCF-7	MDA.231
	IC50 (µM±SEM)	IC50 (µM±SEM)	IC50 (µM±SEM)	IC50 (µM±SEM)
Resin-capped $CuFeO_2$ NPs	21.5±0.43	6.18±0.01	50.60±7.47	66.78±5.91
resin aqueous extract	134.57±0.01	53.4±0.04	2245.7±0.03	93.74±0.02
Resin-capped $CuFe_2O_4$ NPs	26.52±0.24	5.87±0.09	20.19±2.44	29.28±1.87
1% DMSO	No toxicity	No toxicity	No toxicity	No Toxicity

SEM: Standard error of the mean

In conclusion, we have successfully produced biogenic $CuFeO_2$ and $CuFe_2O_4$-NPs using *Boswellia carteri* resin extract. The XRD and SEM results confirm the production of nanocrystalline spinel $CuFe_2O_4$ and ternary $CuFeO_2$ NPs. According to the SEM results, the synthesized CuFe2O4 NPs are uniformly distributed and almost spherical, while $CuFeO_2$ shows orthorhombic cubic. The biological study demonstrates the potent anticancer activity of the prepared nanoparticles $CuFeO_2$ and $CuFe_2O_4$, particularly against K562 leukemia cells. In contrast, MCF-7 and MDA-MB-231 breast cancer cells exhibited reduced sensitivity. The nanoparticles showed remarkable selectivity, suggesting a specific molecular target in leukemia cells. Notably, the NPs demonstrated safety toward normal fibroblast cells, requiring three to five times higher concentrations to inhibit their proliferation than leukemia cells, indicating a favorable therapeutic window. The crystalline structure of the NPs appears to play a critical role in their biological reactivity, offering a promising avenue for minimizing off-target effects in cancer therapy. These findings highlight the potential of biosynthesized $CuFeO_2$ and $CuFe_2O_4$ NPs as cost-effective, safe, and selective anticancer agents, paving the way for further research into their molecular mechanisms and therapeutic applications.

Conflict of Interests

The authors declare no conflicting interests.

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