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RESEARCH ARTICLE

Green Synthesis of CuO Nanoparticles Using Aqueous Extract of (Artemisia Herba Alba) and Their Potential Applications as Antimicrobial Agents

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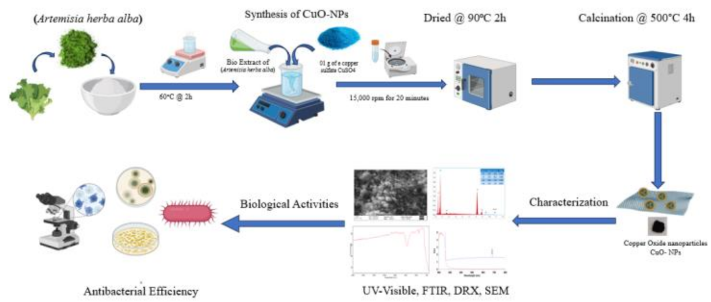
ABSTRACT

The CuO nanoparticles were synthesized using a green synthesis method with the extract of (Artemisia herbaalba) and copper sulfate ($CuSO_4$) as a precursor. After calcination, the relevant properties of the as-synthesized nanoparticles were investigated using X-ray diffraction (XRD), scanning electron microscopy (SEM), and Fourier Transform Infrared (FTIR) Spectroscopy. Further improvement in crystallinity and purification can be attained through a post-calcination process. UV-VIS spectroscopy, FTIR, SEM, EDX, and XRD techniques were used to investigate the pure form of CuO-NPs. The synthesized CuO-NPs were crystalline in nature, with an average size of 22 nm, and exhibited a monoclinic phase according to the XRD pattern. The UV-Visible spectrum analysis characteristic absorption peaks in the 200–800 nm wavelength range. Prominent absorption peak was observed at 682 nm. The FTIR analysis revealed that the ester (C=O), carboxyl (C=O), alkyne (C-H), and (Cu-O) A pronounced peak was observed at 660 cm−1 in the band that arose from the Cu-O stretching vibration, while similar peaks at 490 cm-1. the EDX spectra, shown indicate that copper is the predominant element, accounting for 62.04% of the elemental composition. In addition to copper, the spectra reveal the presence of carbon and oxygen, constituting 22.51% and 5.83% of the composition, respectively.is the average crystalline size of the nanoparticles. As shown in Figure 9, the major XRD 2θ value peaks of the biosynthesized CuO-NPs that appeared at 33.50, 35.00, 38.05, 48.49, 36.15, 43.98, 58.50, 63.97, 67.53, and 68.31, were ascribed to (110), (002), (200), (002), (002), (020), (202), (113), (022), and (221) miller index planes.the CuO-NPs synthesiaed solution exhitied . CuO-NPs at concentrations of 33333μg/Mldisplayedantibacterial and antifungal activity against the plant pathogenic bacteria Xanthomonas sp. and the pathogenic fungus Alternaria sp., respectively.The copper oxide nanoparticles synthesized in this study demonstrate strong antibacterial activity, particularly at higher concentrations. Their effectiveness is comparable to that of conventional antibiotics, making them a viable alternative in combating bacterial infections, especially in the face of growing antibiotic resistance. However, further studies are needed to fully understand their long-term safety and potential applications in various fields.



Graphic abstract

INTRODUCTION

Recently, MnO nanoparticles (NPs) have become some of the most important commercial nanomaterials in the world. This high popularity is due to their multiple applications, such as in light detectors, gas detectors, biosensors, electronics, lasers, transistors, photoelectronics, solar cells, cosmetics, biomedicine, the food industry, anticorrosive coatings, antibacterial and antifungal agents, and photocatalysts.

Nanoparticles are small clusters of atoms with one or more dimensions ranging in size from 1 to 100 nanometers. The term "nano" is derived from the Greek word "nanos," meaning very small. A nanometer is one-billionth of a meter (10^-9 m). The sizes and dimensions of these materials cause them to behave differently from traditional materials with dimensions greater than 1000 nm. Nanomaterials possess unique properties that are not found in larger, conventional materials. They are considered the building blocks of the twenty-first century(Mohamed Sharif Al-Iskandari, April 2010) .

In recent years, scientific community has an interest on nanometer sized materials with unique physical, chemical, and biological properties (Ameer A, et al 2010) . Copper oxide (CuO) is broadly used with wide range of applications in number of fields such as catalyst, sensing materials, ceramics, thermoelectric materials, glass, superconducting materials and anti-microbial activity (Faheem I, Sammia S, Shakeel AK, et al 2016). Nano-copper oxide is very interested because of their potential applications in many fields such as heterogeneous catalysts, anti-microbial, anti-oxidants, imaging agents and drug delivery agents in field of biomedicine (Yallappa S, et al2013). Usually, nanostructures ranges from 1 to 100 nm in size compared to large biological molecules such as enzymes, receptors etc. Nanoparticles (NPs) can interact with biomolecules and it has useful in diagnosis and treatment cancer (Seigneuric R, et al 2010 , Liu Z, et al 2010).

Nanotechnology is a transformative frontier in science and engineering that harnesses the unique attributes of nanoscale materials to create innovative solutions across multiple fields (Bouafia, A et al 2023). One of the most promising advancements in this field is the synthesis and application of nanomaterials, which, owing to their amplified surface area, stability, and conductivity, have diverse applications, ranging from engineering to biomedical instrumentation (Gu, J.et al 2023). The potential hazards associated with chemical synthesis methods, coupled with increasing environmental concerns, have shifted the focus towards green and sustainable approaches to nanoparticle synthesis. The use of biological materials, particularly plant extracts and microorganisms, presents a compelling case for developing ecofriendly, cost-effective, and safe alternatives (Dejen, K.D et al 2023, Prakash, M ,et al 2022). Biological synthesis can be carried out using plant parts, secondary metabolites from plants, (leaf, root, stem, fruit, and flower secondary metabolites), and microbes and their secondary metabolites (Nthunya, L.N et al.2023)In recent years, metals such as copper (Cu), silver (Ag), zinc (Zn), iron (Fe), gold (Au), silicon (Si), nickel (Ni), and platinum (Pt) have been used in the green synthesis of nanoparticles for biomedical applications (Abed, A.S et al.2023) The potential hazards associated with chemical synthesis methods, coupled with increasing environmental concerns, have shifted the focus towards green and sustainable approaches to nanoparticle synthesis. This biological synthesis not only eliminates the need for toxic chemicals, high temperatures, and sophisticated instrumentation but also leverages the intrinsic bioactive compounds present in these natural resources to enhance the miniaturization and stabilization of nanoparticles. Copper (Cu) is an essential nutrient for plant growth, enzymes, photosynthesis, and RNA synthesis (Gemin, L.G et al.2023) With regard to trade, Cu nanoparticles are extensively used to produce antimicrobial, larvicidal, anticancer, and antioxidant products (Naz, S et al.2023) Plants such as Caesalpinia bonducella(Sukumar, S. et al,2020) Punica granatum (Siddiqui, V.U.et al ,2021)Ocimumsanctum(Ramadhan, V.et al ,2019)Saussurealappa(Kolahalam, L.A et al,2022) and Ziziphus spina-christi(Khani, R.et al 2018)have been used for the synthesis of copper nanoparticles (CuO NPs). The synthesis of CuO NPs using plant extracts appears to be a favorable and environmentally friendly approach in the field of nanotechnology (Cuong, H.N et al 2022).CuO NPs exhibit exceptional properties and a wide range of applications in numerous scientific and industrial domains, such as catalysis, electronics, energy storage, and biomedicine (Verma, N.et al 2019). In recent years, the use of plant extracts as reducing and stabilizing agents for the green synthesis of nanoparticles has gained considerable attention due to its sustainable and eco-friendly nature (Adil, S.F et al 2015). Traditional methods of producing CuO nanoparticles frequently comprise the use of hazardous chemicals, high temperatures, and energy-intensive processes, which can have adverse environmental and health impacts (Mohamed, E.A .et al 2023) In contrast, green synthesis methods employ natural plant extracts rich in bioactive compounds, such as phytochemicals, polyphenols, and flavonoids, to enable the size reduction and stabilization of CuO NPs. These plant-derived compounds act as both reducing and capping agents, making the corresponding synthesis method not only more sustainable but also safer for investigators and the environment (Rehana, D.et al 2017). The green synthesis of CuO NPs using plant extracts offers numerous advantages, comprising reduced environmental pollution, lower energy consumption, cost-effectiveness, and the potential for large-scale production. Additionally, the use of plant extracts adds a new dimension to nanoparticle synthesis, as different plant species and their extracts may convey exceptional properties and functionalities to the resulting CuO nanoparticles (Akintelu, S.A.et al .2020) We hypothesize that CuO NPs hold vast potential for a wide range of biological applications. Additionally, the antimicrobial properties of CuO NPs suggest their effectiveness in combatting drug-resistant infections and designing supportive novel therapeutic approaches. We also assume that the controlled functionalization of CuO NPs will permit personalized solutions for precise biological challenges, further enhancing their potential impact on healthcare and life sciences. Through inclusive examination and investigation, we aimed to authenticate our hypothesis and enhance the growing body of knowledge concerning the usage of CuO NPs for biological applications. Our research will not only advance the understanding of these nanoparticles but also enable the development of innovative strategies that may eventually lead to improved patient care, more effective treatments, and enhanced diagnostic techniques in the realm of life sciences. Indigofera linnaei Ali is an annual herb with small trailing branches belonging to the Fabaceae family. It is dispersed throughout India, China, Nepal, Pakistan, Sri Lanka, and Australia, where it is used as an indigenous medicine to treat tumors, liver inflammation, arthritis, and rheumatism (Kumar, R.S.et al.2011 , Kumar, R.S et al 2016) The juice of I. linnaei Ali is known for its diuretic and antiscorbutic properties (Kumar, R.S.; Kumar, S.V et al 2016).

In the present work, the main objective is to investigate the effect of starting precursors on the structural properties of CuO nanostructures synthesized via the green synthesis of CuO

Nanoparticles nanoparticles using aqueous extract of(*Artemisia herba alba*)and their potential applications as antimicrobial activity, The as-prepared precipitates were analyzed using scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier Transform Infrared Spectroscopy (FTIR).Moreover, the potential antimicrobial properties of the as-synthesized CuO NPs were evaluated against various bacterial strains, such as Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis, and Staphylococcus aureus.

Presentation of plants

Most of the atoms found in nature may combine with atoms of their own kind. This phenomenon can form huge molecular structures that contain billions of billions of atoms of the same kind. In most cases, these structures become solid, shiny, plastic bodies called metals. In metals, some electrons can leave their atoms and move through the body of the object. Moving electrons form electric currents, and so metals carry charge. Electrical cables, power transmission lines, and television antennas are all examples of devices in which electric charges move through metal bodies (Ratner, M. A. et al .2023)

Preparation of the studied plant:

The wormwood plant was collected from the southern Algerian desert region, it was washed with a completely distilled water and dried in a shaded place, and then the leaves were removed from the plant and dried after it dried. This work was carried out in the scientific research field for dry and semi-desert areas in Touggourt, where copper oxide nanoparticles were tested using the extract of the wormwood plant (*Artemisia alba herba*). The prepared samples were analyzed, and their different properties were determined using ultraviolet rays, infrared rays, and X-rays. Finally, the effectiveness of the samples as antibacterials was studied (Figure 1).

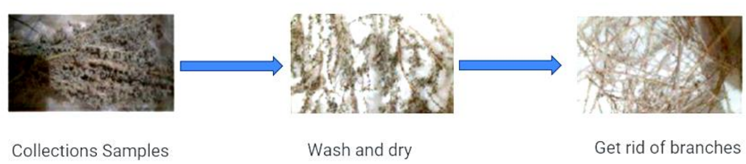


Figure 1: Collect and Preparation the (Artemisia herba alba) plant.

Extract preparation:

The extract of the (*Artemisiaherba alba*) plant was prepared by placing 20 g of the plant leaves in 300 ml ofdistilled water solution at a temperature ranging from 51-61 degrees Celsius for an hour with magnetic shaking according to (Figure 2).

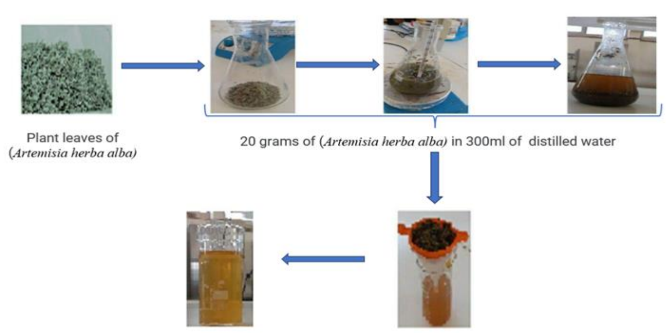


Figure 2: Preparation The extract of the (*Artemisia herba alba*) plant

Preparation of copper oxide nanoparticles:

To synthesize copper oxide nanoparticles (CuO-NPs), 1 g of copper sulfate was weighed and dissolved in 100 mL of deionized water in a beaker. To this solution, 100 mL of wormwood extract was added, and the mixture was stirred at 60°C for 2 hours. After stirring, the solution was centrifuged @15,000 rpm for 20 minutesto separate the components, resulting in a black aqueous precipitate. This precipitate was then dried in a furnace at 90°C for 2 hours. After drying, the material was ground into a fine powder using a mortar and pestle. Finally, the powder was calcined in a furnace at 500°C for 4 hours, yielding copper oxide nanoparticles, as illustrated in (Figure 3).

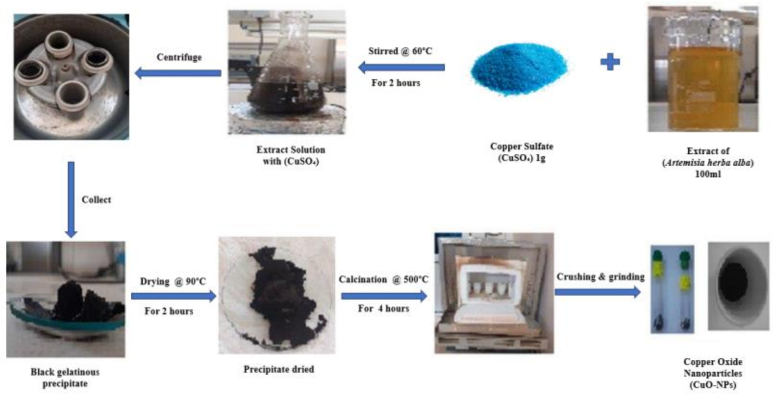


Figure 3: Diagram illustrating the protocol for preparing CuO NPs by leaf extract of (*Artemisia alba herba).*

Experimental Synthesis des nanoparticles.

Mechanism of green synthesis of nanoparticles by leaf extract of plant.

Various plant metabolites, including terpenoids, polyphenols, polysaccharides, alkaloids, phenolic acids, and proteins, play an important role in the biological reduction of metal ions, leading to the production of nanoparticles. (Nobahar, A. et al.2021)

In general, the mechanism of green synthesis of metals nanoparticles using plant extracts involves three major phases: activation phase, growth phase, and termination phase (Figure. 4).

1. The activation phase is the basic step in which metal ions are recovered from their salts by the action of plant metabolites, biomolecules with reductive capabilities. In addition, metal ions change from a mono- or divalent oxidation state to a zero-valence state and transform into nuclei of reduced metal atoms (Song, J. Y .et al .2009)
2. growth phase, which refers to the spontaneous coalescence of nanoparticles in which neighboring small nanoparticles spontaneously combine into larger particles, which is accompanied by an increase in the thermodynamic stability of the nanoparticles (Keat, C. L. et al .2015)
3. The last step of the synthesis process is the termination phase during which the nanoparticles finally reach their maximum possible activity, and this process is affected by the strength and ability of the plant extract to stabilize the metal nanoparticles (Makarov, V. V .et al .2014)

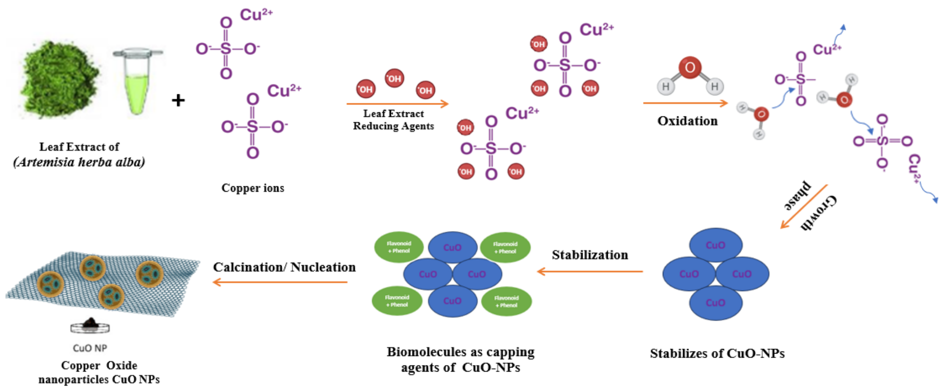


Figure. 4: **Mechanism of plant mediated synthesis of CuO-NPs nanoparticles.**

Characterization of Synthesized Copper oxide Nanoparticles

The initial characterization of copper nanoparticles was conducted using ultraviolet– visible (UV-Vis) spectroscopy (model: UV- 1800, Make: Shimadzu, Kyoto, Japan) in a scanning wavelength range of 200–800 nm as a preliminary confirmation of copper ion reduction. FT-IR (Shimadzu IR Spirit, Kyoto, Japan) was performed in the range from 450 to 4000 cm−1. This analysis was helpful for detecting the biomolecules of the whole-plant *(Artemisia herba alba)*extract in the aqueous extract of plants responsible for the formation of CuO-NPs. Scanning electron microscopy combined (ZEISS, Gemini SEM 360, Jane, Germany) with EDX (Carl Zeiss Microscopy Germany, model: EVO 18) was used to identify the surface morphology, size, and elemental composition of *(Artemisia herba alba)*plant-extract-mediated CuO NPs. X-ray diffraction (XRD) analysis was carried out using a Bruker D8 Advance Diffractometer (Bruker, Ettlingen, Germany) with a copper tube operating at 40 kV and 40 mA. The diffractograms were recorded using monochromatic CuKα radiation (λ = 0.1542 nm) across a 2θ range of 5°–90°, with a step size of 0.05° and a measurement time of 1 second per step. The XRD patterns provided information on the crystalline structure and phase purity of the CuO-NPs. The average crystalline size of the nanoparticles was calculated using the Debye-Scherrer equation, offering insights into the crystalline properties of the synthesized material.

$$D = \left(\frac{k\lambda}{\beta hl \cos\theta} \right)$$

RESULTS AND DISCUSSION

UV-Visible Spectrum Analysis

The CuO-NPs synthesiaed solution exhitied characteristic absorption peaks in the 200–800 nm wavelength range. Prominent absorption peak was observed at 682 nm (Figure 5). Additionally, the absorption peak detected at 682 nm can be correlated with the core electrons of the copper within the Cu-NPs, as reported in a previous study (Nzilu, D.M .et al. 2023) The synthesis of these nanoparticles is fascinating. The generated OH ions, which were derived from water molecules, interacted with copper sulfate, resulting in the formation of copper hydroxides. Subsequently, the decomposition of this nanoparticle conjugate ensued, eventually leading to the production of CuO-NPs (Karuppannan, S.K .et al .2021) The absorption peaks and patterns identified in our study are consistent with the findings of previous reports (Manasa, D.et al 2021, Alhalili, Z.et al 2022, Jayasimha, H. et al .2023) The similarities in the peak wavelengths provided consistent evidence regarding the properties and behavior of the CuO-NPs.

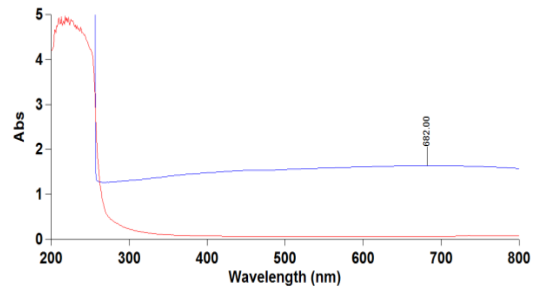


Figure 5: UV-Vis Spectrum of Biosynthesized CuO-NPs

FT-IR Analysis

Fourier-transform infrared spectroscopy (FT-IR) was employed to analyze the functional groups of the plant metabolites present in the aqueous extract of the (*Artemisiaherba alba*) plant plants as well as to identify the functional groups present on the surfaces of the Cu-NPs. In the FT-IR spectra, pronounced peaks were detected at wavenumbers such as 1400, 803, 660, and 490 cm−1 (Figure 6). The peak at 1400 cm−1 indicated strong the presence of C=C and C=O bending vibration modes. The other small, weak peaks at 803 cm−1 are associated with the vibration of C-H groups (Din, M.I. et al .2017).Apronounced peak was observed at 660 cm−1 in the band that arose from the Cu-O stretching vibration, while similar peaks at 490 cm−1 . According to the FT-IR results, acids, polyphenols, proteins, alkaloids, and carboxylic acid functional groups could be found in the Cu-NPs (Thiruvengadam, M.et al .2019)

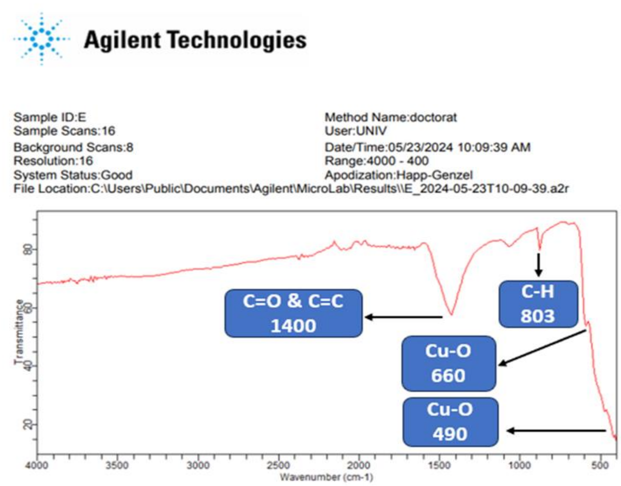


Figure 6: FTIR Spectrum of Biosynthesized CuO-NPs

SEM and EDX Analysis

Scanning electron microscopy (SEM) was employed to examine the surface morphology of the biosynthesized copper oxide nanoparticles (CuO-NPs). The SEM images presented in Figure 7 (a)& (b) offer different resolutions, providing a detailed visualization of the CuO-NPs. The images reveal a mixture of spherical and cuboidal morphologies, though some degree of agglomeration is observed. Such agglomeration is common in nanoparticle synthesis and can be attributed to factors such as high surface energy and van der Waals forces between particles. To further analyze the composition of the nanoparticles, energy-dispersive X-ray (EDX) spectroscopy was conducted. The EDX spectra, shown in Figure 8, indicate that copper is the predominant element, accounting for 62.04% of the elemental composition. In addition to copper, the spectra reveal the presence of carbon and oxygen, constituting 22.51% and 5.83% of the composition, respectively (Figure 8).

The presence of carbon and oxygen suggests that organic molecules, likely phytochemicals from the green synthesis process, are associated with the surface of the nanoparticles. These phytochemicals are believed to act as both reducing and capping agents during synthesis, playing a critical role in the formation and stabilization of the CuO-NPs. The organic molecules contribute to the stability of the nanoparticles by preventing excessive agglomeration, thereby maintaining the unique properties of the CuO-NPs.

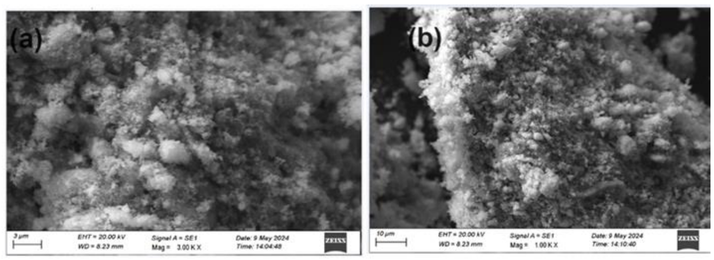


Figure 7. SEM images of Biosynthesized CuO-NPs (a) 3 µm and (b) 10 µm

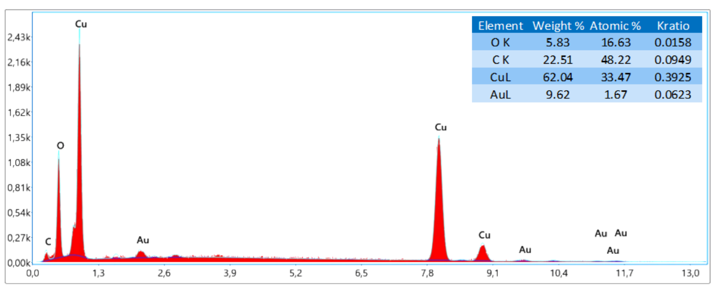


Figure 8: EDX images of Biosynthesized CuO-NPs

The significant presence of copper in the EDX spectra confirms the successful synthesis of CuO-NPs, while the elevated oxygen levels are consistent with the oxidized nature of the nanoparticles. The detection of carbon, likely associated with plant-derived compounds, aligns with previous research, where phytochemicals were found to play crucial roles in nanoparticle synthesis. These compounds not only facilitate the reduction of metal ions but also contribute to the stabilization and capping of the nanoparticles. Collectively, these results highlight the intricate and multifaceted roles that phytochemicals play in the green synthesis of CuO-NPs.

XRD Analysis

The synthesis of the CuO NPs was confirmed via X-ray diffraction (XRD) analysis. The crystal size of the nanoparticles was 113 nm. The average crystalline structure size of (Figure 9). FTIR spectrum of biosynthesized CuO-NPs. 2.3. XRD Analysis The synthesis of the CuO NPs was confirmed via X-ray diffraction (XRD) analysis. The crystal size of the nanoparticles was 113 nm. The average crystalline structure size of the biosynthesized CuO NPs was calculated using Debye Scherrer's equation (Kumar, R.S,et al 2016) where k denotes the shape constant of the geometric factor (0.9), λ represents wavelength, β is the line broadening at half-maximum intensity, θ is the Bragg angle, and D is the average crystalline size of the nanoparticles. As shown in Figure 9, the major XRD 2θ value peaks of the biosynthesized CuO-NPs that appeared at 33.50, 35.00, 38.05, 48.49, 36.15, 43.98, 58.50, 63.97,

67.53,and 68.31, were ascribed to (110), (002), (200), (002), (002), (020), (202), (113), (022), and (221)miller index planes. The sharp peak at 2θ = 38.7 with the diffraction of the (111) plane corresponds to CuO NPs, indicating that the synthesized CuO NPs are crystalline and monoclinic in nature (Tamuly, C.et al .2014)
These unidentified peaks appeared because of the deposition of photo-molecules on the surfaces of the CuO-NPs during synthesis (Sharma, P.et al .2018)

$$D = k\lambda \Big/ \beta \frac{1}{2} \cos\theta$$

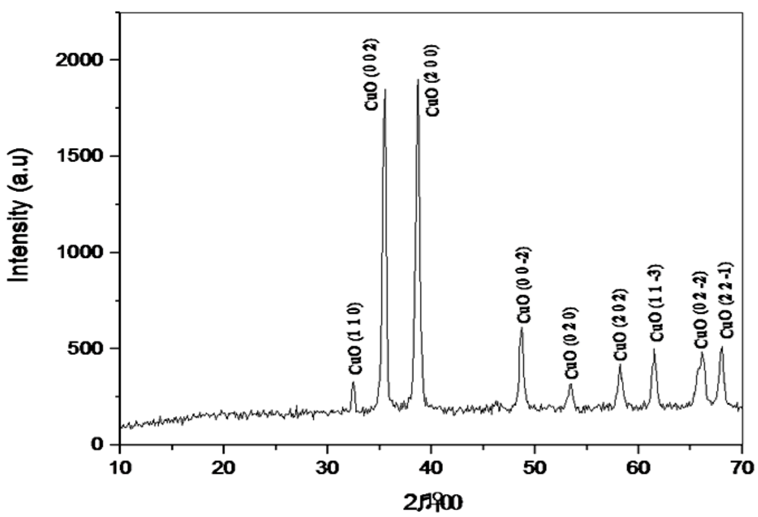


Figure 9. XRD analysis of Biosynthesized CuO-NPs

Antibacterial efficacy study of CuO-NPs

The bacterial culture media were prepared using Hinton-Mueller agar, which was placed in sterile household jars. These jars were then stored in the refrigerator until use. The synthesized copper oxide nanoparticles (CuO-NPs) were dissolved in the organic solvent dimethyl sulfoxide (DMSO) at varying concentrations. Sterilized discs were soaked in each concentration of the CuO-NPs solution. Three types of isolated bacteria were cultured on the prepared media, with each jar labeled according to the bacterial strain it contained. A disc from each concentration, along with a control disc, was then placed in each jar. The cultures were incubated at 37°C for 24 hours in a laboratory incubator (Figure 10).

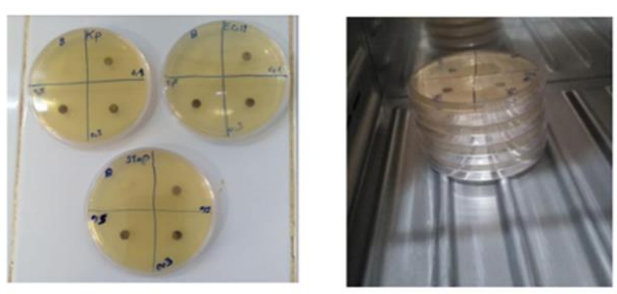


Figure 10: Preparation of bacterial boxes and incubation

Antibacterial Activity

The biosynthesized CuO-NPs exhibited pronounced antibacterial efficacy against a range of bacterial pathogens. Significant zones of inhibition were observed for pathogens such as Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, and Pseudomonas aeruginosa, as shown in Figure 6a (Din, M.I .et al .2017) In contrast, dimethyl sulfoxide (DMSO) was used as a negative control and did not demonstrate any inhibitory activity against bacterial cells, thereby underscoring the antibacterial ability of CuO-NPs (Thiruvengadam, M. et al .2019)

Assay for the Determination of Minimum Inhibitory Concentration (MIC)

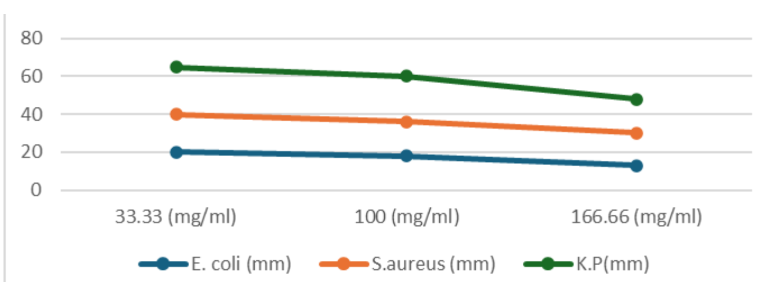
The antimicrobial potency of the biosynthesized CuO-NPs was evaluated across varying concentrations, namely, 150.0, 300.0, 450.0, and 600.0 μg/mL, to establish the minimum inhibitory concentration (MIC) values. As shown in Figure 7, as the concentration of CuO-NPs increased, there was an associated decrease in microbial growth. Specifically, the MIC value for Pseudomonas aeruginosa was determined to be 450.0 μg/mL. Figure 6. (a) Antibacterial activity of CuO-NPs exerted via agar well diffusion and (b) zone of inhibition with bacterial pathogens. A prominent mechanism through which CuO-NPs exert their antibacterial action is believed to be their interaction with bacterial cells, primarily occurring via the thiol groups present in bacterial proteins. This interaction synergistically amplifies the inherent antibacterial properties of CuO-NPs. Moreover, a concentration-dependent trend was observed, where the zone of inhibition expanded with increasing doses of CuO-NPs, suggesting a dose-dependent relationship between their antibacterial efficacies (Figure 11). In the broader spectrum of nanoparticle research, metal oxide nanoparticles such as CuONPs have been spotlighted for their potential in antimicrobial applications. Their mode of action, which involves the disruption of bacterial cell membranes or interference with essential cellular processes, provides an avenue for potential therapeutic applications, especially in an era in which antibiotic resistance is a rising concern (Ali, M.et al .2021, Wu, S.; Rajeshkumar, S. et al .2020)

Results and Discussion of Antibacterial Activity of Copper Oxide Nanoparticles (CuO-NPs)

To demonstrate the antibacterial activity of the copper oxide nanoparticles (CuO-NPs) prepared in this study, we employed the disc diffusion method. Sterile paper discs with a diameter of 6 mm were impregnated with different concentrations of CuO-NPs (33.33, 101.11, and 66.66 mg/mL) and tested against three types of pathogenic bacteria isolated from patients who underwent tests at the Al-Amal laboratory in Ouargla. The bacteria tested included *Escherichia coli* (E. coli), *Klebsiella pneumoniae* (K. pneumoniae), and *Staphylococcus aureus* (S. aureus). The results of the antibacterial activity tests, showing the measured inhibition zone diameters, are presented in Table(01) and graph (01).

Table 01:Diameters of bacterial inhibition by copper oxide nanoparticles

	Concentration (mg/ml)	***E. coli***	***S.aureus***	***K.P***
CuO-NPs	33.33mg/ml	20mm	20mm	25mm
	100.00mg/ml	18mm	18mm	24mm
	166.66mg/ml	13mm	17mm	18mm
	Blanc	No	No	No



Graph (01); Diameters of bacterial inhibition by copper oxide nanoparticles

The results showed a clear correlation between the concentration of CuO-NPs and the diameter of the inhibition zones. As the concentration of CuO-NPs increased, the inhibition zone diameter also increased, indicating enhanced antibacterial activity. This concentration-dependent activity was consistent across all three bacterial strains tested.

- Low Concentration (33.33 mg/mL): Minimal inhibition was observed, with small inhibition zones indicating limited antibacterial activity.
- Medium Concentration (66.66 mg/mL): A noticeable increase in inhibition zone diameter was recorded, reflecting moderate antibacterial effectiveness.
- High Concentration (101.11 mg/mL): The highest concentration demonstrated significant antibacterial activity, with the largest inhibition zones among the tested concentrations.

These findings suggest that the antibacterial efficacy of CuO-NPs is significantly influenced by nanoparticle concentration.

Comparison with Conventional Antibiotics

The antibacterial activity of CuO-NPs was compared to standard antibiotics, including Rifampicin, Levofloxacin, and Amoxicillin + Clavulanic Acid. CuO-NPs at higher concentrations exhibited comparable or even superior inhibition against some bacterial strains when compared to these antibiotics.

- Against *E. coli*: CuO-NPs showed strong activity, particularly at higher concentrations, which was comparable to the efficacy of Levofloxacin.
- Against *K. pneumoniae*: The inhibition zones produced by CuO-NPs were similar to those observed with Rifampicin, indicating good antibacterial potential.
- Against *S. aureus*: CuO-NPs demonstrated significant activity, though slightly less than that of Amoxicillin + Clavulanic Acid.

This comparison highlights the potential of CuO-NPs as an effective alternative to traditional antibiotics, particularly in cases where antibiotic resistance is a concern.

Results and discussion of the antibacterial activity of nanoparticles

The inhibition activity of the prepared copper oxide nanoparticles on E.coli, ,S.aureusand P.K. bacteria showed good results. The higher the concentration, the greater the inhibition diameter, as shown in (Figure 9).

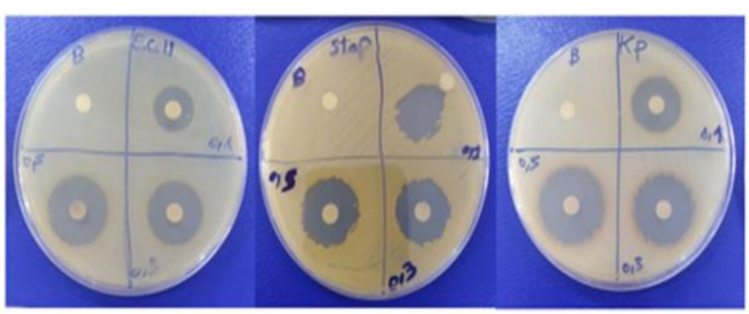


Figure 9 : Results of the Inhibition of bacteria by copper oxide nanoparticles

Comparison between the antibacterial activity of prepared copper oxide nanoparticlesand antibiotics.

The results indicated an increase in the percentage of inhibition by the pectin-capped copper oxide nanoparticles (CuO-NPs); specifically, as the concentration increased, so did the inhibition zone diameter. To compare the antibacterial activity of these pectin-stabilized CuO-NPs with that of conventional antibiotics, we conducted a study at Mohamed Boudiaf Hospital in Ouargla, Algeria. This comparison is illustrated in(Figure 10) and detailed in Table(02).

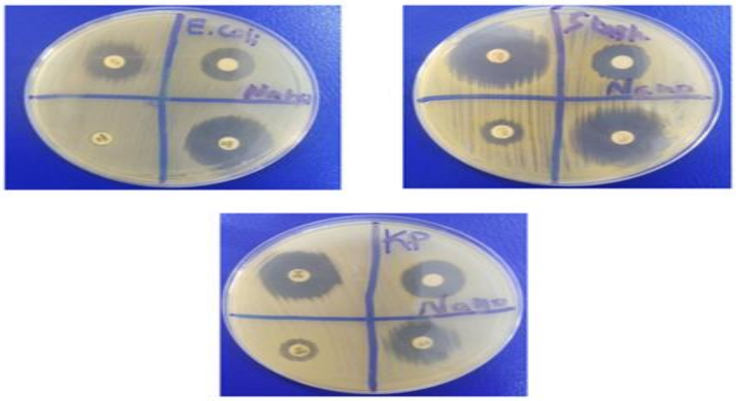


Figure 10: Inhibition diameters S. aureus, E. Coli, KP, bycopper oxide nanoparticules

Results and discussion of Comparison of the activity of prepared copper oxide against E. Coli

Table 02: Comparison of the activity of prepared copper oxide against E. Coli bacteria with antibiotic

Name of isolated bacteria	Antibiotic name	Amount of antibiotic	Inhibition area diameter
E. coli	CuO-NPs	33333 µg	14 mm
	SXT	25 µg	24 mm
	AK	30 µg	17 mm
	AMC	30 µg	05 mm

Results and discussion of the antibacterial activity of nanoparticles

Table (03): Comparison of the activity of prepared copper oxide against P.K bacteria with antibiotic

Name of isolated bacteria	Antibiotic name	Amount of antibiotic	Inhibition area diameter
K.P	CuO-NPs	33333µg	17 mm
	SXT	25 µg	20 mm
	AK	30 µg	20 mm
	AMC	30 µg	09 mm

Results and discussion of Comparison of the activity of prepared copper oxide against S.aureus

Table (04): Comparison of the activity of prepared copper oxide against S.aureus bacteria withantibiotics

Name of isolated bacteria	Antibiotic name	Amount of antibiotic	Inhibition area diameter
S.aureus	CuO-NPs	33333 µg	15 mm
	RIF	5 µg	29 mm
	CMN	2 µg	25 mm
	LVX	5 µg	09 mm

CONCLUSION OF RESULTS AND DISCUSSION ANTIBACTERIAL EFFICACY

In light of the results obtained from this study, which involved evaluating the biological effectiveness of copper oxide nanoparticles (CuO-NPs) against three types of bacteria—Staphylococcus aureus (Gram-positive), Escherichia coli (Gram-negative), and Klebsiella pneumoniae (Gram-negative) and comparing their efficacy with six commonly used antibiotics (Rifampicin, Levofloxacin, Clindamycin, Amoxicillin + Clavulanic Acid, Amikacin, and Sulfamethoxazole + Trimethoprim), the following conclusions were drawn:

Biological Effectiveness: The synthesized CuO-NPs demonstrated significant antibacterial activity against the tested bacteria.

Influence of Particle Size: The particle size of the CuO-NPs played a critical role in their antibacterial effectiveness, with smaller particles exhibiting greater biological activity.

Comparison with Antibiotics: The prepared CuO-NPs showed comparable or superior antibacterial effectiveness relative to the tested antibiotics, particularly against Trimethoprim + Sulfamethoxazole, Rifampicin, Levofloxacin, Clindamycin, and Amikacin.

Conclusions and Outlook

Copper is a less expensive material than other noble metals but is susceptible to microscopic oxidation. Recently, many researchers have begun evaluating the biological activities of nanoparticles containing metal oxides, such as copper oxide (CuO), and have found that they demonstrate superior biological and photophysical activities compared to those derived from metallic nanoparticles. Metallic and metal oxide nanoparticles have attracted significant scientific interest due to their unique physical and chemical properties, which have applications across various fields of science and technology.

Although there are several physical and chemical methods for manufacturing nanoparticles, these protocols are often expensive or toxic due to the use of caustic chemicals and hazardous solvents. This has led to the need for non-hazardous and greener technologies for the synthesis of metals, metal oxides, and nanoparticles. Various biological agents, such as pectin, fungi, and plants, have been claimed to be environmentally safe, offering a cost-effective and rapid method of synthesis. However, despite the widespread use of nanoparticles (NPs), concerns remain about their potential effects on the environment and human health.

While copper is generally considered to have low toxicity to humans, research indicates that the properties and potential toxicity of CuO-NPs are influenced by several factors, including the composition of the biological agent, the precursor used, the synthesis conditions, and the size of the nanoparticles. Optimizing the synthesis process to produce NPs with high antimicrobial activity and low toxicity is a complex but essential task.

In summary, the green synthesis of CuO nanoparticles using an aqueous extract of Artemisia herba-alba and copper sulfate ($CuSO_4$) was successful. UV-VIS, FTIR, XRD, and SEM analyses confirmed the effective formation of CuO-NP nanostructures. The calcination process effectively removed residues and improved the crystallization of CuO.

The study also investigated the biological effectiveness of copper oxide nanoparticles against three types of bacteria: Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae. These results were compared with the effectiveness of six antibiotics: Rifampicin, Levofloxacin, Clindamycin, Amoxicillin + Clavulanic Acid, Amikacin, and Sulfamethoxazole + Trimethoprim. The following conclusions were drawn:

This study highlights the potential of Artemisia herba-alba leaf extract for the green synthesis of CuO-NPs. The formation of CuO-NPs was confirmed by UV-VIS results, which showed distinctive λ-max peaks at 682 nm. FTIR was used to identify specific functional groups in the synthesized CuO-NPs, while XRD confirmed their crystalline nature. SEM and EDX analyses provided insights into the morphology and elemental composition of the nanoparticles. Notably, the green-synthesized CuO-NPs exhibited significant antibacterial activity against pathogenic bacteria, including Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli.

Overall, the findings of this study support the use of Artemisia herba-alba as a green synthesis method for CuO nanoparticles, while also highlighting the multifaceted biomedical applications of CuO-NPs. These results lay the groundwork for further research and potential therapeutic interventions in the medical field.

The overall data indicate that CuO-NP nanocomposites are promising candidates for assessing in vitro and in vivo biological activity, as well as applications in catalysis, wastewater treatment, and especially biomedical fields, due to their safety, cost-effectiveness, and biocompatibility.

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REFERENCES

Abed, A.S.; Khalaf, Y.H.; Mohammed, A.M. Green synthesis of gold nanoparticles as an effective opportunity for cancer treatment. Results Chem. 2023, 5, 100848.

Adil, S.F.; Assal, M.E.; Khan, M.; Al-Warthan, A.; Siddiqui, M.R.H.; Liz-Marzán, L.M. Biogenic synthesis of metallic nanoparticles and prospects toward green chemistry. Dalton Trans. 2015, 44, 9709–9717.

Akintelu, S.A.; Folorunso, A.S.; Folorunso, F.A.; Oyebamiji, A.K. Green synthesis of copper oxide nanoparticles for biomedical application and environmental remediation. Heliyon 2020, 6, e04508.

Alhalili, Z. Green synthesis of copper oxide nanoparticles CuO NPs from Eucalyptus Globoulus leaf extract: Adsorption and design of experiments. Arab. J. Chem. 2022, 15, 103739.

Ali, M.; Ijaz, M.; Ikram, M.; Ul-Hamid, A.; Avais, M.; Anjum, A.A. Biogenic synthesis, characterization and antibacterial potential evaluation of copper oxide nanoparticles against Escherichia coli. Nanoscale Res. Lett. 2021, 16, 148.

Ameer A, Arham SA, Oves M, et al. Size-dependent antimicrobial properties of CuO Nanoparticles against Gram-positive and Gram–negative bacterial strains. Int J Nanomedicine. 2010;7:3527-35.

Bouafia, A.; Meneceur, S.; Chami, S.; Laouini, S.E.; Daoudi, H.; Legmairi, S.; Mohammed Mohammed, H.A.; Aoun, N.; Menaa, F. Removal of hydrocarbons and heavy metals from petroleum water by modern green nanotechnology methods. Sci. Rep. 2023, 13, 5637.

Cuong, H.N.; Pansambal, S.; Ghotekar, S.; Oza, R.; Hai, N.T.T.; Viet, N.M.; Nguyen, V.-H. New frontiers in the plant extract mediated biosynthesis of copper oxide (CuO) nanoparticles and their potential applications: A review. Environ. Res. 2022, 203, 111858.

Dejen, K.D.; Kibret, D.Y.; Mengesha, T.H.; Bekele, E.T.; Tedla, A.; Bafa, T.A.; Derib, F.T. Green synthesis and characterisation of silver nanoparticles from leaf and bark extract of Croton macrostachyus for antibacterial activity. Mater. Technol. 2023, 38, 2164647.

Din, M.I.; Arshad, F.; Rani, A.; Aihetasham, A.; Mukhtar, M.; Mehmood, H. Single step green synthesis of stable copper oxide nanoparticles as efficient photo catalyst material. Biomed. Mater. 2017, 9, 41–48.

Faheem I, Sammia S, Shakeel AK, et al. Green synthesis of copper oxide nanoparticles using Abutilon indicum leaf extract: Antimicrobial, antioxidant and photocatalytic dye degradation activities. Trop J of Phar Res. 2016;16:743-53.

Gemin, L.G.; de Lara, G.B.; Mógor, Á.F.; Mazaro, S.M.; Sant'Anna-Santos, B.F.; Mógor, G.; Amatussi, J.D.O.; Cordeiro, E.C.N.; Marques, H.M.C. Polysaccharides combined to copper and magnesium improve tomato growth, yield, anti-oxidant and plant defense enzymes. Sci. Hortic. 2023, 310, 111758.

Gu, J.; Chen, F.; Zheng, Z.; Bi, L.; Morovvati, H.; Goorani, S. Novel green formulation of copper nanoparticles by Foeniculum vulgare: Chemical characterization and determination of cytotoxicity, anti-human lung cancer and antioxidant effects. Inorg. Chem. Commun. 2023, 150, 110442.

Jayasimha, H.; Chandrappa, K.; Sanaulla, P.; Dileepkumar, V. Green synthesis of CuO nanoparticles: A promising material for photocatalysis and electrochemical sensor. Sens. Int. 2023, 7, 100254. [

Karuppannan, S.K.; Ramalingam, R.; Khalith, S.M.; Dowlath, M.J.H.; Raiyaan, G.D.; Arunachalam, K.D. Characterization, antibacterial and photocatalytic evaluation of green synthesized copper oxide nanoparticles. Biocatal. Agric. Biotechnol. 2021, 31, 101904.

Keat, C. L., Aziz, A., Eid, A. M., &Elmarzugi, N. A. (2015). Biosynthesis of nanoparticles and silver nanoparticles. Bioresources and Bioprocessing, 2(1), 1-11.

Khani, R.; Roostaei, B.; Bagherzade, G.; Moudi, M. Green synthesis of copper nanoparticles by fruit extract of Ziziphus spina-christi (L.) Willd.: Application for adsorption of triphenylmethane dye and antibacterial assay. J. Mol. Liq. 2018, 255, 541–549.

Kolahalam, L.A.; Prasad, K.; Krishna, P.M.; Supraja, N.; Shanmugan, S. The exploration of bio-inspired copper oxide nanoparticles: Synthesis, characterization and in-vitro biological investigations. Heliyon 2022, 8, e09726

Kumar, R.S.; Kumar, S.V.; Rajkapoor, B.; Pravin, N.; Mahendiran, D. Chemopreventive effect of Indigofera linnaei extract against diethylnitrosamine induced hepatocarcinogenesis in rats. J. Appl. Pharm. Sci. 2016, 6, 199–209.

Kumar, R.S.; Rajkapoor, B.; Perumal, P. Antitumor and cytotoxic activities of methanol extract of Indigofera linnaei Ali. Asian Pac. J. Cancer Prev. 2011, 12, 613–618.

Kumar, R.S.; Rajkapoor, B.; Perumal, P.; Kumar, S.V.; Geetha, A.S. Beneficial effects of methanolic extract of Indigofera linnaei Ali. on the inflammatory and nociceptive responses in rodent models. Braz. J. Pharm. Sci. 2016, 52, 113–123.

Liu Z, Kiessling F, Gatjent J. Advanced nanomaterials in multimodel imaging: design, functionalization, and biomedical applications. J Nanomater. 2010;894003.

Makarov, V. V., Love, A. J., Sinitsyna, O. V., Makarova, S. S., Yaminsky, I. V., Taliansky, M. E., &Kalinina, N. O. (2014). "Green" nanotechnologies : synthesis of metalnanoparticlesusing plants. Acta Naturae (англоязычнаяверсия), 6(1 (20), 35-44.

Manasa, D.; Chandrashekar, K.; Kumar, D.M.; Niranjana, M.; Navada, K.M. Mussaendafrondosa L. mediated facile green synthesis of copper oxide nanoparticles–characterization, photocatalytic and their biological investigations. Arab. J. Chem. 2021, 14, 103184.

Mohamed, E.A. Green synthesis of copper & copper oxide nanoparticles using the extract of seedless dates. Heliyon 2020, 6, e03123.

Nanotechnology, Mohamed Sharif Al-Iskandari, April 2010.

Naz, S.; Gul, A.; Zia, M.; Javed, R. Synthesis, biomedical applications, and toxicity of CuO nanoparticles. Appl. Microbiol. Biotechnol. 2023, 107, 1039–1061.

Nobahar, A., Carlier, J. D., Miguel, M. G., & Costa, M. C. (2021). A review of plant metabolites with metal interaction capacity:a green approach for industrial applications. BioMetals, 34(4), 761-793.

Nthunya, L.N.; Mbakop, S.; Makgabutlane, B.; Matlou, G.; Mhlanga, S.; Richards, H. Fungal synthesis of copper nanoparticles and their applications in agri-food, environmental, and biomedical sectors. In Fungal Cell Factories for Sustainable Nanomaterials Productions and Agricultural Applications; Elsevier: Amsterdam, The Netherlands, 2023; pp. 91–114.

Nzilu, D.M.; Madivoli, E.S.; Makhanu, D.S.; Wanakai, S.I.; Kiprono, G.K.; Kareru, P.G. Green synthesis of copper oxide nanoparticles and its efficiency in degradation of rifampicin antibiotic. Sci. Rep. 2023, 13, 14030.

Prakash, M.; Kavitha, H.P.; Abinaya, S.; Vennila, J.P.; Lohita, D. Green synthesis of bismuth based nanoparticles and its applications-A review. Sustain. Chem. Pharm. 2022, 25, 100547.

Ramadhan, V.; Ni'Mah, Y.; Yanuar, E.; Suprapto, S. Synthesis of copper nanoparticles using Ocimumtenuiflorum leaf extract as capping Agent. AIP Conf. Proc. 2019, 2202, 020067.

Ratner, M. A., & Ratner, D. (2003). Nanotechnology: A gentle introduction to the next big idea. Prentice Hall Professional.

Rehana, D.; Mahendiran, D.; Kumar, R.S.; Rahiman, A.K. Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts. Biomed. Pharmacother. 2017, 89, 1067–1077.

Seigneuric R, Markey L, Nuyten DSA, et al. From nanotechnology to nanomedicine: applications to cancer research. Curr Mol Med. 2010;10:640-52.

Sharma, P.; Pant, S.; Poonia, P.; Kumari, S.; Dave, V.; Sharma, S. Green synthesis of colloidal copper nanoparticles capped with Tinospora cordifolia and its application in catalytic degradation in textile dye: An ecologically sound approach. J. Inorg. Organomet. Polym. Mater. 2018, 28, 2463–2472.

Siddiqui, V.U.; Ansari, A.; Chauhan, R.; Siddiqi, W.A. Green synthesis of copper oxide (CuO) nanoparticles by Punica granatum peel extract. Mater. Today Proc. 2021, 36, 751–755.

Song, J. Y., & Kim, B. S. (2009). Rapid biologicalsynthesis of silver nanoparticlesusing plant leafextracts. Bioprocess and biosystems engineering, 32, 79-84.

Sukumar, S.; Rudrasenan, A.; Padmanabhan Nambiar, D. Green-synthesized rice-shaped copper oxide nanoparticles using Caesalpinia bonducella seed extract and their applications. ACS Omega 2020, 5, 1040–1051.

Tamuly, C.; Saikia, I.; Hazarika, M.; Das, M.R. Reduction of aromatic nitro compounds catalyzed by biogenic CuO nanoparticles. RSC Adv. 2014, 4, 53229–53236.

Thiruvengadam, M.; Chung, I.-M.; Gomathi, T.; Ansari, M.A.; Gopiesh Khanna, V.; Babu, V.; Rajakumar, G. Synthesis, characterization and pharmacological potential of green synthesized copper nanoparticles. Bioprocess Biosyst. Eng. 2019, 42, 1769–1777.

Verma, N.; Kumar, N. Synthesis and biomedical applications of copper oxide nanoparticles: An expanding horizon. ACS Biomater. Sci. Eng. 2019, 5, 1170–1188.

Wu, S.; Rajeshkumar, S.; Madasamy, M.; Mahendran, V. Green synthesis of copper nanoparticles using Cissus vitiginea and its antioxidant and antibacterial activity against urinary tract infection pathogens. Artif. Cells Nanomed. Biotechnol. 2020, 48, 1153–1158.

Yallappa S, Manjanna J, Sindhe MA, et al. Microwave assisted rapid synthesis and biological evaluation of stable copper nanoparticles using T. arjuna bark extract. Spectrochim Acta Mol BiomolSpectrosc. 2013;110:108-15.