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A Review of Green Synthesis of Copper Oxide

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ABSTRACT

Copper oxide nanoparticles (CuO NPs) have attracted considerable attention in health-related fields because of their diverse properties and broad applications, particularly as antimicrobial agents. CuO nanoparticles can be synthesized using various physical, chemical, and biological methods. However, conventional chemical and physical methods are costly, resource-intensive, and often require hazardous reagents that pose risks to human health and the environment. In contrast, biological synthesis provides a sustainable and cost-effective alternative by eliminating chemical contaminants and allowing the production of CuO nanoparticles with suitable sizes and shapes. This review article focuses on the green synthesis of CuO nanoparticles using various biological sources, especially plants.

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1. Introduction

Among the various types of nanoparticles employed, metal and metal oxide nanoparticles are considered highly effective due to their exceptional properties, including high stability, biocompatibility, and significant antimicrobial and anticancer activities [1, 2]. Engineered metal oxide nanoparticles rank among the most widely used synthetic nanomaterials. Their size-dependent characteristics—such as unique catalytic properties, high sensitivity and selectivity, superparamagnetic behavior, diverse nonlinear optical features, unusual absorption capabilities, and rapid diffusion—make them pivotal materials in various nanotechnology applications [3].

Research has demonstrated that copper oxide nanoparticles can be utilized as coatings for medical implants, such as prostheses and other devices implanted in the body, to reduce the risk of postoperative infections [4]. These nanoparticles are also employed in the development of antibacterial and anticancer pharmaceuticals, as they can inhibit the proliferation of cancer cells and help mitigate bacterial drug resistance [5].

Copper oxide nanoparticles are used in the fabrication of disinfectant wound dressings that accelerate the healing process. These dressings contribute to the reduction of hospital-acquired infections [6]. Additionally, due to their nanoscale dimensions and high surface interaction capabilities, these nanoparticles are applied in smart drug delivery systems to transport therapeutic agents to specific target sites within the body [7].

Copper is an essential element for humans, plants, and animals [8]. In humans, copper participates in several physiological processes [9,10]. In plants, copper is fundamental to various biochemical and physicochemical pathways and is considered a trace element essential for growth [11].

2. Copper oxide nanoparticles

Copper oxide (CuO) possesses a monoclinic crystal structure and is a p-type semiconductor with a narrow band gap of 1.7eV. The monoclinic crystal system is one of the seven fundamental crystal structures in crystallography, characterized by a distorted parallelepiped shape where only one of the interaxial angles deviates from 90 degrees [12]. Among metal oxide nanoparticles, CuO NPs have recently garnered attention due to their distinctive optical, electrical, magnetic, biological, and catalytic properties [13, 14]. These remarkable features have led to their widespread application in diverse fields such as energy, electronics, cosmetics, biosensors, data storage devices, supercapacitors, catalysis, food and agriculture, and healthcare (Fig. 1) [15, 16]. Furthermore, their antimicrobial and anticancer properties make CuO NPs promising therapeutic agents [17, 18].

In recent years, nanotechnology-based therapeutic approaches have been employed for disease diagnosis, treatment, and novel drug formulations. The antibacterial activity of CuO NPs has been tested against various pathogenic bacterial strains, yielding significant results [19]. Published reports indicate that CuO nanoparticles exhibit high toxicity against most human pathogens [20]. Biogenically synthesized CuO NPs have attracted considerable interest as antibacterial agents due to their unique morphology, size, and biocompatibility [21]. Green-synthesized CuO NPs also demonstrate potent antibacterial activity against both Gram-positive and Gram-negative strains.

The antibacterial efficacy of CuO NPs appears to vary depending on bacterial cell characteristics. For example, the structure of bacterial cell walls influences their susceptibility to CuO NPs, with Gram classification being a key factor. One study revealed that 100% of Gram-negative *E. coli* cells were killed at concentrations above 9.5% CuO NPs, whereas Gram-positive *Staphylococcus aureus* showed lower susceptibility [22]. Another investigation found that CuO NPs inhibited the growth of *E. coli*, *Pseudomonas aeruginosa*, and *S. aureus* in a time-dependent manner, with dosage being the most critical factor [23]. Goyal and colleagues also reported that antimicrobial activity depends on nanoparticle surface properties and size, with smaller particles exhibiting greater antibacterial efficacy due to their larger surface area [24].

These nanoparticles can enhance the generation of Reactive Oxygen Species (ROS), leading to oxidative stress and damage to bacterial proteins, lipids, and DNA [25]. Studies have shown that CuO NPs can affect bacterial genomes by altering DNA sequences, potentially disrupting cellular functions and causing bacterial death [26]. Additionally, CuO NPs can interfere with cellular metabolism by inhibiting enzymatic activities and disrupting metabolic processes, thereby halting bacterial growth and proliferation [27].

2.1. Catalytic applications

CuO NPs are employed in oxidative dehydrogenation of alcohols. They catalyze the oxidation of alcohols into carbonyl compounds (e.g., aldehydes and ketones) using atmospheric oxygen as the oxidant, offering a green alternative to noble-metal-based catalysts [28]. CuO NPs are also used in Ullmann-type coupling reactions, which are essential for synthesizing complex organic molecules [29]. Moreover, CuO NPs synthesized using plant extracts have been applied in catalytic processes such as diphenyl ether synthesis [30].

2.2. Agricultural applications

Research indicates that CuO NPs can alleviate osmotic and oxidative stress caused by drought in plants. Foliar application of CuO NPs at specific concentrations has been shown to increase chlorophyll content and enhance plant growth [31].

2.3. Energy sector applications

Due to their unique properties—such as high surface area, excellent catalytic activity, and tunable electronic characteristics—CuO NPs have significant applications in the energy sector. For instance, they are used in lithium-ion batteries and supercapacitors to improve charge storage capacity and conductivity [32]. CuO NPs are effective in water-splitting reactions (photocatalysis), contributing to hydrogen production as a clean fuel source [33]. They are also incorporated into photovoltaic devices to enhance light absorption and electron transport efficiency [34]. In fuel cells, CuO NPs improve electrochemical reactions and energy conversion efficiency due to their catalytic properties [33].

Copper oxide nanoparticles (CuO NPs) are widely utilized in biosensors due to their excellent electrochemical properties, high surface area, and catalytic activity. They play a crucial role in the detection of biomolecules, making them valuable in medical diagnostics, environmental monitoring, and food safety applications [35].

CuO NPs are employed in non-enzymatic glucose sensors, where they facilitate glucose oxidation and offer a cost-effective and stable alternative to enzyme-based sensors [36]. Their high electrical conductivity enhances electron transfer in biosensing platforms, thereby improving sensitivity and lowering detection limits [37].

CuO-based biosensors have been investigated for the detection of various biomolecules, including proteins and DNA, contributing to disease diagnosis and molecular analysis [38]. Fig. 1 illustrates the diverse applications of copper oxide nanoparticles.

3. Synthesis methods of copper oxide nanoparticles

Research into the synthesis and properties of metallic nanomaterials has emerged as a prominent field in nanotechnology, driven by the diverse applications of these nanoparticles in scientific, technological, pharmaceutical, and biomedical sectors. Various physical and chemical methods have been employed to synthesize CuO nanoparticles [39], which are described below:

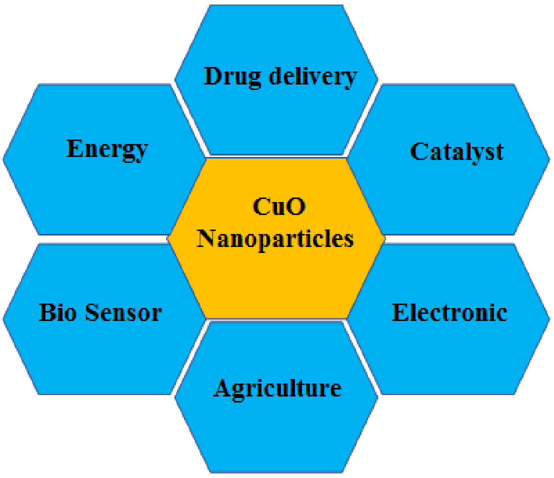

Fig. 1. The applications of CuO nanoparticles

3.1. Physical methods

All physical synthesis techniques follow a top-down approach, where bulk materials are broken down into smaller particles, ultimately forming nanoparticles. Several processes fall under this category:

Ball milling is one of the simplest mechanical methods; it involves grinding bulk copper oxide into nanoscale particles. This technique is widely used due to its simplicity and scalability. Fig.2 illustrates the ball milling process [40, 41]. Laser ablation is another physical method; it uses laser irradiation to decompose precursor materials and generate nanoparticles. Fig. 3 shows the laser-ablation setup [42, 43].

Sputtering: Sputtering has also been reported for CuO NP synthesis. It involves the annealing of thin nanoparticle layers to achieve desired properties [44]. Although physical methods yield high-purity nanoparticles, they typically require expensive equipment and high energy input. Moreover, achieving uniform morphology and particle size remains a significant challenge [45].

3.2. Chemical methods

Chemical synthesis methods adopt a bottom-up approach, where basic molecular units assemble into larger structures, eventually forming nanoparticles. These methods are cost-effective and capable of producing uniform particles at large scale without the need for high-power equipment.

The sol-gel technique is the most popular chemical route for CuO NP synthesis due to its simplicity, scalability, and economic feasibility [46].

Co-precipitation is another efficient and straightforward method. It allows the use of various precursors, resulting in nanoparticles with diverse sizes and morphologies [47]. For instance, Younus Ali et.al synthesized CuO NPs by gradually adding sodium hydroxide to a copper chloride solution, followed by heating to obtain the nanoparticles [48]. Fig. 2 provides a visual comparison of several physical and chemical synthesis methods for CuO nanoparticles. Capping agents, which often function as reducing and stabilizing agents, are typically introduced alongside precursors at the onset of chemical nanoparticle synthesis. These agents fully encapsulate the target particles, serving to prevent aggregation or agglomeration and to enhance chemical stability. Reducing agents, on the other hand, possess electron-donating capabilities that facilitate the conversion of metal cations into electrically neutral nanoparticles [49].

The sonochemical method utilizes ultrasonic waves to synthesize nanoparticles. This approach induces a phenomenon known as cavitation, where microscopic bubbles form within the solution. The rapid collapse of these bubbles generates localized energy, creating transient high-temperature and high-pressure zones that enable chemical reactions to occur under mild bulk conditions [50].

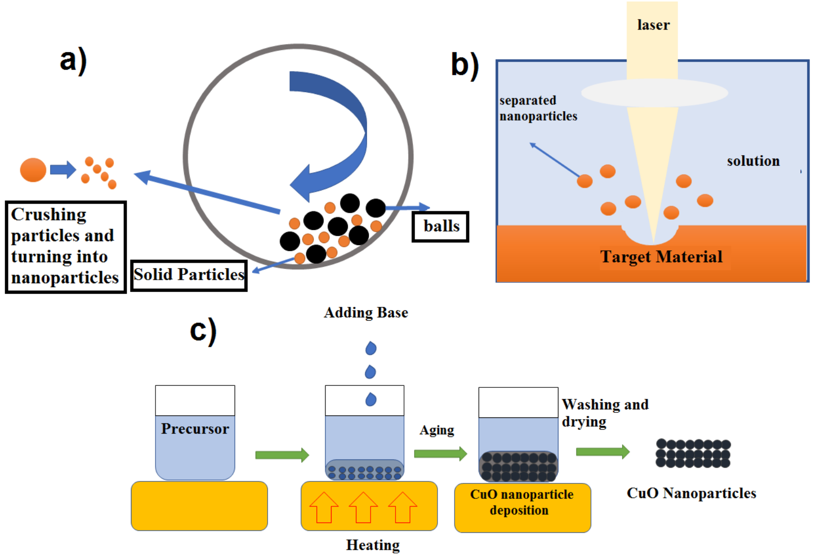

Fig. 2. (a) The ball mill method, which is considered one of the simplest and most applicable physical methods, (b) Laser ablation is a precise technique used to fabricate nanomaterials by vaporizing a target material with high-energy laser pulses, (c) The precipitation method is a simple and cost-effective approach for synthesizing nanomaterials by inducing solid particle formation from a solution through chemical reactions

Despite their effectiveness, chemical synthesis methods are often costly and environmentally unfriendly. They typically rely on non-renewable materials and do not incorporate green chemistry principles or sustainable energy sources, raising concerns about their ecological impact [51].

3.3. Green synthesis of copper oxide nanoparticles

Biogenic or "green" synthesis of nanoparticles involves the use of plant and microbial extracts, such as those derived from bacteria and fungi. This environmentally friendly approach is simple and cost-effective, and unlike conventional physical and chemical methods that rely on hazardous and expensive chemicals (e.g., solvents, reducing agents, and stabilizers), it results in the formation of biocompatible and non-toxic nanoparticles. Green synthesis using plant extracts does not require external capping agents, as secondary metabolites present in the extracts—such as phenolics, alkaloids, flavonoids, terpenoids—and enzymes from microorganisms act both as reducing and capping agents, ultimately producing stabilized nanoparticles [45].

A capping agent, also known as a stabilizer or surfactant, is a molecule used during nanoparticle synthesis to coat the surface of the particles. These agents help prevent aggregation, control particle size and shape, and can modify the physical and biological properties of the nanoparticles [52]. However, microbe-based green synthesis is less attractive due to the need for controlled and sufficient culturing, which makes large-scale commercial production costly. Similarly, algae-based synthesis is considered inefficient due to its slow and time-consuming nature [49].

Overall, biological synthesis of copper oxide nanoparticles represents a promising and sustainable alternative that harnesses nature's capabilities to produce nanoparticles with desirable properties. By addressing the challenges associated with various biological approaches, researchers can optimize and enhance the efficiency of this eco-friendly synthesis method [53].

3.3.1. Fungal-mediated synthesis

In recent years, various fungal species have been employed to synthesize copper oxide and other metallic nanoparticles. Compared to other microorganisms, fungi offer several advantages for nanoparticle synthesis. They exhibit greater tolerance to agitation, shear stress, and other conditions within bioreactors or growth chambers than bacteria.

Fungi are effective candidates for producing metallic and metal oxide nanoparticles via both extracellular (reduction of metal ions using metabolites secreted into the biomass filtrate) and intracellular (uptake of metal ions into fungal cells and reduction via intracellular enzymes) pathways [54]. Nanoparticles synthesized intracellularly tend to be smaller, better dispersed, and more uniform in size compared to those produced extracellularly.

Extracellular synthesis offers several benefits, including the production of nanoparticles free from cellular debris. This pathway is predominantly used in fungal-mediated synthesis, as fungi secrete a wide range of metabolites that act as reducing and stabilizing agents [55].

Among fungi, endophytic strains—those residing within plant hosts without causing disease—are notable for secreting large quantities of secondary metabolites similar in type and activity to their host plants. These metabolites can be utilized to synthesize nanoparticles with varied sizes, stability, and morphologies [56].

3.3.2. Bacterial-mediated synthesis

Bacteria have also been widely used in recent years to produce various nanoparticles, including copper oxide nanoparticles. Through either intracellular or extracellular pathways, bacteria can synthesize nanomaterials with specific morphologies and nanoscale dimensions. They offer advantages such as short production times, ease of cultivation, mild laboratory conditions, high stability, extracellular nanoparticle production, and genetic modifiability [57].

When exposed to environments with high concentrations of toxic metal ions, bacteria can survive by converting these ions into non-toxic metal oxides [58]. In response to oxidative stress, bacteria produce various important compounds containing thiol groups. These molecules act as capping agents during nanoparticle synthesis, preventing oxidation of the metal oxide nanoparticles [59].

3.3.3. Plant extract-mediated synthesis

Previous studies have demonstrated successful synthesis of CuO nanoparticles using a simple approach involving the mixing of copper salts with plant extracts such as bay leaf [60], rosemary [61], yarrow [62], tamarind seed [63], and others. Under standard laboratory conditions, this mixture undergoes reaction within minutes to hours. The presence of phytochemicals, such as polyphenolic compounds, facilitates the reduction of copper salts, leading to nanoparticle formation. The synthesis of CuO nanoparticles is initially confirmed by a noticeable color change in the solution and subsequently characterized using various spectroscopic and microscopic techniques [64]. In general, green synthesis of metallic and metal oxide nanoparticles is illustrated in Fig. 3.

The initial step in green synthesis involves extracting the desired plant material, where different parts of the plant are selected based on their chemical composition. For instance, Kalayan et al. utilized the leaves of Euphorbia heterophylla for extraction. These leaves contain phenolic and polyphenolic compounds, as well as fatty acids such as oxalic acid and palmitic acid [65]. In another study, Rostami used the seeds of Rumex species for extraction, which are rich in flavonoids [66]. Vasantharaj et al. employed the flowers of Tecoma stans, which are known to contain flavonoid compounds and flavonoid acids such as gallic acid [67]. Yugandhar et al. extracted from the fruits of Syzygium alternifolium, which are rich in polyphenolic and phenolic compounds including tannins, flavonoids, and phenolic acids [68].

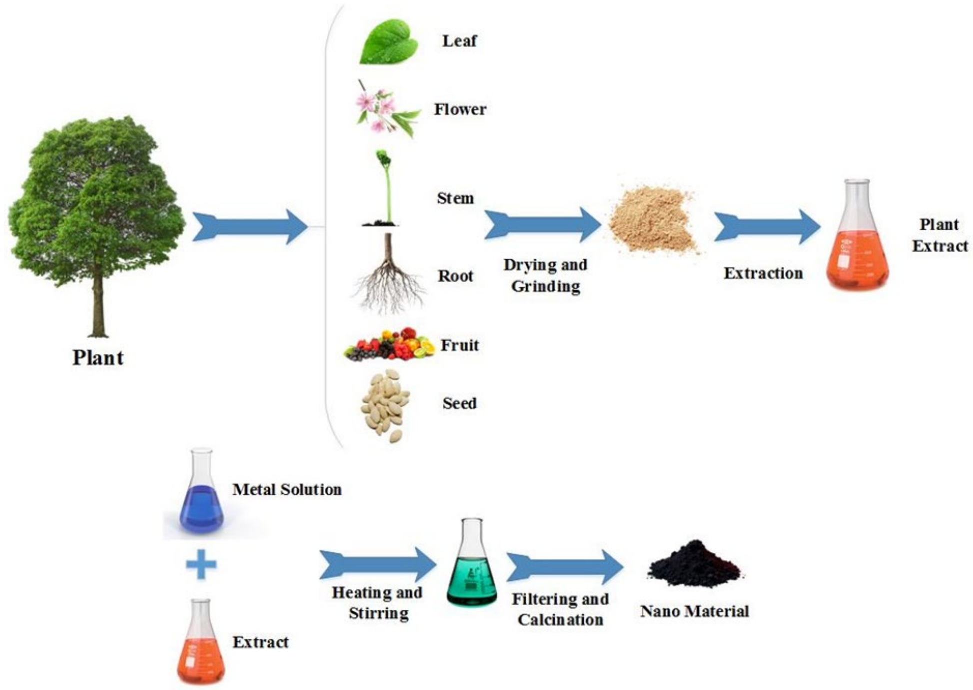

Fig. 3. General schematic of the green synthesis method

Typically, the extraction process involves heating the solution. For example, Jayasimha et al. performed extraction from Giant cane at boiling water temperature [69], whereas Ali et al. conducted extraction from Aegle marmelos at room temperature [70].

The duration and temperature of the mixing stage between the metal salt and the extract vary depending on the plant type and the concentrations of the metal salt and extract. Ali et al. mixed copper sulfate solution with the extract at room temperature for 24 hours [70], while Ogubio et al. carried out this step at 80°C for 2 hours [71].

Following the mixing and separation of the solid precipitate from the solution, the calcination step is performed to convert the precursor into the desired phase, eliminate organic materials and impurities, and enhance crystallinity and stability. This step is generally conducted at temperatures ranging from 300°C to 600°C. For example, Orhan performed calcination at 450°C for 2 hours [72], while Sedighi et al. calcined copper oxide nanoparticles at 300°C for 3 hours [73]. Abdollahzadeh et al. investigated the effect of calcination temperature on the particle size of synthesized nanoparticles. They conducted calcination on four different samples at 400°C, 500°C, 700°C, and 900°C. According to FESEM and DLS analyses, increasing the calcination temperature led to an increase in nanoparticle size, with the average particle size increasing more than fourfold from 400°C to 900°C [74].

Tamer et al. studied the effect of different pH levels on the green synthesis of copper oxide nanoparticles and found that pH significantly influences nanoparticle size. Their research indicated that at lower (acidic) pH levels, nanoparticles tend to aggregate, resulting in larger particle sizes. Conversely, at higher pH levels, nucleation dominates over aggregation [75].

Dehajipour et al. optimized the biosynthesis of copper oxide nanoparticles using pistachio leaf extract. They examined parameters such as temperature, extract-to-copper salt volume ratio, and reaction time, and identified optimal conditions for green synthesis. According to their findings, synthesis at 80°C with an extract-to-salt ratio of 1:4 yielded better results [76].

4. Characterization of copper oxide nanoparticles

Various techniques are available for the characterization of copper oxide nanoparticles. One of the fundamental methods is X-ray Diffraction (XRD), which is a core tool for analyzing the crystalline structures of solid materials. This technique is based on the principles of X-ray diffraction as the rays pass through crystal lattices. By analyzing the angle and intensity of the diffracted beams, precise information can be obtained regarding the crystal structure, unit cell dimensions, phase types, and crystal orientation. Using the data derived from this analysis and the Debye-Scherrer equation, the crystallite size can be calculated.

Debye-Scherrer Equation [77]:

$$D = \frac{K\lambda}{\beta . \cos\theta} \tag{1}$$

where, D is Crystallite size, K (dimensionless) depends on the shape of the crystallites and is typically taken as 0.9 for spherical particles with cubic symmetry. β is wavelength of the X-ray radiation used (in nanometers or angstroms). B is Full Width at Half Maximum (FWHM) of the diffraction peak (in radians). This value reflects the broadening of the peak due to small crystallite size. θ is bragg angle (in degrees or radians). It is half of the diffraction angle (2θ) where the peak occurs. A widely used and practical method for observing the surface morphology of nanoparticles is Scanning Electron Microscopy (SEM). This technique is among the most powerful tools for examining the surface and microscopic structure of materials. By utilizing electrons instead of light, SEM can produce images with extremely high magnification and remarkable resolution.

Another advanced technique for nanoparticle observation is Transmission Electron Microscopy (TEM). TEM is one of the most sophisticated and powerful imaging methods for investigating the internal structure of materials at the nanoscale. Unlike SEM, which examines the surface of a sample, TEM allows visualization of the internal features of the material.

To identify the chemical bonds within nanoparticles, Fourier Transform Infrared Spectroscopy (FTIR) is commonly employed. This analytical method investigates how a material absorbs infrared light, thereby revealing the functional groups and chemical bonds present. In the case of copper oxide nanoparticles, the characteristic stretching vibrations of the Cu–O bond typically appear in the range of 400–600 cm^{-1}. Fig. 4 displays images obtained from these four characterization techniques.

Observed discrepancies between crystallite sizes derived from the Debye–Scherrer equation and particle sizes measured by TEM are common in studies of green-synthesized CuO nanoparticles and merit explicit discussion. XRD Scherrer estimates report the coherent diffraction domain (crystallite) size and therefore reflect crystallite aggregation, internal strain, and instrumental broadening rather than the full physical particle diameter. In contrast, TEM images report the projected geometric size of particles, which may be single crystals, polycrystalline aggregates, or core–shell entities coated by organic residues from plant extracts. Consequently, Scherrer sizes are often smaller than TEM diameters when particles are polycrystalline or when several crystallites are fused into a single observed particle; conversely, strain, defects, or broadened peaks caused by small coherent domains can make Scherrer estimates appear anomalously small relative to well-resolved TEM particles. In summary, a table of studies conducted on the green synthesis of copper oxide nanoparticles is presented in Table 1.

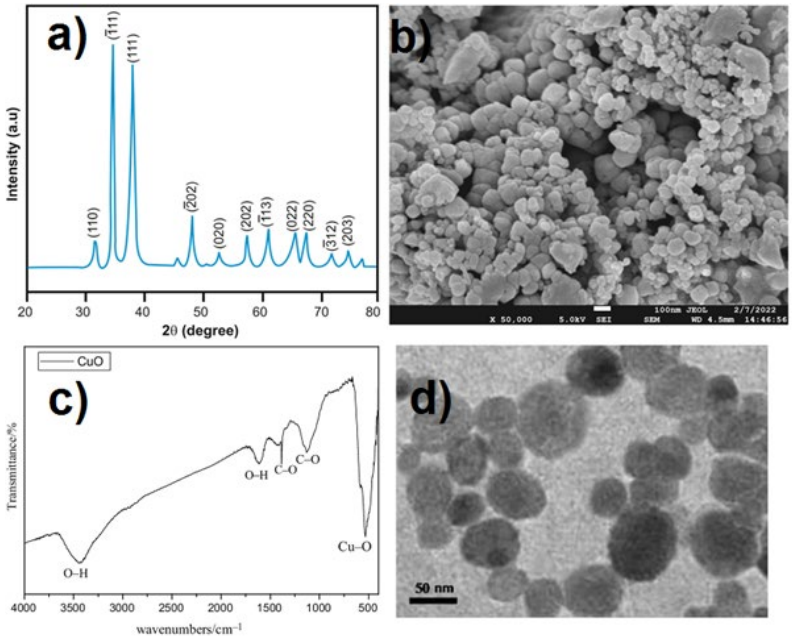

Fig. 4. (a) XRD pattern [78], (b) SEM image [79], (c) FTIR pattern [80], and (d) TEM [81] image of copper oxide nanoparticles

Table 1. Summary of research conducted on the green synthesis of copper oxide nanoparticles

Plant name	Plant component	Precursor	Particle size (nm)	Reference
Euphorbia heterophylla	Leaf	Copper nitrate	108	[65]
Laurus nobilis	Leaf	Copper sulfate	90	[60]
Okra	Fruit	Copper nitrate	25	[82]
Cistus creticus	Leaf	Copper chloride	230	[83]
Rosmarinus officinalis	Leaf	Copper nitrate	Not specified	[61]
Jojoba	Leaf	Copper sulfate	31	[84]
Malva sylvestris	Leaf	Copper nitrate	26	[85]
Quercus sp.	Fruit	Copper sulfate	20–30	[86]
Carica papaya L.	Leaf	Copper chloride	20	[87]
Citrus medica L.	Leaf	Copper sulfate	20	[88]
Gymnema sylvestre	Leaf	Copper sulfate	65	[89]
Coffea arabica L.	Leaf	Copper sulfate	260	[90]
Cedrus deodara	Leaf	Copper sulfate	16	[91]
Caesalpinia bonducella	Seed	Copper sulfate	13	[92]
Hylotelephium telephium	Flower	Copper nitrate	83	[93]
Beta vulgaris	Leaf	Copper sulfate	60	[94]
Acanthospermum hispidum	Leaf	Copper sulfate	Not specified	[95]
Pterolobium hexapetalum	Leaf	Copper sulfate	10-50	[96]
Terminalia catappa	Leaf	Copper sulfate	30–100	[97]
Citrus limon	Leaf	Copper chloride	60–100	[98]
Eucalyptus sp.	Leaf	Copper sulfate	38	[99]
Juglans regia	Leaf	Copper sulfate	80	[100]
Zea mays L.	Leaf	Copper sulfate	36–73	[101]
Enicostemma littorale Blume	Leaf	Copper sulfate	30	[102]
Populus ciliata Wall. ex Royle	Leaf	Copper nitrate	50–60	[103]
Solanum lycopersicum	Leaf	Copper sulfate	20–40	[104]
Azadirachta indica A. Juss	Leaf	Copper chloride	38	[105]

Stronger reducing agents (high-total-phenolic or high-flavonoid extracts) accelerate initial reduction of Cu^{2+} ions, increasing the nucleation rate relative to growth and yielding smaller primary crystallites. Weaker or slower reductants favour fewer nuclei and extended growth, producing larger particles or polydisperse samples. Selective surface binding and shape control: Different classes of phytochemicals bind preferentially to specific crystal facets. Molecules with multiple hydroxyl groups (many flavonoids, tannins) can selectively adsorb and inhibit growth on particular facets, promoting anisotropic shapes (rods, sheets) or exposing high-energy facets that influence catalytic and antibacterial activity [106].

5. Antibacterial application

The most common application of these nanoparticles in the reviewed studies pertains to antibacterial activity, particularly against Escherichia coli and Staphylococcus aureus. The extent of antibacterial efficacy of copper oxide nanoparticles has typically been assessed using the Zone of Inhibition (ZOI) method, which measures the diameter of the growth-free halo around the sample (Fig. 5). Selected results from these studies are summarized in Table 2.

Table 2. Measurement of the diameter of the growth inhibition zone of Escherichia coli and Staphylococcus aureus bacteria

Plant Extract	ZOI for Staphylococcus aureus (mm)	ZOI for Escherichia coli (mm)	Reference
Acer palmatum	18	14	[107]
Plectranthus amboinicus	13	13	[108]
Portulaca oleracea	17	18	[109]
Morinda citrifolia	13	13	[110]
Spinacia oleracea	9	18	[111]
Macroptilium lathyroides	14	13	[112]
Centratherum punctatum	20	18	[113]
Annona glabra	19	20	[114]
Psidium guajava	21	20	[115]
Euphorbia heterophylla	30	34	[65]
Coelastrella terrestris Algal	22	17	[116]
Sesbania grandiflora	15	14	[117]

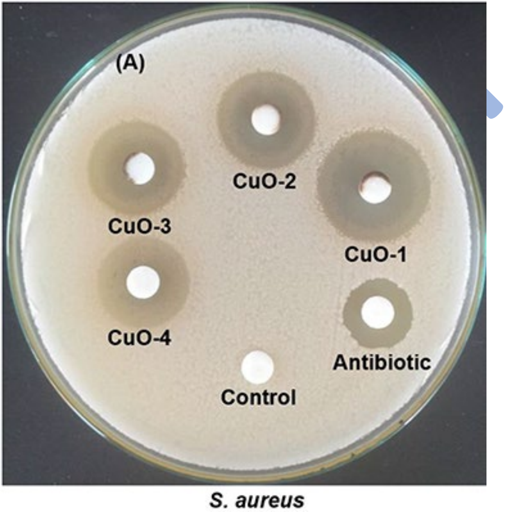

Fig. 5. Diameter of the growth inhibition zone of Escherichia coli in the presence different concentrations of copper oxide nanoparticles [116]

A comparative mechanistic discussion that links specific phytochemical profiles to nanoparticle properties and antibacterial outcomes is necessary to explain the superior ZOI values observed for Euphorbia heterophylla-derived CuO NPs. Euphorbia species are rich in phenolics, tannins, cardiac glycosides and other bioactive secondary metabolites; several reports document that E. heterophylla extracts contain relatively high amounts of phenolics and tannins compared with many other plant extracts, which can increase both reducing capacity and surface functionalization of formed nanoparticles.

These phytochemicals influence three key synthesis parameters that determine nanoparticle antibacterial performance: (1) nucleation vs growth kinetics (which control particle size), (2) surface capping and charge (which control colloidal stability and surface reactivity), and (3) surface-bound organic moieties (which modulate interaction with bacterial membranes). Stronger reducing agents (polyphenols, flavonoids) promote fast nucleation and typically produce smaller CuO crystallites with higher surface area; simultaneous adsorption of tannins and phenolic residues on particle surfaces acts as a stabilizing capping layer that prevents aggregation and can increase exposure of reactive Cu sites.

Smaller, well-dispersed CuO NPs with reactive surfaces generate more reactive oxygen species (ROS) and provide greater direct contact with bacterial envelopes, two established antibacterial mechanisms for CuO nanoparticles. Therefore, an extract such as E. heterophylla with high phenolic/tannin content plausibly yields CuO NPs that are both smaller and more surface-functionalized, explaining the larger ZOI (30mm S. aureus, 34mm E. coli) reported in Table 2 compared with other plant-derived samples.

6. Challenges, limitations, and future directions

Despite the promising potential of green synthesis for copper oxide nanoparticles (CuO NPs), several challenges and limitations hinder its scalability and broader industrial adoption. Addressing these issues is essential for advancing the field and unlocking new applications.

6.1. Challenges and limitations

Green synthesis protocols vary widely depending on the biological source, extraction method, and reaction conditions. This variability leads to inconsistent nanoparticle size, shape, and yield, complicating reproducibility and comparative analysis across studies.

Also, compared to chemical and physical methods, green synthesis often offers less precise control over nanoparticle morphology and crystallinity. This can affect the performance of CuO NPs in applications like catalysis and sensing [118].

While plant-based synthesis is cost-effective and eco-friendly, scaling up for industrial production remains challenging due to batch-to-batch variability, seasonal availability of plant materials, and the need for large volumes of extract. The exact biochemical pathways and roles of phytochemicals in nanoparticle formation are not fully understood. This limits the ability to optimize synthesis conditions and predict outcomes**.** Green-synthesized CuO NPs may contain residual organic compounds from plant extracts, which can affect their stability, shelf-life, and performance in biomedical applications [119].

6.2. Future directions

Future research should focus on elucidating the molecular mechanisms of nanoparticle formation, including the role of specific phytochemicals and enzymes. This could enable more targeted and efficient synthesis strategies . Combining green synthesis with controlled physical or chemical techniques (e.g., microwave-assisted green synthesis) may improve particle uniformity and scalability while retaining eco-friendly benefits.

Developing standardized, validated protocols for plant extract preparation and nanoparticle synthesis will enhance reproducibility and facilitate regulatory approval for biomedical applications [118].

Integration of high-resolution techniques such as Atomic Force Microscopy (AFM), X-ray Photoelectron Spectroscopy (XPS), and zeta potential analysis can provide deeper insights into nanoparticle surface chemistry and stability. Tailoring synthesis conditions to meet the requirements of specific applications—such as drug delivery, biosensing, or energy storage will help translate lab-scale success into real-world impact.

Recent advances in synthetic biology and bioprocess engineering are likely to reduce current barriers to microbial and algal nanoparticle production, complementing plant-based green synthesis. Targeted genetic engineering can enhance microbial strains to overexpress specific reductases, metal-binding peptides, or secretion pathways that increase reduction kinetics, control nucleation, and yield more uniform nanoparticles. Parallel progress in scalable bioreactor design—continuous flow reactors, membrane bioreactors, and immobilized cell systems—can mitigate batch variability, decrease cultivation time, and enable tighter control of physico-chemical parameters (pH, redox potential, metabolite feed) that govern particle size and morphology. Together, these tools could transform fungal and bacterial "nano-factories" from costly laboratory curiosities into robust, tunable platforms that offer complementary advantages to plant extracts, particularly for applications requiring narrow size distributions, tailored surface chemistry, or high-throughput production [120, 121].

7. Conclusion

This review explored studies on the green synthesis of CuO nanoparticles. The findings highlight that biological synthesis has gained considerable attention due to its rapid, environmentally friendly, cost-effective, non-toxic, and straightforward nature. Unlike physical and chemical methods, green synthesis does not require toxic reagents, high temperatures, pressure, or intensive energy input. Various biological sources—including plants, bacteria, and fungi—have successfully provided natural reducing and stabilizing agents for the fabrication of CuO nanoparticles, enabling their application across a wide spectrum of fields such as biomedicine, catalysis, and etc.

Nomenclature

Symbols	
D	Crystallite size (nm)
K	Scherer constant (dimensionless)
Greek symbols	
λ	Wavelength of the X-ray radiation (nm)
β	Full Width at Half Maximum (in radians)
θ	Bragg angle (in degrees or radians)

Abbreviation	
AFM	Atomic Force Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
FWHM	Full Width at Half Maximum
ROS	Reactive Oxygen Species
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
XPS	X-ray Photoelectron Spectroscopy
ZOI	Zone of Inhibition

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