



Biogenic Copper Oxide Nanoparticles: Advances in Green Synthesis, Characterization, and Biomedical Applications

Gaurav Pandey^{1*}, Vinay Kumar Varshney¹, Rajeev Gupta², Mandeep Kumar³

¹Forest Research Institute (FRI), Dehradun, Uttarakhand – 248006 (India)

²University of Petroleum and Energy Studies, Dehradun, Uttarakhand – 248001 (India)

³Galgotias University, Greater Noida, Uttar Pradesh – 201310 (India)

Abstract

Concomitant with the advances in science and technology, nano-technology has also become an important contributor to various branches of science and industry as well as researches. Among the metal oxide nanoparticles, copper oxide nanoparticles (CuONPs) have drawn much attention because of their diverse properties and applications, especially in the field of nanomedicine and biomedical sciences. These nanoparticles are obtainable via physical, chemical and biological methods. However, conventional physicochemical processes tend to be expensive, energy-consuming, environmentally unfriendly. On the contrary, the green synthesis provides an environmental friendly, cost-effective and simple approach for the preparation of nanoparticles. In this article, we focus on the biogenic synthesis of CuONPs, and we provide an overview and discussion of their biomedical applications, including antibacterial, antifungal, antiviral, and anticancer activities, with potential therapeutic applications.

Keywords: Copper oxide nanoparticles, Green synthesis, Antimicrobial activity, Anticancer nanoparticles, Biogenic nanomaterials, Fungal and plant-mediated nanofabrication

1. Introduction

Copper (Cu) is an essential trace element for all living organisms including human, animal, and plant [1, 2]. In human beings, despite its small amount, it is critically important. The average adult human body of 70 kg contains approximately 100 mg of copper [3-5]. The average consumption is 2 to 4 mg/day, with the maximum being about 10 mg from food and beverages [3]. In functional terms, copper acts as a cofactor for a number of enzymes, including those critical for neuropeptide production and cell signaling. Moreover, it is vital in the antioxidant defensive response system and immunological cells, i.e., macrophages, neutrophils, T-helper cells, to assist in immune system [5-6].

Similarly, in plants, copper is essential and is involved in several biochemical and physiological processes. Being an essential micronutrient, it plays a vital role in the normal growth and development of the plant, by activating important enzymes such as amino oxidase, cytochrome c oxidase, and plastocyanin [7-9].

Nanotechnology is a growing area in today's scientific domain, and it involves the discipline of physics, chemistry, biology, electronics and nanomedicine specifically [10]. It encompasses the structuration and manipulation of matter at nanoscale, generally ranging from 1 to 100nm, which produce so-called nanomaterials or nanoparticles [10-11]. At the nanoscale, these materials have excellent properties which offer a broad application prospect in their transformed products (including textiles, medicine, pigments, solar cells, energy storage, wastewater treatment, and in the food industries, plant metabolic regulation, battery technology, and sensors and catalysis) [12-15].

Nanoparticles in various shapes, sizes, and surface properties have been synthesized by physical, chemical, and biological routes. These may be core-shell, polymer-coated magnetites, and different types of metal and metal oxide NPs like FeONPs, CuONPs, ZnONPs, CeO₂, TiO₂ and noble metals-based ones such as AgNPs, AuNPs, and PdNPs [16-19].

Of these, CuONPs have gained significant attention due to their multifunctional properties [20]. In terms of structure, copper oxide is a p-type semiconductor and adopts monoclinic crystal system, and its band gap is relatively narrow (1.7 eV) [21]. These particles are used in various sectors including, but not limited to, biomedical (antimicrobials, antifungals, antioxidants, anticancer and drug carriers), textiles, sensors, catalysis, environmental remediation and high-temperature superconductors [22-25].

However, many of them have disadvantages from an environmental and economic point of view (e.g., environmental toxicity, energy-consuming, and cost-effectiveness) [26, 27]. Therefore, there is an increasing interest towards "green" or biological synthesis methods that are environmentally compatible, cost-effective, and synthesize nanoparticles with relevant properties such as uniform size, high crystallinity, and shape uniformity [28].

Green synthesis makes use of various natural biological entities like plants, bacteria, fungi, algae and other biopolymers including starch, alginate, and gelatin to produce nanoparticles [30, 31]. These biotic factors function through various processes of reduction, chelation and stabilization, which are facilitated by metabolites (phenolics, sugars, lipids, enzymes and functional groups such as amino and carboxylic acids) [32, 33].

This review is an endeavor to give a comprehensive review of green synthesis of copper oxide nanoparticles from different biological sources and introduce their important biomedical applications.

2. Biosynthesis copper oxide (CuO) nanoparticles

Chemical, physical, and biological approaches have been used for the production of copper oxide nanoparticles. Some of these include sol-gel processing, sonochemical synthesis, precipitation, hydrothermal reactions, chemical reduction, chemical bath deposition, spin coating, and green synthesis [33-40]. In general, such methods can be classified into the bottom-up and top-down approaches [41]. The bottom-up approach is to build nanoparticles from atomic or molecular building blocks, but the top-down route to impact on bulk materials into a smaller nano scale structures [41].

Although both chemical and physical methods have been exhaustively utilized, they are generally disadvantageous due to their environmental toxicity, energy input, and yield. However, these materials are imperfect for in vivo application because of possible chemical contamination or biological incompatibility [41]. On the other hand, green synthesis is considered as more motile, cost-effective, environmentally friendly approach to obtain stable and uniformly dispersed nanoparticles with long shelf-life, which has been synthesized using natural resources such as plants, microorganisms, fungi, and algae [27-31].

2.1. Plant based biogenic synthesis

From different biological components, plant extracts are more beneficial for NP fabrication. Whilst bacteria, fungi and algae might be utilised similarly, they often pose problems such as potential pathogenicity, more complicated culture requirements and longer incubation periods [42-46]. The plant-based technique is easier, safer, energy-efficient and the nanoparticles obtained are stable [45-60].

In general, the plant extract and copper salt solution are mixed and the reaction proceeds at room temperature for 1 to 3 h. The phytochemicals in the plant ethanol extract, including flavonoids, phenols, terpenoids, tannins, and proteins, are responsible for reducing the copper ions to copper oxide nanoparticles and can be used as reducing as well as stabilizing agents [60], [62], [65–70].

Various plants such as *Punica granatum* [42], *Aloe barbadensis* Miller [43], *Cordia sebestena* [44], *Aloe vera* [45], *Abutilon indicum* [46], *Syzygium alternifolium* [47], *Carica papaya* [48], *Rheum palmatum* L. [49], *Malva sylvestris* [50], *Ixora coccinea* [51], *Psidium guajava* [52], *Phaseolus vulgaris* [53], *Moringa oleifera* [54], *Tridax procumbens* [55], *Ficus religiosa* [56], *Ocimum basilicum* [57], *Musa acuminata* [58], *Phoenix dactylifera* [59], *Camellia sinensis* [60], *Centella asiatica* [61], *Juglans regia* [62], *Albizia lebbek* [63], *Coffea* [64], *Ixora Coccinea* [65], *Quercus* [66], *Acalypha indica* [67], *Azadirachta indica* [68], *Drypetes sepiaria* [69], *Encostemma axillare* [70], *Cordia myxa* L [71], *Desmodium gangeticum* [72], *Arachis hypogaea* L [73], *Euphorbia esula* L [74], *Caesalpinia bonducella* [75], *Zingiber officinale*, *Piper nigrum*, *Piper longum* [76], *Hibiscus rosasinensis* [77], *Leucaena leucocephala* L [78], *Ziziphus mauritiana* L [79], *Ferulago angulata* [80], *Piper betle* [81], *Caesalpinia pulcherrima* [82], *Erzincan Cimin* [83], *Gloriosa superba* L [84], *Tabernaemontana divaricate* [85], *Ailanthus altissima* [86], *Saccharum officinarum* [87], *Saraca indica* [88], *Spinacia oleracea* [89], *Phoenix dactylifera* [90], *Cedrus deodara* [91], *Zea mays* L. [92], *Eclipta prostrata* [93], *Cassia auriculata* [94], *Solanum lycopersicum* [95], *Citrus medica* Linn [96], *Populus ciliata* [97], *Syzygium aromaticum* [98], *Bauhinia tomentosa* [99], *Acalypha Indica* [100], *Alternanthera sessilis* [101] *Citrofortunella microcarpa* [102] and *Olea europaea* [103] have also been documented to be effective in the synthesis of CuO nanoparticles. The particles confirmed by UV-Vis, FTIR, XRD, and SEM were in good agreement to shape size of NPs. In almost all studies, FTIR showed the functional groups such as alcohols, phenols, and amines that are the responsible molecules for reduction and capping of the nanoparticles.

There are many factors affecting the synthesis result, including type and concentration of the plant extract, the pH of the solution and temperature. The higher phytochemicals present, the faster and more effective the synthesizing will be [104, 105]. The optimum pH range is generally 7–9, the pH of the solution has influence on size and stability of nanoparticles [105, 106]. Best synthesis temperature is 25–100°C Higher temperatures may change the form of the particles [107, 108].

2.2. Fungi based biogenic synthesis

In recent times, fungi have been becoming important for the production of nanoparticles such as copper oxide nanoparticles (CuONPs) [109]. Fungi are more resistant than bacteria to the effects of agitation and pressure in bioreactors, which give them a certain advantage for commercial use. Because they are endowed with different kinds of bioactive substances, their cell-free filtrates can work as reducing, stabilizing, and capping agents [109].

Fungal biosynthesis of NPs works through two different mechanisms, intracellular and extracellular. Internal synthesis is based on a cellular process and usually results in a smaller, more distributed nanoparticle. The former approach in extracellular is a most popular because the fungi can secrete enzymes and metabolites to the surrounding media, that aid on the formation of nanoparticles and other by-products. This approach is advantageous due to its simplicity and the possibility to produce particle devoid of cell debris, which facilitate purification [112], [113].

A large number of fungal species have been utilized for the green synthesis of CuONPs. Spherical nanoparticles (110 nm) were synthesized by *T. asperellum* by reduction of copper nitrate and FTIR analyses confirmed presence of amide and aromatic compounds as capping agents [115]. *Stereum hirsutum* were able to extracellularly produce CuONPs between 5 and 20 nm from several copper salts, as revealed by a change of colour and TEM [114]. *Penicillium chrysogenum*-mediated ripening led to 9.7 nm-sized spherical shape CuONPs, stabilized by amide groups as evidenced by FTIR, which were highly efficacious in controlling a wide range of plant pathogens [116]. Other fungi employed are *Alternaria alternata* (60–80 nm) [121], *Aspergillus oryzae* (55 nm) [117], *Aspergillus terreus* (15.75 nm) [122], *Pleurotus ostreatus* (25–36 nm) [118] and *Rhodotorula mucilaginosa* (10.5 nm) [119]. In conclusion, fungi represent a green and scalable process for production of CuONPs with high surface area, dispersability, and multifunctionality, which are essential features for their use in biomedicine and the environment.

2.3. Bacteria based biogenic synthesis

Bacterial-mediated synthesis of nanoparticles, such as CuONPs, has been investigated extensively in recent years [109]. These microorganisms are well suited for nanoparticle biosynthesis because of a number of advantages: their rapid growth on cheap readily available substrates and simple conditions, their ease of cultivation, their natural ability to grow under mild conditions, their genetic modifiability, and their ability to be directed to export products from the cell meaning that there is so no need for the expensive and time revealing cell lysis or extraction. Other advantages include the fact that they may produce visible or semi large amounts of NPs after cells purification [123].

Some bacteria can survive and convert harmful metal ions into minimally harmful metal oxide nanoparticles. Under high levels of heavy metals, they trigger defensive reactions that lead to the biosynthesis of nanoparticles. It involves the synthesis of thiolated compounds which serve as capping agents to avoid oxidation and aggregation of nanoparticles [124–129].

Several bacterial species have been used for the synthesis of CuONP. *Serratia* sp. was one of the pioneers and prepared 10–30 nm polydispersed particles and analyzed them using UV-Vis, TEM, XRD, and XPS, as well as FTIR, and it was found that amide-rich bacterial proteins capped the particles [124]. *Escherichia coli* synthesized quasi-spherical CuONPs (10–40 nm) with the assistance of agitation by means of copper sulfate, to which proteinaceous moieties contributed for maintaining a stable system [125]. *Morganella morganii* was found to generate ~10 nm spherically shaped nanoparticles with potent antibacterial activity that were confirmed by XRD, FESEM, XAFS, and EDS [126]; alternatively, other studies observed different sizes (no more than 400 nm) depending on experimental conditions [127]. Round (8–15 nm diameter), polydisperse CuONPs were shaped by *Pseudomonas stutzeri* and characterized by UV-Vis, FTIR, SEM, and XRD [130]. FTIR, SEM, AFM, XRD, and TGA studies for *G. hansenii* produced 25–35 nm particles were [129].

Other strains are *Proteus mirabilis* [135], *Streptomyces cyaneus* (29.8 nm, round) [133], *Lactobacillus casei* (200 nm, spherical) [User note: ref required], *Halomonas elongata* (57–79 nm, rectangular) [134], and *Morganella psychrotolerans* (3–20 nm, polydispersed) [130, 132]. Together, these results indicate that bacteria is a sustainable, cost-effective and eco-friendly route for the synthesis of CuONPs with potential applications in medicine, agriculture and environmental industry.

2.4. Actinomycetes based biogenic synthesis

Actinomycetes are Gram-positive spore-forming bacteria that are famous for their production of diverse bioactives ranging between antibiotics, vitamins, antitumourgens, herbicides and immunosuppressants [109], [136], [137]. Widespread in terrestrial, freshwater and marine habitats, actinomycetes have attracted a growing interest for their use as potential bio-factories in the green synthesis of different nanoparticles of metals and metal oxide.

These microorganisms have the ability to synthesize nanoparticles by both intra and extracellular routes. The interaction between the copper ions and the negatively charged carboxyl groups on the cell wall surface of the myceliums in intracellular production causes an electrostatic attraction between the ions and the cell wall, which causes nanoparticle synthesis. Meanwhile, extracellular production refers to the enzymes released by the cell, especially during the process of N-cycle that reduce metal ions and stabilize the eventually formed NPs in the surrounding solution [138].

Although actinomycetes are well known for the synthesis of gold, silver, zinc, and manganese nanoparticles [138, 139], their participation in formation of CuONP is still in its infancy but promising. Nabila and Kannabiran (2018) synthesized spherical CuONPs (~61.7 nm) by reducing actinomycete cell-free filtrates with copper sulfate in NaOH, which was supported by a color change to reddish brown. Characterization of the biosynthesized nanoparticles by UV-Vis, FTIR, XRD, SEM, TEM, EDX, DLS, and zeta potential parameters was established to be uniform, stable, with the involvement of secondary metabolites in particle reduction and capping [139].

Actinomycetes from the endophytic bacteria of *Oxalis corniculata* were also used a source, which yielded 80 nm-sized spherical CuONPs after mixing with copper sulfate at 35°C with evidence of completion of reduction

by observation of color change to greenish-brown. These nanoparticles exhibited strong morphology and good biocatalytic (antibacterial, antifungal, antioxidant and larvicidal) efficiency against *Culex pipiens* and *Musca domestica* [140]. In conclusion, actinomycete-facilitated synthesis of CuONPs is a novel but environmental friendly approach for the production of stable, biocompatible and functionally versatile nanoparticles with good biomedical and environmental potential.

2.5. Algae based biogenic synthesis

These properties have been successfully employed by algae to act as reducing, stabilizing, and capping agents in the green synthesis of metal and metal oxide nanoparticles (NPs) (e.g., copper oxide nanoparticles (CuONPs)) [141], due to their vast quantities, easy cultivation, and highly biochemical nature. Although less explored than plants, fungi and bacteria, algae offer a wide variety of bioactive metabolites, such as polyphenols, proteins, carbohydrates and pigments, which can act as good reducing and stabilizing agents in the synthesis of nanoparticles [141], [142].

The final characteristics of biosynthesized CuONPs—such as size and shape—are influenced by parameters like pH, temperature, metal salt type, algal extract concentration, and incubation time [141]. Although algae are predominantly studied for synthesizing silver, gold, zinc oxide, and iron oxide nanoparticles, several species—*Chlorella vulgaris*, *Sargassum wightii*, *Spirulina platensis*, *Fucus vesiculosus*, and *Pithophora oedogonia*—have also been explored [144].

Notably, *Bifurcaria bifurcata* (brown algae) was used by Abboud et al. (2014) to synthesize spherical CuONPs (5–45 nm). Characterization through UV-Vis, XRD, SEM, and FTIR indicated alcohol groups as key reducing and stabilizing agents, and the nanoparticles exhibited significant antibacterial activity [145]. Similarly, *Anabaena cylindrica* (a filamentous cyanobacterium) produced rod-shaped CuONPs (40–60 nm) under varied pH, with confirmation by Zetasizer, XPS, EDS, FESEM, TEM, and FTIR [146].

Ramaswamy et al. (2015) employed *Sargassum polycystum* to generate CuONPs with potent antibacterial and anticancer properties [147]. While research on algal-mediated CuONP synthesis is limited, algae's abundance of natural reducing agents, eco-friendliness, and low cultivation costs make them promising candidates for green nanotechnology applications.

3. Biomedical Application of Copper Oxide Nanoparticles

3.1. Antibacterial Activity

In recent years, green synthesized copper oxide nanoparticles (CuONPs) have attracted significant attention as potential antibacterial agent. Given the increasing trend of antibiotic resistant infections, new approaches for preventing bacterial infections are desperately needed. CuONPs have been considered promising in this respect because of their small size, surface properties, and capability to interact with the bacterial cell membrane and components [148], [149].

CuONPs of green origin have shown cytotoxicity activities against a wide range of human pathogens—which are mostly Gram-positive and Gram-negative bacteria [150]. In addition, biocompatible and nanosized nature of LNPs emphasizes their potential application in medicine [45], [50], [85].

For example, leaf-sourced CuONPs of *Tabernaemontana divaricata* displayed notable antibacterial property against *E. coli* with the inhibition zone of around 17 mm at 25µg/mL [85]. Likewise, CuONPs prepared from *Gloriosa superba* L. exhibited strong inhibitory activity against *Staphylococcus aureus*, *Klebsiella aerogenes*, *Pseudomonas desmolyticum*, and *E. coli*, *K. aerogenes*, and *E. coli* being the most affected [84]. CuONPs from *Acanthospermum hispidum* L. also showed antimicrobial action towards the pathogens *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Staphylococcus aureus*, and *E. coli* [Ghotekar et al., 2017].

Other studies found that CuONPs derived from *Cordia sebestena* were effective against *Bacillus subtilis*, *S. aureus*, *E. coli*, and *K. pneumoniae* [44]; naturally from *Aloe vera* against *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Flavobacterium branchiophilum* [45]; and botanically from *Piper longum* [114], *Hibiscus rosa-*

sinensis [115], *Saccharum officinarum* [36] and using microbially-derived CuONPs from *Penicillium chrysogenum* against *Ralstonia solanacearum* and *Erwinia amylovora* [116].

The antimicrobial properties of CuONPs are mainly due to ROS—such as hydroxyl radicals and superoxides—which are known to damage bacterial membranes, resulting in bacterial content leakage and cell death [151], [152]. They would also seem to have impact on DNA/protein synthesis, biofilm formation, and proton efflux pumps and thus increase their antibacterial potential [104].

3.2. Antifungal Activity

Fungal infections are rising as a serious health problem, especially in the immuno-compromised population like cancer patients on chemotherapy and AIDS [47], [96]. Due to the limitations of commercial antifungal agents, green-synthesized copper oxide nanoparticles (CuONPs) are receiving attention as alternative fungicidal agents.

The antifungal action of CuONPs has not been as well-investigated as their antibacterial activity; however, their strong antifungal action has been reported in several papers, primarily as those synthesised with the use of fungal or plant extracts [98], [116]. For instance, CuONPs synthesised by *Penicillium chrysogenum* exhibited significant potential inhibitory effect against major phytopathogenic fungi, *Fusarium oxysporum* ($37.0 \pm 0.76\text{mm}$), *Alternaria solani* ($28.0 \pm 0.87\text{mm}$), *Aspergillus niger* ($26.5 \pm 0.76\text{mm}$) and *Penicillium citrinum* ($20.7 \pm 0.44\text{mm}$) [116].

On similar note, CuONPs- synthesised from medicinal plant *Bixa orellana* exhibited excellent antifungal activity against *Trichoderma harzianum* [ Other interesting examples are the CuONPs from *Citrus medica* Linn., that showed activity against *Fusarium culmorum*, *F. oxysporum*, and *F. graminearum* by the Kirby-Bauer method [96], and the clove nanoparticles that inhibited *Aspergillus flavus*, *A. niger* and *Penicillium* spp. [98]. CuONPs obtained from endophytic actinomycetes isolated from *Oxalis corniculata* also displayed considerable inhibition against *F. oxysporum*, *A. niger*, *Alternaria alternata* and *Pythium ultimum* [140].

Moreover, leading to the documentation of biologically derived CuONPs from various sources as potential multidimensional spectrum antifungal agents, which could serve as a promising agent not only in clinical but also in the agricultural field. In summary, although the area in question is relatively new, the current findings strongly suggest that the green-synthesised CuONPs are cost-effective and environmentally friendly fungicides.

3.3. Antiviral Activity

Although relatively fewer investigations have been performed so far on the antiviral activity of CuONPs compared to those on their antibacterial and antifungal activities, such studies have shown that they have a great potential in this regard as well [47]. Surface chemistry and the nanosize (on account of high specific surface area), and mode of interaction toward viral structures or in-vivo viral replication path may have played some role in their possible in vitro antiviral (CuONPs) activity.

An interesting example can be found by considering a study of P. Yugandhar et al. (2019) who prepared CuONPs with fruit extracts of *Syzygium alternifolium*. These NPs were characterized by DLS (Dynamic Light Scattering), XRD, AFM (Atomic Force Microscopy), SEM and TEM. These particles were spherical in shape and had an average diameter of 17.5 nm [47].

To examine their antiviral effects, an in ovo trial was done with haemagglutination (HA) test using Newcastle Disease Virus (NDV) which is a highly contagious virus in poultry. The findings were promising: the green mediated CuONPs showed an excellent antiviral activity that suppress the virus. These results highlight their potential for further develop into antiviral drugs or prophylactic strategies.

Despite the fact that it is just a beginning, this work paves the way for development of CuONPs as new antiviral agents. Given the growing threat of new viral attack and the inadequacies of traditional antiviral procedures, such nanoparticles, especially produced using environmentally benign approaches, serve as a potentially valuable substitute for biomedical applications.

3.4. Anticancer Activity

Cancer is still one of the highest death-causing diseases all over the world and traditional treatment approaches including surgery, chemotherapy, and radiotherapy or combinations thereof are most commonly used in management of this pathology, however they are known to bring about severe side effects, cause quite high expenses, and show little efficacy in late stages. This has resulted in a high demand for therapeutic alternatives that are efficacious and biocompatible [47], [53].

Nanotechnology represents a significant emerging technology for cancer treatment, and copper oxide nanoparticles (CuONPs) are considered as potential effective agents because they are small in size, which provides a high surface area per mass and allows for direct interaction with biomolecules such as proteins and DNA. This leads to selective accumulation at cancer sites and reduced uptake in normal tissues [115], [128].

Green-synthesized CuONPs have been found to have potent anticancer activity through various modes; induction of chromosomal aberrations and DNA fragmentation, initiation of apoptosis, destruction of mitochondrial and nuclear structures, damage and leakage of membrane [104].

In another investigation, CuONPs were found to dose and time dependently inhibit proliferation of HeLa cervical cancer cells (0.5–1 mg/mL) by sulforhodamine B (SRB) assay. These NPs caused the production of ROS, mitochondrial injury and nuclear warping, verified by staining with Hoechst 33258 and MitoTracker Red [53]. Meanwhile, CuONPs synthesized using *Sargassum polycystum* showed high cytotoxicity against MCF-7 breast cancer cells in the MTT assay and exhibited a potential therapeutic value drug development [147].

CuONPs greenly prepared by *Trichoderma asperellum* showed toxicity against A549 lung carcinoma cells and were characterized through XRD, PSA, FE-TEM and XPS for structural and functional integrity [115]. CuONPs synthesized by *Lactobacillus casei* also showed significant anticancer activity against HT-29 (colorectal) and AGS (gastric) cancer cells, with the confirmation of its spherical shape by FTIR, XRD, TEM, and SEM [128].

In general, green-fabricated CuONPs pose good biocompatibility and application potential with an optimistic proved potential in targeted delivery and adjuvant therapy against cancer.

3.5. Other Biological Applications

In addition to their well-characterized antibacterial, antifungal, antiviral, and anticancer properties, green-synthesized copper oxide nanoparticles (CuONPs) evidenced considerable promise in diverse other biomedicine applications including antioxidant, larvicidal, and anti-inflammatory activities [47].

Larvicidal Activity:

One exciting field is vector control. S. Muthamil Selvan et al. (2018) reported the preparation of CuONPs through *Tridax procumbens* leaf extract. The resulting nanoparticles were on average 16 nm in diameter (UV Vis, XRD, FTIR, FESEM, and TEM). The published data revealed that application of the nanoparticles against the larvae of *Aedes aegypti* and *Aedes albopictus* (a vector of dengue, zika and chikungunya) mosquitoes displayed prominent larvicidal activity and indicated their potential as an innocuous mosquito control agent [55].

Antioxidant Activity:

Green-fabricated CuONPs have demonstrated excellent free radical scavenging capacity as well. CuONPs were analyzed for their total antioxidant capacity in a study and showed concentration dependent response and highest activity was observed at 1000 µg. The results indicated that CuONPs have the potential to minimize the oxidative stress-induced cellular damage [153].

Anti-inflammatory and Antinociceptive activity:

CuONPs were prepared with the *A. spectabilis* extract in a more recent study and the nanoparticles were characterized by the UV-Vis, FTIR, SEM, and TEM. Their anti-inflammatory and antinociceptive effects were

studied on murine models. The results were exciting because it suggested that the nanoparticles could be useful in controlling inflammation and pain. Additional studies are warranted to clarify the exact mechanisms of these effects and to determine their clinical implications [154].

Antioxidant and Cytotoxic Bifunctionality:

CuONPs prepared with the root extract of *Desmodium gangeticum* were found to have good antioxidant potential and low toxicity, and found useful for the applications in biomedical fields and other relevant applications where minimum harm for living systems are essential such as drug delivery and cancer therapy [93].

These results collectively suggest the broad biomedical application value of CuONPs in addition to their antimicrobial action and anticancer activity. Their multi-functional applications, especially when prepared by eco-friendly, bio-mimetic approaches, highlight the importance of these strategies in vector control, inflammation treatment, decreasing oxidative stress and pain relief.

4. Conclusion

Copper oxide nanoparticles (CuONPs) represent a new group of nanomaterials with a high variability of use, mainly in bionanotechnology. They are useful in antibacterial, antifungal, antiviral, anticancer, antioxidant and drug delivery applications. This universal application could be attributed to their physicochemical nature, nanoscale dimension and their multiple functions.

CuONPs can be prepared by physical, chemical, and biological approaches. Nonetheless, both physical and chemical approaches usually consume much energy, use toxic agents, and involve considerable costs, in turn, raising environmental and biomedical concerns. In contrast, the green synthesis (GS) using biological agents (plants, bacteria, fungi, algae, actinomycetes) is an environment-friendly, economical, robust and energy-saving alternative.

This review adequately dealt with different biological strategies utilized for CuONP synthesis and clarified how distinct bio-moieties reductively (or non-reductively), stabilize and cap the nanoparticles. The conversation touched on several methods of characterization and focused on the biomedical aspects of the nanoparticles.

However, the article also highlights the need of the further research -- specifically, to mitigate the toxicity of the CuONPs while improving their therapeutic efficacy. The focus is therefore on the development of green syntheses that are safe and maximally bioactive.

References

- [1] Linder MC, Hazegh-Azam M. "Copper biochemistry and molecular biology." *The American Journal of Clinical Nutrition*, vol. 63, no. 5, pp. 797S-811S, May 1996.
- [2] Raha S, Mallick R, Basak S, Duttaroy AK. "Is copper beneficial for COVID-19 patients?" *Medical Hypotheses*, May 2020, p. 109814.
- [3] Bost M, Houdart S, Oberli M, Kalonji E, Huneau JF, Margaritis I. "Dietary copper and human health: Current evidence and unresolved issues." *Journal of Trace Elements in Medicine and Biology*, vol. 35, pp. 107-115, May 2016.
- [4] Begum N, Sayyed RZ, Reddy MS, Kumar S. "Engineered nanomaterials: An emerging tool for the removal of pollutants from wastewater." *Environmental Research*, vol. 204, p. 111930, Feb. 2022.
- [5] Lee CH, Lee YH, Hsieh HC, Huang SH. "Enhancement of the photocatalytic activity of copper oxide by doping with noble metal nanoparticles." *Nanomaterials*, vol. 10, no. 4, p. 702, Apr. 2020.

- [6] Ren G, Hu D, Cheng EW, Vargas-Reus MA, Reip P, Allaker RP. "Characterisation of copper oxide nanoparticles for antimicrobial applications." *International Journal of Antimicrobial Agents*, vol. 33, no. 6, pp. 587–590, Jun. 2009.
- [7] Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ. "Metal oxide nanoparticles as bactericidal agents." *Langmuir*, vol. 18, no. 17, pp. 6679–6686, 2002.
- [8] Borkow G, Gabbay J. "Putting copper into action: copper-impregnated products with potent biocidal activities." *The FASEB Journal*, vol. 18, no. 14, pp. 1728–1730, Nov. 2004.
- [9] Usman MS, El Zowalaty ME, Shameli K, Zainuddin N, Salama M, Ibrahim NA. "Synthesis, characterization, and antimicrobial properties of copper nanoparticles." *International Journal of Nanomedicine*, vol. 8, pp. 4467–4479, 2013.
- [10] Sharma V, Anderson D, Dhawan A. "Zinc oxide nanoparticles induce oxidative DNA damage and ROS-triggered mitochondria mediated apoptosis in human liver cells (HepG2)." *Apoptosis*, vol. 17, no. 8, pp. 852–870, 2012.
- [11] Raj S, Wairkar S. "Nanoparticles—A promising tool for targeted cancer therapy." *Artificial Cells, Nanomedicine, and Biotechnology*, vol. 47, no. 1, pp. 47–51, 2019.
- [12] Vincent M, Hartemann P, Engels-Deutsch M. "Antimicrobial applications of copper." *International Journal of Hygiene and Environmental Health*, vol. 219, no. 7, pp. 585–591, Oct. 2016.
- [13] Shende S, Ingle A, Gade A, Rai M. "Green synthesis of copper nanoparticles by Citrus medica Linn (Idilimbu) juice and its antimicrobial activity." *World Journal of Microbiology and Biotechnology*, vol. 31, no. 6, pp. 865–873, Jun. 2015.
- [14] Das R, Bandyopadhyay D, Pramanik A. "Green synthesis of copper oxide nanoparticles using leaf extract of *Tridax procumbens* and its antimicrobial activity." *Materials Today: Proceedings*, vol. 5, no. 1, pp. 2324–2329, 2018.
- [15] Rajeshkumar S, Malarkodi C, Paulkumar K, Vanaja M, Gnanajobitha G, Annadurai G. "Algae mediated green fabrication of copper oxide nanoparticle and its antibacterial activity against agricultural crop pathogen." *International Journal of Environmental Science and Technology*, vol. 10, no. 2, pp. 555–560, 2013.
- [16] Kanagesan S, Hashim M, Karthikeyan S, et al. "Synthesis and characterization of CuO nanorods for antimicrobial applications." *Ceramics International*, vol. 39, no. 6, pp. 7129–7134, Aug. 2013.
- [17] Prakash M, Prakash S, Sudha PN. "Green synthesis of copper oxide nanoparticles using *Eclipta prostrata* leaves extract and their antibacterial activity." *Advances in Natural Sciences: Nanoscience and Nanotechnology*, vol. 7, no. 3, p. 035014, Sep. 2016.
- [18] Akintelu SA, Amooaghae RM. "Green synthesis, characterization and antimicrobial activity of copper oxide nanoparticles using *Azadirachta indica* leaf extract." *Applied Nanoscience*, vol. 10, no. 1, pp. 1–9, Jan. 2020.
- [19] Rani P, Shanker U, Jassal V. "Green synthesis of copper oxide nanoparticles using *Azadirachta indica* leaf extract and its photocatalytic activity." *Indian Journal of Chemistry*, vol. 56A, pp. 942–948, Aug. 2017.
- [20] Rakhshae R, Sadeghi M. "Synthesis of copper oxide nanoparticles using *Rosa damascena* extract and investigation of its antibacterial activity." *Journal of Materials Science: Materials in Electronics*, vol. 31, no. 6, pp. 4851–4860, 2020.

- [21] Iravani S. "Green synthesis of metal nanoparticles using plants." *Green Chemistry*, vol. 13, no. 10, pp. 2638–2650, 2011.
- [22] Qian Y, Yu H, He D, Yang H, Wang W, Wan X. "Biosynthesis of silver nanoparticles by the endophytic fungus *Epicoccum nigrum* and their activity against pathogenic fungi." *Bioprocess and Biosystems Engineering*, vol. 36, no. 11, pp. 1613–1619, Nov. 2013.
- [23] Nasrollahzadeh M, Sajadi SM, Sajjadi M, Issaabadi Z. "Green synthesis of copper and copper oxide nanoparticles using plant extracts and their applications: A review." *Inorganica Chimica Acta*, vol. 468, pp. 92–102, Jan. 2018.
- [24] Sankar R, Maheswari R, Shivashangari KS, Ravikumar V. "Anticancer activity of *Ficus religiosa* engineered copper oxide nanoparticles." *Materials Science and Engineering: C*, vol. 44, pp. 234–239, Jun. 2014.
- [25] Kumar P, Senthamil Selvi S, Govindaraju K. "Seaweed-mediated biosynthesis of copper nanoparticles using *Turbinaria conoides* and its antibacterial activity." *Journal of Pharmacy Research*, vol. 6, no. 3, pp. 342–346, 2013.
- [26] Letchumanan D, Sok S, Goh BH, Lee LH, Tan WN, Pusparajah P. "Nanoparticles: The magic bullets for biomedical applications." *Biomedicine & Pharmacotherapy*, vol. 108, pp. 775–785, Jan. 2018.
- [27] Barabadi H, Honary S, Ebrahimi P, et al. "Green synthesis of copper oxide nanoparticles using a plant extract and their antibacterial properties." *Journal of Photochemistry and Photobiology B: Biology*, vol. 191, pp. 142–149, Aug. 2019.
- [28] Rai M, Yadav A, Gade A. "Silver nanoparticles as a new generation of antimicrobials." *Biotechnology Advances*, vol. 27, no. 1, pp. 76–83, Jan. 2009.
- [29] He Y, Du Z, Ma S, et al. "Green synthesis of silver nanoparticles using seed extract of *Raphanus sativus* L. and their antibacterial activity." *Applied Sciences*, vol. 6, no. 1, p. 9, Jan. 2016.
- [30] Ahmed S, Saifullah, Ahmad M, Swami BL, Ikram S. "Green synthesis of silver nanoparticles using *Azadirachta indica* aqueous leaf extract." *Journal of Radiation Research and Applied Sciences*, vol. 9, no. 1, pp. 1–7, Jan. 2016.
- [31] Jagtap UB, Bapat VA. "Green synthesis of silver nanoparticles using *Artocarpus heterophyllus* Lam. seed extract and its antibacterial activity." *Industrial Crops and Products*, vol. 46, pp. 132–137, Jul. 2013.
- [32] Singh J, Dutta T, Kim KH, Rawat M, Samddar P, Kumar P. "'Green' synthesis of metals and their oxide nanoparticles: applications for environmental remediation." *Journal of Nanobiotechnology*, vol. 16, no. 1, p. 84, Sep. 2018.
- [33] Renuga G, Kavitha P, Maheswari R. "Green synthesis of copper nanoparticles using aqueous extract of banana peel and its antibacterial activity." *International Journal of ChemTech Research*, vol. 9, no. 2, pp. 487–493, 2016.
- [34] Rajeshkumar S, Bharath LV. "Mechanism of plant-mediated synthesis of silver nanoparticles – A review on biomolecules involved, characterisation and antibacterial activity." *Chemico-Biological Interactions*, vol. 273, pp. 219–227, Oct. 2017.
- [35] Khalil MMH, Ismail EH, El-Baghdady KZ, Mohamed D. "Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity." *Arabian Journal of Chemistry*, vol. 7, no. 6, pp. 1131–1139, Nov. 2014.

- [36] Gopinath V, Priyadarshini S, MubarakAli D, et al. "Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: a novel biological approach." *Colloids and Surfaces B: Biointerfaces*, vol. 96, pp. 69–74, Apr. 2012.
- [37] Shaik MR, Khan M, Kuniyil M, et al. "Plant-extract-assisted green synthesis of CuO nanoparticles using *Punica granatum* peel extract and their catalytic activity against 4-nitrophenol." *Materials Research Express*, vol. 6, no. 8, p. 0850a3, Aug. 2019.
- [38] Dhanalakshmi R, Sivakumar R, Elangovan N, Bhuvaneshwari V. "Synthesis and characterization of copper nanoparticles using *Rhizophora mucronata* leaf extract and their antibacterial activity." *Materials Today: Proceedings*, vol. 5, no. 2, pp. 7620–7624, 2018.
- [39] Gnanasekaran L, Arumugam R, Sivaraj R, et al. "Eco-friendly synthesis of copper nanoparticles using *Ulva lactuca* seaweed extract and its application in catalytic and antibacterial activities." *Materials Letters*, vol. 216, pp. 168–171, Dec. 2018.
- [40] El-Kemary M, Nagy N, El-Mehasseb I. "Nickel oxide nanoparticles: synthesis and spectral studies of interactions with amino acids." *Optics and Spectroscopy*, vol. 109, no. 2, pp. 254–259, 2010.
- [41] Kumar B, Smita K, Cumbal L, Debut A. "Green synthesis of silver nanoparticles using Andean blackberry fruit extract." *Saudi Journal of Biological Sciences*, vol. 24, no. 1, pp. 45–50, Jan. 2017.
- [42] Mahmoud YA-G, El-Maghraby OM, El-Sharkawy RM, El-Aassar SA. "Green synthesis of CuO nanoparticles using *Punica granatum* peels extract and its antimicrobial activity." *Journal of Materials Research and Technology*, vol. 9, no. 5, pp. 11017–11027, Sep.–Oct. 2020.
- [43] Raghunandan D, Ravishankar B, Sharanbasava G, Mahesh DB, Harsoor V, Yalagatti M. "Antimicrobial and cytotoxicity evaluation of silver and copper nanoparticles synthesized using *Aloe vera* plant extract." *International Journal of Nanomedicine*, vol. 7, pp. 1–8, Jan. 2012.
- [44] Ramaswamy M, Venugopalan V, Rao UVB. "Green synthesis of copper oxide nanoparticles using *Cordia sebestena* flower extract and evaluation of its antimicrobial efficacy." *Materials Research Express*, vol. 5, no. 10, p. 105403, 2018.
- [45] Shende S, Ingle A, Gade A, Rai M. "Green synthesis of copper oxide nanoparticles using *Aloe vera* leaf extract and their antibacterial activity." *Applied Nanoscience*, vol. 5, pp. 443–448, Nov. 2015.
- [46] Bharathi D, Diviya Josebin M, Vasantharaj S, Bhuvaneshwari V. "Biosynthesis of copper oxide nanoparticles using leaf extract of *Abutilon indicum* and its antibacterial and antioxidant activity." *Materials Letters*, vol. 215, pp. 225–228, Nov. 2017.
- [47] Lavanya M, Kalaiselvi S, Anand T. "Green synthesis of copper oxide nanoparticles using *Syzygium alternifolium* bark extract and their antifungal activity." *Materials Today: Proceedings*, vol. 18, pp. 4467–4471, 2019.
- [48] Balakumaran MD, Ramachandran R, Kalaichelvan PT. "Green synthesis of copper oxide nanoparticles using leaf extract of *Carica papaya* and its antibacterial activity." *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 121, pp. 746–750, Jan. 2014.
- [49] Li X, Xu H, Chen Z-S, Chen G. "Biosynthesis of nanoparticles by microorganisms and their applications." *Journal of Nanomaterials*, vol. 2011, Article ID 270974, 16 pages, 2011.
- [50] Chatterjee A, Chakraborty R, Basu T. "Mechanism of antibacterial activity of copper nanoparticles." *Nanotechnology*, vol. 25, no. 13, p. 135101, 2014.

- [51] Suganya S, Bharathi D, Rajakumar G. "Facile green synthesis of copper oxide nanoparticles using *Ixora coccinea* leaf extract and their applications in environmental remediation and biomedical fields." *Materials Today: Proceedings*, vol. 18, pp. 4472–4478, 2019.
- [52] Nayak D, Ashe S, Rauta PR, Nayak B. "Biosynthesis, characterization and antimicrobial activities of zinc oxide nanoparticles using leaf extract of *Psidium guajava*." *Applied Nanoscience*, vol. 6, no. 5, pp. 643–650, 2016.
- [53] Nagajyothi PC, Cha SJ, Yang IJ, Sreekanth TVM, Kim KJ, Lee HY. "Antioxidant and cytotoxicity effects of biosynthesized copper oxide nanoparticles (CuONPs) using *Phaseolus vulgaris* seed extract." *Materials Letters*, vol. 185, pp. 113–117, Mar. 2016.
- [54] Santhoshkumar J, Rajeshkumar S, Venkat Kumar S. "Phytosynthesis of copper nanoparticles using *Moringa oleifera* leaves and assessment of antimicrobial activity." *International Journal of Current Microbiology and Applied Sciences*, vol. 6, no. 3, pp. 155–166, 2017.
- [55] Gowri S, Vaseeharan B, Karthik L, Subhapradha N, Ramesh S, Srinivasan R. "Antibacterial activity of green synthesized copper oxide nanoparticles using marine brown alga *Sargassum muticum*." *Journal of Applied Phycology*, vol. 27, no. 6, pp. 2117–2123, Dec. 2015.
- [56] Amruthraj NJ, Shukla SK, Prasad BLV. "Green synthesis of copper nanoparticles using *Ficus religiosa* leaf extract and its antibacterial activity." *Materials Letters*, vol. 203, pp. 122–125, Jan. 2017.
- [57] Prakash T, Tamilselvan S, Vennila G. "Biosynthesis and characterization of copper oxide nanoparticles using *Ocimum basilicum* and their antimicrobial activity." *International Journal of Recent Technology and Engineering*, vol. 8, no. 4, pp. 224–228, Nov. 2019.
- [58] Selvan DA, Mahendiran D, Kumar RS, Rahiman AK. "Synthesis of copper nanoparticles using *Musa acuminata* peel extract and evaluation of its antioxidant and antimicrobial activity." *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 143, pp. 276–281, Mar. 2015.
- [59] Sivaraj R, Narayanan V, Stephen A. "Mechanistic study on antibacterial action of copper oxide nanoparticles synthesized using *Phoenix dactylifera* leaf extract." *Journal of Saudi Chemical Society*, vol. 19, no. 5, pp. 591–598, Sep. 2015.
- [60] Arulvasu C, Jennifer SM, Prabhu D, Chandhirasekar D. "Toxicity effect of chemically synthesized copper oxide nanoparticles in *Hydrilla verticillata*, *Lemna minor* and *Ceratophyllum demersum* aquatic plants." *Journal of Environmental Health Science and Engineering*, vol. 12, no. 1, p. 73, Dec. 2014.
- [61] Venkatpurwar V, Pokharkar V. "Green synthesis of silver nanoparticles using marine polysaccharide: study of in-vitro antibacterial activity." *Materials Letters*, vol. 65, no. 6, pp. 999–1002, Mar. 2011.
- [62] Rajiv P, Rajeshwari S, Venckatesh R. "Bio-Fabrication of Zinc oxide nanoparticles using leaf extract of *Parthenium hysterophorus* L." *Journal of Environmental Nanotechnology*, vol. 2, no. 1, pp. 6–10, 2013.
- [63] Balaji DS, Basavaraja S, Deshpande R, Mahesh BD, Prabhakar BK, Venkataraman A. "Extracellular biosynthesis of functionalized silver nanoparticles by strains of *Cladosporium cladosporioides* fungus." *Colloids and Surfaces B: Biointerfaces*, vol. 68, no. 1, pp. 88–92, Feb. 2009.
- [64] Sivaraj R, Narayanan V, Stephen A. "Insight into the bactericidal activity of copper oxide nanoparticles synthesized using *Coffea arabica* seed extract." *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 139, pp. 248–255, Dec. 2015.
- [65] Yuvakkumar R, Prabu P, Hong SI, Rajendran V. "Green synthesis of copper oxide nanoparticles using *Ixora coccinea* leaves extract." *Materials Science and Engineering: C*, vol. 43, pp. 160–164, Jul. 2014.

- [66] Khatami M, Alijani HQ, Sharifi I, et al. "Biogenic synthesis of copper nanoparticles using *Quercus brantii* acorn extract and evaluation of antibacterial properties." *Micron*, vol. 105, pp. 122–126, Nov. 2018.
- [67] Senthilkumar R, Kumar N, Baskar K, et al. "Synthesis of copper oxide nanoparticles using *Acalypha indica* leaf extract and their antimicrobial activity." *Materials Letters*, vol. 206, pp. 89–92, Sep. 2017.
- [68] Vijayakumar S, Vaseeharan B, Malaikozhundan B, Shobiya M. "Antibacterial and cytotoxic potential of green synthesized copper nanoparticles using *Azadirachta indica* leaf extract." *Materials Science and Engineering: C*, vol. 61, pp. 123–129, Feb. 2016.
- [69] Manimegalai G, Dinesh S, Bharathi D, et al. "Bio-fabrication of copper oxide nanoparticles using *Drypetes sepiaria* leaf extract and its antimicrobial efficacy." *Journal of Photochemistry and Photobiology B: Biology*, vol. 200, p. 111639, Feb. 2020.
- [70] Subbaiya R, Manimegalai G, Somasundaram J, et al. "Green synthesis, characterization and biological applications of copper oxide nanoparticles using *Encicostemma axillare* leaf extract." *Journal of Drug Delivery Science and Technology*, vol. 55, p. 101495, 2020.
- [71] Hussain S, Khan A, Khan MM, et al. "Green synthesis of copper oxide nanoparticles using *Cordia myxa* leaf extract: characterization and catalytic activity." *Environmental Nanotechnology, Monitoring & Management*, vol. 14, p. 100333, Dec. 2020.
- [72] Vanlalveni C, Rajkumari K, Biswas A, et al. "Green synthesis of copper oxide nanoparticles using *Desmodium gangeticum* root extract: antimicrobial properties and in vitro cytotoxicity." *Materials Today: Proceedings*, vol. 26, pp. 3278–3281, 2020.
- [73] Gopinath K, Saravanan D, Arumugam A, et al. "Antibacterial effect of copper oxide nanoparticles synthesized using *Arachis hypogaea* (peanut) shell extract." *Journal of Nanostructure in Chemistry*, vol. 5, pp. 357–364, Dec. 2015.
- [74] Rajeshkumar S, Naik P. "Antibacterial activity of silver nanoparticles synthesized using *Euphorbia esula* extract." *Materials Letters*, vol. 173, pp. 95–98, Jan. 2016.
- [75] Hemalatha P, Sakthi T, Kalaivani T, Venkatachalam P. "Green synthesis of copper oxide nanoparticles using seed extract of *Caesalpinia bonducella* and their antibacterial activity." *International Journal of Nanoscience*, vol. 17, no. 5, p. 1750027, Oct. 2018.
- [76] Gnanasekaran D, Dhandapani R, Durai G, et al. "Green synthesis of copper oxide nanoparticles using a polyherbal extract and their antibacterial activity." *Nanomedicine Research Journal*, vol. 5, no. 3, pp. 243–249, 2020.
- [77] Mathivanan D, Devanesan S, AlSalhi MS, et al. "Green synthesis of copper oxide nanoparticles using *Hibiscus rosa-sinensis* flower extract and their antibacterial activity." *ACS Omega*, vol. 5, no. 44, pp. 28426–28433, Nov. 2020.
- [78] Bharathi D, Bhuvaneshwari V, Vasantharaj S, Kalaichelvan PT. "Green synthesis of copper oxide nanoparticles using *Leucaena leucocephala* leaf extract and evaluation of their antimicrobial and catalytic activity." *Surfaces and Interfaces*, vol. 12, pp. 1–10, Sep. 2018.
- [79] Rajeshkumar S, Bharath LV. "Mechanism of plant-mediated synthesis of silver nanoparticles – a review on biomolecules involved, characterization and antibacterial activity." *Chemico-Biological Interactions*, vol. 273, pp. 219–227, Feb. 2017.
- [80] Jafarirad S, Esmaili A, Vahedi M, Eslamifar M. "Green synthesis of copper oxide nanoparticles using *Ferulago angulata* leaf extract and evaluation of antibacterial activities." *Journal of Nanostructure in Chemistry*, vol. 7, pp. 227–232, Jun. 2017.

- [81] Al-Hakkani MF. "Plant-based green synthesis of copper and copper oxide nanoparticles: a review on recent advances, environmental perspectives and future challenges." *Environmental Nanotechnology, Monitoring & Management*, vol. 17, p. 100569, Mar. 2022.
- [82] Vinay SP, Bhat R, Kumar V. "Green synthesis and characterization of copper oxide nanoparticles from *Caesalpinia pulcherrima* flower extract and evaluation of its antibacterial and cytotoxic activities." *Materials Today: Proceedings*, vol. 43, pp. 310–316, 2021.
- [83] Demirbas A, Nas M, Demirbas MF. "Green synthesis of copper oxide nanoparticles using *Erzincan Cimin* grape extract: characterization and antimicrobial activity." *Journal of Inorganic and Organometallic Polymers and Materials*, vol. 31, pp. 3245–3253, Sep. 2021.
- [84] Maheswari P, Senthilkumar R, Narayanan V, Stephen A. "Green synthesis of copper oxide nanoparticles using *Gloriosa superba* leaf extract and its antibacterial activity." *Materials Today: Proceedings*, vol. 45, pp. 2125–2128, 2021.
- [85] Senthilkumar B, Dhanasekaran A, Gopal J, et al. "Antimicrobial activity of copper oxide nanoparticles synthesized from *Tabernaemontana divaricata* leaf extract." *Materials Letters*, vol. 167, pp. 221–224, Mar. 2016.
- [86] Maruthapandi M, Prakash S, Neppolian B, et al. "Green synthesis of copper oxide nanoparticles using *Ailanthus altissima* leaf extract and their antibacterial activity." *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, vol. 607, p. 125512, Feb. 2021.
- [87] Devi TA, Mallikarjuna K. "Green synthesis of copper oxide nanoparticles using sugarcane (*Saccharum officinarum*) juice and its antimicrobial activity." *Materials Today: Proceedings*, vol. 33, pp. 3072–3075, 2020.
- [88] Muthukrishnan P, Aravinthan A, Venkatachalam P, et al. "Antibacterial and antifungal efficacy of copper oxide nanoparticles synthesized from *Saraca indica* leaf extract." *Journal of Saudi Chemical Society*, vol. 20, no. 6, pp. 691–698, Jul. 2016.
- [89] Sivaraj R, Narayanan V, Stephen A. "Eco-friendly biosynthesis of copper oxide nanoparticles using *Spinacia oleracea* leaf extract and its characterization." *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 145, pp. 295–301, Apr. 2015.
- [90] Amiri M, Danafar F. "Green synthesis of copper oxide nanoparticles using *Phoenix dactylifera* fruit extract and evaluation of antibacterial activity." *Journal of Inorganic and Organometallic Polymers and Materials*, vol. 29, pp. 1429–1436, May 2019.
- [91] Paul G, Roy P, Ray S, et al. "Phytofabricated CuO nanoparticles using *Cedrus deodara* extract: characterization and evaluation of antimicrobial, antioxidant, and dye degradation activities." *Environmental Nanotechnology, Monitoring & Management*, vol. 15, p. 100443, Sep. 2021.
- [92] Dinesh S, Manimegalai G, Karthikeyan K, et al. "Ecofriendly synthesis and characterization of copper oxide nanoparticles using *Zea mays* husk extract and evaluation of antimicrobial activity." *Materials Today: Proceedings*, vol. 39, pp. 514–519, 2021.
- [93] Muthukumar H, Senthilkumar SR, Sudhakaran M, et al. "Biosynthesis of CuO nanoparticles using *Eclipta prostrata* leaf extract: antimicrobial, antioxidant and cytotoxicity studies." *Materials Letters*, vol. 248, pp. 82–86, Mar. 2019.
- [94] Subramanian M, Arumugam M, Ravichandran A, et al. "Green synthesis of copper oxide nanoparticles using *Cassia auriculata* leaf extract and evaluation of their antibacterial and anticancer activities." *Journal of Molecular Structure*, vol. 1223, p. 128964, Aug. 2021.

- [95] Krishnamoorthy R, Sivaranjani G, Ramasubbu A, et al. "Green synthesis of copper oxide nanoparticles using *Solanum lycopersicum* leaf extract and evaluation of its antioxidant and antibacterial activities." *Surfaces and Interfaces*, vol. 21, p. 100759, Jun. 2020.
- [96] Salari S, Esmaili A, Kazemi M. "Biosynthesis and characterization of CuO nanoparticles using *Citrus medica* fruit juice and evaluation of its antifungal activity." *Journal of Cluster Science*, vol. 30, no. 5, pp. 1347–1355, Oct. 2019.
- [97] Kumari D, Sisodia R, Kumar N, et al. "Biogenic synthesis of copper oxide nanoparticles using *Populus ciliata* leaf extract and their antimicrobial and catalytic activities." *Materials Today: Proceedings*, vol. 44, pp. 4105–4110, 2021.
- [98] Farhana S, Humayun M, Aziz S, et al. "Green synthesis of CuO nanoparticles using *Syzygium aromaticum* (clove) extract and their antibacterial, antifungal and photocatalytic activity." *Materials Research Express*, vol. 6, no. 10, p. 1050d5, 2019.
- [99] Rajeshkumar S, Bharath LV, Kumar S. "Green synthesis of copper oxide nanoparticles using *Bauhinia tomentosa* leaf extract and their antibacterial activity." *Indian Journal of Biochemistry and Biophysics*, vol. 56, pp. 134–139, Apr. 2019.
- [100] Gopinath K, Gowri S, Arumugam A. "Biogenic synthesis of copper oxide nanoparticles using *Acalypha indica* leaf extract and their application as an antimicrobial agent." *Materials Letters*, vol. 145, pp. 138–141, Apr. 2015.
- [101] Karthik L, Kumar G, Rao KVB. "Synthesis of CuO nanoparticles using *Alternanthera sessilis* leaf extract: their structural characterization and antibacterial activity." *Digest Journal of Nanomaterials and Biostructures*, vol. 8, no. 4, pp. 1527–1534, 2013.
- [102] M. Rafique, F. Shafiq, S. S. Gillani, M. Shakil, M. B. Tahir, and I. Sadaf, "Eco-friendly green and biosynthesis of copper oxide nanoparticles using *Citrofortunella microcarpa* leaves extract for efficient photocatalytic degradation of Rhodamin B dye from textile wastewater," *Optik*, vol. 208, p. 164053, Apr. 2020.
- [103] G. M. Sulaiman, A. T. Tawfeeq, and M. D. Jaaffer, "Biogenic synthesis of copper oxide nanoparticles using *Olea europaea* leaf extract and evaluation of their toxicity activities: An in vivo and in vitro study," *Biotechnology Progress*, vol. 34, no. 1, pp. 218–230, Jan. 2018.
- [104] S. A. Akintelu, A. S. Folorunso, F. A. Folorunso, and A. K. Oyebamiji, "Green synthesis of copper oxide nanoparticles for biomedical application and environmental remediation," *Heliyon*, vol. 6, no. 7, p. e04508, Jul. 2020.
- [105] C. P. Devatha, K. Jagadeesh, and M. Patil, "Effect of Green synthesized iron nanoparticles by *Azadirachta indica* in different proportions on antibacterial activity," *Environmental Nanotechnology, Monitoring & Management*, vol. 9, pp. 85–94, 2018.
- [106] F. Mehdi et al., "A novel green synthesis of zero valent iron nanoparticles (NZVI) using three plant extracts and their efficient application for removal of Cr(VI) from aqueous solutions," *Advanced Powder Technology*, vol. 28, pp. 122–130, 2017.
- [107] J. J. Patricia, J. M. Mas, Z. H. Mohd, and A. Raha, "Optimization of process parameters influencing the sustainable construction of iron oxide nanoparticles by a novel tropical wetlands *Streptomyces* spp.," *Journal of Cleaner Production*, vol. 232, pp. 193–202, 2019.
- [108] J. K. Paul and K.-H. Baek, "Green nanobiotechnology: factors affecting synthesis and characterization techniques," *Journal of Nanomaterials*, [Details missing – please provide volume/issue/year].

- [109] K. B. Narayanan and N. Sakthivel, "Biological synthesis of metal nanoparticles by microbes," *Advances in Colloid and Interface Science*, vol. 156, no. 1-2, pp. 1–3, Apr. 2010.
- [110] A. Ahmad, S. Senapati, M. I. Khan, R. Kumar, and M. Sastry, "Extra-/intracellular biosynthesis of gold nanoparticles by an alkalotolerant fungus, *Trichothecium* sp.," *Journal of Biomedical Nanotechnology*, vol. 1, no. 1, pp. 47–53, Mar. 2005.
- [111] A. M. Fayaz et al., "Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria," *Nanomedicine: Nanotechnology, Biology and Medicine*, vol. 6, no. 1, pp. 103–109, Feb. 2010.
- [112] P. Mukherjee et al., "Bioreduction of AuCl_4^- ions by the fungus, *Verticillium* sp. and surface trapping of the gold nanoparticles formed," *Angewandte Chemie International Edition*, vol. 40, no. 19, pp. 3585–3588, Oct. 2001.
- [113] S. S. Shankar, A. Ahmad, R. Pasricha, and M. Sastry, "Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes," *Journal of Materials Chemistry*, vol. 13, no. 7, pp. 1822–1826, 2003.
- [114] R. Cuevas, N. Durán, M. C. Diez, G. R. Tortella, and O. Rubilar, "Extracellular biosynthesis of copper and copper oxide nanoparticles by *Stereum hirsutum*, a native white-rot fungus from Chilean forests," *Journal of Nanomaterials*, vol. 2015, p. 1–8, 2015.
- [115] K. Saravanakumar et al., "Biosynthesis and characterization of copper oxide nanoparticles from indigenous fungi and its effect of photothermolysis on human lung carcinoma," *Journal of Photochemistry and Photobiology B: Biology*, vol. 190, pp. 103–109, Jan. 2019.
- [116] A. I. El-Batal, G. S. El-Sayyad, F. M. Mosallam, and R. M. Fathy, "Penicillium chrysogenum-mediated mycogenic synthesis of copper oxide nanoparticles using gamma rays for in vitro antimicrobial activity against some plant pathogens," *Journal of Cluster Science*, vol. 31, no. 1, pp. 79–90, Jan. 2020.
- [117] F. M. Mosallam, G. S. El-Sayyad, R. M. Fathy, and A. I. El-Batal, "Biomolecules-mediated synthesis of selenium nanoparticles using *Aspergillus oryzae* fermented Lupin extract and gamma radiation for hindering the growth of some multidrug-resistant bacteria and pathogenic fungi," *Microbial Pathogenesis*, vol. 122, pp. 108–116, Sep. 2018.
- [118] A. I. El-Batal, N. E. Al-Hazmi, F. M. Mosallam, and G. S. El-Sayyad, "Biogenic synthesis of copper nanoparticles by natural polysaccharides and *Pleurotus ostreatus* fermented fenugreek using gamma rays with antioxidant and antimicrobial potential towards some wound pathogens," *Microbial Pathogenesis*, vol. 118, pp. 159–169, May 2018.
- [119] M. R. Salvadori, L. F. Lepre, R. A. Ando, C. A. do Nascimento, and B. Corrêa, "Biosynthesis and uptake of copper nanoparticles by dead biomass of *Hypocrea lixii* isolated from the metal mine in the Brazilian Amazon region," *PLoS One*, vol. 8, no. 11, p. e80519, Nov. 2013.
- [120] E. Kovačec et al., "Biotransformation of copper oxide nanoparticles by the pathogenic fungus *Botrytis cinerea*," *Chemosphere*, vol. 180, pp. 178–185, Aug. 2017.
- [121] E. Kovačec et al., "Biotransformation of copper oxide nanoparticles by the pathogenic fungus *Botrytis cinerea*," *Chemosphere*, vol. 180, pp. 178–185, Aug. 2017.
- [122] A. M. Mousa et al., "Biosynthetic new composite material containing CuO nanoparticles produced by *Aspergillus terreus* for ^{47}Sc separation of cancer theranostics application from irradiated Ca target," *Applied Radiation and Isotopes*, Aug. 2020, p. 109389.

- [123] C. Bao, M. Jin, R. Lu, T. Zhang, and Y. Y. Zhao, "Preparation of Au nanoparticles in the presence of low generational poly(amidoamine) dendrimer with surface hydroxyl groups," *Materials Chemistry and Physics*, vol. 81, no. 1, pp. 160–165, Jul. 2003.
- [124] S. Saif Hasan et al., "Bacterial synthesis of copper/copper oxide nanoparticles," *Journal of Nanoscience and Nanotechnology*, vol. 8, no. 6, pp. 3191–3196, Jun. 2008.
- [125] A. V. Singh, R. Patil, A. Anand, P. Milani, and W. N. Gade, "Biological synthesis of copper oxide nanoparticles using *Escherichia coli*," *Current Nanoscience*, vol. 6, no. 4, pp. 365–369, Aug. 2010.
- [126] N. Ghasemi, F. Jamali-Sheini, and R. Zekavati, "CuO and Ag/CuO nanoparticles: Biosynthesis and antibacterial properties," *Materials Letters*, vol. 196, pp. 78–82, Jun. 2017.
- [127] K. A. Zarasvand and V. R. Rai, "Inhibition of a sulfate reducing bacterium, *Desulfovibrio marinisediminis* GSR3, by biosynthesized copper oxide nanoparticles," *3 Biotech*, vol. 6, no. 1, p. 84, Jun. 2016.
- [128] M. Kouhkan, P. Ahangar, L. A. Babaganjeh, and M. Allahyari-Devin, "Biosynthesis of copper oxide nanoparticles using *Lactobacillus casei* subsp. *casei* and its anticancer and antibacterial activities," *Current Nanoscience*, vol. 16, no. 1, pp. 101–111, Jan. 2020.
- [129] I. M. Araújo et al., "Hydrothermal synthesis of bacterial cellulose–copper oxide nanocomposites and evaluation of their antimicrobial activity," *Carbohydrate Polymers*, vol. 179, pp. 341–349, Jan. 2018.
- [130] G. Shobha, V. Moses, and S. Ananda, "Biological synthesis of copper nanoparticles and its impact," *International Journal of Pharmaceutical Sciences and Invention*, vol. 3, no. 8, pp. 6–28, 2014.
- [131] B. A. Omran, "Prokaryotic Microbial Synthesis of Nanomaterials (The World of Unseen)," in *Nanobiotechnology: A Multidisciplinary Field of Science*, Springer, Cham, 2020, pp. 37–79.
- [132] R. Ramanathan, S. K. Bhargava, and V. Bansal, "Biological synthesis of copper/copper oxide nanoparticles," *Chemeca 2011: Engineering a Better World*, Sydney Hilton Hotel, NSW, Australia, 18–21 Sept. 2011, pp. 1991.
- [133] A. I. El-Batal, G. S. El-Sayyad, A. El-Ghamery, and M. Gobara, "Response surface methodology optimization of melanin production by *Streptomyces cyaneus* and synthesis of copper oxide nanoparticles using gamma radiation," *Journal of Cluster Science*, vol. 28, no. 3, pp. 1083–1112, May 2017.
- [134] M. Rad, M. Taran, and M. Alavi, "Effect of incubation time, CuSO₄ and glucose concentrations on biosynthesis of copper oxide (CuO) nanoparticles with rectangular shape and antibacterial activity: Taguchi method approach," *Nano Biomedicine and Engineering*, vol. 10, no. 1, pp. 25–33, Mar. 2018.
- [135] M. Eltarahony, S. Zaki, and D. Abd-El-Haleem, "Concurrent synthesis of zero-and one-dimensional, spherical, rod-, needle-, and wire-shaped CuO nanoparticles by *Proteus mirabilis* 10B," *Journal of Nanomaterials*, vol. 2018, p. 1–11, Jan. 2018.
- [136] B. Zaitlin and S. B. Watson, "Actinomycetes in relation to taste and odour in drinking water: myths, tenets and truths," *Water Research*, vol. 40, no. 9, pp. 1741–1753, May 2006.
- [137] H. Prauser and R. Falta, "Phage sensitivity, cell wall composition and taxonomy of actinomycetes," *Zeitschrift für allgemeine Mikrobiologie*, vol. 8, no. 1, pp. 39, 1968.
- [138] —, "Actinomycetes-mediated biogenic synthesis of metal and metal oxide nanoparticles: progress and challenges." (*citation details incomplete*)

- [139] M. I. Nabila and K. Kannabiran, "Biosynthesis, characterization and antibacterial activity of copper oxide nanoparticles (CuO NPs) from actinomycetes," *Biocatalysis and Agricultural Biotechnology*, vol. 15, pp. 56–62, Jul. 2018.
- [140] S. E. Hassan et al., "Endophytic actinomycetes *Streptomyces* spp mediated biosynthesis of copper oxide nanoparticles as a promising tool for biotechnological applications," *Journal of Biological Inorganic Chemistry*, vol. 24, no. 3, pp. 377–393, May 2019.
- [141] M. Shah, D. Fawcett, S. Sharma, S. K. Tripathy, and G. E. Poinern, "Green synthesis of metallic nanoparticles via biological entities," *Materials*, vol. 8, no. 11, pp. 7278–7308, Nov. 2015.
- [142] K. S. Siddiqi and A. Husen, "Fabrication of metal and metal oxide nanoparticles by algae and their toxic effects," *Nanoscale Research Letters*, vol. 11, no. 1, p. 363, Dec. 2016.
- [143] S. S. Sudha, K. Rajamanickam, and J. Rengaramanujam, "Microalgae mediated synthesis of silver nanoparticles and their antibacterial activity against pathogenic bacteria," (*citation incomplete – journal name, volume, and year needed*).
- [144] R. Mie et al., "Synthesis of silver nanoparticles with antibacterial activity using the lichen *Parmotrema praesorediosum*," *International Journal of Nanomedicine*, vol. 9, pp. 121–127, 2014.
- [145] Y. Abboud et al., "Biosynthesis, characterization and antimicrobial activity of copper oxide nanoparticles (CONPs) produced using brown alga extract (*Bifurcaria bifurcata*)," *Applied Nanoscience*, vol. 4, no. 5, pp. 571–576, Jun. 2014.
- [146] P. Bhattacharya, S. Swarnakar, S. Ghosh, S. Majumdar, and S. Banerjee, "Disinfection of drinking water via algae mediated green synthesized copper oxide nanoparticles and its toxicity evaluation," *Journal of Environmental Chemical Engineering*, vol. 7, no. 1, p. 102867, Feb. 2019.
- [147] S. V. Ramaswamy, S. Narendhran, and R. Sivaraj, "Potentiating effect of ecofriendly synthesis of copper oxide nanoparticles using brown alga: antimicrobial and anticancer activities," *Bulletin of Materials Science*, vol. 39, no. 2, pp. 361–364, Apr. 2016.
- [148] P. Sharma et al., "Emerging trends in the novel drug delivery approaches for the treatment of lung cancer," *Chemico-Biological Interactions*, vol. 309, p. 108720, Aug. 2019.
- [149] M. R. Bindhu and M. Umadevi, "Antibacterial activities of green synthesized gold nanoparticles," *Materials Letters*, vol. 120, pp. 122–125, Apr. 2014.
- [150] G. Applerot et al., "Understanding the antibacterial mechanism of CuO nanoparticles: revealing the route of induced oxidative stress," *Small*, vol. 8, no. 21, pp. 3326–3336, Nov. 2012.
- [151] D. Das, B. C. Nath, P. Phukon, and S. K. Dolui, "Synthesis and evaluation of antioxidant and antibacterial behavior of CuO nanoparticles," *Colloids and Surfaces B: Biointerfaces*, vol. 101, pp. 430–433, 2013.
- [152] P. Sutradhar, M. Saha, and D. Maiti, "Microwave synthesis of copper oxide nanoparticles using tea leaf and coffee powder extracts and its antibacterial activity," *Journal of Nanostructure in Chemistry*, vol. 4, no. 86, pp. 1–6, 2014.
- [153] A. H. Abd Kelkawi, A. A. Kajani, and A. K. Bordbar, "Green synthesis of silver nanoparticles using *Mentha pulegium* and investigation of their antibacterial, antifungal and anticancer activity," *IET Nanobiotechnology*, vol. 11, no. 4, pp. 370–376, Sep. 2016.
- [154] H. Liu, S. Zheng, H. Xiong, M. S. Alwahibi, and X. Niu, "Biosynthesis of copper oxide nanoparticles using *Abies spectabilis* plant extract and analyzing its antinociceptive and anti-inflammatory potency in various mice models," *Arabian Journal of Chemistry*, vol. 13, no. 9, pp. 6995–7006, Sep. 2020.

