A Review on the Green Synthesis of Metal Oxide Nanoparticles for Antimicrobial Activity Using Different Plant Parts

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Abstract

In recent years, green chemistry has witnessed a surge towards sustainable approaches for nanoparticle synthesis. Researchers are now exploring the potential of diverse plant extracts to produce ecological nanoparticles. Biomedical sciences increasingly focus on synthesizing various metal oxide nanoparticles (MONPs) mediated by plants due to their extensive biological applications. Plant-mediated biogenic synthesis of MONPs is a sustainable, less harmful, and low-cost method; additionally, it has advantages for biological analysis regarding antifungal and antibacterial activities. Plants contain diverse phytochemicals, including amino acids, terpenoids, polyphenols, and flavonoids, which can act as both reducing and stabilizing agents. This report focuses on the plant-mediated synthesis of nanoparticles (NPs), such as zinc oxide (ZnO), titanium dioxide (TiO_2), iron oxide (FeO), nickel oxide (NiO), and copper oxide (CuO), offering essential insights into their antimicrobial activity against various bacterial strains in different concentrations. It explores their structural properties, such as shape and size, analysed through advanced techniques and their antimicrobial effectiveness against various microbe strains.

Keywords: Green synthesis, Metal Oxide Nanoparticles, Plant extracts, Antimicrobial Activity.
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Introduction

Microbial infections are disorders resulting from the invasion of harmful bacteria into the body's tissues. These infections can manifest in various forms, ranging from small, localized problems to severe, life-threatening diseases [1–3]. In addition, the efficacy of traditional pharmaceutical interventions for microbial disorders declines over time [4]. This scenario occurs due to antimicrobial resistance to conventional treatments and the high utilization or improper usage of these therapies [5]. Additionally, bacteria acquire resistance by employing genetic alterations or mobile genetic components that facilitate the transfer of resistance genes. There is an urgent need to explore novel methods for addressing antimicrobial issues [6–9]. Researchers have focused on establishing environmentally beneficial solutions using nanotechnology [10,11]. The utilization of MONPs has gained attention due to its cost-effectiveness and environmentally friendly nature [12,13]. The review explores advanced green synthesis techniques that are environmentally friendly for making MONPs from plants. The effectiveness and environmental benefits of the green manufacture of MONPs are emphasized in this article, highlighting their potential as antibacterial agents. The article explains new, plant-based approaches to producing NPs and investigates their varying antimicrobial effects at different concentrations.

Green Synthesis

Green synthesis is an approach that adheres to sustainable principles by reducing the utilization of harmful substances, resulting in advantages for both human well-being and the environment. [14–16]. The synthesis of NPs through green chemistry principles involves three phases: using environment-friendly solvent mediums, reducing agents, and stabilization agents. Plant extracts from leaves, seeds, and stems can synthesize nanoparticles using their stabilizing or reducing characteristics [17,18]. The synthesis of MONPs using plant extracts follows a sequential processing approach, as depicted in Fig 1.

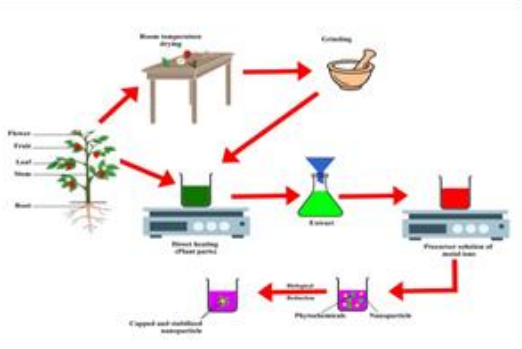

Figure 1: Illustration depicting synthesizing MONPs using natural plant extracts.

The green route involves the following steps. Initially, selected plant parts are soaked in distilled water to eliminate debris. Subsequently, the cleaned plant parts are dried and converted into powder by grinding or cutting. Later, the powder was boiled at a particular temperature in the desired solvent, filtered, and refrigerated for further use [19,20]. Filtration is carried out to isolate the phytochemicals, like amino, carboxylic, hydroxyl, allyl, alkoxy, and sulfhydryl groups [21]. The resultant plant extracts were combined with the respective salt solutions to synthesize NPs. A change in colour of the solution can indicate the early stages of NPs formation. Green synthesized NPs have extensive applications in contaminant remediation and antibacterial, antifungal, high catalytic, and photochemical activities [22]. The resulting plant-based metal oxide nanoparticles exhibit diverse beneficial properties [23,24].

MONPs Mechanism of Antimicrobial Activity

MONPs have attracted significant attention recently owing to their distinct physical and chemical properties and their diverse applications in disciplines such as environmental remediation, medicine, and electronics [25]. Currently, MONPs are being investigated as potential treatments for microbial infections due to their numerous applications. MONPs have several advantages over conventional antibiotics [26]. This review focuses on the antimicrobial effects of some MONPs. The efficacy of NPs is impacted by consistency, shape, and size [27]. Research indicates that the NPs interact with bacteria and fungi, altering the membrane shape and restricting their growth. Its disruption impedes proper mobility during plasma membrane formation, ultimately resulting in cell death [28]. The produced MONPs, smaller in size compared to micro-sized particles and having a high surface-to-volume, come into contact with sulphur or phosphorus in the DNA, causing cessation of protein synthesis and subsequent cell death. Reactive Oxygen Species (ROS) are crucial in promoting antimicrobial actions, as they are integral to the regular metabolic processes of living organisms [29]. The destruction of cells is attributed to the generation of ROS, which forms highly reactive radicals and breaks down cell wall proteins, the cytoskeleton system, and DNA [30]. Moreover, the electrostatic interaction of nanoparticles with the membrane may induce charge imbalances (+, -, and o), disrupting the membrane's equilibrium and forming hole pairs near its surface. eventually leading to the release of proteins RNA, DNA, and lipids, which culminates in cell death [31], as shown in Fig. 2.

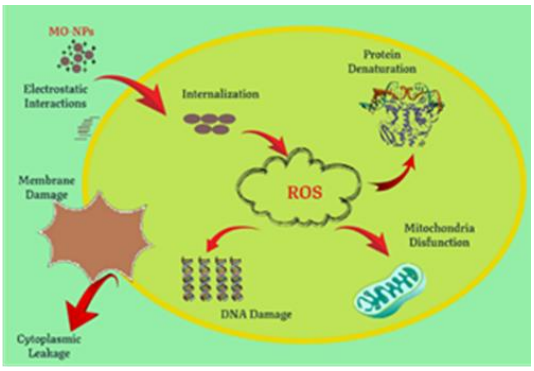

Figure 2: MONPs mechanism of antimicrobial activity.

Table 1 summarizes the green synthesis of MONPs using various extracts, highlighting their morphology, size, and notable antimicrobial activity against microbial species as reported in several publications [32-46]. Muthu et al. prepared ZnO nanoparticles using an extract derived from *Pisonia alba* leaves, identifying them as hexagonal crystalline structures with an average size of 48 nm through SEM, TEM, and XRD. FTIR studies suggested that phenolic compounds, alkaloids, terpenoids, and proteins in the extract are essential for the nucleation and stability of the ZnO Nps. The antibacterial activity was tested at 100 µg/µL, showing a 20 mm zone of inhibition (ZOI) against *S. aureus* (gram-positive) and 11 mm ZOI against *K. pneumoniae* (gram-negative). The NPs were more effective against gram-positive bacteria, which have thicker peptidoglycan cell walls, while gram-negative bacteria possess more complex cell walls [47]. Suresh et al. described the synthesis of ZnO NPs with sizes ranging from 5 to 15 nm utilizing *Cassia fistula* leaf extract, which was tested against four pathogenic Gram-negative bacteria: *K. aerogenes* (ZIO = 7.33 ± 0.33, 9.67 ± 0.33mm, respectively), *E. coli* (ZOI = 3.67 ± 0.33, 4.67 ± 0.33 mm) *P. desmolyticum* (ZOI = 3.00 ± 0.00, 4.00 ± 0.00 mm) and Gram-positive bacteria *S. aureus* (ZOI = 2.67 ± 0.33, 4.67 ± 0.33 mm) at different dosage 500 µg/µL, 1000 µg/µL [48]. Ansari *et al.* synthesized TiO_2 NPs using a leaf extract from *Acorus calamus*. The SEM analysis revealed that the NPs were spherical, with an average size ranging from 15 - 40 nm. The antimicrobial efficacy was assessed using the disc diffusion method, divulging inhibitory effects against gram-negative bacteria such as *P. aeruginosa* (ZOI: 6 ± 0.2, 8 ± 0.3 mm) and *E. coli* (9 ± 0.3, 10 ± 0.2 mm), as well as gram-positive bacteria including *B. subtilis* (12 ± 0.4, 14 ± 0.5 mm) and *S. aureus* (10 ± 0.3, 12 ± 0.3 mm) at

nanoparticle concentrations of 10 µg/mL and 20 µg/mL, respectively. The biosynthesized TiO_2 NPs shown enhanced antibacterial activity against gram-positive bacteria compared to gram-negative bacteria and exceeded the

Table 1: A brief summary of the antimicrobial activity of green-synthesized MONPs.

MONPs	Reducing agent	Part of plant	Morphology	Size	Microbial name	Applications	Ref.
ZnO	*Moringa Oleifera*	Leaf	Irregular shape	25 nm	*Pseudomonas, Bacillus*	Antibacterial	[32]
ZnO	*Artemisia pallens*	Leaf	Hexagonal	100 nm	*S. aureus, B. subtilis, E. coli,*	Antibacterial	[33]
ZnO	*Andrographis alata*	whole plant	Flake	35-53nm	*B. subtilis, S.pyogenes, S. aureus, C. diphtheria, S.typhi, E. coli, K. pneumonia, P. aeruginosa,*	Antimicrobial	[34]
CuO	*Luffa acutangula*	Peel	Rectangular	26 nm	*E. coli, K. pneumoniae, S. aureus, B. subtilis*	Antibacterial	[35]
TiO_2	*Acorus calamus*	Leaf	Spherical	11–30nm	*E. coli, P. aeruginosa, B. subtilis, S. aureus*	Antibacterial	[36]
TiO_2	*Coleus aromaticus*	Leaf	Spherical	12–33 nm	*Shigella boydii, Vibrio cholerae, B. cereus, A. hydrophilia, E. faecalis, B. megatarium*	Antibacterial	[37]
TiO_2	*Luffa acutangula*	Leaf	Hexagonal	10 - 49 nm	*B. subtilis, E. faecalis, P. aeruginosa, S. aureus, K. pneumonia*	Antimicrobial	[38]
CuO	*Bougainvillea*	Leaf	Spherical	8–20 nm	*E. coli, E. faecallis S. aureus*	Antibacterial	[39]
CuO	*Ocimum americanum*	Leaf	Spherical	67.7 nm	*E. coli, V. cholera S. typhimurium K. pneumoniae A. hydrophila P. aeruginosa*	Antibacterial	[40]
FeO	*Phoenix dactylifera*	Seed	Spherical	50 nm	*K. pneumonia S. epidermidis P. aeruginosa*	Antibacterial	[41]
NiO	*Averrhoa bilimbi*	Fruit	Spherical	100–120 nm	*E. coli, S. aureus*	Antibacterial	[42]
NiO	*Pometia pinnata*	Leaf	Irregular	10–30 nm	*E. coli, S. aureus,*	Antibacterial	[43]
NiO	*Solanum trilobatum*	Leaf	Cylindrical	25- 30nm	*S. aureus, E. coli, S. pnemoniae E. hermannii*	Antibacterial	[44]
NiO	*Berberis balochistanica*	Leaf	Irregular	23nm	*S. aureus P. vulgaris*	Antibacterial	[45]
NiO	*Clitoriaternatea*	Fruit	Hexagonal	10nm	*S. aureus, E. coli*	Antibacterial	[46]

antimicrobial efficacy of bare TiO_2 NPs. The findings suggest that biosynthesized TiO_2 NPs have potential as therapeutic agents for bacterial infections due to their significant in vitro antibacterial effectiveness [36]. Anbumani et al. prepared TiO_2 NPs using *L. acutangula* leaf extract as an affordable, eco-friendly method. They characterized the NPs using XRD, FT-IR, UV, FE-SEM-EDAX, and TEM techniques to determine their form, size, and structure. The antibacterial activity of TiO_2 NPs against various microorganisms, including *B. subtilis* (15 ± 0.46,16

± 0.38, 18 ± 0.56 mm), *E. faecalis* (13 ± 0.35,18 ± 0.32, 21 ± 0.41mm), *S. aureus* (21 ± 0.53, 33 ± 0.48, 42 ± 0.13 mm) and *P. aeruginosa* (33 ± 0.33, 36 ± 0.35, 42 ± 0.45 mm) with varying NPs concentrations 20, 30, 40 µg/mL. The antibacterial mechanism was ascribed to the interaction between positively charged TiO_2 NPs and negatively charged bacterial cell walls. This connection generated an electromagnetic attraction that caused oxidative stress in the microorganisms, resulting in their demise. All the microorganisms exhibited a ZOI, and the highest dosage showed the maximum ZOI [38].

Majid et al. utilized *P. dactylifera* to synthesize spherical FeO NPs. The effectiveness of these green iron oxide NPs in killing bacteria was assessed against three different bacterial strains. The ZOI measurements for *K. pneumonia*, *S. epidermidis*, and *P. aeruginosa* were acquired as 16mm, 15 mm, and 21 mm, respectively, utilizing a 100 µg/mL dosage of NPs. Furthermore, imperfections in nanoparticle surface shape may lead to membrane disruption or misalignment. This may enhance the antibacterial efficiency of nanoparticles by up to tenfold [41]. Haritha et al. synthesized spherical NiO NPs utilizing *A. bilimbi* fruit extract, resulting in an average 100-120 nm size range. The antibacterial efficacy of *A. bilimbi*-enhanced NiO NPs was evaluated employing the disc diffusion technique. The NPs were evaluated against *S. aureus* (ZOI: 6.1 mm) and *E. coli* (ZOI: 7 mm) at a dose of 150 µg/µL. The produced NPs exhibited enhanced antibacterial activity against gram-negative bacteria owing to their capacity to penetrate the complex cell membrane structure. Moreover, the production of reactive oxygen species (ROS) caused interactions with cellular components, including the cytoplasmic membrane, peptidoglycan layer, lipids, proteins, and DNA, disrupting multiple physiological processes. The interaction between cationic nickel molecules and anionic microbial cell membranes leads to the release of proteins and other intracellular components, ultimately resulting in cell destruction. This process is essential for improving biological applications. Prabhu et al. synthesized hexagonal-shaped NiO NPs utilizing *C. ternatea* fruit extract with a mean size of 10 nm and the antibacterial efficacy of NiO NPs towards *E. coli* (ZOI =15 ± 1.0, 17±1.0, and 22 ± 1.0 mm), and *S. aureus* (ZOI=13±1.0, 16±1.0, and 19 ± 1.0 mm) was studied. The antibacterial activity improved as the concentration of NiO NPs increased. At 200 mg/mL, the largest inhibition zone for *E. coli* was 22 ± 1.0mm, and the highest inhibition zone for *S. aureus* was 19 ± 1.0 mm. NiO NPs have a positively charged surface and a negatively charged surface for bacterial cell walls. As a result, it induces an electromagnetic interaction that destroys the bacterial cytoplasm and border. Previous research indicates that NiO NPs exert a pronounced effect on gram-negative bacteria such as *K. pneumoniae* and *P. mirabilis*, showcasing substantial inhibition zones of 32 mm and 28 mm, respectively [46]. Ramzan et al. employed *Cedrus deodara* leaf extract to produce spherical CuO NPs with an average diameter of approximately 20 nm. CuO NPs exhibited strong antibacterial effects, achieving notable ZOI at 125, 150 mg/mL concentrations. The ZOI ranged from 20–29 mm in *E. coli*, 6–16 mm in *S. aureus*, 12–20 mm in S. enterica, and 8–24 mm in *L. monocytogenes*. CuO NPs are vulnerable to various diseases and possess production processes and compatibility with living organisms inspired by biological systems [49].

Conclusion and Future Prospective

The green synthesis of MONPs using natural sources offers a safer, more eco-friendly alternative to traditional synthesis methods, effectively addressing the environmental and health concerns associated with toxic reagents and byproducts. Phytochemical-rich biological sources, such as plant extracts, facilitate the efficient production of nanoparticles with well-defined characteristics and significant antibacterial properties. MONPs synthesised through green methods have demonstrated superior efficacy, particularly in biomedical applications. The future of green-synthesized MONPs lies in expanding their use in advanced biotechnological fields, including nano-sensors, food packaging, targeted drug delivery, and cancer therapy. However, challenges such as scalability, safety concerns, and regulatory standards must be addressed. Further research is needed to refine synthesis methods, explore novel applications, and develop affordable analytical tools like ICP-AES, NMR, and HPLC for improved characterisation of phytochemicals. Innovations in this field can enhance the stability and effectiveness of MONPs, providing solutions to global challenges, including pandemic management and sustainable development.

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