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Plant-Mediated Green Synthesis of Nanoparticles for Biomedical Applications

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ABSTRACT: Plant-mediated green synthesis of nanoparticles has emerged as a sustainable and eco-friendly alternative to conventional physical and chemical synthesis methods. Plant-derived phytochemicals, including flavonoids, phenolics, alkaloids, terpenoids, and proteins, act as natural reducing, capping, and stabilizing agents, enabling the synthesis of biocompatible metallic and metal oxide nanoparticles under mild conditions. These green-synthesized nanoparticles exhibit remarkable physicochemical properties and have demonstrated significant biomedical potential, including antimicrobial, anticancer, antioxidant, anti-inflammatory, drug delivery, and tissue engineering applications. In addition, their environmentally benign synthesis minimizes hazardous chemical use and energy consumption. Despite these advantages, challenges related to standardization, reproducibility, large-scale production, comprehensive toxicity assessment, and regulatory approval remain barriers to clinical translation. This review summarizes recent advances in plant-mediated nanoparticle synthesis, characterization techniques, biomedical applications, safety considerations, and future perspectives, highlighting their potential to advance sustainable nanomedicine and support the development of safe, effective, and environmentally responsible therapeutic technologies.

1. INTRODUCTION

Nanotechnology is one of the most rapidly developing interdisciplinary research fields, which offers solutions to medical, biotechnology, pharmaceutical and materials science problems through the development of nanoparticles with dimensions between 1 and 100 nm. Due to their unique properties, such as high surface-area-to-volume ratio, increased catalytic activity, better optical properties and interactions with cells, nanoparticles are well suited for various applications, including drug delivery, bioimaging, biosensing, antimicrobial therapy, tissue engineering and cancer therapy (1). There are several methods for the synthesis of nanoparticles, including physical methods and chemical methods; most of these methods require high temperature, high pressure, expensive instruments and the use of dangerous reducing and stabilizing agents, which may generate hazardous by-products, and are not suitable for biomedical applications and environmental sustainability (1,2). For these reasons, green synthesis has become an environmentally friendly, sustainable, and economical alternative that conforms to the tenets of green chemistry by reducing the amount of hazardous materials used and energy consumption (2). However, among the biological method, the synthesis using the plant-based method is gaining much attention as there are plenty of bioactive phytochemicals in the group of medicinal and aromatic plants like flavonoids, phenolic compounds, tannins, terpenoids, alkaloids, proteins, polysaccharides, and organic acids which can act as reducing, capping, and stabilizing agents during the synthesis of the nanoparticles (2,3). The naturally occurring properties of these biomolecules help to reduce metal ions to stable nanoparticles and also increase the physicochemical stability, biocompatibility, and biological activity of nanoparticles (3). In contrast to microbial synthesis, the plant-mediated synthesis is relatively simpler, faster, less expensive, is easily scalable, and does not require the maintenance of microbial culture (which helps to prevent microbial contamination), making it more suitable for industrial production. Natural-origin metal and metal oxide nanoparticles, in particular silver, gold, zinc oxide, copper oxide, iron oxide, titanium dioxide, and selenium nanoparticles, have remarkable antimicrobial, antioxidant, anti-inflammatory, antiviral, anticancer, and wound-healing properties, which are attributed to their nanoscale size and the synergistic effect of phytochemical surface functionalization. Furthermore, they have been identified as having the potential to revolutionize the fields of targeted drug delivery, regenerative medicine, biosensing, diagnostic imaging, and precision therapeutics, offering great promise in the future for biomedical applications (3). Although these achievements have been made, there are still challenges to their clinical translation, such as variability in the phytochemical composition of plants, lack of understanding of the mechanisms of nanoparticle synthesis, batch-to-batch variability, large-scale production, thorough toxicological assessment, and the absence of standards for regulatory guidelines in different countries. Advancements in standardized, safe, and clinically effective plant-mediated nanoparticles for future biomedical applications will rely on advances in phytochemistry, nanotechnology, materials science, computer modeling, and biomedical engineering.

2. PLANT-MEDIATED GREEN SYNTHESIS OF NANOPARTICLES

Green synthesis approach with the use of medicinal plants and bioactive phytochemicals containing inherent reducing and stabilizing properties of plants has captured a lot of attention in recent years owing to the synthesis of green (natural), eco-friendly and economically viable metallic and metal oxide nanoparticles. Various parts of the plant like leaves, flowers, fruits, seeds, roots, stems and bark contain high concentrations of plant secondary metabolites, which are important in the synthesis of nanoparticles including flavonoids, phenolic acids, tannins, terpenoids, alkaloids, saponins, proteins, amino acids, polysaccharides, and reducing sugars (4–6). These phytochemicals donate electrons to the metal ions to reduce the charge on the metal ions to zero resulting in the formation of stable nuclei. The resulting nanoparticles are then subjected to the principle of controlled crystal growth, leading to nanoparticles with a clearly defined size, shape and crystallinity, in which hydroxyl, carbonyl, carboxyl and amino functional groups are intrinsically present as capping/stabilizing agents, preventing them from aggregating and, consequently, enhancing the colloidal stability and biocompatibility (4,5). The overall process of plant-based synthesis can be broken down into four main steps: reducing the metal ions, nucleation of the reduced metal atoms, growth of nanoparticles by a controlled crystal architecture and capped by molecules of biological origin, resulting in minimum production footprint in terms of the environment. An overview of overall green synthesis of nanoparticles using plants is shown in Figure 1 (4).

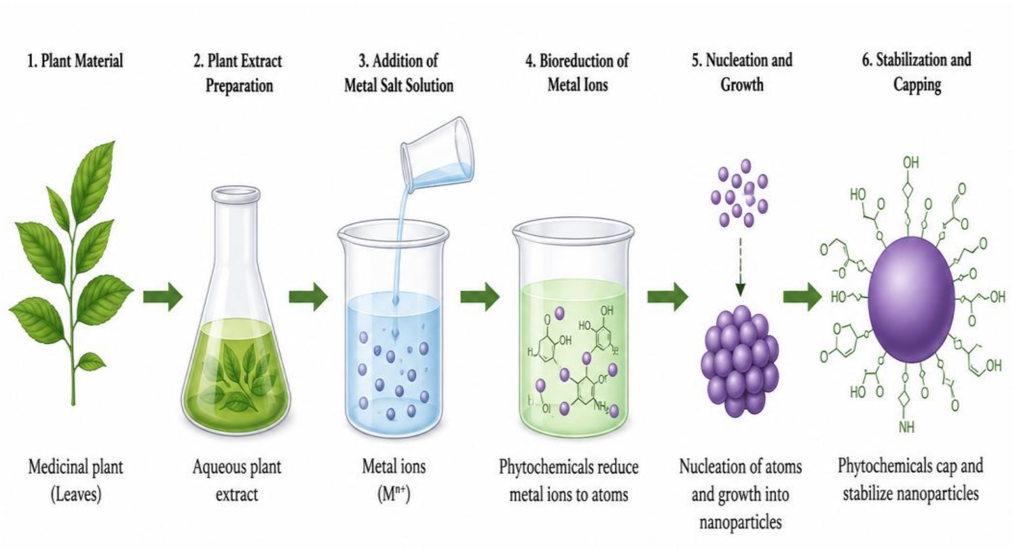

Figure 1. Mechanism of Plant-Mediated Green Synthesis of Nanoparticles

The physicochemical properties of the nanoparticles obtained are greatly influenced by the reaction parameters. The reducing efficiency and stabilization capacity are dependent on the species of the plant and phytochemical constituents, while the particle size, morphology, and reaction kinetics of the particle synthesis are dependent on the pH of the reaction (4-6). The rate of the reaction, which is determined by temperature, determines the maturation and stability of the nanoparticles, and the rate of reduction and growth of the crystals depends on the temperature. Concentration of plant extract, concentration of metal precursors, extract-to-metal ion ratio, mixing conditions of precursor metals and plant extraction, and incubation conditions, similarly, also influence the nucleation rate, the dispersion of the particles, the amount of synthesized particles, and the biological performance (5,6). Optimal values of these parameters allow to obtain high stability nanoparticles with desirable physicochemical and biological properties and a narrow size distribution. Due to its simplicity, scalability, low cost, and green chemistry principles, the plant-mediated synthesis is becoming popular in the synthesis of biocompatible nanomaterials and applications in antimicrobial therapy, drug delivery, cancer therapy, tissue engineering, biosensing, and regenerative medicine using the least amount of hazardous chemicals and environmental pollution.

3. CHARACTERIZATION OF GREEN-SYNTHESIZED NANOPARTICLES

The physicochemical characterization of the green synthesized nanoparticles is essential to understand the properties of nanoparticles such as their stability, morphology, crystallinity, surface chemistry and biological performance, which would enable to ensure the suitability of the nanoparticles for biomedical applications. Usually, spectroscopic, microscopic, structural and surface analysis methods are used, since there is no single method that provides all of the information necessary for the characteristics of nanoparticles (9). The confirmation of the formation of nanoparticles and determination of the phytochemicals responsible for their reduction and stabilization is mainly achieved by the use of spectroscopic techniques. For surface plasmon resonance monitoring, the ultraviolet–visible (UV–Vis) spectroscopy is a common method and for the evaluation of the synthesis of nanoparticles, Fourier-transform infrared (FT-IR) spectroscopy is used for detecting functional groups involved in capping and stabilization, like hydroxyl, carbonyl, amino, and carboxyl groups. Raman spectroscopy and X-ray photoelectron spectroscopy (XPS) also give information on molecular interactions, elemental composition, oxidation state, and surface chemical environment (8). The critical role of microscopic techniques in determining the morphology, size distribution, aggregation behavior, and surface architecture of nanoparticles. The surface morphology of particles can be determined by scanning electron microscopy (SEM), and the particle size, shape, crystal boundaries, and lattice arrangements at high resolution can be determined by transmission electron microscopy (TEM). Furthermore, the ability to visualize particle size and surface topography at the nanoscale with high resolution using TEM (high-resolution TEM, HRTEM) and atomic force microscopy (AFM) (8) makes it possible to assess the characteristics of the material with high precision. The X-ray diffraction (XRD), which uses analysis of diffraction patterns to identify crystalline phase, crystal structure, lattice parameters, and average crystallite size, is generally employed for structural characterization, while selected area electron diffraction (SAED) is used to determine crystallinity at the individual nanoparticle level (7,). Dynamic light scattering (DLS) is used to determine the hydrodynamic particle size and polydispersity index, while zeta potential analysis is used to determine the surface charge and colloidal stability, which give an indication of the dispersion of the nanoparticles and the long-term stability of the storage. (9) A common application of electron microscopy is to combine it with energy-dispersive X-ray spectroscopy (EDX) for purposes of elemental composition and purity determination for synthesized nanoparticles. The biomedical significance and principles of the major characterization techniques are summarized in Table 1. These complementary analytical approaches, when used collectively, enabled the characterization of the quality, reproducibility, physicochemical characteristics, and biological activity of the green-synthesized nanoparticles, which in turn aided in the development of standardized, safe, and highly effective nanomaterials in biomedical, pharmaceutical, and diagnostic applications (10).

Table 1. Characterization Techniques and Their Applications

Technique	Purpose	Application
UV–Vis Spectroscopy	Confirms nanoparticle formation	Surface plasmon resonance analysis
FTIR Spectroscopy	Identifies functional groups	Detects phytochemicals involved in capping and stabilization
SEM	Examines surface morphology	Determines particle shape and aggregation
TEM	Measures particle size and morphology	High-resolution nanoparticle imaging
XRD	Determines crystal structure	Confirms crystallinity and phase purity
DLS	Measures hydrodynamic size	Evaluates size distribution and dispersion
Zeta Potential	Measures surface charge	Assesses colloidal stability
EDX	Determines elemental composition	Confirms nanoparticle purity and elemental analysis

4. BIOMEDICAL APPLICATIONS

The unique biocompatibility, functionalization of the surfaces of nanoparticles with phytochemicals, and multifunctional biological activity, are some of the reasons why plant-mediated green-synthesized nanoparticles are becoming versatile nanomaterials for biomedical applications. Their most intensively explored use is in antimicrobial applications, which show a wide range of bacterial, fungal and viral activities against microbial cell membranes, production of reactive oxygen species (ROS), intracellular protein and nucleic acid damage, and inhibition of microbial biofilm formation, and thus they hold potential as alternatives for overcoming antimicrobial resistance (11). The green synthesized nanoparticles have also exhibited excellent anticancer properties by selectively inducing oxidative stress, mitochondrial dysfunction, DNA damage, apoptosis, autophagy and cell cycle arrest in cancer cells, without causing much damage to the normal cells (12). Furthermore, phytochemical coated nanoparticles bring synergistic effects and targeted intracellular delivery of conventional chemotherapeutic drugs, resulting in reduced systemic toxicity of the drug and better treatment efficiency (12).They are also biologically active owing to their antioxidant and anti-inflammatory properties, which are mainly attributed to the phytochemicals that are bound to the surface of the nanoparticles. They can also scavenge free radicals, modulate oxidative stress, block pro-inflammatory cytokines, block inflammatory pathways such as NF-κB, and have tissue repair effects, making them potential candidates for the treatment of chronic inflammatory diseases and oxidative stress-related diseases (11). Furthermore, drug delivery has been a hot topic of research as nanoparticles are capable of transporting therapeutic agents, with control over drug loading and sustained release; the high surface area of the nanoparticles increases the bioavailability of the therapeutic agent carried by the nanoparticles and the surface can be modified to achieve site-specific delivery of the therapeutic agent. Surface functionalization can be done to further enhance cellular uptake and targeted delivery, allowing for reduced off-target toxicity and increased therapeutic efficacy (13). The application of these nanoparticles in tissue engineering and regenerative medicine has been growing, where the use of these nanoparticles in biodegradable scaffolds, hydrogels, materials for wound dressings, and biomaterials for implantation enhances the adhesion, proliferation, angiogenesis, collagen deposition, and tissue healing, and inhibits microbial infection (11).

The different biomedical applications of plant-based nanoparticles, like antimicrobial, anticancer, antioxidant, anti-inflammatory, drug delivery, and tissue engineering applications, are summarized in Figure 2. To conclude, the multifunctional properties highlight the tremendous potential of plant-mediated nanoparticles as next-generation nanotherapeutics in precision medicine and translational biomedical research (14).

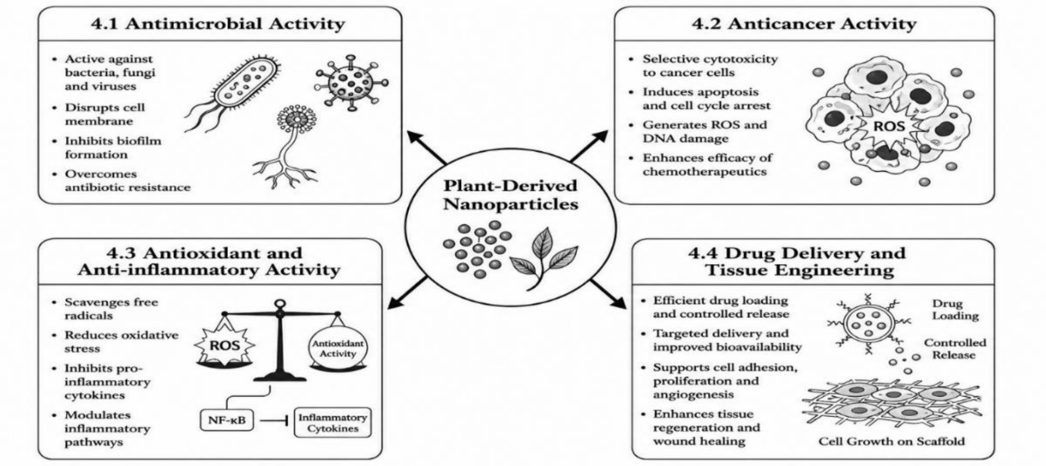

Figure 2. Biomedical Applications of Plant-Derived Nanoparticles

5. SAFETY, TOXICITY, AND REGULATORY ASPECTS

The clinical translation of plant-mediated green-synthesized nanoparticles is not only dependent on their therapeutic efficacy, but also on a thorough assessment of their safety, toxicity, environmental impact and regulatory compliance. For chemically synthesized nanoparticles, it has been demonstrated that toxicity increases with decreasing size, increasing concentration, increasing duration of exposure, and depending on the route of exposure and on their chemical composition, shape and surface charge (15). Incorporating biocompatibility in the assessment of nanoparticles requires in vitro and in vivo toxicity testing to determine the nanoparticles' effect on cellular uptake, metabolism, and long-term accumulation in tissues, as well as its effect on oxidative stress, hemocompatibility, and immunogenicity, to ensure that the nanoparticles do not cause an adverse biological response or organ toxicity (16). Hence, the risk of over-exposure of the ROS and subsequent impairment of mitochondrial function, DNA damage, and induction of inflammatory pathways is significant and dose optimization and thorough toxicological evaluation are imperative before biomedical application (17). Concern over the potential for bioaccumulation and ecological toxicity in aquatic and terrestrial environments via human exposure has led to the environmental impact of nanoparticles becoming a major concern, with it being thought that the release of these particles into the environment could hurt microorganisms, plants, invertebrates, and higher organisms. Therefore, it is increasingly suggested to evaluate long-term impacts of the production, use, and disposal of nanoparticles by life-cycle assessment, environmental fate studies, biodegradability, and ecotoxicological research (18). There is a consensus among regulatory agencies that there is a need for standardisation of evaluation protocols for ensuring quality, safety, efficacy, and reproducibility of nanomaterials for medical application. Although guidance documents from international organizations, including the Organisation for Economic Co-operation and Development (OECD), the International Organization for Standardization (ISO), the United States Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have been developed for physicochemical characterization of nanomaterials, toxicological assessment, quality control and risk assessment of plant-mediated nanoparticles are limited at the global level (19). The important toxicological parameters, evaluation methods, and recommendations for the regulation of green synthesized nanoparticles are summarized in Table 2. To facilitate commercialization and clinical use of plant-derived nanoparticles, standardized approaches for manufacturing, the development of preclinical and clinical safety studies, environmentally friendly production methods, and the establishment of internationally agreed-upon regulatory guidelines for safe and effective biomedical application are necessary (20).

Table 2. Toxicological Evaluation and Regulatory Guidelines for Plant-Mediated Green-Synthesized Nanoparticles

Aspect	Evaluation Parameter	Purpose
Biocompatibility	Cell viability, hemocompatibility	Evaluates compatibility with biological systems
Cytotoxicity	MTT, LDH, apoptosis assays	Determines cellular toxicity
Genotoxicity	Comet assay, micronucleus test	Assesses DNA damage
Oxidative Stress	ROS generation, antioxidant enzymes	Evaluates oxidative injury
Biodistribution	Tissue accumulation and clearance	Determines in vivo fate
Environmental Impact	Ecotoxicity, biodegradability	Assesses environmental safety
Quality Assessment	Particle size, zeta potential, stability	Ensures reproducibility and product quality
Regulatory Frameworks	ISO, OECD, FDA, EMA	Guides safety testing and clinical approval

6. FUTURE PERSPECTIVES

It is likely that one of the most important approaches which can be employed for developing green nanomedicine will be the green synthesis of nanoparticles using plants. Future research is needed to reveal the molecular mechanism of nanoparticle formation process from phytochemicals, standardize the process and achieve reproducible large scale production. The design and optimization of nanoparticles can be speeded up by the help of AI, machine learning, computational modeling, and high throughput screening. Furthermore, the application of multifunctional nanoparticles having targeted delivery, diagnostic and therapeutic activities should be explored. Multi-disciplinary work of researchers, clinicians, industry and regulatory bodies will help bring safe, effective and environmentally friendly plant-based nanomaterials to the clinical arena and to market.

7. LIMITATIONS

While there are some positive developments, there are also some drawbacks with the application of green-synthesized nanoparticles with plants. The size, shape and biological activity of these nanoparticles will change depending on the type of plant species and extraction method, which will result in variations in the quality and characteristics of the nanoparticles produced. However, there are still a lack of mechanisms understanding, poor batch to batch repeatability, and process scale-up challenge. In addition, there is a lack of long-term toxicity, pharmacokinetic, and environmental safety studies. Synthesis dynamic standards are not established, and there are no regulatory standards that are harmonized throughout the world, which further delays the clinical translation process. This is essential to solve the problem to make the green nanotechnology replicable, safe and commercially available.

8. CONCLUSION

Green synthesis with plant is an environment-friendly and sustainable technology for the preparation of biocompatible nanoparticles for various applications in biomedical field. In the process of phytochemical mediated synthesis, toxic chemicals are not used and nanoparticles are more stable and biologically active. Green synthesized nanoparticles have been reported with different antimicrobial, anticancer, antioxidant, anti-inflammatory, drug delivery and tissue engineering properties. However, several key considerations are needed for successful clinical translation including standardisation of the manufacturing process, safety evaluation and well established regulatory frameworks. The development of safe, efficient and clinically viable plant based nanomaterials in the field of precision medicine and healthcare would be expected to benefit from further advances in the development of nanotechnology, phytochemistry, computational and biomedical engineering.

ABBREVIATIONS

AgNPs, Silver nanoparticles; AuNPs, Gold nanoparticles; ZnO NPs, Zinc oxide nanoparticles; ROS, Reactive oxygen species; FTIR, Fourier-transform infrared spectroscopy; UV–Vis, Ultraviolet–visible spectroscopy; XRD, X-ray diffraction; SEM, Scanning electron microscopy; TEM, Transmission electron microscopy; DLS, Dynamic light scattering; EDX, Energy-dispersive X-ray spectroscopy.

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Data Availability: No new data were generated or analyzed during this study; all information is derived from published literature.

Ethical Approval: Not applicable, as this study is based exclusively on previously published literature and did not involve human participants or experimental animals.

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