# Nanotechnology in Plants

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**Abstract** The integration of nanotechnology in medicine has had a tremendous impact in the past few decades. The discovery of synthesis of nanomaterials (NMs) and their functions as versatile tools promoted various applications in nano-biotechnology and nanomedicine. Although the physical and chemical methods are still considered as commonly used methods, they introduce several drawbacks such as the use of toxic chemicals (solvent, reducing, and capping agents) and poor control of size, size distribution, and morphology, respectively. Additionally, the NMs synthesized in organic solvents and hydrophobic surfactants rapidly aggregate in aqueous solutions or under physiologic conditions, limiting their applications in medicine. Many of the phase-transfer strategies were developed and applied for the transfer of NMs into aqueous solutions. Although great efforts have been put into phase transfers, they mostly include expensive, time-consuming, intensive labor work, multi steps, and complicated procedures.

Use of plant extracts in the biological synthesis method offers stark advantages over other biomolecules (protein, enzyme, peptide, and DNA). Plant extracts have been commonly used for food, medicine, NM synthesis, and biosensing. There are many viable techniques developed for the production of plant extracts with various contents based on their simplicity, cost, and the type of extract content. In this chapter, we conduct a comparative study for extract preparation techniques, the use of extracts for metallic single and hybrid nanoparticle (NP) synthesis, and their antimicrobial properties against pathogenic and plant-based bacteria.

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**Graphical Abstract**
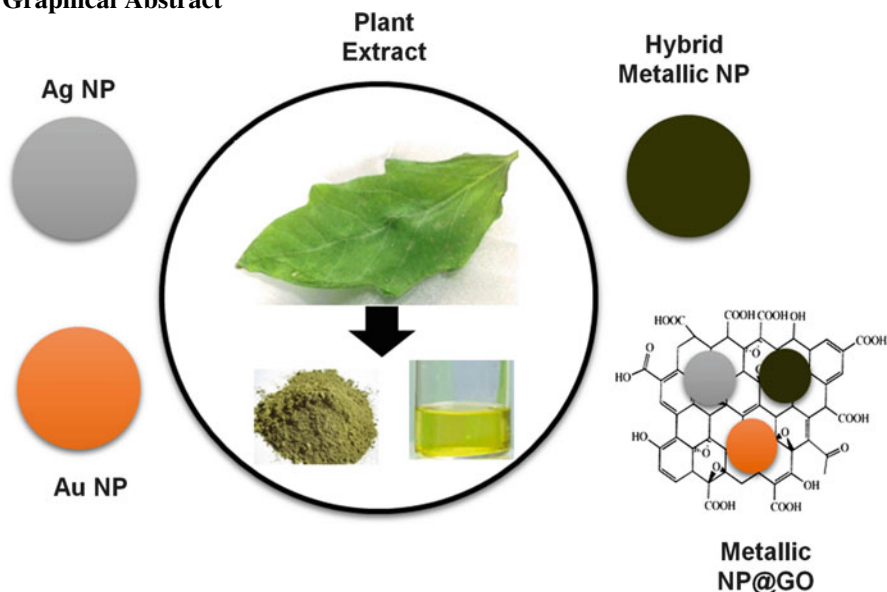


**Keywords** Disease and antimicrobial property, Metallic NP, Microorganisms, Plant extract

**Contents**

# 1   Introduction

In recent years, nanotechnology has been increasingly utilized for the synthesis, engineering, and designing of various nanomaterials (NMs) used as antioxidants, antimicrobials, anticancer agents, therapeutics, and diagnostics agents, and in the fabrication of nanosensors. The NMs have been intensively applied in many

different scientific and industrial fields. However, production of biogenic NMs in nanotechnology and their uses in medicine have become the fastest developing and most attractive research. The use of plant extracts is prominent, not only because of their easy production and cheap cost compared to other biomolecules (protein, enzyme, peptide, and DNA), but also because they provide large-scale and environmentally benign NMs, which can widen their medical applications.

## 2 A Comparative Study of Extraction Methods for Medicinal Plants

Medicinal plants have been practically used as effective and traditional drugs or biocides for various disorders for a long time. The research has focused on the elucidation of the chemical structures of these plant extracts. The physicochemical properties of plants are investigated at the following steps: (1) authentication, (2) extraction, (3) separation, (4) isolation, (5) characterization of isolated compounds, and (6) quantitative evaluation. The methods vary in simplicity, cost, efficiency, and degree of extracted or isolated molecule damage. It is worth mentioning that each extract needs its own characteristic extraction method for production with greater efficiency. For instance, while essential oils as volatile compounds in aromatic plants are extracted using distillation methods, solvent extraction methods are viable and suitable for obtaining other volatile compound-rich extracts.

In addition to conventional extraction methods, some modern extraction methods including microwave-assisted extraction (MAE), ultrasonication-assisted extraction (UAE), supercritical fluid extraction (SFE), and solid-phase micro-extraction (SPME) have been developed and are actively used. They display certain advantages over conventional methods. Although classical methods are fairly simple, standard, and have widespread use, they consume large quantities of organic solvents, cause degradation of heat-labile constituents, produce extracts with a low yield, and have time-consuming and labor-intensive procedures. The use of these classical extraction methods allows the benefits of production efficiency, selectivity, and the elimination of additional steps of modern extraction methods before chromatographic analysis allows them to be used intensively and preferentially. The extraction procedures can also be redesigned to obtain the desired molecules by manipulating experimental parameters. Additionally, the extraction method selection to isolate targeted components with the highest yield and highest purity is dependent upon the plant source [1, 2]. Thus, the development of modern extraction methods plays an important role in the overall effort of ensuring and providing high-quality herbal products [3].

# 3   Preparation of Extract for Nanoparticle (NP) Synthesis

The extraction of plant contents has received considerable attention owing to the use of plant contents in medicine, nanoparticle (NP) synthesis, and biosensing. Three major extraction methods for NP synthesis are: (1) solvent extraction, (2) micro-wave-assisted extraction, and (3) maceration extraction. The ideal extraction method should be cost-effective, simple, less time-consuming, and simply conducted in any laboratory.

## 3.1   Solvent-based Extraction Methods

The solid-liquid extraction provides soluble components in the solid material to be integrated with the solvent. The mass transfer ratio decreases as the concentration of the active principle in the solvent increases. This process results in the solvent and solid material reaching an equilibrium concentration when a mass transfer of the active components from plant material to solvent occurs. There are different types of this technique: cold percolation, hot percolation, and concentration [4, 5].

### 3.1.1   Cold Percolation

The extraction of the plant contents is carried out in a percolator that is connected to a condenser and a receiver for removing the solvent from the mixture. The powdered material is in contact with the percolator along with a suitable solvent until equilibrium is reached.

### 3.1.2   Hot Percolation

The principle of hot percolation is based on increasing the temperature of the solvent, which increases the solubility. The extract is permanently passed into a tubular heat exchanger by steam heating.

## 3.2   Microwave-assisted Extraction Technique

Microwave-assisted extraction (MAE) allows the materials to reach the given energy that is associated with the dielectric susceptibility of both the solvent and the solid plant material, through rapid heating [6, 7].

### 3.2.1 Maceration

The maceration process can be completed in three steps: (1) grinding of plant materials into small particles, (2) choosing the appropriate solvent, which is added in a closed vessel, and (3) filtration for the separation of the liquid phase from the plant pulp. The mixture of the plant powder and solvent should be shaken to provide proper extraction with a high efficiency [8, 9].

## 4 Isolation of Specific Molecules from Plant Extracts Using Appropriate NPs

It is well known that titanium dioxide ($TiO_2$), with a certain crystal form, potentially reacts with phosphorylated biomolecules including peptides, proteins, and glycoproteins, under the proper acidic experimental conditions. The phosphate moieties of those molecules specifically attach to the surface of $TiO_2$ NPs and retain their surfaces, which provides the isolation or enrichment of the corresponding molecules.

Recently, $TiO_2$ NPs have been integrated into plant nanobiology for isolation of specific molecules from plants. In a recent studies, $TiO_2$ NPs acted as nano-harvesting agents to isolate bioactive compounds from living cells. For instance, Kurepa et al. [10] used phosphorylated anatase $TiO_2$ NPs with a 20-nm diameter to capture the specific flavonoids from Arabidopsis plants. This work showed that quercetin and kaempferol as enediol and catechol groups containing flavonoids can successfully bind to the phosphorylated $TiO_2$ NPs, and they were isolated from the plant matrix.

## 5 Synthesis of NPs Using Plant Extracts

The production of colloidal metallic NPs has become one of the fastest developing and most exciting fields of research and has had an enormous impact on the evolution of nanotechnology over the past decades. The size, shape, and composition-dependent electronic, optical, luminescent, and magnetic features of the NPs with great enhancement have found a wide spectrum of applications in scientific and technical fields [11–16]. In general, three major methods, chemical, physical, and biological, have been actively and extensively used for the synthesis of NPs. Although chemical methods are used the most for the synthesis of high-quality NPs with a narrow size distribution, the use of toxic organic solvents as well as reducing and stabilizing agents greatly limits the applications for NPs, especially in biomedicine and bioanalytics [16–19]. Additionally, in order to use NPs synthesized in organic solvents in biologically-related applications, phase transfer is an indispensable step for introducing the NPs into aqueous solutions. The NPs can be made

water-soluble with two common surface-engineering procedures: (1) ligand exchange and (2) ligand polymerization [20–25].

Physical methods include simple one-step procedures and provide large-scale production in a short time. However, those methods almost always result in a lack of size, shape, and size distribution of the NPs [26, 27]. To address the drawbacks encountered in chemical and physical methods, researchers have recently focused on biological methods called "green methods" for NP synthesis [28, 29].

The main principle of green methods is to use nontoxic biomolecules including DNA, proteins, enzymes, carbohydrates, and plant extracts for the synthesis of biocompatible metallic NPs through the reduction of metal ions in aqueous solution [30–38]. Although DNA, proteins, and enzymes have been employed as scaffolds for nucleation and growth of metallic NPs with unique crystalline structures [39–43], those biomolecules are quite costly, easily decomposable, and can be contaminated. In contrast to those molecules, plant extracts are easily reachable, quite affordable, and very stable against environmental conditions (temperature, pH, and salt concentration).

Plant extracts are a rich source of polyphenols, flavonoids, sugars, enzymes, and/or proteins, and can be utilized as reducing and stabling agents for the biosynthesis of metallic NPs. In the potential proposed mechanism, hydroxyl, amine, and thiols groups existing on particular extracts of plants may bind to metal ions, canalize electron flow from the extracts to metal ions, and lead to the completion of the eventual NP synthesis [44–49]. Numerous numbers of plant extract-directed metallic NPs have been synthesized and used in various fields [47–49]. Using plant extracts for the rapid reduction and formation of metallic NPs was discovered by Sastry and co-workers. They used lemongrass plant extract to synthesize the spherical gold NPs and triangular gold nanoprisms [50]. In addition to the aforementioned unique properties of plant extracts, plant-based biogenic synthesis can provide cost-effective, environmentally-friendly, simple, less labor intensive, and large-scale production procedures.

## 5.1 Synthesis of Silver and Gold NPs

Silver (Ag) and gold (Au) are two commonly synthesized plasmonic NPs, due to their unique intrinsic properties. Ag NPs have been considered as effective and universal germicidal agents against various microbes. Their use has also been recognized in nanomedical and industrial applications [51–54]. As stated above, the plant extract-based synthesis method for Ag NPs provides simple, one-step, and rapid procedures compared to other synthesis methods. The extracts produced from different parts of the plant such as leaves, roots, seeds, and fruit act as potential reducing and stabilizing agents to form Ag NPs of various sizes and shapes. For instance, square, spherical, triangular, and hexagonal-shaped Ag NPs with diameters ranging from 10 to 90 nm were synthesized using leaf extracts obtained from plants [55–57]. In our view, different plants contain different contents in the extract, which

may lead to the formation of Ag NPs of various sizes and shapes. The synthesis of Ag NPs of various morphologies was systematically reported using extracts of roots, seeds, and fruit [58–60].

Similar strategies have been used for the synthesis of Au NPs. Au NPs have received considerable attention due to their attractive optical and non-toxic properties, which market them for use in a wide variety of scientific areas including nanomedicine, nanoelectronics, nanobiosensing, and catalysis [61–66]. Similar to Ag NPs, the extracts obtained from different parts of plants reacted with Au ions to reduce them and to eventually form Au NPs. For instance, while leaf extract from the *Menta piperita* plant resulted in the formation of spherical Au NPs with a diameter of 150 nm, triangular-, hexagonal-, and pentagonal-shaped Au NPs with a size ranging from 5 to 500 nm were synthesized from the extracts of *Coriandum sativum, Memecylon edule, and Magnolia kobus* plants [55, 67, 68].

# 6  Plant Disease Treatment

Various techniques have been developed and applied to control microorganism-caused diseases in plants. For instance, the antibiotic streptomycin, as part of a chemical technique, was used in the 1950s to prevent the proliferation of *Xanthomonas vesicatoria* found on plants. However, those bacterial strains developed resistance against streptomycin and thus made it ineffective [69]. Copper-based (Cu) bactericides incorporated ethylene-bis-dithiocarbamate (EBDC) fungicides (e.g., maneb or mancozeb). These fungicides (e.g., maneb or mancozeb) have acted as potential biocides in order to effectively manage the diseases existing on plants. Nevertheless, Cu-resistant bacterial strains have been observed due to their frequent use and the resulting drastic reduction in antimicrobial activity of those biocides [70–72].

Research has focused on investigation and development of bacteriophages and systemic-acquired resistance (SAR) inducers as alternative disease-management techniques over the last decade [73, 74]. As an example, acibenzolar-S-methyl (ASM) was used as an SAR inducer agent, to activate and enhance plant defense systems by increasing the transcription of stress-related genes against bacterial tomato spot [73]. Although bacteriophages have been introduced as biological alternatives to Cu-based bactericides, real-time use in the field reduced their viability and then their use was highly limited due to environmental conditions [75, 76]. It is worth mentioning that only very few chemical techniques are available and there is an urgent need to develop effective, biocompatible, and economical materials for disease management.

No reports have fully explained the mechanism underlying the antimicrobial activity of NPs, and the mechanism is still under debate. Recently, various types of single-component metallic NPs and metal-graphene oxide (GO) NPs have been synthesized and used as novel and effective antimicrobial agents for the management of agricultural crop diseases. The key point in the use of NPs is their toxicity, which

can adversely influence environmental and human health [77–80]). For instance, Paret et al. studied antibacterial properties of light-activated titanium dioxide ($TiO_2$) and metal-doped hybrid $TiO_2$ NPs ($TiO_2$/Ag, $TiO_2$/Zn) against *Xanthomonas perforans*, which causes bacterial tomato spot disease. This study demonstrated that $TiO_2$ did not show any antimicrobial function under non-illuminated conditions and only $TiO_2$/Ag exhibited some antimicrobial activity due to the intrinsic antimicrobial property of Ag. In contrast, all $TiO_2$-based NPs effectively inhibited bacterial growth when exposed to an incandescent light intensity of $3 \times 10^4$ lux. The combination of the photocatalytic activity of $TiO_2$ and the natural germicidal activity of Ag introduced the best antimicrobial activity under illuminated conditions [80].

## 6.1   *Antimicrobial Properties of Silver Nanoparticles*

Silver NPs have been considered to be the strongest and most universal biocides compared to other metallic NPs. The one logical proposed mechanism offered is that Ag NP may interact with some functional groups (thiol, carboxyl, hydroxyl, amino, and phosphate groups) existing on bacterial membranes, with membrane degradation then leading to serious structural deformation. In addition to that, some Ag NPs can be internalized through the membranes and may inactivate or distort the working function of enzymes, which may lead to cell death [81, 82]. However, when Ag NPs are aggregated, their antimicrobial activities are weakened and can be lost. Most recent works show that Ag-GO nanocomposites overcome the limitations of bare Ag NPs. Ag-GO nanocomposites display extraordinary antibacterial activity that results in rapid killing [83, 84].

## 7   Conclusion

The type of extraction method used varies according to the type of content in the extracts. The use of plant extracts has advantages over other biomolecules (proteins, enzymes, peptides, and DNA) in terms of the biosynthesis of metallic NPs, because they are inexpensive, easily producible, and accessible. They provide environmentally friendly NPs with the ability for large-scale production. For these reasons, plant extract-directed NPs can potentially be used in various bioanalytical and biomedical applications as antioxidants, antimicrobial agents, anticancer agents, therapeutics, diagnostic tools, and drug-vehicle agents.

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