

Green Synthesis and biological Applications of Zinc Oxide Nanoparticles: A Plant-Mediated Nanobiotechnological Approach

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Abstract

Green synthesis is an emerging field in the domain of modern nanotechnology that provides ecofriendly and sustainable nanoparticles which are stable in nature and have varying size and shapes. In the case of zinc oxide (ZnO) nanoparticles, these are particularly remarkable anticancer activity largely due to their high antibacterial activities and such activity is mainly as a result of the surface reactivity and their activity is increased when the size of particles is reduced. As a result of decreasing size, the surface area of these nanoparticles increases and, therefore, their interaction with bacteria is improved. ZnO is also notorious as biocompatible and non-toxic, which means that zno is safe for biomedical applications. One of its major mechanisms is production of reactive oxygen species (ROS) which may permeabilize microorganisms' membranes and damage their genetic material and thus kill them. Due to these properties, ZnO nanoparticles are being pursued as effective antimicrobial agents, especially for killing multidrug-resistant (MDR) bacteria. This review is dedicated to the green synthesis of ZnO NPs via numerous plant based sources, and describes their attractive activities for healthcare and other related fields.

Keywords: Zinc Oxide Nanoparticles, Green Synthesis, Plant Extracts, Antibacterial Activity, Reactive Oxygen Species (ROS), Nanobiotechnology

1. Introduction

The increase of drug-resistant microorganisms infections has become an urgent global public health problem with considerable morbidity and mortality. This is the effect of having pathogens such as bacteria, fungi, viruses, and protozoa become resistant to traditional antimicrobial treatments, such as antibiotics, antifungals, antivirals, and antiprotozoals [1]. With conventional drugs becoming increasingly ineffective against these strains, there is a rising demand for new strategies to control and clear these antibiotic resistant strains [2].

Nanotechnology, the study of manipulating matter at the nanometer (nm 1–100 nm) scale, constitutes a potential platform for meeting these challenges. Nanoparticles display special physical-chemical, optical and magnetic characteristics, which are quite different from the corresponding properties of the bulk material, and they are mostly associated with their large surface area to volume ratio as well as, their nanometers scale size [3]. One of these types of nanomaterials, which has recently attracted much attention in antibacterial actions, are the ZnO nanoparticles because of their multifunctional characteristics and high potential to go for antibacterial actions [4].

Currently, ZnO nanoparticles have been widely used for various purposes such as biomedicine, electronics, diagnosis and environmental science. Their part in antibacterial application is particularly prominent, since they can perturb bacterial membranes, induce oxidative stress and inhibit vital intracellular enzymes and all of these can lead to the inactivation of the microorganism 5. In this context, the ZnO nanoparticles are emerging as a promising bioactive agent compared to antibiotics, especially in cases of the multidrug-resistant (MDR) organisms.

2. The Green Method of the Synthesis of Zinc Oxide (ZnO) Nanoparticles

The green synthesis is considered as an interesting method to synthesize zinc oxide (ZnO) nanoparticles from the chemical method. The plant-derived methodology is environmentally friendly, scalable and economical, as well as being free from harmful chemicals, safer for human health and environmental habitats. ZnO nanoparticles, in particular, have been increasingly recognized for their utility in gas sensing, photocatalysis, optoelectronics, and more importantly for having strong and effective antimicrobial activity [7].

ZnO is a water-insoluble white powder that is structurally characterized by very strong antibacterial activity. One of its important functions is that it can produce reactive oxygen species (ROS) to attack the membranes of microorganisms and decompose toxic organic pollutants (e.g., pesticides and herbicides) [8]. The principle of green synthesis is based on the bioactive molecules present in the plant extracts like polyphenols, alkaloids, flavonoids, terpenoids, proteins etc., that serve as natural reducing and stabilizing agents at the time of nanoparticle formation [9].

The synthesis generally involves the selection of plant material (usually leaves, stems, root, flowers) followed by a series of processing. These components are washed initially with tap water and subsequently distilled water to remove dust and impurities, shade dried for 10-15 days. The dried plant materials are then pulverized to a fine powder with a blender. The powder is dissolved in Milli-Q water and is boiled with stirring with the aid of a magnetic stirrer. The filtrate is filtered and the clear extract is methanolic extract.

This extract is mixed with a zinc precursor including zinc nitrate ($Zn(NO_3)_2$) or zinc sulfate ($ZnSO_4$), and with sodium hydroxide (NaOH). The solution is warmed at a gentle temperature of 30° to 35°C for 1 to 4 hours. A color change in the solution, usually yellow, can be observed, which corresponds to the ZnO nanoparticles being formed. Such particles are harvested by centrifugation and further characterized based on UV-visible spectrophotometry for revealing their characteristic extinction peaks [9].

Different plants have been employed as effective precursors for the synthesis of ZnO nanoparticles of different size and shape. For example, ZnO nanoparticles with an average size of 18 nm are prepared from azadirachta indica leaves, whereas aloe vera leaf extract can lead to particles of size within 8-20 nm with spherical, anisotropically oval, or hexagonal shape. Likewise, the granular-shaped particles ($d = 24$ nm) derived from Moringa oleifera and uniform shape spherical particles ($d = 13.3$ nm) obtained from the fruit extract of Rosa canina extract were produced. The variation in particle shapes across species is also observed in species such as Agathosma betulina, which produces quasi-spherical agglomerates and Santalum album, that forms nanorods [10].

The green synthesis approach is a rapid, simple, cost effective and eco-friendly method for the synthesis of ZnO nanoparticles that could be utilised for various biomedical, environmental and industrial applications.

3. Parameters Influencing Green Synthesis of Zinc Oxide (ZnO) Nanoparticles

Different experiment conditions are quite crucial in controlling the quality, dimension, shape and stability of ZnO nanoparticles in green synthesis. Within these, pH value, temperature, and time of reaction and composition of plant extract are the most contributing parameters.

3.1 Effect of pH

The size and morphology of ZnO-NPs are found to be influenced by the pH. The pH of the reaction medium plays a vital role for both the size and structure of ZnO-NPs. Under more acidic circumstances, (lower pH) the nanoparticles continue to aggregate and to compose larger assemblies. In alkaline condition (higher pH), zinc salts can be reduced more efficient to produce smaller and dispersed nanoparticles. For instance, agglomeration of as-synthesized ZnO nanoparticles using Sargassum myriocystum extract was observed at pH 5-7, while complete reduction and uniform dispersion of particles were achieved at pH 8 [14].

3.2 Temperature and Time Effects

Temperature is an important parameter in the synthesis of green nanoparticle. The reaction usually works effectively at below 100°C For the synthesis of ZnO nanoparticles from *Sargassum myriocystum*, the optimal temperature was 80°C, and the formed ZnO particles were well-defined with an UV-visible absorption peak at 376 nm—a proof of successful formation. Opposite temperature values resulted in not satisfactory neither quality nor clearness of nanoparticle production [14].

This is thought to be one of the significant factors in the overall yield of nanoparticle synthesis. Time of incubation depends on the different type of plant extract. For example, the reaction time (± 6 h) to obtain complete development and stabilization of ZnONPs, synthesized from *Parthenium hysterophorus* leaf extract, was needed [15].

3.3 Plant Extract Formulation

The bio-chemical composition of the plant extract employed during the synthesis has a marked effect on the nature of the fabricated nanoparticles. The extracts with a high content of the functional groups (hydroxyl, carboxyl, ketone and amine groups) help reducing and capping the zinc ions. For instance, leaf extract of *Azadirachta indica* harbors several active groups that contribute to synthesis of small spherical nanoparticles. However, SEM images also showed unevenness in shape. *Agathosma betulina*, containing a lot of hydroxyl groups, may form quasi-spherical agglomerates instead [16].

These observations reflect the necessity to heedfully optimize reaction conditions, such as pH, temperature, duration, and plant biochemistry, to obtain reproducibly desired properties of ZnO nanoparticles. Controlling the size and shape of nanoparticles is critical for designing them according to a particular biomedical and environmental need [5-7].

4. Applications of Zinc Oxide (ZnO) Nanoparticles

Zinc oxide (ZnO) nanoparticles are known for their strong antibacterial potential, which mainly arises from the capability of (ROS) production, generating hydrogen peroxide (H_2O_2). These ROS are able to cross the bacterial membrane and damage essential cellular components, and consequently induce bacterial killing [18].

The generation of hydrogen peroxide by ZnO NPs governs one of the major antimicrobial action. It breaks through the bacterial cell wall, disrupting the interior construction and making it difficult for the organism to replicate or repair itself. For example, approximately 13 nm ZnO nanoparticles have been demonstrated to inhibit *Staphylococcus aureus* growth up to 95% at 80 $\mu\text{g}/\text{mL}$ via ROS-induced membrane destruction [19]. Larger size particles, such as those ~60 nm, showed less activity, suggesting that smaller particle size increases bactericidal effectiveness [19].

ZnO NPs have shown an effectiveness towards Gram-positive as well as Gram-negative bacteria. At 400 $\mu\text{g}/\text{mL}$, 40 nm-sized particles eliminated nearly 99% of *E. coli* and *S. aureus* populations via bacterial membrane disruption [20]. This action is partially due to the abrasive nature of NPs and interaction with lipid bilayer of microbial cells [21].

Apart from their antimicrobial activities, ZnO nanoparticles have been widely utilized for medicinal and healthcare products. Traditionally, they have been utilized to cure diseases including diarrhea, epilepsy by oral treatment [22]. In recent times, ZnO nanoparticles are being added into sunscreens for its capability to absorb harmful UVA and UVB rays as well in dermatological formulations, for the treatment of inflammation, itching and healing of wounds [23].

They are also used in dusting powders, dietary supplements, and dental pastes. Due to their oxidizing nature, they are also employed in disinfectants and for the degradation of pollutants, such as in water treatment systems. More recently, ZnO-nanoparticles are also being studied for bioimaging, biosensing, and gene therapy due to its high surface reactivity and biocompatibility [24].

In industry, ZnO nanoparticles are used to enhance thermal conductivity and as cross-link reactions in rubber production. In the textile field, they are used as ultraviolet (UV) neutrals and air-permeable agents to improve fabrics protection factor [25].

5. Conclusion

Nanoparticles are in milli-, micro- or nanometer and have large specific surface area, so that they are more likely used on a specific job. Of a variety of metal-based nanomaterials, zinc oxide (ZnO) nanoparticles are particularly interesting from the versatile activity and simple preparation by green routes. Using plant extracts in ZnO nanoparticle synthesis is an efficient, green and cost-effective alternative to the conventional methods as it does not involve the use of hazardous chemicals in synthesis and reducing the hazardous effect on environment.

The green synthesis of GNPs incorporating the antioxidant, phytochemicals, are essential due to their use as a natural reducing, stabilizing, and capping agent, which is responsible for the size, shape, and stability of the particles. A variety of plants has been effectively employed to synthesize ZnO-nanoparticles of different size, shape and suited requirements.

ZnO nanoparticles have shown strong antibacterial ability and can be the potential agents against multi-drug resistant (MDR) pathogens. They used their oxygen capacity to generate reactive oxygen species (ROS), particularly peroxide, to disrupt the microbial membrane and cell structures. In the present study, nanoparticles of ZnO are shown to be particularly potent against both Gram +ve and Gram -ve bacteria such as *E. coli*, *Staphylococcus aureus*. For instance, at a concentration of 400 µg/mL, ZnO NPs inhibited microbial growth by 99% and 95% against *E. coli* and *S. aureus*, respectively.

Their MICs are also moderately higher compared to some other particles; however, ZnO is still an attractive antibacterial agent because of its selective toxicity to the microbes and general safety in humans as well as in animals. In addition to antibacterial properties, ZnO nanoparticles show potential in cosmetic industry, environmental treatment, food safety, and biomedical engineering among " which the drug release and wound healing and UV absorption are some of the few applications. In conclusion, green-prepared ZnO nanoparticles are a sustainable and efficient response to the novel microbial challenges, also helping to make UV-light balls for various fields.

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