



Eco-friendly synthesis of zinc oxide nanoparticles: A comprehensive review of methods and applications

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Abstract

Recent research in nanoscience has increasingly focused on the green synthesis of plant-derived materials at the nanoscale. Zinc oxide nanoparticles (ZnO-NPs) represent one of the most popular areas of study. Because of their wide band gap and high exciton binding energy, ZnO nanoparticles (ZnO-NPs) are considered highly suitable for a range of applications, including antibacterial and anticancer therapies. They also demonstrate notable anti-inflammatory, antioxidant, and photocatalytic activities, especially under harsh or toxic environmental conditions. Conventional methods for producing ZnO-NPs, including ball milling, hydrothermal techniques, sol-gel processes, chemical vapour deposition, precipitation and co-precipitation, frequently rely on toxic chemicals and harsh conditions. In contrast, green synthesis approaches employ plant extracts and other eco-friendly materials, making them an attractive, sustainable alternative to hazardous physicochemical methods. This review offers a comprehensive overview of the various approaches to the green synthesis of ZnO nanoparticles reported over the past five years. Initially, the review offers a concise overview of the conventional methods commonly employed for synthesizing ZnO-NPs. The review will subsequently provide a detailed examination of the various plant-based extracts employed in the green synthesis of ZnO-NPs.

Keywords: Zinc oxide nanoparticles (ZnO NPs), Green synthesis, Plant extracts, Eco-friendly nanotechnology, Biomedical Applications

Introduction

Advanced multidisciplinary research in both science and engineering, and the field of nanotechnology is concerned with manipulating and designing materials, and structures and systems on an atomic or molecular level; typically these are between 1-100 nanometres (nm) in dimension ^[1, 2]. ZnO nanoparticles possess excellent photocatalytic and biomedical properties. Therefore, they have attracted significant attention from researchers in various scientific fields ^[3]. The majority of traditional methods used to produce metal nanoparticles (MNPs) utilize toxic and harmful chemicals, which present serious environmental risks. To obtain nanoparticles with regulated particle size and structure, a variety of synthetic approaches have been developed.

Although nanoparticle synthesis using these processes produces excellent quality nanoparticles, the need for a better understanding of the synthesis process continues to be important for successful commercial or industrial use. A common way to describe how nanoparticles are produced is through either a "bottom-up" method (as shown in Fig. 1) or "top-down" method. The "top-down" method, which includes traditional preparation techniques, has included methods such as ball milling, sputtering, lithography methods and etching ^[4]. Green synthesis of metal oxide nanostructures is an easier process than microbial (e.g., fungus and algae) approaches. Since many reactions are influenced by both the time required for the reaction and the environment of the reaction, it can be much simpler to predict the size, shape, etc. of metal oxide nanoparticles produced via biological extraction from medicinal plants and algae compared to microorganisms. The chemical

structure of the phytochemicals contained in these biological systems includes a wide variety of functional groups, including carboxylic acids, esters, phenol, aldehyde, ketone and terpene, which will significantly influence the synthesis of metal oxides ^[5].

The top-down process of developing nanoparticles is developed by taking larger amounts of material and then decreasing their particle size, in many cases using techniques including Laser Ablation, Lithography, Ball Milling and Pulsed Electrochemical Etching. The opposite is true for the bottom-up method, where a number of small building blocks (atoms, ions or molecules) come together to form nanoparticles. Methods such as sol-gel processing, hydrothermal synthesis, co-precipitation, and thermal decomposition are widely employed for the preparation of nanoparticles with controlled size and morphology ^[6].

The novelty of this paper is that it examines all recently developed green synthesis processes for creating zinc oxide (ZnO) nanoparticles from plant-based materials, as well as the application of these nanomaterials for photocatalysis and various biomedical activities; i.e., antimicrobial, anticancer, anti-inflammatory, and antioxidant effects.

Green Synthesis of ZnO Nanoparticles

Zinc oxide nanoparticles may be prepared through physical, chemical, or biological synthesis techniques. Depending on the synthesis strategy, these methods are broadly classified into top-down and bottom-up approaches. In the top-down approach, bulk materials are broken down into nanosized particles using mechanical techniques such as milling, grinding, cutting, or attrition. This method is generally employed for producing small amounts of nanoparticles ^[7].

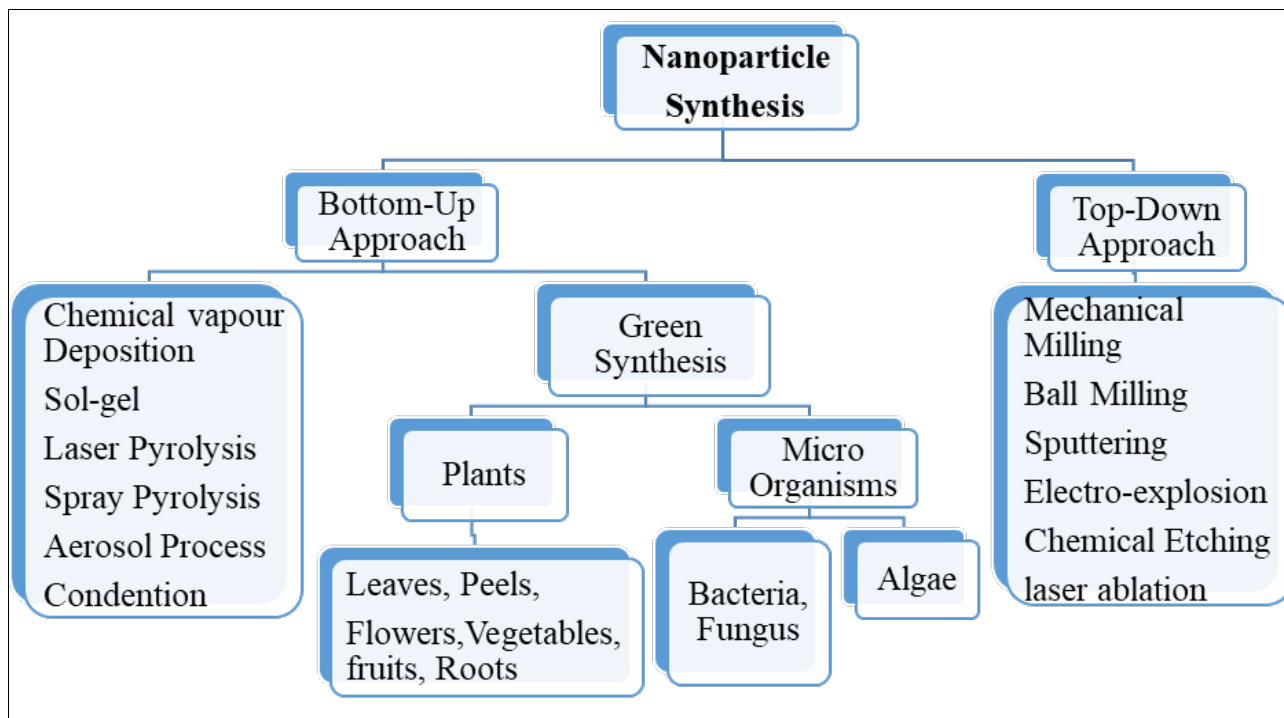


Fig 1: Green Synthesis of ZnO Nanoparticles

1. Top-Down Approach

Mechanical milling, Ball milling, Sputtering, Electro-explosion, Chemical etching, Laser ablation are all forms of the top-down process [8]. The top-down approach employs many different types of processes (mechanical, chemical, physical) to break down large materials into small nanoparticles. Top-down can be described as an aggressive form of synthesis where it uses mechanical force to "carve" out a material in order to create the desired shape at the nano level. This method converts larger molecules and deconstructs it to smaller components that will eventually become useful nanoparticles [9]

2. Bottom-Up Approach

The bottom-up methodology (also referred to as the "building up" strategy) is just the opposite of the top-down method. This method includes gathering atoms and molecules together to create an array of various nanoparticles. Methods for producing this type of nanoparticle include biological agents, aerosols, sol-gel techniques, layer pyrolysis, and chemical vapour deposition. Due to their environmental compatibility and practical advantages, green or biological bottom-up approaches have gained considerable attention among researchers in recent years. These methods utilize microorganisms such as bacteria, yeasts, and fungi for the synthesis of nanoparticles. In addition, this approach is regarded as both economical and environmentally friendly [10].

Typical Synthesis of ZnO nanoparticles

Green synthesis: This section briefly outlines the synthesis of ZnO nanoparticles through both top-down and bottom-up approaches. Top-down methods primarily include ball milling, while bottom-up techniques involve sol-gel, hydrothermal, and precipitation methods.

1. Ball milling

Ball milling in material synthesis has gained significant attention because of its cost-effectiveness and environmentally friendly characteristics that can address challenges associated with the use of dangerous chemical processes, high-temperature; and high-pressure; while also providing opportunities for mass production. Ball milling is one of the most direct, fastest and easiest techniques to produce nanoparticles of varying dimensions. The process begins when a milling vial is charged with a specified amount of powder sample and several milling balls. Following, the grinding action repeatedly strikes the surface of the milled material, reducing the average diameter of the particles to obtain nanoparticles [11]. ZnO nanoparticles have been the focal point of this investigation for their application to biological systems. The same preparation method was employed to generate ZnO nanoparticles (at varying calcination temperature from 400 to 900°C) using an identical mill configuration. The 20-hour milling of the ZnO powder generated desolvated ZnO particles.

2. Sol-gel method

The reactions taking place during the formation of oxide nanostructures prepared through a microwave-assisted sol-gel technique are still not well understood. In addition, analysing the chemical structure of the generated material may help researchers develop new theoretical models for the use of these materials in various applications. Plant-based systems for generating nanoparticles has been an emerging trend in recent years due to the fact that they are safer, quicker and lower cost than other biological alternatives (e.g., bacteria, algae, fungi). As shown in Fig 2, plant extracts possess elevated levels of bioactive constituents, including phenolic acids, flavones and carotenoids, with demonstrated antioxidant activity [12].

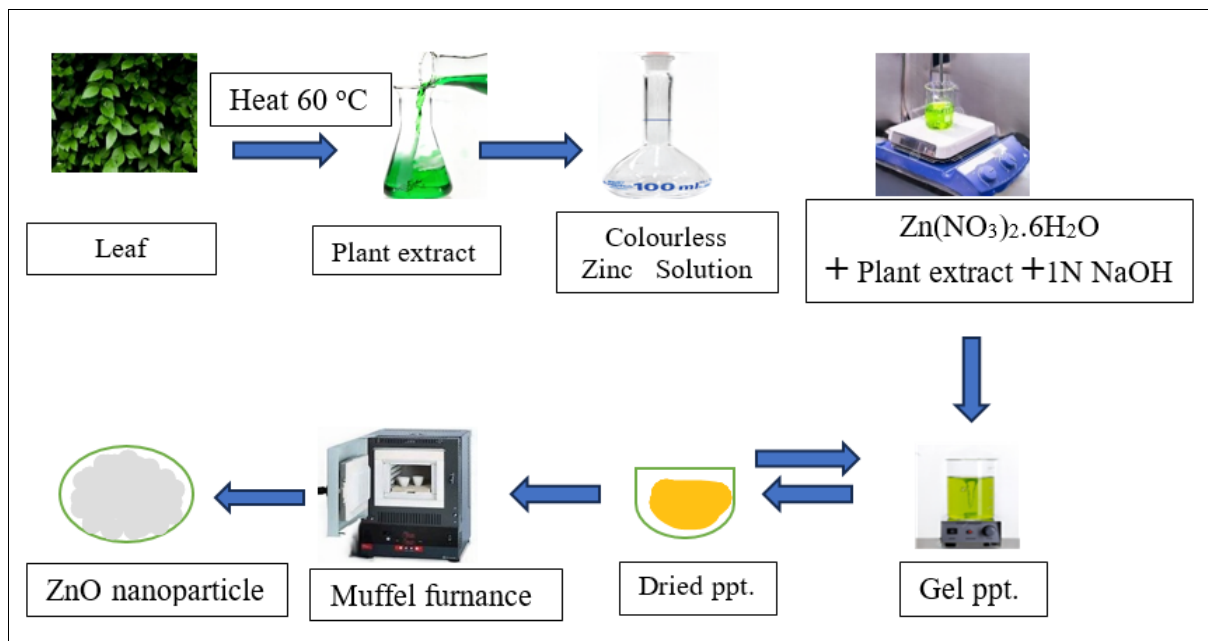


Fig 2: Eco-friendly synthesis of ZnO nanoparticles using the sol-gel technique

The sol-gel process uses either colloids or a chemical solution (sol), which is used to create a network (gel) of molecules in an organic or aqueous solvent. There are five stages to the sol-gel process as illustrated by fig. These include hydrolysis and polycondensation reactions, gel formation, maturation, dehydration, and crystal growth.^[13] It is a flexible low temperature process that has been developed to produce highly specialized materials having well defined nanoscale structures, especially metal oxide. The sol-gel process converts molecular precursors into solids via a series of chemical reactions that occur from solutions^[14]. Metal oxide synthesis begins by forming precursor materials, usually metal alkoxide or inorganic salt. Metal oxides, including ZnO, zinc hexanitrate and zinc acetate, are preferred due to their high reactivity which results in formation of an oxide network through hydrolysis. Additionally, a variety of other inorganic salts (such as various nitrates and acetates) can be synthesized if characteristics, such as cost, stability, or water solubility need to be considered [14-15].

Biological method of Zinc Oxide nanoparticles synthesis

The various plant parts are used in the reduction and stabilization of nanoparticles (NPs). Several investigators have utilized eco-friendly methods of generating metal and metal oxide nanoparticles by employing a number of different components of plants, e.g., leaves, stems, roots, and fruits. Fig shows examples of green production of NPs from a variety of plant resources. Plant extracts include an array of biomolecules; among these biomolecules there are proteins, coenzymes, and carbohydrates that assist in transforming metal salts into nanoparticles. Additionally, microorganisms like fungi, bacteria, and yeast may be used as reducing agents because they can reduce metal ions to generate nanomaterials. Various bacterial species have been utilized for the synthesis of metal nanoparticles. This technique utilizes both the extracts of plants and microorganisms to increase nanoparticle formation^[16]. These economical and eco-friendly approaches produce ZnO nanoparticles in a variety of sizes and morphologies, enabling their use in fields such as optoelectronics,

biomedical applications, environmental cleanup, and catalysis. The following section presents a comprehensive overview of these synthesis techniques.

1. Preparation of ZnO by plant extract

The plant is a good example of an inexpensive and environmentally friendly means of producing chemicals using natural processes. Additionally, there are many examples of how effectively plants remove and store heavy metals from the soil. Although trace levels of toxic metals can pose serious risks to humans and animals, the ability of plants to absorb and process these substances is one reason why they are so useful in cleaning up polluted environments. When compared to all other biologically-synthesized nanoparticles (fungi, bacteria, actinomycetes and algae), the advantages of synthesizing nanoparticles with plant extracts include simplicity and large-scale feasibility^[17]. Plant-based materials such as seeds, root, leaf, flower, and fruit have chemical properties that can serve as both effective reducers and caps for metal ions, forming nanoparticles. A typical method of preparing ZnO nanoparticles from an aqueous solution of a plant extract involves obtaining the desired plant. The plant extract is cleaned through washing it with de-ionised (DI) water, dried, ground into a powder, mixed with DI water and filtered to eliminate contaminants. Following filtration the precipitate is collected and purified. The purified ZnO nanoparticle is formed when the precipitated material is annealed^[18].

1.1 Green synthesis of zinc oxide nanoparticles using plant leaves Extracts

A water-based extract obtained from the leaves of *Delonix elata* was used to synthesize ZnO nanoparticles (NPs) by the sol-gel technique in 2025. These particles had a mean diameter of ~ 30.1 nm and were fully characterized for their antibacterial properties^[49]. Triparna Das *et al.* also studied the use of *Prosopis juliflora* leaf extract for the preparation of ZnO NPs by zinc acetate as a precursor, and evaluated their photocatalytic activities^[50]. In addition, a ZnO nanoparticle sample was prepared from the leaves of

Artemisia annua with zinc acetate as a precursor; this particle sample could be tuned to an average size of about 26nm, and its main application area is against parasitic diseases ^[19]. Additionally, it was demonstrated that the incorporation of these synthesized particles can significantly increase the rate of photodegradation of MB dye compared to samples without the incorporation of such particles, and therefore the degradation rates of MB dye ranged from 89.6% up to 96.4%. Finally, to show additional examples of green synthesis methods, Veronika Kolotygina *et al.* have used a water-based leaf extract of *Prunus* (plum) for the preparation of ZnO nanoparticles ^[20].

1.2 Green synthesis of zinc oxide nanoparticles using flower Extracts:

In recent times, there has been significant focus in using flowers for the green preparation of ZnO nanoparticles (ZnO NPs). The aqueous extract of *Senna auriculata* flowers has been used to prepare ZnO NPs exhibiting antimicrobial, cytotoxic, and anti-diabetic properties ^[71]. For photocatalytic dye degradation, Rao KJ *et al.* demonstrated the production of ZnO NPs using banana flower extract ^[21]. Similarly, Yadav RS *et al.* prepared ZnO nanoparticles using extracts from *Chrysanthemum* spp. flowers for photocatalytic degradation ^[22].

Table 2: The size of green ZnO nanoparticles is influenced by zinc types and floral extract. Additionally, this site mentions the application areas

Sr. No.	Plant	Plant's Part	Zinc Salt	Particle size (nm)	Applications	Ref.
1	Viola	Flower	Zn (CH ₃ COO) ₂ .2H ₂ O	20-50	-	23
2	Cassia fistula	Flower	Zn (CH ₃ COO) ₂ .2H ₂ O	-	Antibacterial activity	24
3	Mirabilis Jalapa	Flower	Zn (NO ₃) ₂ .6H ₂ O	50	Photocatalytic activity	25
4	Clitoria ternatea	Flower	Zn (NO ₃) ₂ .6H ₂ O	45-86	Antimicrobial, antioxidants, anticancer	26
5	Nyctanthes arbortristis	Flower	Zn (CH ₃ COO) ₂ .2H ₂ O	115.40	Anti-cancer activity	27
6	Lantana camara	Flower	Zn (NO ₃) ₂ .6H ₂ O	-	Anti-inflammatory activity	28

1.3 The Preparation of ZnO NPs using fruit extract

As shown in Table 3, the use of fruit extracts from different plants as reducing and capping agents for the preparation of zinc oxide nanoparticles (ZnO NPs) has been well established. ZnO NPs with an average size of 41.23 nm were produced using fruit juice from the *Myristica fragrans* plant; these particles were nearly spherical or elliptical ^[80]. An aqueous fruit extract of *Capsicum chinense* was used to prepare ZnO NPs, which showed both cytotoxic and antioxidant activities ^[29]. According to this study, *Citrullus lanatus* fruit extract is a suitable medium for producing spherical, monodisperse, highly crystalline ZnO NPs with a broad band gap ^[30].

Application of ZnO-NPs in biomedical

The biological applications of ZnO nanoparticles are illustrated in Fig. 5. Nanoparticles play a significant role across various aspects of daily life due to their wide range of uses. However, their analysis remains a complex task. They function as molecular-scale tools for addressing medical challenges, utilizing molecular-level data to support and improve human health. In a related study, Fernández Llamosas employed *Azoarcus* for the synthesis of selenium nanoparticles ^[31].

1. Antibacterial Activity

The antibacterial potential of the ZnO NPs was tested with two bacteria, *E. coli* and *S. aureus*. In order to test their antibacterial activity, we used a broth dilution procedure. We preserved the bacteria on trypticase soy agar (TSA), so they could grow for 18–24 hours in an incubator at 37°C. After this, we grew the bacteria again before using them as part of our tests. We used 10⁶ CFU/mL for all of our tests of bacterial concentrations; we did this by taking some of the bacterial solution, mixing it with water until it had a turbidity that matched that of 0.5 McFarland standards. Then, The bacterial cultures were treated with a ZnO nanoparticle solution (0.025 mg/mL) for 8 hours under conditions without UV light exposure ^[32]. The antibacterial activity of the ZnO-NPs was also investigated using the disc diffusion method against both

Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria ^[33]. The bacterial strains were swabbed onto nutrient agar plates, which were then treated individually with distilled water, gentamicin, *Rhamnus prinoides* plant extract, and ZnO NPs. The plates were incubated for 24 hours, and the zones of inhibition were measured in millimetres at the end of the incubation period ^[34].

2. Anti-cancer activity

The preparation of ZnO nanoparticles through a simple precipitation method with starch acting as a non-toxic stabilizer has been studied. The size of the crystallites produced via this process is approximately 32.7nm according to the Scherrer equation and from the FWHM of the peaks observed in X-ray diffraction. While ZnO nanoparticles have shown great promise in terms of their ability to treat various forms of cancer, the mechanism by which ZnO induces apoptosis in cancerous cells is still being investigated. Furthermore, since ZnO nanoparticles can be toxic to many types of cells, including those found in mammals, it will also be necessary to determine the potential toxicity of these particles.

3. Anti-Oxidant activity

According to Nozipho P. *et al.*, preparation of ZnO NPs was superior to the reference material in terms of IC₅₀ values and displayed a substantial percentage inhibition; therefore, they demonstrated a significant cytotoxic and antioxidant action. Likewise, Maqbool *et al.* found that ZnO NPs exhibit antioxidant properties that help alleviate oxidative stress in cellular model systems of numerous infections. In addition, these particles can provide protection to brain cells against oxidative injury in neurodegenerative disorders like Alzheimer's and Parkinson's disease ^[35].

4. Anti-inflammatory activity

The anti-inflammatory effects of zinc oxide nanoparticles (ZnO NPs), with different sizes and morphologies, have been widely explored. Evidence suggests that their anti-inflammatory activity mainly occurs through the

suppression of inflammatory marker genes such as TNF- α , IL-6, IL-1 β , and IL-10. ZnO NPs also decrease the expression of cyclooxygenase-2 (COX-2), an important enzyme involved in the progression of inflammation. Furthermore, these nanoparticles regulate protein release and inhibit inflammatory mediators by suppressing the NF- κ B signaling pathway. According to Zahoor S. *et al.*, inflammation represents a complex physiological response to environmental stress and plays a significant role in the onset of chronic diseases [36]. In another study, Hussien *et al.* investigated the anti-inflammatory efficacy of CBE-ZnO NPs using RAW 264.7 cells. The nanoparticles exhibited 99.18% cell viability at a concentration of 0.05 mg/mL, while viability decreased to 34.07% at 500 mg/mL. Their anti-inflammatory potential was determined by evaluating nitric oxide inhibition in LPS-stimulated RAW 264.7 cells, resulting in an IC₅₀ value of 69.3 μ g/mL [37].

5. Anti-diabetic activity

Zinc oxide nanoparticles, as well as ethanol-, or methanol-based plant extracts, were tested in an α -glucosidase assay to determine their anti-diabetic potential according to. Diabetes is still one of the biggest public health concerns today, which explains why there is so much focus by researchers into finding new and better treatments for diabetes. As many studies have been done on diabetes, the lack of newer and more efficient anti-diabetic drugs has caused the continued need for research into diabetes [38].

M. Zubair *et al.* investigated the comparative anti-diabetic activity of *Acacia nilotica*'s solvent-based extracts versus zinc oxide nanoparticles (ZnO NPs) via an α -glucosidase assay. For their study on ZnO NPs, they examined three different concentrations of ZnO NPs; i.e., 50 μ g/mL, 100 μ g/mL, and 250 μ g/mL. Many authors have suggested that acarbose be used as a positive control for studies focused on anti-diabetic activities because it has been shown to exhibit established anti-diabetic activity. Similarly, other research was conducted by Achasih Q. *et al.* who assessed the anti-diabetic effects of ZnO NPs prepared from *H. cymosum* through examining both glucose uptake and utilization in both C3A liver cell lines and L6 muscle cell lines. The C3A cells treated with ZnO NPs demonstrated increased glucose utilization after 24 hours of treatment when exposed to 15, 30, and 60 μ g/mL of ZnO NPs [39].

Conclusion

In recent times, there is an increasing number of researchers investigating the possibility of using plant extracts as a method for creating eco-friendly Zinc Oxide Nanoparticles (ZnO NPs). This approach is advantageous when considering costs, toxic chemical free products and the environmental benefits associated with this form of nanotechnology. Several methods of synthesizing ZnO NPs have also been reported and reviewed to analyze the potential of eco-friendly synthesized ZnO NPs in application areas that include; opto-electronic devices, renewable energy, healthcare, remediation technologies, and photo-catalytic processes. Many different approaches to prepare ZnO NPs via plant mediated green synthesis have been reported in the literature, which provide insight into controlling particle size, shape and morphology. Although there are many benefits to utilizing plant mediated green synthesis, One significant challenge is the large-scale synthesis of uniform ZnO NPs that maintain structural and functional

characteristics. However, plant mediated ZnO NPs may present exciting possibilities in medicine, drug delivery, healthcare, agriculture, catalysis, energy and photo-catalytic technology. Plant-mediated ZnO NPs are naturally coated with bioactive compounds on their surface thereby providing them with improved antibacterial, anticancerous, anti-inflammatory, and antidiabetic activity.

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Punam D. Bagul: Writing – review & editing.

Prafulla S. Patil: Project administration, Conceptualisation.

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On behalf of all authors, the corresponding author states that there is no conflict of interest.

Abbreviations

NPs (Nanoparticles), TSA (Trypticase soy agar), ROS (Reactive oxygen species), DNA (Deoxyribonucleic acid), MDR (Multidrug-resistant), FWHM (Full width at half maximum), TNF (Tumor necrosis Factor), IL (Interleukin), EBT (Eriochrome Black T), VB (Valence band), CB (Conduction band), and NCI (National Cancer Institute)

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