

Review Article

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Plant mediated-green synthesis of zinc oxide nanoparticles: An insight into biomedical applications

<https://doi.org/10.1515/ntrev-2023-0112>

received March 16, 2023; accepted August 3, 2023

Abstract: Green synthesis of zinc oxide (ZnO) nanoparticles (NPs) using various plant extracts as reducing and capping agents has gained attention in recent research. The green synthesis of ZnO NPs offers several advantages such as being simple, eco-friendly, safe, cost-effective, and reproducible approach with high stability. Hence, this article provides an overview of zinc metal and ZnO compounds, and traditional chemical and physical synthesis of ZnO NPs with primary focuses on the green synthesis of ZnO NPs. This study discusses various plant extracts used and the proposed mechanisms in the green synthesis of ZnO NPs. Additionally, it explores the cytotoxic mechanisms of the green-synthesized ZnO NPs and addresses the various biomedical applications of ZnO NPs, including antibacterial, anticancer, antidiabetic, antioxidant, antifungal, antiviral, antiparasitic, anti-inflammatory, and wound healing. Moreover, the review critically discusses the toxicity of ZnO NPs and emphasizes the need for more toxicological studies to ensure the safety and facilitate the risk assessments and risk management of ZnO NPs. Furthermore, this review underlines the challenges associated with the translation process of ZnO NPs from bench to market, including the complex and time-consuming regulatory approval process for ZnO NPs, which requires a multidisciplinary approach involving scientists, regulators, and manufacturers.

Keywords: antibacterial activity, anticancer activity, antioxidant activity, antidiabetic activity, toxicity, wound healing, antifungal activity

1 Introduction

Nanotechnology is the science of design, synthesis, characterization, and development of nanomaterials [1,2]. Nanoparticles (NPs) are objects that range in size from 1 to 100 nm [3,4]. They are used to target drugs to a specific site in the body by passive or active targeting strategies, which helps reduce the side effects of drugs [5]. Presently, metal oxide NPs (MONPs) are synthesized and are used in many biomedical applications [6,7]. These MONPs include iron oxide NPs (Fe_2O_3 NPs and Fe_3O_4 NPs), magnesium oxide NPs, zinc oxide NPs (ZnO NPs), titanium dioxide NPs, and copper oxide NPs [8–10]. MONPs are being used in diverse fields, including medical treatments, industries like solar and oxide fuel batteries for energy storage, cosmetics and sunscreens, and in textiles [11,12].

Among other MONPs, ZnO NPs have received significant attention due to their distinct physicochemical properties and potential biomedical applications such as antibacterial, anticancer, antidiabetic, antioxidant, antifungal, antiviral, antiparasitic, anti-inflammatory, and in wound healing [13–17]. The characteristic properties of ZnO NPs including size, crystallinity, morphology, and chemical composition showed great potential in biomedical applications [18,19]. The nanoscale size of ZnO NPs can modify their mechanical, chemical, structural, morphological, electrical, medicinal, and optical properties [19].

Recently, the green synthesis approach has been adopted to provide biocompatible and biodegradable ZnO NPs, making them suitable for various biomedical applications [15,20]. The green synthesis approach avoids the production of unwanted or harmful by-products, offering a reliable, sustainable, and eco-friendly synthesis approach [21,22]. This approach minimizes the waste and environmental pollution resulting from toxic substances generated during the chemical synthesis process. In addition, it reduces the use of toxic solvents or chemical agents required for chemical synthesis, forming biocompatible and environmentally friendly NPs [23]. Moreover, green synthesis emerges as an inexpensive and simple

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approach where no sophisticated pieces of equipment are required [24]. Finally, reducing and capping agents, essential for the stabilization of the green-synthesized ZnO NPs, are obtained from natural compounds available in the plant extracts, limiting the use of toxic and expensive reagents [25,26].

The growing interest in the green synthesis of ZnO NPs, its potential applications in various fields, including biomedicine, with the intrinsic properties of the environmentally friendly nature and cost-effectiveness makes it an attractive choice to be reviewed. Therefore, this review aims to provide deep insight information of plant-mediated green synthesis of ZnO NPs recently described in the literature to clarify the unique properties of ZnO NPs that can achieve their optimal bioactivities. This article provides a comprehensive review of the synthesis methods of ZnO NPs, with a particular emphasis on green synthesis using plant extracts. The authors explore the mechanisms involved in the green synthesis of ZnO NPs, the cytotoxic mechanisms underlying their bioactivities, and a thorough discussion of their potential biomedical applications. Additionally, a concise discussion is made on the toxicological studies of green-synthesized ZnO NPs, emphasizing the need for safety, risk assessment, and risk management strategies. Moreover, the authors address the challenges associated with the translation process of ZnO NPs from basic research to successful clinical applications and commercial products, where a multidisciplinary collaboration involving scientists, regulators, and manufacturers is needed.

2 An overview of zinc and ZnO

Zinc is an essential mineral for the body and is present in the brain, bone, muscles, skin, hair, nails, and immune system [27]. Additionally, Zn is a cofactor of >300 enzymes in the body that help in the synthesis of proteins and nucleic acids, maintaining DNA replication and repair, and cell cycle progression and apoptosis [28]. Moreover, Zn plays an important role in host defense against the initiation and progression of cancer [28]. Furthermore, Zn exhibits an antioxidant activity *via* the catalytic action of copper/Zn peroxide dismutase, stabilizes membrane structure by competing with iron and copper which are redox active metals and protects proteins from oxidation by binding to sulfhydryl groups. Further, Zn upregulates the metal-binding protein metallothionein expression which is very rich in cysteine and is an excellent scavenger of the hydroxy free radical ($\cdot\text{OH}$) [29–31]. Finally, Zn acts as an anti-inflammatory agent by regulating the tumor nuclear factor-kappa B (NF- κB) transcription *via* the anti-inflammatory

protein A20 and the receptor signaling pathway activated by peroxisome proliferator- α [31–33]. Deficiency in Zn may lead to the release of vitamin A from the liver due to the reduced synthesis of plasma retinol-binding protein [33,34].

ZnO has been listed by the US Food and Drug Administration as “generally recognized as safe (GRAS)” [35]. Hence, it can be used as a food additive, where Zn is considered an essential trace element that plays a vital role in the growth and development of humans and animals [35,36]. Additionally, ZnO is a II–VI semiconductor, where Zn and O are found in groups 2 and 6 on the periodic table, respectively [37]. ZnO has distinctive properties including chemical, sensing, optical, semiconducting, and electrical conductivity [38]. Moreover, ZnO is characterized by a direct wide band gap of 3.3 eV in the near UV spectrum, high exciton binding energy of 60 meV at room temperature, and a natural n-type electrical conductivity [39]. These characteristic properties make ZnO a potential material to be used for a broad range of applications in electronics, optoelectronic devices, and photocatalysis [40].

Although ZnO has a slight covalent characteristic, it has a strong ionic interaction between Zn and O with higher selectivity and heat resistance, compared with other organic/inorganic compounds. The small size of ZnO improves the Zn^{2+} dissolution rate, making it more biocompatible with normal body cells and a potential antibacterial, anti-inflammatory, and anti-tumor agent [41].

3 Chemical and physical methods for the synthesis of ZnO NPs

The multitudinous applications of ZnO NPs in various fields have raised their production. It has been reported that the global production of ZnO NPs was 570 tons/year in 2010 [42] and between 32,000 and 36,000 tons/year in 2014 [43]. Additionally, it was expected that the production would be between 1,600 and 58,000 tons/year by 2020 [42]. Yashni *et al.* [44] estimated the production cost of ZnO NPs (<100 nm) synthesized from orange peel extract to be 20.25 USD per kg, which was considered a cost-effective method.

Traditional chemical and physical methods have been employed in the synthesis of ZnO NPs [16,45,46]. Chemically, ZnO NPs can be synthesized by sol–gel [47,48], solvothermal [49], vapor deposition [50], co-precipitation [51], hydrothermal [52,53], thermal decomposition [54], and electrochemical reduction methods [55]. Whereas physically, ZnO NPs can be synthesized by laser ablation [56], ultrasonication [57], photoirradiation [58], ball milling [59], evaporation

condensation [60], microwave-assisted combustion [61], sonochemical [62], and mechanochemical methods [63] (Figure 1). These methods have many disadvantages such as high cost, high impurities, instability, and limitations in reproducibility and reliability [64,65]. In addition, they require toxic chemicals that are hazard to the environment and medical applications [66]. Therefore, to avoid these disadvantages, alternative eco-friendly and cost-effective green methods have recently been employed to synthesize ZnO NPs [67–69].

4 Green synthesis of ZnO NPs

The green synthesis of ZnO NPs exhibited many advantages like simplicity, eco-friendly, and biologically safe [70–72]. The green synthesis method reduces the need of using toxic chemicals and increases the limit to produce pure NPs [73]. By using plants or unicellular microorganisms (algae, bacteria, fungi, yeasts, and viruses) for NP synthesis (Figure 1), the addition of stabilizing agents can be reduced [74]. This is because plant phytochemicals (e.g., alkaloids, flavonoids, and polyphenols) and proteins secreted by microorganisms act as reducing and stabilizing agents which provide colloidal stability and prevent agglomeration of NPs [75–77]. It has been shown that plant leaves are the best source for the synthesis of metal and MO NPs. This is because the phytochemicals, especially those present in plant leaf extracts, play a dual role in reducing the metal ions and stabilizing the NPs [20,21]. Additionally, plant waste products or by-products are safe and eco-friendly because they are composed of the leftovers of natural plant extracts [21,78].

Moreover, other green resources such as egg albumin, L-alanine, and starch have been used to synthesize ZnO NPs [45]. Ijaz *et al.* [79] reported that the best method for the synthesis of NPs is one that is not harmful to the environment. Thus, the green method is the most preferable method. Table 1 summarizes the advantages and limitations of the chemical, physical, and green synthesis of ZnO NPs.

The use of plant extracts was found to be more valuable than other biological organisms since it reduces the duration of the reactions from days to hours and prevents the risk and complicated process of preserving cell cultures [86]. The green synthesis using plant extracts provides ZnO NPs with an antibacterial effect that cannot be achieved when using physical and chemical methods. This is because the green synthesis coats the surface of the NPs with many pharmacologically active biomolecules such as flavonoids, organic acids, ketones, aldehydes, amides, quinones, or polysaccharides which enhance the ligand-based conjugation of NPs to the bacterial membrane receptor [72,87,88].

In plant-mediated-green synthesis, the use of “ideal solvents” and plant extracts (as natural resources) is essential to achieve biocompatible NPs, where the utilization of plant extracts is a rather simple and easy process to produce NPs at a large scale relative to bacteria- and/or fungi-mediated synthesis [21,89]. The “ideal solvents” such as water, carbon dioxide (CO₂), and ionic liquids such as hexafluorophosphate (PF₆) or tetrafluoroborate (BF₄) are employed in the green synthesis of MONPs [21,90]. Water is always considered an “ideal solvent” as it is the cheapest, non-toxic, non-explosive, environmentally friendly, and most available solvent [21,91]; whereas, CO₂ is a universal solvent that is available as liquid or supercritical solvent (fluid at temperature

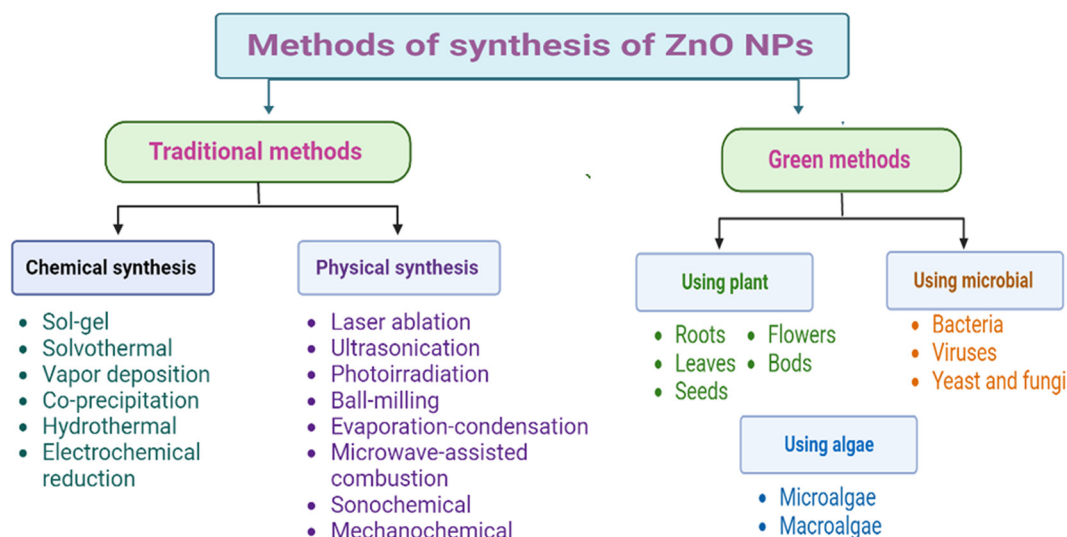


Figure 1: Methods employed in the synthesis of ZnO NPs.

Table 1: Advantages and limitations of the chemical, physical, and green synthesis methods of ZnO NPs

Method	Advantages	Limitations
Chemical synthesis [80–82]	– Controlled size – High purity and quality – NPs are considerably stable	– Use of toxic chemicals – Use of organic solvents – Hazard – Low purity – Capping agents and stabilizers are required to control the size and avoid agglomeration – Some of the capping and stabilizing agents might be toxic – Irreversible pollution of the environment
Physical synthesis [82,83]	– No use of toxic ingredients – High-speed method – High production rates – High purity and quality – Uniform size and shape	– High cost – High energy – Low stability – Irreversible pollution of the environment
Green synthesis [81,84,85]	– Safe and toxic free – Simple and cost-effective – No expensive equipment – No toxic chemicals – Eco-friendly – Biocompatible – Energy efficient	– Difficult to control the particle size and shape – NPs are relatively less stable

and pressure above critical point) [90]. Ionic liquids, which are composed of ions with melting points $<100^{\circ}\text{C}$, are among the best solvents used in green synthesis. This is because ionic liquids act as reducing and protective agents, making the NP synthesis process simpler [21,90].

Although the green synthesis of ZnO NPs, using plant extracts, has attracted attention due to their broad range of applications, there has been a concern regarding their reproducibility and repeatability. This is attributed to the variation in the chemical composition of the phytochemicals of plant extracts of the same species owing to their collection from different parts of the world. This might significantly affect the reproducibility and repeatability of ZnO NPs [92]. Therefore, identifying the phytochemicals present in the plant extracts is crucial to enhance the reproducibility and repeatability of ZnO NPs. Sharma *et al.* [93] used ZnO NPs synthesized greenly from the seed extract of *Carica papaya* (*C. papaya*) to investigate the electrochemical sensing of silymarin molecules which decrease serum transaminases in viral hepatitis patients. The reproducibility of nanocomposite ZnO NPs was tested using three electrochemical sensors independently in 0.2 mM silymarin solution. The nanocomposite ZnO NPs showed promising reproducibility of a relative standard deviation (RSD) of 1.92% ($n = 6$). In addition, a repeatability of RSD of 2.31% ($n = 6$) was reported when the nanocomposite ZnO NPs were investigated for 0.1 mg l⁻¹ silymarin. Moreover, Muthuchamy *et al.* [94] fabricated glucose biosensors immobilized into ZnO NP-

embedded nitrogen-doped carbon sheets for monitoring glucose. The ZnO NPs were synthesized using the peach fruit extract. The reproducibility and repeatability were determined by measuring the amperometric response against 3 mM glucose in phosphate buffer saline (PBS, pH 7). The ZnO NP biosensor showed good reproducibility (RSD = 2.99%, $n = 5$) and repeatability (RSD = 2.86%, $n = 5$).

Several factors may impact the green synthesis of ZnO NPs including temperature, pH, mixing speed, reaction time, plant extract/chemical precursor ratio, calcination temperature, and precursor concentration. These parameters may ultimately affect the shape, size, and phase of ZnO NPs [95]. It has been shown that the higher the temperature of the reaction, the smaller the size of ZnO NPs [96]. Naseer *et al.* [96] used a relatively high temperature of 70°C to greenly synthesize ZnO NPs from the leaf extracts of *Cassia fistula* (*C. fistula*) and *Melia azedarach* (*M. azedarach*). The average nano-size was between 68.1 and 3.62 nm, respectively. In a recent study, Jayachandran *et al.* [97] prepared ZnO NPs using a leaf extract of *Cayratia pedata* (*C. pedata*) at different reaction temperatures (55, 65, and 75°C). A reaction temperature of 65°C confirmed the production of ZnO NPs of 52.2 nm. Hassan Basri *et al.* [98] investigated the effect of the reaction temperature on the size and shape of ZnO NPs greenly synthesized using the peel extract of pineapple. When the reaction temperature was maintained at 28°C , the size of ZnO NPs was between 8 and 45 nm with a mixture of spherical and rod shapes.

However, when the reaction was increased to 60°C, the size of ZnO NPs was between 73 and 123 nm with a flower rod shape.

Padalia *et al.* [99] demonstrated the effect of pH on the size and morphology of ZnO NPs synthesized using the leaf extract of *Salvadora oleoides* (*S. oleoides*). Based on the transmission electron microscope (TEM) analysis, ZnO NPs synthesized at pH 5 were spherical in shape with an average size of 26.6 nm, whereas, at pH 8, irregular shape ZnO NPs with an average size of 38.6 nm were formed. Additionally, ZnO NPs synthesized at pH 5 showed higher antibacterial activity against Gram-positive and Gram-negative bacteria, compared to pH 8, indicating the importance of pH on the biological activity of ZnO NPs. In another study, the effect of pH (8–14) on the green synthesis of ZnO NPs prepared from the leaf extract of *Raphanus sativus* var. *Longipinnatus* was investigated [100]. The authors showed that no UV-vis absorption peaks were observed at pH 8–10 and pH 14. At pH 12, an absorption peak was observed at 369 nm, indicating the formation of ZnO NPs with an average size of 209 nm. Mohammadi and Ghasemi [101] used different pH values (4–10), temperatures (25, 60, and 90°C), and precursor concentrations of zinc nitrate (0.005, 0.02, 0.05, and 0.3 M) to greenly synthesize ZnO NPs using the cherry leaf extract. The results showed that the size of ZnO NPs increased from 87.5 to 116 nm as the temperature increased. Additionally, the optimum synthesis conditions of pH 8, temperature 25°C,

zinc nitrate concentration of 0.005 M, and incubation time of 12 h confirmed the formation of ZnO NPs with an average size of 20.2 nm and spherical morphology.

All physical features of ZnO NPs like size, morphology, and surface properties can be assessed using various characterization tools such as UV-vis spectroscopy, X-ray powder diffraction (XRPD), Fourier transform infrared (FTIR), energy-dispersive X-ray analysis, TEM, scanning electron microscopy (SEM), and dynamic light scattering [102]. Figure 2 shows the various characterization analyses of green-synthesized ZnO NPs.

5 Mechanism of green synthesis of ZnO NPs

The proposed mechanism of the green synthesis of ZnO NPs is related to the presence of phytochemicals, particularly polyphenols such as flavonoids, tannins, anthocyanins, and phenolic acids, which act as reducing/capping agents [92,104]. The phenolic rings rich in hydroxyl groups act as chelating agents, forming complexes with Zn^{2+} . A hydrolysis reaction transforms the intermediate chelated compound into zinc hydroxide $\text{Zn}(\text{OH})_2$, which is then transformed into ZnO during calcination. Finally, ZnO undergoes a growth phase by electrostatic interaction,

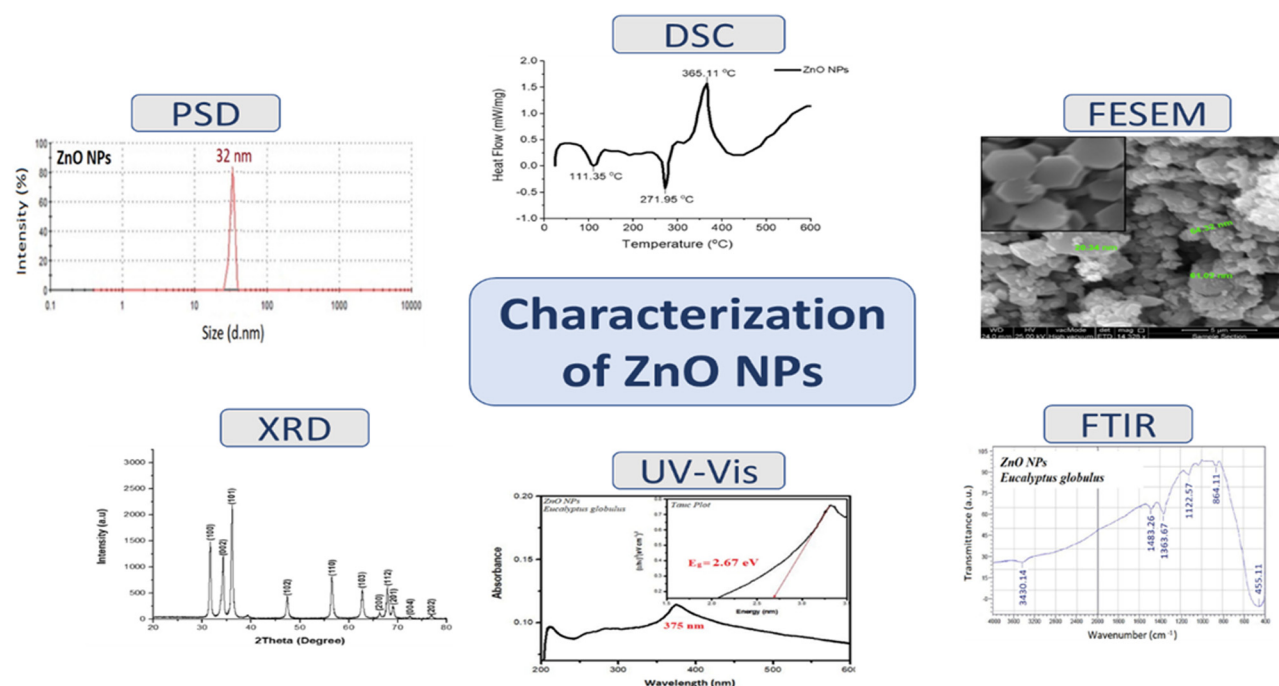


Figure 2: Characterization analyses of green-synthesized ZnO NPs including particle size distribution, differential scanning calorimetry, field emission scanning electron microscopy, X-ray diffraction (XRD), UV-vis spectroscopy (UV-vis), and FTIR [103].

forming ZnO NPs [104,105]. Another proposed mechanism has been reported in the literature which involves the formation of ZnO NPs upon the reduction of Zn^{2+} ions, by various phytochemicals in the aqueous extract, to nano-scale zinc particles (Zn^0) which react with dissolved oxygen to form ZnO, followed by capping with active phytochemicals to prevent agglomeration and increase the stability of ZnO NPs [105].

The redox potential of the phytochemicals is very important. It determines the extent of reduction that can occur. In the case of ZnO NP synthesis, the redox potential is usually not high enough to completely reduce Zn^{2+} ions to zerovalent Zn, resulting in the formation of ZnO NPs. The redox potential of the phytochemicals can be influenced by various factors such as the presence of functional groups, the presence of electron-donating or electron-withdrawing substituents, and the overall chemical reaction conditions (reaction time, temperature, pH, types of phytochemicals, and plant extract and metal salt concentrations) [106,107]. The other attribute is related to the stability and complexation of Zn^{2+} complexes [107]. Phytochemicals often form stable complexes with metal ions. In the case of ZnO NP synthesis, these complexes help in the stabilization of ZnO NPs by preventing their aggregation and facilitating controlled growth. The presence of functional groups in

phytochemicals, such as hydroxyl ($-\text{OH}$), carboxyl ($-\text{COOH}$), or amino ($-\text{NH}_2$) groups, allows for the binding and complexation with Zn^{2+} ions, leading to the formation of ZnO NPs. Furthermore, the pH of the reaction medium plays a significant role in the formation of ZnO NPs. Most green synthesis methods involve alkaline conditions ($\text{pH} > 7$), which promote the formation of ZnO. Under these alkaline conditions, $\text{Zn}(\text{OH})_2$ is formed, which subsequently reacts to produce ZnO NPs [108].

Alamdari *et al.* [109] describe the mechanism of the green synthesis of ZnO NPs using the *Sambucus ebulus* (*S. ebulus*) leaf extract (Figure 2). The authors proposed a reaction between flavonoid/phenolic molecules with Zn^{2+} ions via the donor–acceptor mechanism, resulting in Zn^{2+} complexes and the formation of ZnO NPs (Figure 3).

6 Cytotoxic mechanisms of ZnO NPs

ZnO NPs are one of the most commonly used MONPs in biomedical applications as antibacterial and anticancer agents and in the cellular imaging fields [110–112]. The cytotoxic effect of ZnO NPs is mainly concerned with

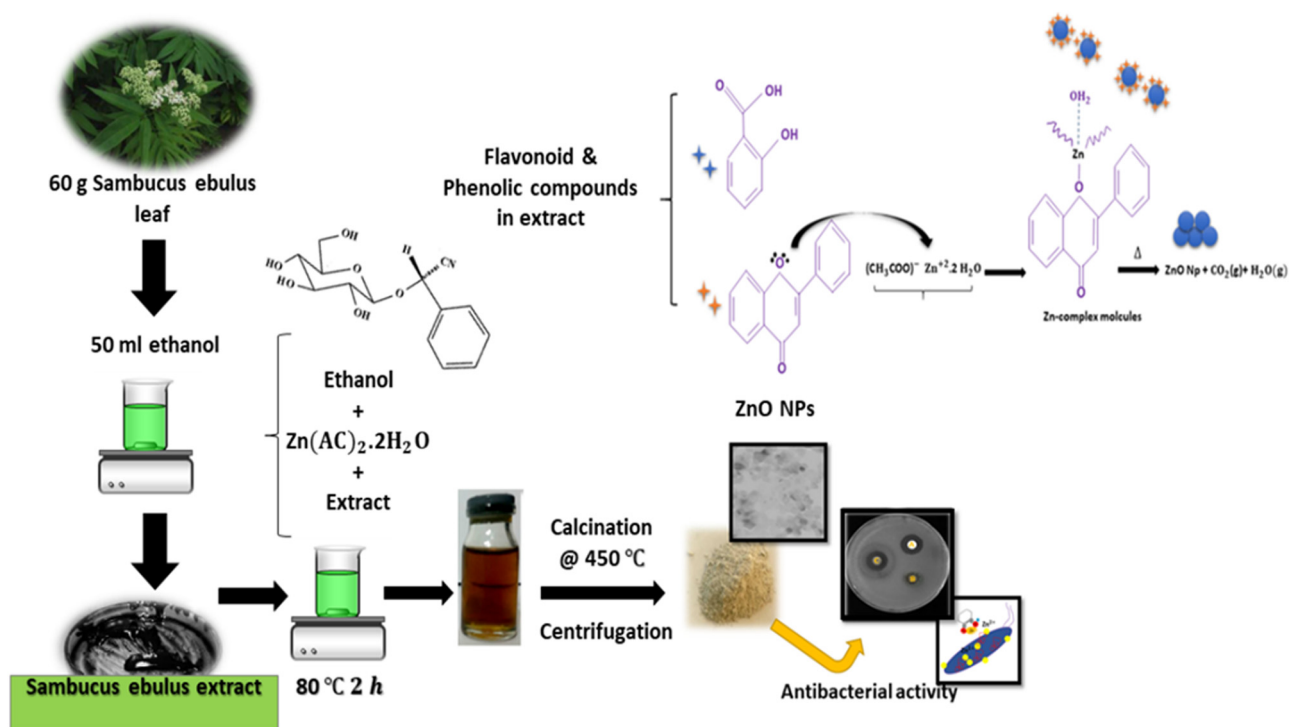


Figure 3: A schematic diagram representing the experimental work and the possible mechanism of the green synthesis of ZnO NPs using the leaf extract of *S. ebulus* [109].

changing the cytoskeleton and nucleoskeleton of proteins and/or generating reactive oxygen species (ROS) in cells exposed to ZnO NPs [113]. Ultimately, three mechanisms are involved simultaneously in the cytotoxicity of ZnO NPs including the release of Zn^{2+} , production of ROS, and direct interaction between ZnO NPs and cells [28].

6.1 Release of Zn^{2+} ions from ZnO NPs

One of the main mechanisms of ZnO NP cytotoxicity is that these NPs dissolve into free Zn^{2+} in the lysosomal acidic media, causing lysosomal destabilization, mitochondrial dysfunction, and disruption of cellular zinc homeostasis, leading to cell death [114]. The release of Zn^{2+} into the cells enables the dynamic transport of membrane, and mitochondrial and DNA damage [115]. The dissolved Zn^{2+} induced lactate dehydrogenase (LDH) leakage into the cell culture medium, which indicates cell membrane damage. Additionally, it was found that there is a significant relationship among the Zn^{2+} content, cell viability, and the LDH level [115]. Murali *et al.* [15] discussed the potential cytotoxicity of ZnO NPs that stems from the release of Zn^{2+} and hence their role in different biomedical applications including antibacterial, antidiabetic, antifungal, anticancer, anti-inflammatory, and antioxidant. It has been reported that Zn^{2+} is released due to the dissolution of ZnO NPs which may occur either extracellularly or intracellularly [116]. In the extracellular dissolution of ZnO NPs and due to their small size, Zn^{2+} can enter the cytoplasm *via* the cell membrane, inducing the production of ROS that leads to oxidative stress in many cells. However, the intracellular dissolution of ZnO NPs involves the release of Zn^{2+} due to the acidic environment of lysosomes after they were internalized by endocytosis, inducing oxidative stress and apoptosis [117]. Additionally, Zn^{2+} is one of the main contributors to the antibacterial activity of ZnO NPs, where Zn^{2+} interacts with proteins in bacteria and oxidize their amino acids, leading to protein denaturation and loss of enzyme activity. This results in the blockage of the metabolic and growth functions [118]. To summarize, the released Zn^{2+} in high levels contributed to the cytotoxicity of ZnO NPs.

6.2 Production of ROS

The second mechanism of ZnO NP cytotoxicity involves the production of ROS. When ZnO NPs enter the cells, the defense mechanism begins inside the cells, generating ROS. If the

generation of ROS exceeds the limit of the antioxidant capacity of cells, ROS produces pro-inflammatory cytokines, which initiate inflammation. Inflammation induces mitochondrial disturbance that leads to an impairment in the cellular membrane, cellular components, and DNA, in addition to an increase in LDH from necrosis or apoptosis, leading to cell death [119,120]. Additionally, the generation of the intracellular ROS and the entrance of the anticancer agent into cancer cells are the main causes of destroying the electron transport chain [121]. A high amount of ROS leads to mitochondrial damage and loss in protein activity, leading to cell apoptosis [122–124].

In 2018, Attia *et al.* [125] studied the neurotoxicity of ZnO NPs when given orally to rats in different doses. They reported that ZnO NPs may reach the brain when administered orally. In addition, the exposure of cells to ZnO NPs decreases the concentration of antioxidants such as glutathione (GSH), catalase, and superoxide dismutase, where these antioxidants generally prevent or repair the damage caused by ROS and regulate the redox-sensitive signaling pathways. Thus, exposure to ZnO NPs resulted in DNA damage confirmed by increasing the percentage of DNA tail, length, and intensity. This indicates that ZnO NPs deteriorate and damage the antioxidant system *via* developing ROS in the brain, elevating the inflammatory response, cytokines, DNA fragmentation, and apoptosis [125]. Therefore, the green-synthesized ZnO NPs displayed oxidative stress-mediated cytotoxic effects.

6.3 Direct interaction between ZnO NPs and cells

It has been shown that there is a direct interaction between ZnO NPs and cells, resulting in a change in the cell morphology, disruption of the membrane, damage of mitochondria, and spillage of intracellular structure [126,127]. In addition, exposure to ZnO NPs changed the cellular distribution of lipid biosynthetic enzymes and the structure of the endoplasmic reticulum (ER), mitochondria, and ER-mitochondria encounter structure complex, leading to cell death [126]. Altogether, the interaction of ZnO NPs with cells caused cell death by affecting the integrity of the cell wall, homeostasis of ER, and the generation of ROS and saturated free fatty acids [126]. Yu *et al.* [127] showed that exposure to ZnO NPs leads to cell death *via* the accumulation of autophagic vacuoles and the damage of the mitochondria in normal skin cells *via* inducing the generation of ROS. Figure 4 illustrates the three mechanisms involved in the cytotoxicity of ZnO NPs.

7 Biomedical applications of ZnO NPs

The use of eco-friendly, safe, inexpensive, and dexterous ways for the green synthesis of ZnO NPs has offered a revolution in various biomedical fields [111,128,129]. ZnO NPs have been employed in many life applications including drug delivery, cosmetics, medical installations (as antibacterial paints in hospitals), dentistry (for blocking microbial leakage), and orthopedics (as a reinforcing material) [35]. Recently, ZnO NPs showed promising biomedical applications such as antibacterial, anticancer, antidiabetic, antioxidant, antifungal, antiviral, and anti-inflammatory, and in wound healing [13–16]. Biomedically, several studies have proven the nontoxic effect of the surface-modified ZnO NPs, using hyaluronan, PEG, and Triton X-100, on normal human cells, without affecting the anticancer effects of ZnO NPs [111].

7.1 Antibacterial activity of ZnO NPs

The green-synthesized ZnO NPs exhibited a substantial antibacterial activity for wide-spectrum bacteria, whereby

interacting with the bacterial cell surface and its core, ZnO NPs can enter inside the cells and display their bactericidal effect [130–132]. Therefore, the antibacterial activity of ZnO NPs revealed a promising potential to be linked with antibiotic functions, overcoming antimicrobial resistance [36]. In addition, the toxic interaction between ZnO and bacteria has proved as an antibacterial agent in the food industry [102]. This is attributed to their high specific surface area and high activity to block a wide range of pathogenic bacteria [14,133]. Recently, the antibacterial activity of ZnO NPs was proved by their ability to generate ROS such as hydroxyl radicals ($\cdot\text{OH}$), hydrogen peroxide (H_2O_2), superoxide anions ($\text{O}_2^{\cdot-}$), and singlet oxygen ($^1\text{O}_2$). The four ROS displayed different levels of antibacterial activity. It has been shown that $\text{O}_2^{\cdot-}$ and H_2O_2 exhibited lower antibacterial activity than $\cdot\text{OH}$ and $^1\text{O}_2$. This is because $\text{O}_2^{\cdot-}$ and H_2O_2 are less reactive and can be detoxified by endogenous antioxidants that are induced by oxidative stress. However, no enzyme can detoxify $\cdot\text{OH}$ or $^1\text{O}_2$, making them more toxic [134]. Additionally, it has been reported that the negative charge of hydroxyl radicals ($\cdot\text{OH}$) and superoxide ($\text{O}_2^{\cdot-}$) prevents their penetration into the bacterial cell wall. However, the direct contact between these ROS and bacterial membranes can cause bacterial death. Whereas, hydrogen peroxide (H_2O_2) can penetrate through the

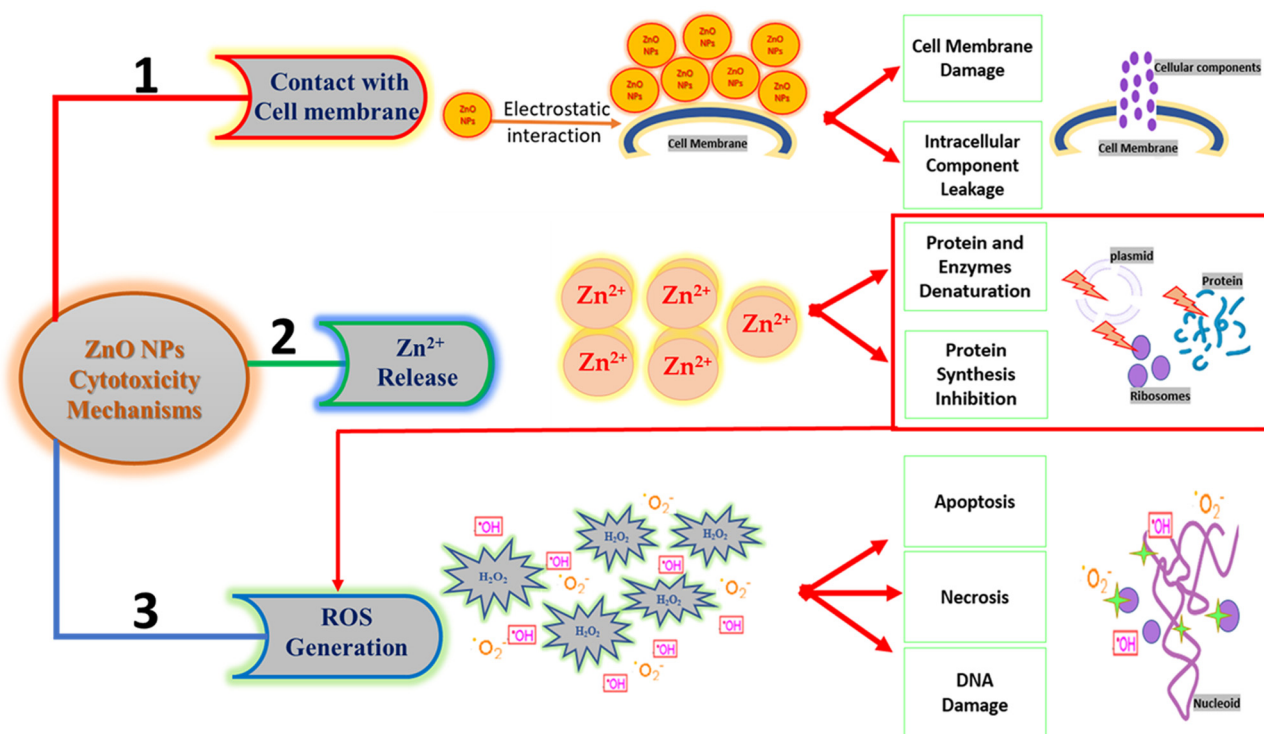


Figure 4: The three mechanisms involved in the cytotoxicity of ZnO NPs: (1) direct contact with the cell membrane, (2) release of Zn^{2+} , and (3) generation of ROS.

bacterial cell wall, getting internalized in the bacterial cell, causing cell death [104,135,136]. Additionally, the antibacterial activity involves the accumulation of ZnO NPs in the outer membrane or in the cytoplasm of the bacterial cells which triggers the release of Zn^{2+} , causing disintegration of the cell membrane, damaging the membrane proteins, and instability of bacterial genomics, resulting in bacterial cell death [137,138]. Because ZnO has an amphoteric nature, it can react with acids and bases, releasing Zn^{2+} which binds to the bacterial biomolecules [139]. Figure 5 shows a schematic diagram of the antibacterial mechanisms of ZnO NPs.

Although ZnO NPs showed antibacterial activity against both Gram-positive and Gram-negative bacteria [135], the antibacterial activity of ZnO NPs depends on the sensitivity of the microorganism and the difference in the cell wall structure of Gram-positive and Gram-negative bacteria [140]. The membrane of Gram-positive bacteria is composed of a thick peptidoglycan layer (20–80 nm) that is covalently attached to teichoic, lipoteichoic acids, and surface proteins [135]. This thick layer acts as a physical barrier that protects the cells from the exterior environment, whereas the membrane of the Gram-negative bacteria is composed of a thin peptidoglycan layer (~8 nm) and a thick lipopolysaccharide external layer (1–3 μm) [135,136].

Reddy *et al.* [141] reported that the minimum inhibitory concentration (MIC) of ZnO NPs (13 nm) in the Gram-positive (*Staphylococcus aureus*, *S. aureus*) and Gram-negative (*Escherichia coli*, *E. coli*) was 1 and 3.4 mg ml^{-1} , respectively. This indicates that the inhibition of Gram-positive bacteria required a lower concentration of ZnO NPs than the Gram-negative bacteria. This was attributed to the fact that the peptidoglycan layer of *S. aureus* can promote the

attack of ZnO NPs inside the cell, whereas the cell wall components of *E. coli*, particularly lipopolysaccharides, can impede this attack [135]. Similar results were reported by Tayel *et al.* [142], who studied the antibacterial activity of ZnO powder and ZnO NPs against nine bacterial strains. The study showed that ZnO NPs were more efficient as antibacterial agents than the powder and that Gram-positive bacteria were more sensitive to ZnO NPs than Gram-negative bacteria. Contrarily, Shinde *et al.* [140] showed that the antibacterial activity of ZnO microspheres against *S. aureus* was lower than *E. coli*. Researchers suggested that the difference in the cell wall structure of Gram-positive and Gram-negative bacteria is responsible for this outcome, where the thick peptidoglycan layer of *S. aureus* acts as a physical barrier, preventing the penetration of ZnO NPs through the cell wall. However, the thinner peptidoglycan layer of *E. coli* allows the penetration of ZnO NPs, rupturing the cell wall [140].

Moreover, d'Agua *et al.* [143] evaluated the antibacterial activity of ZnO NPs containing cotton fibers against Gram-positive bacteria (*S. aureus*, *S. epidermidis*, and *Propionibacterium acnes* [*P. acnes*]) and Gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa* [*P. aeruginosa*]). The agar diffusion and absorption methods were used in the study. The results showed that ZnO NPs containing cotton fibers exhibited higher antibacterial activity against Gram-positive bacteria than Gram-negative bacteria. The lower sensitivity of ZnO NPs toward Gram-negative bacteria can be attributed to the complex structure of the bacterial cell wall, where the thick outer lipopolysaccharide membrane surrounds the thin peptidoglycan layer and acts as a barrier, protecting against ZnO NPs. The

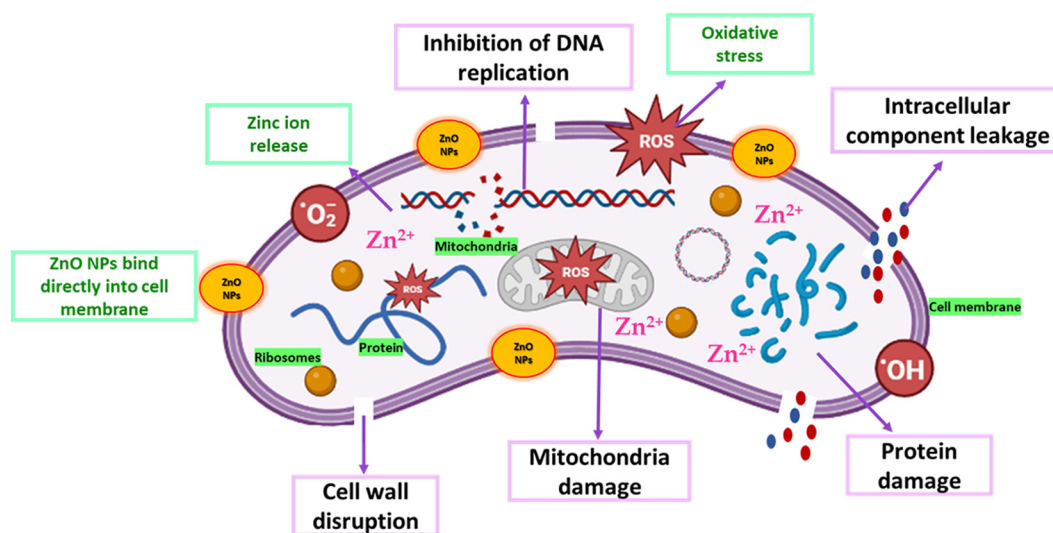


Figure 5: Schematic diagram representing the antibacterial mechanisms of ZnO NPs.

authors reported that the antimicrobial studies of ZnO NPs against Gram-positive and Gram-negative bacteria showed contradictory results, where some studies have shown a stronger antibacterial effect of ZnO NPs on Gram-positive bacteria, whereas other studies have shown that Gram-negative bacteria are more sensitive to ZnO NPs [143].

Jiang *et al.* [144] studied the interaction between ZnO NPs and the Gram-negative bacteria *E. coli* to examine the antibacterial mechanism of ZnO NPs. It was found that using ZnO NPs (30 nm) and the direct contact between ZnO NPs and the phospholipids bilayer of *E. coli* membrane led to a disruption in the *E. coli* membrane, measured by quantifying the leakage of the intracellular K^+ from the bacterial membrane, causing cell death. Although mannitol, vitamin E, and GSH were used as radical scavengers to quench the release of ROS generated from ZnO NPs and thereby suppress their antibacterial activity, the antibacterial activity of ZnO NPs against *E. coli* indicates the generation of ROS, particularly the ($\cdot$ OH) radicals.

Agarwal *et al.* [102] reviewed the mechanism of the antibacterial activity of the green-synthesized ZnO NPs. The results showed that ZnO NPs, due to their large surface area to volume ratio, can bind to the bacterial cell surface via a large number of ligand binding sites like proteins, lipids, phospholipids, and lipoteichoic acid. Additionally, the authors reported that Gram-negative bacteria have less resistance to ZnO NPs due to their thin layer of peptidoglycan, making ZnO NPs more toxic to this type of bacterium. The antibacterial mechanisms of ZnO NPs involve the disruption of cell membranes, release of Zn^{2+} resulting in ROS generation, DNA disruption, protein oxidation, lipid peroxidation, and metabolic enzyme inhibition which blocks food or respiratory pathways, causing cell death.

The cytotoxicity of ZnO NPs depends on different parameters of NPs such as size, shape, surface charge, and concentration. In addition, the pH and temperature of the culture medium impact the antibacterial activity of ZnO NPs, where the acidic pH from 4 to 5 and high temperatures increased the antibacterial activity of ZnO NPs. This is because acidic media enhanced the dissolution rate of ZnO NPs, hence increasing the concentration of Zn^{2+} in the media and improving the activity against the microorganisms [102,145]; however, high temperatures enhanced the production of ROS [146,147].

Several studies have confirmed the relationship between the size and morphology of ZnO NPs and their antibacterial activities [104,118,148]. Recently, Sharma *et al.* [148] studied the antibacterial activity of different sizes and shapes of ZnO NPs, prepared from the plant extract of Aloe vera. The size of ZnO NPs ranged between 40 and 180 nm with hexagonal, spherical, cylindrical, and cuboidal shapes. The cuboidal

ZnO NPs exhibited higher antibacterial activity against *S. aureus*, *B. subtilis*, and *E. coli*, compared to the spherical and hexagonal ZnO NPs. Another study by Álvarez-Chimal *et al.* [118] investigated the effect of the size of the green-synthesized ZnO NPs (7–130 nm), prepared from the *Dysphania ambrosioides* (*D. ambrosioides*) extract, on the antibacterial activity against various pathogenic bacteria. It was found that the smaller the size of ZnO NPs (4–10 nm), the higher the antibacterial potential, particularly against *S. aureus* and *E. coli*. A very recent study by Fouda *et al.* [104] demonstrated the high antimicrobial potential of spherical ZnO NPs (10–45 nm), prepared from the peel aqueous extract of *Punica granatum* (*P. granatum*), against Gram-positive and Gram-negative bacteria, and unicellular fungi with an MIC of 12.5–6.25 $\mu\text{g mL}^{-1}$. The authors attributed the high antimicrobial potential to the high generation of ROS when pathogenic microbes are treated with smaller sizes of ZnO NPs.

Additionally, it has been shown that the interaction between NPs and bacterial membranes is influenced by the shape of NPs, where the triangular-shaped NPs showed better interaction with cell membranes than the rod or spherical ones, enhancing the antibacterial effect [102]. Moreover, the shape of ZnO NPs affects the release of Zn^{2+} , where the shape influences the surface area of NPs and hence their solubility [102]. For instance, it has been shown that the spherical-shaped ZnO NPs showed better solubility and higher Zn^{2+} release than the rod-shaped ones [149].

The surface charge of ZnO NPs is one of the important parameters that determine their antibacterial activity, where a correlation was established between the zeta potential of NPs, the surface charge of bacterial cells, and the antibacterial activity [150,151]. For instance, Mendes *et al.* [152] stated that the positively charged ZnO NPs showed better binding toward the negatively charged surface cell membrane via electrostatic interactions, improving the toxicity of NPs against Gram-positive and Gram-negative bacteria. Teichoic acid in the peptidoglycan layer and lipoteichoic acid in the membrane, responsible for the negative charge of the bacterial cell membrane, act as chelating agents for Zn^{2+} which is carried by passive diffusion across membrane proteins [152].

Thi *et al.* [153] reported the synthesis of spherical ZnO NPs (20 nm) from the orange fruit peel extract and zinc acetate dihydrate which were used as a reducing agent and a chemical precursor, respectively. The antibacterial activity of ZnO NPs was studied against *E. coli* and *S. aureus*. The results showed a strong bactericidal effect of >99.9% against *E. coli* and 89.4–98.1% against *S. aureus* at an NP concentration of 0.025 mg mL^{-1} and 8 h of incubation.

Additionally, the mechanism of the bactericidal activity of ZnO NPs was based on the generation of ROS and their interaction with cell membranes, resulting in DNA and cell wall damage. In the same year, Umavathi *et al.* [154] synthesized green spherical ZnO NPs from the *Parthenium hysterophorus* (*P. hysterophorus*) leaf extract by a single-step process. ZnO NPs were characterized by UV-Vis spectroscopy and FTIR to confirm the presence of flavonoids, phenolics, tannins, phytic acid, and ZnO, whereas the TEM and SEM micrographs revealed the spherical shape and the nanosized range of 10 nm of ZnO NPs. The XRPD results confirmed the formation of the hexagonal wurtzite structure. ZnO NPs exhibited strong antibacterial activity against Gram-positive and Gram-negative organisms and fungal strains with a concentration-dependent effect. For instance, it was found that 10 mg of ZnO NPs was sufficient to exhibit a maximum inhibitory concentration against *E. coli*. Moreover, Safavinia *et al.* [155] used *Daphne oleoides* (*D. oleoides*) leaf extract, which contains five polyphenolic compounds, to prepare ZnO NPs and embed them into silica gel (SG) matrix, forming ZnO/SG nanocomposites. The antibacterial activity of ZnO/SG nanocomposites was evaluated against pathogenic bacteria and compared with the unembedded ZnO NPs. Data showed that ZnO NPs were stabilized by the SG matrix which prevents their agglomeration. Additionally, the surface area of ZnO/SG nanocomposites was higher than the unembedded ZnO NPs, demonstrating greater antibacterial activity against pathogens than the unembedded ZnO NPs.

The antibacterial activity of green-synthesized ZnO NPs was compared to their chemical counterparts. Gunalan *et al.* [133] found that the antimicrobial activity of green-synthesized ZnO NPs (40 nm), prepared from aloe leaf extract, was higher than that of the chemical ZnO NPs (25 nm). The antimicrobial activity was tested against different types of bacteria and fungi at various concentrations. In another study [156], the antibacterial activity of ZnO NPs, prepared from the leaf extract of *Sesbania grandiflora* (*S. grandiflora*), was compared to that of the chemical ZnO NPs, prepared by the co-precipitation method. The study showed that the green-synthesized ZnO NPs exhibited higher antibacterial activity against Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacteria, in agreement with Gunalan *et al.* [133]. Similarly, a recent study by Bekele *et al.* [157] compared the antibacterial activity of the green (11–21 nm) and chemically synthesized (30–40 nm) ZnO NPs against *Bacillus subtilis* (*B. subtilis*), *S. aureus*, and *Salmonella typhimurium* (*S. typhimurium*). The green and chemical ZnO NPs showed a strong antibacterial activity with zones of inhibition of 15–24 and 7–15 mm, respectively, suggesting that the green-synthesized ZnO NPs showed promising antibacterial activity.

7.2 Anticancer activity of ZnO NPs

Scientists are putting a lot of effort to develop effective treatments to kill cancer cells with minimum side effects. Nowadays, numerous studies have been performed to advance the use of green-synthesized ZnO NPs to treat different types of cancers [158–160]. The role of ZnO NPs involves the anticancer activity on different types of cancers and targeted drug delivery by using ZnO NPs as nano-carriers for chemotherapeutic drugs.

7.2.1 Role of ZnO NPs in anticancer activity

ZnO NPs cause oxidative stress in cancer cells, leading to their damage, due to the rapid dissolution of ZnO NPs into Zn^{2+} at acidic pH [70]. The low endosomal pH of 6.3, 5.5, and 4.7 in the early endosome, late endosome, and endosome, respectively, induce the release of soluble Zn^{2+} , causing lysosomal destabilization and cell death [28]. The higher selectivity of ZnO NPs toward cancer cells, compared to normal cells, is attributed to the electrostatic interaction between the positively charged ZnO NPs, under physiological conditions, and the anionic phospholipids on the outer membrane of cancer cells which are present at high concentration. This interaction increases the cellular uptake of ZnO NPs by cancer cells and hence their cytotoxicity [161]. Hanley *et al.* [162] showed that ZnO NPs have 28–35 times more selective cytotoxicity against cancer cells compared to normal cells. Additionally, they reported that altering the surface properties of ZnO NPs may further enhance their cytotoxicity against cancer cells [162]. Moreover, cancer cells possess higher levels of ROS, owing to their rapid metabolism, when compared to normal cells. Hence, when cancer cells are treated with ZnO NPs, more ROS are produced, resulting in severe oxidative stress that promotes cell death [28,161]. Finally, the enhanced permeation and retention effect in cancer cells to ZnO NPs, due to their small size and surface properties, enhances the cytotoxicity of ZnO NPs against cancer cells compared to normal cells [28].

Sharmila *et al.* [163] used the *Tecoma castanifolia* (*T. castanifolia*) leaf extract for the green synthesis of spherical ZnO NPs (70–75 nm) to evaluate their anticancer activity against lung cancer. The results demonstrated that ZnO NPs exhibited antibacterial, antioxidant, and anticancer activities. Additionally, it was found that an increase in the ZnO NP concentration proportionally increases the radical scavenging activity. ZnO NPs exhibited an anticancer activity with half-maximal inhibitory concentration (IC_{50}) around $65 \mu\text{g ml}^{-1}$, confirmed by the cytotoxic effect on the

proliferation of human lung carcinoma (A549) cells, tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Thus, ZnO NPs can be used as nano-delivery systems in various biomedical applications. Ruangtong *et al.* [164] used banana peel crude extract as a reducing and capping agent and zinc acetate as a chemical precursor to synthesize ZnO NPs with rod-like or sheet-like structure, where the shape of NPs was varied based on the crude extract concentration used in the reaction. The ZnO NPs and nanosheets were investigated for their antibacterial and anticancer activities against skin, colorectal, and liver cancers. The ZnO nanosheets inhibited the growth of Gram-negative (*E. coli*) and Gram-positive *B. subtilis* and *Staphylococcus epidermidis* (*S. epidermidis*) bacteria. In addition, ZnO nanosheets demonstrated potent anticancer activity against colorectal (SW620), skin (A431), and liver cancer cells (HepG2) without affecting the normal cells. Similarly, Jevapatarakul *et al.* [165] synthesized green ZnO NPs (200 nm) and nanosheets (500 nm) from the crude extract of *Cratogeomys formosum* (*C. formosum*) leaves to assess their anticancer activity against non-melanoma skin cancer cells. The shape of ZnO NPs varied based on the concentration of the crude extract and the synthesis process. The cytotoxic activity of ZnO NPs nanosheets was evaluated by testing the cell viability on epidermoid carcinoma cell line (A431), normal kidney fibroblasts (Vero), and liver cancer cells (HepG2). ZnO NPs at $120 \mu\text{g ml}^{-1}$ concentration significantly inhibited the cell viability of A431 cells without affecting Vero cells. Contrarily, a higher concentration of ZnO NPs ($300 \mu\text{g ml}^{-1}$) showed a weak cytotoxic activity when applied to the HepG2 liver cell line. The ZnO NPs nanosheets showed greater cytotoxicity against A431 cells when compared to the spherical ZnO NPs (~200 nm). Additionally, the spherical ZnO NP nanosheets inhibited the growth of *E. coli*, *B. subtilis*, and *S. epidermidis*. Moreover, ZnO NP nanosheets altered several transcript pathways responding to the generation of ROS and hydrogen peroxides that are involved in the cytotoxic effect on A431, causing cell death due to oxidative stress and inflammatory response [165].

Naser *et al.* [72] synthesized ZnO NPs using the root hair extract of *Phoenix dactylifera* (*P. dactylifera*) and 0.6 M zinc acetate dihydrate to assess their anticancer activity against lung and breast cancers. The spherical ZnO NPs (31–48 nm) were 45% more cytotoxic than doxorubicin (DOX) hydrochloride alone. The ZnO NPs proved their cytotoxic efficacy by reducing the cell viability of the triple negative breast cancer cell line (TNBC) to 9.0% and showed 82.3% cytotoxic efficacy against lung cancer cell line (A549), confirming that ZnO NPs exhibited a potent anticancer activity. Moreover, ZnO NPs showed higher antibacterial activity against various microorganisms than those of

penicillin, gentamycin, and tetracycline. A follow-up study conducted by the same research group evaluated the loading of these ZnO NPs into transdermal patches for breast cancer therapy [71]. Patches were prepared from the film-forming polymer Carbopol, backing layer ethylene-vinyl acetate, and ZnO NPs (88 nm) with a zeta potential of -16.63 mV . Patches were clear, transparent, and flexible with uniform thickness and weight of 0.25 mm and 0.42 g, respectively, and a surface pH of 6.25. The content of ZnO NPs in the patches was 91.7–94.4%. In addition, ZnO NPs from patches exhibited a sustained release profile over 25 h. Moreover, the cytotoxic effect of ZnO NPs against the TNBC cell line was dose-dependent ranging from 0.16 to $2.5 \mu\text{g ml}^{-1}$ with IC_{50} of $0.42 \mu\text{g ml}^{-1}$, significantly lower than the IC_{50} of DOX ($4.58 \mu\text{g ml}^{-1}$) ($p < 0.05$). Whereas the IC_{50} on human dermal fibroblast (HDF) was $1.61 \mu\text{g ml}^{-1}$, demonstrating a stronger effect of ZnO NPs in inhibiting the growth of cancerous cells, compared to that of DOX. Thus, ZnO NP-loaded patches may offer a potential transdermal delivery platform for breast cancer treatment by overcoming the limitations of invasive chemotherapy delivery.

In 2020, Duan *et al.* [166] synthesized ZnO NPs from *Cardiospermum halicacabum* (*C. halicacabum*) using an eco-friendly green method. The spherical shape of ZnO NPs (10–20 nm) was determined by TEM and evaluated against skin melanoma. The antitumor activity and apoptosis for the prepared ZnO NPs were explored using a human melanoma cell line (A375). The ZnO NPs exhibited cytotoxic activity against cancer cells. In addition, the exposure to ZnO NPs resulted in an elevation in the ROS level and apoptotic markers (caspases 3, 8, and 9) and stimulation of apoptotic cell necrosis in the tumor cells. Furthermore, Selim *et al.* [167] synthesized ZnO NPs from the aerial part of *Deverra tortuosa* (*D. tortuosa*) to examine their anticancer activity against colorectal and lung cancers that are associated with the greatest mortality. The plant was collected from a natural ecosystem in Egypt. The resulting ZnO NPs (15.2 nm) showed selective cytotoxic activity against two cancer cell lines, the human lung cancer cell line (A594) and the human colon cancer cell line (Caco-2), providing a safer alternative platform to conventional cancer therapy.

The research on the green synthesis of ZnO NPs has been continued. Recently, Vakayil *et al.* [168] synthesized ZnO NPs from rhizomes of the *Acorus calamus* (*A. calamus*) to examine their cytotoxic effect against skin cancer. The ZnO NPs were then coated with cotton fabrics to reduce skin infections. The cotton-coated ZnO NPs showed a cytotoxic and antiproliferative activity against the SK-MEL-3 skin cancer cell line which was proved by the morphological changes in cells such as shrinkage, rounding,

irregular shape, and detachment in cancer cells. In addition, the MTT assay test showed a suppression in cell viability against the SK-MEL-3 cell line with an IC_{50} value of $17.50 \mu\text{g ml}^{-1}$ of the coated ZnO NPs. Finally, the cotton-coated ZnO NPs efficiently inhibited the growth of bacteria, demonstrating a potential antibacterial activity. Thus, the cotton-coated ZnO NPs could be utilized in the medical field in the future.

A recent study by Chandrasekaran *et al.* [169] compared the anticancer activity of ZnO NPs, prepared by green and chemical methods, against the MCF-7 breast cancer cell line. The green-synthesized ZnO NPs were prepared using the *Vinca rosea* (*V. rosea*) leaf extract, whereas chemical ZnO NPs were prepared by the precipitation method. The results showed that the anticancer activity of the green-synthesized ZnO NPs (16–41 nm) with spherical shape was higher compared to chemically synthesized counterparts [169].

7.2.2 Role of ZnO NPs in targeted drug delivery

The combination between ZnO NPs and the chemotherapeutic agents demonstrated a potential effect in treating cancer. George *et al.* [170] studied the therapeutic effect of quercetin (QE), extracted from onion peel waste and formulated as chitosan hydrogel. Using melon seeds, the QE hydrogel matrix was loaded with green-synthesized ZnO NPs forming QE-loaded nanohybrid hydrogels. The release of QE from the hydrogels was the highest in an acidic environment (pH 5), thus suitable for anticancer applications. The growth of *S. aureus* and *Trichophyton rubrum* (*T. rubrum*) strains was inhibited by the commercial QE and QE-loaded nanohybrid hydrogels. The cytotoxicity and anticancer activity of QE-loaded nanohybrid hydrogels were studied against skin cancer using normal murine fibroblast cells (L929) and human dermal carcinoma cell lines (A431), respectively. It was found that the QE-loaded nanohybrid hydrogels were biocompatible toward healthy cells and exhibited enhanced anticancer activity toward A431, compared to commercial QE. The kinetic drug release mechanism followed Fickian diffusion. This suggests that the combination of ZnO NPs and QE in the QE-loaded nanohybrid hydrogels showed synergistic antibacterial and anticancer activities *via* enhancing the cellular uptake of QE, highlighting their potential applications in the biomedical fields. Recently, Chelladurai *et al.* [171] synthesized amine-functionalized ZnO NPs that were covalently linked to mupirocin using an organosilane linker. Mupirocin is a crotonic acid derivative extracted from *Pseudomonas fluorescens* (*P. fluorescens*) used to treat superficial skin infections like impetigo and microbial

infections caused by Gram-positive and Gram-negative bacteria. The ZnO NPs were green synthesized from the *Alpinia calcarata* (*A. calcarata*) rhizome extract. The effect of mupirocin-loaded ZnO NPs on skin cancer and skin infection was investigated. The green spherical ZnO NPs (19 nm) exhibited a crystallite grain size of 24.75 nm. The conjugated NPs showed a strong antiproliferative effect of 63% on human epidermoid carcinoma cells (A431), providing a new nanotechnology platform for skin cancer therapy. Moreover, mupirocin-loaded ZnO NPs demonstrated potency as antioxidant agents and significantly inhibited the growth of *Vibrio cholerae* (*V. cholerae*), *Enterococcus faecalis* (*E. faecalis*), and *Listeria monocytogenes* (*L. monocytogenes*).

Additionally, it has been shown that the biocompatibility and biomedical activity of coated and embedded ZnO NPs were further improved compared to their uncoated and unembedded counterparts [155,172]. For instance, Batool *et al.* [172] green synthesized ZnO NPs (20–40 nm) using the *Aloe barbadensis* (*A. barbadensis*) leaf extract. The ZnO NPs were loaded with the chemotherapeutic agents, doxorubicin (DOX) and gemcitabine (GEM), and were studied for their antitumor cytotoxic activity against breast cancer (the leading cause of cancer death among females), surface-PEGylation, and drug loading capacity. PEGylated ZnO NPs (PEG-ZnO NPs) and their non-PEGylated counterparts (ZnO NPs) showed higher DOX loading capacity and encapsulation efficiency compared to GEM. In addition, DOX/PEG-ZnO NPs and DOX/ZnO NPs exhibited significant anticancer activity against TNBC, with DOX/PEG-ZnO NPs showing greater anticancer activity. The loading of DOX into green-synthesized ZnO NPs has been investigated by Vimala *et al.* [173], who used the palm fruit extract *Borassus flabellifer* (*B. flabellifer*) to treat breast and colon carcinoma. The results proved that DOX/ZnO NPs exhibited high efficacy against breast and colon cancer, and thus are considered a promising drug delivery system. Therefore, plant-mediated green-synthesized ZnO NPs could have huge applications in cancer treatment and thus become a major area of research.

Finally, the anticancer potential of different parts of a plant might vary based on the specific plant species and the phytochemicals present in those parts. Because it is difficult to make a general statement about which part of plants consistently shows the best anticancer potential, different parts of plants have been studied extensively for their anticancer activity. Leaves, roots, stems, flowers, and even certain fruits of various plants have demonstrated anticancer activity [174]. It is important to note that the presence and concentration of phytochemicals might vary among different plant parts. Additionally, the extraction methods used to obtain these compounds can affect their potency. In some cases, leaves have been found

to possess high levels of phytochemicals such as polyphenols, flavonoids, and alkaloids, which exhibit anticancer activity [175]. Leaves are often rich in phytochemicals due to their primary role in photosynthesis [176]. However, this does not necessarily mean that leaves always show the best anticancer potential compared to other plant parts. Contrarily, the roots of certain plants have also been extensively studied for their anticancer activity [177,178]. Roots may contain a range of phytochemicals such as alkaloids, terpenoids, and polysaccharides, which contribute to their therapeutic potential [179]. Ultimately, the choice of the plant part to be investigated for its anticancer potential depends on the specific plant species, desired phytochemicals, and traditional or scientific knowledge about the plant's medicinal properties. Further research is still necessary to compare the effectiveness of different plant parts and their phytochemicals in combating cancer cells.

In the context of anticancer applications, the association of phytochemicals with ZnO NPs can enhance their cytotoxic activity against cancer cells [20,180]. Phytochemicals, such as polyphenols and flavonoids, have shown anticancer activity on their own [181]. When attached to the surface of ZnO NPs, these compounds can potentially exert synergistic effects, leading to increased cytotoxicity against cancer cells [182]. For example, a study by Gobinath *et al.* [183] demonstrated that the metabolites present in the leaf extract of *Calotropis gigantea* (*C. gigantea*) enhanced the cytotoxicity of ZnO NPs against human breast, cervical, and hepatic cancer cells.

7.3 Antidiabetic activity of ZnO NPs

Zinc is a vital key factor in the glucose metabolic process which improves hepatic glycogenesis [184]. In addition, Zn reduces gluconeogenesis and glycogenolysis processes by inhibiting glucagon excretion [185]. Rajakumar *et al.* [186] used the leaf extract of *Andrographis paniculata* (*A. paniculata*) as a reducing and capping agent to synthesize ZnO NPs and study their antioxidant, antidiabetic, and anti-inflammatory activities. The antidiabetic activity was investigated by evaluating the α -amylase inhibitor activity. The proteins, polyphenols, alkaloids carboxylic acids, and flavonoids in the leaf extract, which contained free amino acids and carboxylic groups in their structures, influenced the formation of ZnO NPs by interacting with Zn^{2+} . The FTIR results demonstrated the role of the phenolics, terpenoids, and proteins of the *A. paniculata* leaf extract in the synthesis and stabilization of ZnO NPs. The XRPD pattern revealed that ZnO NPs were in the form of nanocrystals. The ZnO

NPs were spherical (96–115 nm) and hexagonal (57 ± 0.3 nm) in shape, as corroborated by SEM and TEM studies, respectively. The results of the antioxidant, antidiabetic, and anti-inflammatory studies showed that ZnO NPs can decrease the sugar level and inflammations, possessing strong biological activities which could be utilized in pharmaceutical and biomedical applications.

Argade *et al.* [187] used the *Albizzia lebbbeck* (*A. lebbbeck*) aqueous bark extract for the synthesis of ZnO NPs. The ZnO NPs were evaluated for antidiabetic activity *in vitro* using the α -amylase inhibitor activity and glucose uptake by yeast cells. The α -amylase inhibitory activities by the *A. lebbbeck* bark extract, ZnO NPs, and the antidiabetic drug Acarbose (used as a control) were found to be 73.3, 54.7, and 46.7%, respectively. The IC_{50} of the α -amylase activity of the *A. lebbbeck* bark extract, ZnO NPs, and Acarbose were 4.9, 9.6, and $3.9 \mu\text{g ml}^{-1}$, respectively. The data demonstrated that Acarbose exhibited effective antidiabetic activity compared with the plant bark extract and ZnO NPs. In addition, the glucose uptake by yeast cells showed a dose-dependent manner which was directly proportional to the sample concentration. The ZnO NPs at $1 \mu\text{g ml}^{-1}$ concentration enhanced the glucose uptake by 89.2%, thus higher than that of the plant bark extract and the antidiabetic drug metformin (used as a control) of 62.5 and 94.2%, respectively, at the same concentration. Additionally, ZnO NPs were evaluated *in vitro* for their antioxidant activity using the reducing power ability and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay tests. The ZnO NPs showed powerful DPPH scavenging activity of 66.7% and IC_{50} of $7.0 \mu\text{g ml}^{-1}$. Moreover, the results revealed that ascorbic acid (used as a control) exhibited higher reducing power ability compared to the bark extract and ZnO NPs. Taking together, the results of these studies demonstrated that the green-synthesized ZnO NPs acted as a potent antidiabetic agent as evidenced by the decreasing glucose level.

7.4 Antioxidant activity of ZnO NPs

One of the promising biomedical applications for ZnO NPs is their antioxidant activity [15]. The antioxidant activity of ZnO NPs can be determined by the DPPH assay method. This method is associated with a change in the color of the DPPH methanolic solution from deep violet to a stable pale yellow, upon the addition of ZnO NPs. This indicates the scavenging activity of ZnO NPs by donating an electron from the oxygen atom to the odd electron of the nitrogen atom, resulting in the formation of a stable DPPH molecule [15,188].

Suresh *et al.* [189] synthesized ZnO NPs using *Artocarpus gomezianus* (*A. gomezianus*). ZnO NPs showed substantial antioxidant activity against DPPH free radicals with IC_{50} of 10.8 mg ml^{-1} . The same research group synthesized green ZnO NPs using the aqueous plant extract of *C. fistula*, employing the solution combustion method [190]. The results showed that ZnO NPs exhibited a potential antioxidant activity with IC_{50} of 2.8 mg ml^{-1} via scavenging DPPH free radicals [190]. Nagajyothi *et al.* [191] evaluated the antioxidant activity of ZnO NPs, prepared from the root extract of *Polygala tenuifolia* (*P. tenuifolia*), using DPPH free radical assay. The results showed that ZnO NPs established moderate antioxidant activity by scavenging 45.5% DPPH at 1 mg ml^{-1} . Siripireddy *et al.* [192] used the *Eucalyptus globulus* (*E. globulus*) leaf extract to synthesize spherical ZnO NPs (12 nm) under ambient conditions. ZnO NPs possessed antioxidant activity of 82% scavenging potential against DPPH, compared to ascorbic acid (used as a reference) with IC_{50} of $46.6 \text{ } \mu\text{g ml}^{-1}$. In another study [85], ZnO NPs were green-synthesized using the aqueous stem extract of *Ruta graveolens* (L.) (*R. graveolens* L.). The results showed that ZnO NPs (28 nm) possessed DPPH free radical scavenging activity with IC_{50} of 9.3 mg ml^{-1} . Additionally, the antioxidant activity of ZnO NPs, prepared from the leaf extract of *Ceropegia candelabrum* (*C. candelabrum*), showed DPPH free radical scavenging activity ranging from 0 to 55% with IC_{50} of $95.1 \text{ } \mu\text{g ml}^{-1}$, compared to 75% inhibition by ascorbic acid (used as a positive control) at $50 \text{ } \mu\text{g ml}^{-1}$ [193]. Moreover, it was found that the antioxidant activity increased with an increase in the ZnO NP concentration. Khan *et al.* [194] investigated the antioxidant activity of ZnO NPs, prepared from the *Trianthema portulacastrum* (*T. portulacastrum*), using the DPPH assay method. The authors reported that the antioxidant activity of ZnO NPs is attributed to the small size, large surface area, surface charge density, and capping materials present on the surface of ZnO NPs. Additionally, it was found that the free radical inhibition of ZnO NPs was concentration-dependent [194]. Alamdari *et al.* [109] revealed that ZnO NPs (17 nm), green-synthesized from the leaf extract of *S. ebulus*, exhibited H_2O_2 free radical scavenging activity with IC_{50} of $43 \text{ } \mu\text{g ml}^{-1}$. The researchers reported that the presence of Zn^{2+} could enhance the antioxidant activity of ZnO NPs. Recently, Faisal *et al.* [195] green-synthesized ZnO NPs (66 nm) from the aqueous fruit extract of *Myristica fragrans* (*M. fragrans*). The antioxidant activity of ZnO NPs was proved using four assay methods (total antioxidant capacity, total reduction power, 2,2'-azino-bis(3-ethylbenzothiazoline 6-sulfonic acid), and DPPH free radical scavenging assay). In another recent study, Abdelbaky *et al.* [26] demonstrated the antioxidant activity of ZnO NPs, prepared from *Pelargonium odoratissimum* (L.)

(*P. odoratissimum* L.) with IC_{50} of $28.1 \text{ } \mu\text{g ml}^{-1}$. Therefore, the results of these studies revealed that the green-synthesized ZnO NPs possessed antioxidant activity by scavenging DPPH free radicals.

7.5 Antifungal activity of ZnO NPs

In addition to the aforementioned biomedical applications, the green-synthesized ZnO NPs demonstrated a potential antifungal activity toward several types of fungi [15,74,135]. The antifungal mechanism was described by the ability of ZnO NPs to enter the fungal membrane by diffusion and endocytosis. In the cytoplasm, ZnO NPs interfere with the mitochondrial function, initiating the production of ROS and the release of Zn^{2+} . The overproduction of ROS and Zn^{2+} resulted in irreversible DNA damage and cell death [15,135]. Padalia and Chanda [196] reported the green synthesis of ZnO NPs (17.3 nm) using the leaf extract of *Ziziphus nummularia* (*Z. nummularia*). ZnO NPs showed higher antifungal activity against *Candida albicans* (*C. albicans*) than the azole antifungal agents. Miri *et al.* [197] used *Prosopis farcta* (*P. farcta*) to synthesize ZnO NPs (40–80 nm) and investigated their antifungal activity against *C. albicans* with MIC of $128 \text{ } \mu\text{g ml}^{-1}$ and minimal fungicidal concentration (MFC) of $256 \text{ } \mu\text{g ml}^{-1}$. Recently, Yassin *et al.* [198] studied the antifungal activity of ZnO NPs (22.8 nm) synthesized greenly from the peel extract of pomegranate. The antifungal activity of ZnO NPs was tested against three types of candida strains, *C. albicans*, *C. tropicalis*, and *C. glabrata*. A $100 \text{ } \mu\text{g}$ of ZnO NPs per disk demonstrated antifungal activity against *C. albicans*, *C. tropicalis*, and *C. glabrata*, with zones of inhibition of 24.18, 20.17, and 26.35 mm, respectively. The MIC and MFC of ZnO NPs against *C. tropicalis* were 10 and $20 \text{ } \mu\text{g ml}^{-1}$, respectively. Further, ZnO NPs demonstrated a synergistic efficiency against *C. albicans* with the antifungal drugs fluconazole, nystatin, and clotrimazole; whereas terbinafine, nystatin, and itraconazole showed a potential synergism against *C. glabrata* with ZnO NPs.

Additionally, the green-synthesized ZnO NPs exhibit antifungal activity against plant pathogenic fungi [199,200]. Jamdagni *et al.* [201] synthesized ZnO NPs using the *Nyctanthes arbortristis* (*N. arbortristis*) flower extract. The ZnO NPs (12–32 nm) were tested against five phytopathogens *Alternaria alternata* (*A. alternata*), *Fusarium oxysporum* (*F. oxysporum*), *Aspergillus niger* (*A. niger*), *Penicillium expansum* (*P. expansum*), and *Botrytis cinerea* (*B. cinerea*), which showed MICs of 64, 64, 16, 128, $128 \text{ } \mu\text{g ml}^{-1}$, respectively. Zhu *et al.* [202] synthesized ZnO NPs from *Cinnamomum camphora* (L.)

(*C. camphora* L.) at pH values 7, 8, and 9 with corresponding sizes of 13.9, 15.2, and 21.1 nm, respectively. The antifungal activity was evaluated against *A. alternata*. The MIC of ZnO NPs was 20 mg l⁻¹ at pH 7, which showed the best antifungal effect. It was found that ZnO NPs induced excessive accumulation of malondialdehyde in *A. alternata*, causing damage to the cell membrane and thereby leakage of protein and nucleic acid. Despite that ZnO NPs exhibited antimicrobial activity, it was noticed that their sensitivity against fungi was lower compared to bacteria. This is attributed to the ability of fungi to form resistant spores when they grow under aggressive conditions [203].

7.6 Antiviral activity of ZnO NPs

The green synthesis of ZnO NPs demonstrated antiviral activity against many viruses. Melk *et al.* [204] synthesized ZnO NPs (32.58 ± 7.98 nm) from the alcoholic extract of *Plumbago indica* (*P. indica*), a valuable source of alkaloids, phenolics, and saponins. The results showed that ZnO NPs and the plant extract exhibited inhibitory effects against herpes simplex virus type 1 (HSV-1), which could be considered a promising adjuvant to improve the efficacy of HSV-1 drugs. In another study, Melk *et al.* [205] synthesized ZnO NPs (38.29 ± 6.88 nm) from the alcoholic extract of the flowering aerial parts of *Plumbago auriculata* Lam (*P. auriculata* Lam). The ZnO NPs and the plant extract possessed a significant antiviral activity against avian metapneumovirus subtype B, which would be beneficial for controlling the spreading of the virus in infected birds. Moreover, the green synthesis nanocomposites of ZnO NPs/activated carbon (ZnO NPs/AC) (30–70 nm) were prepared using the water hyacinth (*Pontederia crassipes*) [206]. In addition to the antiviral activity against the HSV1 virus, the ZnO NPs/AC nanocomposites showed antibacterial and antifungal activities. Studies revealed that the mechanism of the antiviral activity of ZnO NPs is attributed to the damage of the lipid membrane and RNA, thus inactivating the virus [207]. Hamdi *et al.* [208] conducted a computational analysis to investigate the interaction between ZnO NPs and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) targets. It was found that the hexagonal and spherical ZnO NPs with a crystallite size of ~11 nm and positive zeta potential exhibited the highest antiviral activity. Recently, Alrabayah *et al.* [209] used the alcoholic leaf extract of *Cestrum diurnum* L. (*C. diurnum* L.) and zinc acetate to prepare green-synthesized ZnO NPs (3.4–4.9 nm) to evaluate their antiviral activity against human corona-229E (HCoV-229E). The *C. diurnum* L. leaf extract contained catechin, ferulic acid, chlorogenic acid, and syringic acid. Both

ZnO NPs and the *C. diurnum* L. extract showed antiviral activity against HCoV-229E with IC₅₀ of 7.01 and 61.15 µg ml⁻¹; however, their combination showed higher activity than individually with IC₅₀ of 2.41 µg ml⁻¹.

7.7 Antiparasitic activity of ZnO NPs

The antiparasitic activity of the green-synthesized ZnO NPs has been reported in the literature [17]. The antiparasitic mechanism of ZnO NPs involves the disruption of the parasite membrane, damaging the parasite DNA, inhibition of protein synthesis, and formation of free radicals [17]. Dorostkar *et al.* [210] tested the anthelmintic activity of ZnO NPs and FeO NPs against *Toxocara vitulorum* (*T. vitulorum*) using different concentrations of NPs (0.004, 0.008, and 0.012% w/v). The results showed that both NPs increased the mortality rate of worms and increased the levels of malondialdehyde (MDA) and nitric oxide (NO). The levels of MDA and NO increased with an increase in the exposure time and concentration of NPs. The anthelmintic activity of both NPs was attributed to the induction of oxidative/nitrosative stress, resulting in the generation of ROS. Saleh *et al.* [211] studied the antiprotozoal effects of the metallic NPs (gold, silver, and ZnO) against *Ichthyophthirius multifiliis* (*I. multifiliis*), a ciliated protozoan ectoparasite of fish. The results showed that silver and ZnO NPs at 10 and 5 ng m⁻¹ killed 100 and 97% of theronts, respectively, and inhibited the production of tomonts after 2 h of exposure. However, the antiparasitic effect of gold NPs was lower compared to that of silver and ZnO NPs, killing 80 and 78% of tomonts and theronts, respectively, at 20 ng ml⁻¹ after 2 h of exposure. Recently, spherical and elliptical ZnO NPs (34.2 nm), green synthesized using the aqueous extract of *Himalayan Columbine* (*Aquilegia pubiflora*), were evaluated for their anti-leishmanial activity. The results showed a dose-dependent cytotoxic effect of ZnO NPs against *Leishmania tropica* (KWH23) with IC₅₀ of 48 and 51 µg ml⁻¹ for promastigote and amastigote, respectively [212]. Likewise, Najoom *et al.* [213] green-synthesized ZnO NPs (70–90 nm) using the leaf extract of *Rhazya stricta* (*R. stricta*) as capping and reducing agents. ZnO NPs showed antiplasmodial activity against plasmodium parasites with IC₅₀ of 3.41 µg ml⁻¹. Similarly, spherical or elliptical-shaped ZnO NPs (66 nm) were prepared from the aqueous fruit extracts of *Myristica fragrans* (*M. fragrans*) and evaluated for their antiparasitic activity [195]. The results showed potential leishmanicidal activity against two forms of parasites (promastigote and amastigote) with a mortality rate of 71.5 and 61.4%, respectively. Hence, these studies demonstrated that the green-synthesized ZnO NPs could be effective against parasites.

7.8 Anti-inflammatory activity of ZnO NPs

The green-synthesized ZnO NPs showed excellent activity toward inflammation [15]. The anti-inflammatory mechanism involves the dissolution of ZnO NPs, releasing Zn^{2+} ions. The release of Zn^{2+} can be involved in several pathways, such as suppressing the release of the pro-inflammatory cytokines (interleukin-1 (IL-1), IL-1 β , IL-13, and tumor necrosis factor- α (TNF- α)) in mast cells. Additionally, Zn^{2+} release can suppress the expression of the lipopolysaccharide (LPS)-induced cyclooxygenase-2 (COX-2) gene in macrophages, inducible nitric oxide synthase (iNOS), myeloperoxidase, and NF- $\kappa\beta$. Moreover, Zn^{2+} release may block the caspase-1 enzyme in activated mast cells [15].

Nagajyothi *et al.* [191] green-synthesized ZnO NPs using the root extract of *P. tenuifolia*. The anti-inflammatory activity of ZnO NPs was evaluated in LPS-stimulated RAW 264.7 murine macrophages. The results showed a dose-dependent suppression effect of ZnO NPs on mRNA and protein expression of iNOS and pro-inflammatory cytokines. Rajakumar *et al.* [186] green-synthesized ZnO NPs (57–115 nm) from the leaf extract of *Andrographis paniculata* (*A. paniculata*). By using heat to induce albumin denaturation, the results showed an inhibition in the protein denaturation after exposure to ZnO NPs. Recently, the green-synthesized ZnO NPs, prepared from the leaf extract of *Kalanchoe pinnata* (*K. pinnata*), showed anti-inflammatory activity by inhibiting the production and release of the pro-inflammatory cytokines IL-1 β , IL-6, TNF- α , and COX-2 [214]. Additionally, Mohammad *et al.* [215] evaluated the anti-inflammatory activity of spherical ZnO NPs, prepared from the *Hyssops officinalis* L. (*H. officinalis* L.) extract, using the mouse paw edema test. The results showed a decrease in edema when treated with a dose of 5 mg/kg ZnO NPs. Similarly, Liu *et al.* [216] proved the anti-inflammatory activity of ZnO NPs, greenly synthesized from the leaf extract of *Vernonia amygdalina* (*V. amygdalina*), against inflammation-induced mice model by reducing the inflammatory response and pro-inflammatory cytokines level in mice. Recently, Abdelbaky *et al.* [26] proved the anti-inflammatory activity of ZnO NPs (34.1 nm), synthesized by employing the aqueous leaf extract of *P. odoratissimum* L. as a reducing agent. The anti-inflammatory effect of ZnO NPs was evaluated via an *in vitro* model of human red blood cells (RBCs) which involves hypotonicity-induced hemolysis. ZnO NPs were able to maximally stabilize the membrane by 95.6% at a dose of 1,000 $\mu\text{g ml}^{-1}$, compared with the standard indomethacin. Finally, a very recent study by Lopez-Miranda *et al.* [217] evaluated the anti-inflammatory activity of ZnO NPs, prepared from the *sargassum* extract. The results revealed that ZnO NPs could inhibit protein denaturation and exhibited higher anti-inflammatory activity than the reference drug diclofenac. Thus, these studies suggested

that the green-synthesized ZnO NPs showed promising anti-inflammatory activity.

7.9 Wound healing of ZnO NPs

ZnO NPs showed wound-healing activity [46,218]. Mahdavi *et al.* [219] investigated the cutaneous wound-healing activity of ZnO NPs (32 nm) prepared using the *Ziziphora clinopodioides* (*Z. clinopodioides*) leaf extract. The wound healing of ZnO NPs was confirmed by increasing the level of fibrocytes/fibroblasts, hydroxyl proline, hexosamine, hexuronic acid, and fibrocytes, and decreasing the wound area, total cells, and lymphocytes compared to control groups in rats. Additionally, green and chemical ZnO NPs, loaded into gels, were investigated for wound healing [220]. ZnO NPs were prepared by green synthesis using the *Lawsonia inermis* (*L. inermis*) leaf extract. The green and chemical ZnO NP-loaded gels showed promising wound-healing properties with reduced healing time in treated groups when compared with the control group and the green ZnO NPs gels were more effective than the chemical ones. Moreover, Shao *et al.* [221] reported that the green-synthesized ZnO NPs using the *Barleria gibsoni* (*B. gibsoni*) leaf extract and loaded into Carbopol gels showed wound-healing properties due to burns in rats.

Furthermore, the literature reported the effective wound-healing activity of ZnO NPs prepared using aqueous leaf extracts of *Coleus amboinicus* (*C. amboinicus*) [222], *A. barbadensis* [223], and *Azadirachta indica* (*A. indica*) neem leaves [224] as reducing, capping, and stabilizing agents. In addition, the literature reported an improved treatment of infected wounds caused by resistant bacteria by the embedding of green ZnO NPs prepared using the *Alternanthera sessilis* (*A. sessilis*) leaf extract into cotton fabrics [225], incorporation of ZnO NPs prepared using *Ilex paraguariensis* (*I. paraguariensis*) leaves into polymeric electrospun fibers of polyacrylic acid and polyallylamine hydrochloride [226], and gel formulations loaded with green synthesis silver NPs and ZnO NPs, prepared from the *Annona squamosa* (*A. squamosa*) leaf extract [227]. Altogether, these studies highlighted more promising biomedical applications of the green-synthesized ZnO NPs. Plants, part of the plants, chemical precursors, and size and biological activity of the green-synthesized ZnO NPs reported in Sections 7.1–7.9 are summarized in Table 2.

8 Toxicity of ZnO NPs

Although bulk ZnO has been GRAS [35], there is still doubt regarding the safety of ZnO NPs on human health that

Table 2: Plants, part of plants, chemical precursors, size, and biological activity of green-synthesized ZnO NPs

Plant	Part of the plant	Chemical precursor	Size (nm)	Biological activity	Ref.
Aloe vera	Leaf extract	Zinc acetate	40–180	Antibacterial	[148]
<i>Dysphania ambrasoides</i> (<i>D. ambrasoides</i>)	Leaf extract	Zinc nitrate hexahydrate	7–130	Antibacterial	[118]
<i>Punica granatum</i> (<i>P. granatum</i>)	Peel extract	Zinc acetate	10–45	Antibacterial	[104]
Orange fruit	Peel extract	Zinc acetate	20	Antibacterial	[153]
<i>Parthenium hysterophorus</i> (<i>P. hysterophorus</i>)	Leaf extract	Zinc nitrate	10	Antibacterial	[228]
<i>Daphne oleoides</i> (<i>D. oleoides</i>)	Leaf extract	Zinc nitrate hexahydrate	30–44	Antibacterial	[155]
Aloe vera	Leaf extract	Zinc nitrate	40	Antibacterial	[133]
<i>Sesbania grandiflora</i> (<i>S. grandiflora</i>)	Leaf extract	Zinc acetate	45	Antibacterial	[156]
Avocado, papaya, and mango fruit	Fruit extract	Zinc nitrate hexahydrate	11–21	Antibacterial	[157]
<i>Tecoma castanifolia</i> (<i>T. castanifolia</i>)	Leaf extract	Zinc sulfate	70–75	Anticancer	[163]
Banana fruit	Fruit extract	Zinc acetate	NR*	Antibacterial and anticancer	[164]
<i>Cratogeomys formosus</i> (<i>C. formosus</i>)	Leaf extract	Zinc acetate	200–500	Anticancer	[165]
<i>Phoenix dactylifera</i> (<i>P. dactylifera</i>)	Root hair extract	Zinc acetate	31–48	Anticancer	[72]
<i>Cardiospermum halicacabum</i> (<i>C. halicacabum</i>)	Leaf extract	Zinc acetate	10–20	Anticancer	[166]
<i>Deverra tortuosa</i> (<i>D. tortuosa</i>)	Aerial extract	Zinc nitrate hexahydrate	9–31	Anticancer (lung and colon cancer)	[167]
<i>Acorus calamus</i> (<i>A. calamus</i>)	Rhizomes extract	Zinc acetate	50–100	Anticancer and antibacterial	[168]
<i>Vinca rosea</i> (<i>V. rosea</i>)	Leaf extract	Zinc nitrate hexahydrate	16–41	Anticancer, antidiabetic, and antimicrobial	[169]
Onion peel	Waste extract	Zinc acetate	NR*	Anticancer and antimicrobial	[170]
<i>Alpinia calcarata</i> (<i>A. calcarata</i>)	Rhizomes extract	NR*	19	Anticancer	[171]
<i>Aloe barbadensis</i> (<i>A. barbadensis</i>)	Leaf extract	Zinc nitrate	20–40	Anticancer and wound healing	[172]
<i>Borassus flabellifer</i> (<i>B. flabellifer</i>)	Palm fruit extract	Zinc nitrate	55	Anticancer	[173]
<i>Ilex paraguariensis</i> (<i>I. paraguariensis</i>)	Leaf extract	Zinc acetate and zinc nitrate	18–389	Anticancer	[229]
<i>Andropogon paniculata</i> (<i>A. paniculata</i>)	Leaf extract	Zinc nitrate hexahydrate	57–115	Antidiabetic, antioxidant, and anti-inflammatory	[186]
<i>Albizia lebeck</i> (<i>A. lebeck</i>)	Bark extract	Zinc acetate	NR*	Antidiabetic and antioxidant	[187]
<i>Artocarpus gomezianus</i> (<i>A. gomezianus</i>)	Fruit extract	Zinc nitrate hexahydrate	NR*	Antioxidant	[189]
<i>Cassia fistula</i> (<i>C. fistula</i>)	Leaf extract	Zinc nitrate hexahydrate	5–15	Antioxidant and antibacterial	[190]
<i>Polygala tenuifolia</i> (<i>P. tenuifolia</i>)	Root extract	Zinc nitrate hexahydrate	NR*	Antioxidant and anti-inflammatory	[191]
<i>Eucalyptus globulus</i> (<i>E. globulus</i>)	Leaf extract	Zinc nitrate hexahydrate	12	Antioxidant	[192]
<i>Ruta graveolens</i> (<i>R. graveolens</i>)	Stem extract	Zinc nitrate	20–30	Antioxidant and antibacterial	[85]
<i>Ceropegia candelabrum</i> (<i>C. candelabrum</i>)	Leaf extract	Zinc nitrate hexahydrate	12–35	Antioxidant and antibacterial	[193]
<i>Trianthema portulacastrum</i> (<i>T. portulacastrum</i>)	Fruit extract	Zinc sulfate	2–8	Antioxidant	[194]
<i>Sambucus ebulus</i> (<i>S. ebulus</i>)	Leaf extract	Zinc acetate	17	Antioxidant and antibacterial	[109]
<i>Myristica fragrans</i> (<i>M. fragrans</i>)	Fruit extract	Zinc acetate	66	Antioxidant, antibacterial, and antidiabetic	[195]
<i>Pelargonium odoratissimum</i> (<i>P. odoratissimum</i>)	Leaf extract	Zinc acetate	76	Antioxidant antibacterial antiparasitic anti-inflammatory	[26]
<i>Prosopis farcta</i> (<i>P. farcta</i>)	Aerial extract	Zinc sulfate	40–80	Antifungal	[197]
<i>Pomegranate</i>	Peel extract	Zinc nitrate hexahydrate	22.8	Antifungal	[198]
<i>Nyctanthes arbortristis</i> (<i>N. arbortristis</i>)	Flower extract	Zinc acetate	12–32	Antifungal	[201]
<i>Cinnamomum camphora</i> (<i>C. camphora</i>)	Leaf extract	Zinc acetate	14–21	Antifungal	[202]
<i>Plumbago indica</i> (<i>P. indica</i>)	Aerial extract	Zinc acetate	33	Antiviral	[204]

(Continued)

Table 2: Continued

Plant	Part of the plant	Chemical precursor	Size (nm)	Biological activity	Ref.
<i>Plumbago auriculata</i> Lam (<i>P. auriculata</i> Lam)	Aerial extract	Zinc acetate	38	Antiviral	[205]
<i>Water hyacinth</i> (<i>Pontederia crassipes</i>)	Raw water hyacinth	Zinc chloride	30–70	Antiviral antimicrobial	[206]
<i>Cestrum diurnum</i> L. (<i>C. diurnum</i> L.)	Leaf extract	Zinc acetate	3–5	Antiviral	[209]
<i>Aquilegia pubiflora</i> (A. pubiflora)	Leaf extract	Zinc acetate	34	Antiparasitic	[212]
<i>Rhazya stricta</i> (R. stricta)	Leaf extract	Zinc chloride	70–90	Antiparasitic	[213]
<i>Kalanchoe pinnata</i> (K. pinnata)	Leaf extract	Zinc acetate	24	Anti-inflammatory	[214]
<i>Hyssops officinalis</i> L. (<i>H. officinalis</i> L.)	Leaf and flower extract	Zinc acetate	20–40	Anticancer anti-angiogenesis, and anti-inflammatory	[215]
<i>Vernonia amygdalina</i> (V. amygdalina)	Leaf extract	Zinc acetate	20–40	Anti-inflammatory	[216]
<i>Sargassum</i>	Plant extract	Zinc acetate	20–200	Anti-inflammatory	[217]
<i>Ziziphora clinopodioides</i> (Z. clinopodioides)	Leaf extract	Zinc nitrate hexahydrate	32	Wound healing	[219]
<i>Lawsonia inermis</i> (L. inermis)	Leaf extract	Zinc nitrate hexahydrate	NR*	Wound healing	[220]
<i>Barleria gibsoni</i> (B. gibsoni)	Leaf extract	Zinc nitrate hexahydrate	30–80	Wound healing	[221]
<i>Coleus amboinicus</i> (C. amboinicus)	Leaf extract	Zinc nitrate	15–20	Wound healing	[222]
<i>Azadirachta indica</i> (A. indica)	Leaf extract	Zinc acetate	52	Wound healing	[224]
<i>Alternanthera sessilis</i> (A. sessilis)	Leaf extract	Zinc acetate	NR*	Wound healing	[225]
<i>Ilex paraguariensis</i> (I. paraguariensis)	Leaf extract	Zinc acetate and zinc nitrate	18–389	Wound healing and anticancer	[226]
<i>Annona squamosa</i> (A. squamosa)	Leaf extract	NR*	NR*	Wound healing	[227]
<i>Calotropis gigantea</i> (C. gigantea)	Leaf extract	Zinc nitrate	16–27	Anticancer	[183]
<i>Salvadora oleoides</i> (S. oleoides)	Leaf extract	Zinc nitrate	12–27	Anticancer and antifungal	[99]
<i>Azadirachta indica</i> (L.) (A. Indica)	Leaf extract	Zinc nitrate hexahydrate	NR*	Antibacterial	[230]
<i>Aloe barbadensis miller</i> (Aloe vera)	Leaf extract	Zinc nitrate	25–40	NR*	[67]
<i>Hibiscus subdariffa</i> (H. subdariffa)	Leaf extract	Zinc acetate	16–60	Antibacterial and antidiabetic	[231]
<i>Ocimum basilicum</i> L. var. <i>purpurascens</i> Benth.-Lamiaceae	Leaf extract	Zinc nitrate	50	NR*	[232]
– <i>Beta vulgaris</i> (B. vulgaris)	Whole plant extract	Zinc nitrate	20	Antibacterial antifungal	[87]
– <i>Cinnamomum tamala</i> (C. tamala)					
– <i>Cinnamomum verum</i> (C. verum)					
– <i>Brassica oleracea</i> var. <i>Italica</i> (B. oleracea)					
<i>Atalantia monophylla</i> (A. monophylla)	Leaf extract	Zinc acetate dihydrate	33	Antibacterial	[233]
<i>Thymbra spicata</i> L. (T. spicata L.)	Leaf extract	Zinc acetate dihydrate	6.5–7.5	Antibacterial, antioxidant protection against DNA damage	[234]
<i>Stevia</i>	Leaf extract	Zinc acetate	10–90	Antibacterial	[235]
<i>Cassia auriculata</i> (C. auriculata)	Leaf extract	Zinc acetate	20–30	Antibacterial	[236]
<i>Phoenix dactylifera</i> (P. dactylifera)	Date pulp waste extract	Zinc hexahydrate	30	Antibacterial	[88]
<i>Chamomile flower</i> (<i>Matricaria chamomilla</i> L. <i>M. chamomilla</i> L.), olive leaves (<i>Olea europaea</i> , <i>O. europaea</i>), and red tomato fruit (<i>Lycopersicon esculentum</i> M, <i>L. esculentum</i> M)	Flower, leaf, and fruit extract	ZnO	48	Antibacterial	[237]
<i>Beta vulgaris</i> (B. vulgaris)	Fruit extract	Zinc nitrate	20	Anticancer	[238]
<i>Punica granatum</i> (P. granatum) (pomegranate)	Leaf and flower extract	Zinc nitrate hexahydrate	NR*	Antibacterial	[239]

(Continued)

Table 2: Continued

Plant	Part of the plant	Chemical precursor	Size (nm)	Biological activity	Ref.
<i>Justicia adhatoda</i> (J. adhatoda)	Leaf extract	Zinc acetate, zinc sulfate, and zinc nitrate	15–20	Antibacterial	[240]
<i>Mussaenda frondosa</i> L. (M. frondosa L.)	Leaf, stem, and callus extracts	Zinc nitrate hexahydrate	5–20	Antibacterial, anti-inflammatory, anticancer, antidiabetic and antioxidant	[241]
<i>Parthenium hysterophorus</i> (P. hysterophorus)	Leaf extract	NR*	NR*	Antibacterial	[130]
<i>Raphanus sativus</i> var. Longipinnatus (R. Sativus)	Leaf extract	Zinc acetate dihydrate	209	Anticancer	[100]
<i>Citrus sinensis</i> (C. sinensis)	Peel extract	Zinc nitrate hexahydrate	NA	Antibacterial	[242]
<i>Eucalyptus globules</i> (E. globules)	Leaf extract	Zinc nitrate hexahydrate	52–70	Antifungal	[243]
<i>Salvia officinalis</i> (S. officinalis)	Leaf extract	NR*	NR*	Antifungal	[128]
<i>Ziziphium nummularia</i> (Z. nummularia)	Leaf extract	Zinc nitrate	17	Antifungal and anticancer	[196]
<i>Syzygium cumini</i> (S. cumini)	Leaf extract	Zinc acetate	30	Antioxidant and anticancer	[92]
<i>Cissus quadrangularis</i> (C. quadrangularis)	Stem extract	Zinc acetate dihydrate	75–90	Antibacterial and anticancer	[244]
<i>Sabal blackburniana</i> (S. blackburniana)	Leaf, fruit, and pollen grains extracts	Zinc acetate dihydrate	2–50	Acetylcholinesterase inhibitory activity and anti-Alzheimer	[245]
<i>Amygdalus scoparia</i> (A. scoparia)	Stem bark extract	Zinc acetate dihydrate	15–40	Anticancer, antidiabetic, and antibacterial	[246]
<i>Cnidioscolus aconitifolius</i> (C. aconitifolius)	Leaf extract	Zinc acetate	100	Antioxidant, anti-inflammatory, and antibacterial	[247]
<i>Solanum rantonnetii</i> (S. rantonnetii)	Leaf extract	NR*	12	Antifungal activity	[248]

NR*: not reported.

warrants further investigation [249]. Because ZnO NPs are commonly used in broad biomedical applications, the advantages of these types of NPs must be wisely weighed against possible toxic effects [250]. It has been shown that the concentration and exposure time of NPs and cell type play a vital role in posing toxic effects [251]. Additionally, the toxicity of NPs is determined by their physicochemical properties such as size, shape, composition, and surface properties [252].

Moatamed *et al.* [253] compared the impact of ZnO NPs (39 nm) and bulk ZnO (5 μm) on the liver function of Wistar rats. Three groups of rats received three dose levels of ZnO NPs according to body weight (bw) (25, 50, and 100 mg/kg bw). It was found that the group that received 100 mg/kg bw ZnO NPs showed the most significant changes in liver enzymes, histopathological structure, and oxidative stress markers, indicating a dose-dependent hepatic toxicity of ZnO NPs. However, the group that received 100 mg/kg bw of bulk ZnO showed no significant effects on liver function. This can be explained by the increase in the Zn^{2+} concentration in the hepatic tissues of rats treated with ZnO NPs, while no significant change in Zn^{2+} concentration was found in rats that received the same dose of bulk ZnO. A study by Salami *et al.* [254] determined the hemolytic toxic effects of ZnO NPs and their bulk counterparts on isolated RBCs. A concentration range of 0.01–1 mM of ZnO (bulk and NPs) was used in this study. The results demonstrated that ZnO NPs caused toxic hemolytic effects due to the generation of ROS, lipid peroxidation, and GSH depletion, whereas bulk ZnO did not show any toxic hemolytic effects. Additionally, Roy *et al.* [255] assessed the impact of ZnO NPs *versus* bulk ZnO on the activity of murine macrophages. The study revealed that ZnO NPs were more potent in activating the proinflammatory cytokine production by macrophages, whereas the small size of ZnO NPs may help in the evasion of the macrophage response.

The toxicity studies of green-synthesized ZnO NPs are limited. Saranya *et al.* [256] assessed the *in vitro* cytotoxicity of three green-synthesized NPs including ZnO NPs, FeO NPs, and copper NPs (Cu NPs), prepared using the aqueous leaf extract of *Musa ornate* (*M. Ornate*) and *Zea mays* (*Z. mays*). The cytotoxicity of NPs was assessed in three cell lines, the African green monkey kidney cell line (Vero), pig kidney cell line (PK15), and Madin Darby bovine kidney (MDBK) at 24 and 48 h. The results showed that the cytotoxic effect of green NPs varied based on the concentration and exposure time of NPs and cell type. Additionally, the green ZnO NPs exhibited higher cell viability at lower concentrations (10 $\mu\text{g}/100\ \mu\text{L}$) when compared to green FeO NPs and Cu NPs in all cell types [256]. Additionally, the toxicity of the ZnO NPs, prepared from the aqueous extract of *Amaranthus*

caudatus (*A. caudatus*), was evaluated using zebrafish embryos [257]. The toxicity of ZnO NPs showed a concentration-dependent pattern, where the ZnO NP concentration of $\leq 10\ \text{mg}\ \text{mL}^{-1}$ showed no toxic effect, whereas at 50–100 $\text{mg}\ \text{mL}^{-1}$ high levels of mortalities were observed [257].

The toxicity of chemically synthesized ZnO NPs was evaluated. Earlier, Najim *et al.* [258] investigated the cytotoxicity of ZnO NPs of different sizes in both normal and cancer cell lines. The results showed that ZnO NPs of 85.7 and 190 nm displayed cytotoxic effects on all cell lines derived from the lung tissue (Hs888Lu), neuron-phenotypic cells (SH-SY5Y), neuroblastoma (SH-SY5Y), human histiocytic lymphoma (U937), with no effect on lung cancer (A549). Additionally, it was found that the toxicity of ZnO NPs depends on the particle size, concentration, and exposure time. Khan *et al.* [259] assessed the toxic effect of ZnO NPs at different concentrations (50, 100, 250, and 500 ppm) on human erythrocytes. ZnO NPs showed a concentration-dependent hemolytic activity in red blood cells. Additionally, the genotoxic potential of ZnO NPs, investigated by the *in vitro* alkaline comet assay, showed concentration-dependent DNA damage at 250 and 500 ppm concentrations. The toxic effects of ZnO NPs were attributed to the generation of ROS that resulted from oxidative stress. Moreover, the *in vitro* cytotoxicity of ZnO NPs (25 nm) on an *in vitro* model cell line, obtained from the gill tissue of Wallago attu (WAG), was evaluated by MTT, neutral red uptake, and LDH assay tests [260]. Researchers found that ZnO NPs induced acute toxicity to WAG cells with IC_{50} of 5.7 ± 0.1 , 3.1 ± 0.1 , and $5.6 \pm 0.12\ \text{mg}\ \text{l}^{-1}$, respectively. Uzar *et al.* [261] studied the nephrotoxic potential of ZnO NPs (10–50 nm) against rat kidney epithelial cells (NRK-52E) at 25–100 $\mu\text{g}\ \text{mL}^{-1}$ exposure concentration. The IC_{50} on NRK-52E was $73\ \mu\text{g}\ \text{mL}^{-1}$. Comet assay, used to assess the genotoxic effect of ZnO NPs at 12.5–50 $\mu\text{g}\ \text{mL}^{-1}$, showed significant DNA damage in NRK-52E cells. Additionally, Saranya *et al.* [256] assessed the *in vitro* cytotoxicity of three green-synthesized NPs including ZnO NPs, FeO NPs, and Cu NPs, prepared using the aqueous leaf extract of *Musa ornate* (*M. Ornate*) and *Zea mays* (*Z. mays*). The cytotoxicity of NPs was assessed in three cell lines, the African green monkey kidney cell line (Vero), pig kidney cell line (PK15), and MDBK at 24 and 48 h. The results showed that the cytotoxic effect of green NPs varied based on the concentration and exposure time of NPs and cell type. Additionally, the green ZnO NPs exhibited higher cell viability at lower concentrations (10 $\mu\text{g}/100\ \mu\text{L}$) when compared to green FeO NPs and Cu NPs in all cell types. Furthermore, Yousef *et al.* [262] confirmed the hepatic and renal toxicity of ZnO NPs in male Wistar rats, when ZnO NPs were daily administered orally for 75 days. Despite these studies, there is no standard methodology for assessing the toxicity of ZnO NPs [263].

Therefore, more toxicological studies are still needed to provide information on the safety, risk assessments, and risk management of ZnO NPs.

9 Challenges associated with the translation process of ZnO NPs from bench to market

The translation process of ZnO NPs from bench to market is facing several potential bottlenecks and challenges. The green synthesis of ZnO NPs may suffer from batch-to-batch reproducibility and large-scale production including plant selection, synthesis conditions, NPs' size, and applications [24,264]. For instance, plants used for the green synthesis of NPs are commonly selected based on their local availability and abundance [264]. This, in turn, makes it difficult to green-synthesize NPs globally, using specific plant extracts. Additionally, the time of plant collection hinders their use, where plants are either collected during flowering or fruiting seasons [265]. Moreover, the green synthesis of NPs requires extremely high or low temperatures, long reaction and extraction times, inert conditions (*i.e.*, prepared under an inert atmosphere of argon or nitrogen), and the use of chemical precursors (such as zinc nitrate and zinc acetate) [264]. In addition, the specific reaction mechanism of the green synthesis of NPs is still difficult to elucidate, and the mass balance and stoichiometric ratio that scale up the process is a major challenge. Furthermore, the size of NPs, synthesized from different plant extracts, is heterogeneous with irregular shapes [264].

The safety profile of ZnO NPs needs to be thoroughly investigated and assessed before their use. It was found that ZnO NPs may cause hepatic, nephron, neuronal, pulmonary, and reproductive toxicity [70]. ZnO NPs can enter the biological systems through three primary routes: dermal, inhalation, and ingestion, followed by circulating in the bloodstream [266]. The way zinc interacts with proteins, nucleic acids, and cells plays a crucial role in determining the application of ZnO NPs. Additionally, factors such as size, physicochemical properties, dose, and duration of exposure are important considerations in assessing the toxicity of ZnO NPs [251,252].

The regulatory approval process for novel NPs can be complex and time-consuming. This is because meeting the regulatory requirements for safety, efficacy, and quality can be a challenging and costly process, often leading to a delay in market entry. Moreover, moving from laboratory-scale production to large-scale manufacturing can be

a significant hurdle, where ensuring efficient and cost-effective production methods while maintaining product integrity can be a challenge. For example, NPs can be susceptible to changes in the physicochemical properties over time, such as aggregation, agglomeration, or degradation. Hence, ensuring long-term stability and adequate shelf-life for NP-based products is crucial for commercial viability [267]. Therefore, addressing these challenges requires a multidisciplinary approach involving scientists, regulators, and manufacturers. Additionally, thorough research, robust safety assessments, adherence to regulatory guidelines, and efficient manufacturing processes are essential for the successful translation and commercialization of ZnO NPs.

10 Conclusion and future perspectives

Scientists are actively dedicating their efforts to enhance the green synthesis of ZnO NPs for various biomedical applications. The green-synthesized ZnO NPs have gained significant importance due to their eco-friendly, biocompatibility, and cost-effectiveness than those prepared by chemical and physical methods. The variety of plant extracts with their phytochemicals such as flavonoids, terpenoids, polyphenols, tannins, and alkaloids can be considered a potential source for the green synthesis of ZnO NPs that contribute to the stability and discovery of new physicochemical properties for ZnO NPs. Research studies have shown that the green-synthesized ZnO NPs exhibited superior bioactivities due to the presence of various phytochemicals on their surfaces. The antibacterial activity of ZnO NPs holds promise as a potential solution for the treatment of infections caused by multidrug-resistant bacteria and as an alternative to antibiotics. Moreover, as an anticancer agent, ZnO NPs localize in cancer cells through enhanced permeability and retention effects and electrostatic interactions, leading to cell death through the release of Zn^{2+} ions and/or generation of ROS. This suggests that ZnO NPs are a promising anticancer agent in targeting drug delivery. The green-synthesized ZnO NPs may offer a remedy for diabetes as an antidiabetic agent and an increase in the radical scavenging activity as an antioxidant agent. Therefore, ZnO NPs hold vast potential for pharmaceutical and biomedical applications, making them a major area of research. Furthermore, ZnO NPs exhibited antifungal, antiviral, antiparasitic, and anti-inflammatory activities, expanding their use in health care products such as sunscreen lotions for skin acne, blemishes, and

UV/visible light protection. However, it is crucial to address the limitations of ZnO NP toxicity toward biological systems and the need for evidence-based research exploring the bioactivities of ZnO NPs, which require further investigation. Currently, to our knowledge, the green-synthesized ZnO NPs have not been introduced into the market, and no clinical trials have been reported. Hence, additional *in vivo* studies on the biomedical applications of green-synthesized ZnO NPs are necessary for future clinical use.

Acknowledgments: The authors are thankful to their respective departments/universities for the successful completion of this review.

Funding information: The authors state no funding involved.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conflict of interest: The authors state no conflict of interest.

References

- [1] Contera S, Bernardino de la Serna J, Tetley TD. Biotechnology, nanotechnology and medicine. *Emerg Top Life Sci.* 2020;4(6):551–4.
- [2] Garg R, Rani P, Garg R, Khan MA, Khan NA, Khan AH, et al. Biomedical and catalytic applications of agri-based biosynthesized silver nanoparticles. *Environ Pollut.* 2022;310:119830.
- [3] Saxena SK, Nyodu R, Kumar S, Maurya VK. Current advances in nanotechnology and medicine. *NanoBioMedicine.* United Kingdom: Springer; 2020. p. 3–16.
- [4] Fouda A, Eid AM, Guibal E, Hamza MF, Hassan SE-D, Alkhalifah DHM, et al. Green synthesis of gold nanoparticles by aqueous extract of zingiber officinale: Characterization and insight into antimicrobial, antioxidant, and in vitro cytotoxic activities. *Appl Sci.* 2022;12(24):12879.
- [5] Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. *Arab J Chem.* 2019;12(7):908–31.
- [6] Mohamed AA, Fouda A, Abdel-Rahman MA, Hassan SE-D, El-Gamal MS, Salem SS, et al. Fungal strain impacts the shape, bioactivity and multifunctional properties of green synthesized zinc oxide nanoparticles. *Biocatal Agric Biotechnol.* 2019;19:101103.
- [7] Abu-Huwajir R, Al-Assaf SF, Mousli F, Kutkut MS, Al-Bashtawi A. Perceptive review on properties of iron oxide nanoparticles and their antimicrobial and anticancer activity. *Sys Rev Pharm.* 2020;11(8):418–31.
- [8] Nikolova MP, Chavali MS. Metal oxide nanoparticles as biomedical materials. *Biomimetics.* 2020;5(2):27.
- [9] Fouda A, Hassan SE-D, Eid AM, Awad MA, Althumayri K, Badr NF, et al. Endophytic bacterial strain, *Brevibacillus brevis*-mediated green synthesis of copper oxide nanoparticles, characterization, antifungal, in vitro cytotoxicity, and larvicidal activity. *Green Process Synth.* 2022;11(1):931–50.
- [10] Fouda A, Eid AM, Abdel-Rahman MA, El-Belely EF, Awad MA, Hassan SE-D, et al. Enhanced antimicrobial, cytotoxicity, larvicidal, and repellence activities of brown algae, *Cystoseira crinita*-mediated green synthesis of magnesium oxide nanoparticles. *Front Bioeng Biotechnol.* 2022;10:84992.
- [11] Ealia SAM, Saravanakumar M, editors. A review on the classification, characterisation, synthesis of nanoparticles and their application. *IOP conference series: materials science and engineering.* IOP Publishing; 2017.
- [12] Abu Hajleh MN, Abu-Huwajir R, Al-Samydai A, Al-Halaseh LK, Al-Dujaili EA. The revolution of cosmeceuticals delivery by using nanotechnology: A narrative review of advantages and side effects. *J Cosmet Dermatol.* 2021;20(12):3818–28.
- [13] Jiang J, Pi J, Cai J. The advancing of zinc oxide nanoparticles for biomedical applications. *Bioinorg Chem Appl.* 2018;2018:1062562.
- [14] Al-Shalabi R, Abu-Huwajir R, Hamed R, Abbas MM. The antimicrobial and the antiproliferative effect of human triple negative breast cancer cells using the green synthesized iron oxide nanoparticles. *J Drug Deliv Sci Technol.* 2022;75:103642.
- [15] Murali M, Kalegowda N, Gowtham HG, Ansari MA, Alomary MN, Alghamdi S, et al. Plant-mediated zinc oxide nanoparticles: advances in the new millennium towards understanding their therapeutic role in biomedical applications. *Pharmaceutics.* 2021;13(10):1662.
- [16] Alhujaili M, Albukhaty S, Yusuf M, Mohammed MK, Sulaiman GM, Al-Karagoly H, et al. Recent advances in plant-mediated zinc oxide nanoparticles with their significant biomedical properties. *Bioengineering.* 2022;9(10):541.
- [17] Bajwa HUR, Khan MK, Abbas Z, Riaz R, Rehman TU, Abbas RZ, et al. Nanoparticles: Synthesis and their role as potential drug candidates for the treatment of parasitic diseases. *Life Sci Rep.* 2022;12(5):750.
- [18] Babayevska N, Przysiecka Ł, Iatsunskyi I, Nowaczyk G, Jarek M, Janiszewska E, et al. ZnO size and shape effect on antibacterial activity and cytotoxicity profile. *Sci Rep.* 2022;12(1):8148.
- [19] Sirelkhatim A, Mahmud S, Seeni A, Kaus NHM, Ann LC, Bakhor SKM, et al. Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism. *Nanomicro Lett.* 2015;7(3):219–42.
- [20] Marslin G, Siram K, Maqbool Q, Selvakesavan RK, Kruszka D, Kachlicki P, et al. Secondary metabolites in the green synthesis of metallic nanoparticles. *Materials.* 2018;11(6):940.
- [21] Singh J, Dutta T, Kim K-H, Rawat M, Samddar P, Kumar P. Green synthesis of metals and their oxide nanoparticles: applications for environmental remediation. *J Nanobiotechnology.* 2018;16(1):1–24.
- [22] Rana A, Yadav K, Jagadevan S. A comprehensive review on green synthesis of nature-inspired metal nanoparticles: Mechanism, application and toxicity. *J Clean Prod.* 2020;272:122880.
- [23] Ren J, editor. Research on Green Synthetic Iron Nanoparticles. *IOP Conference Series: Materials Science and Engineering.* IOP Publishing; 2019.
- [24] Kaur K, Sidhu AK. Green synthesis: An eco-friendly route for the synthesis of iron oxide nanoparticles. *Front Nanosci.* 2021;3:655062.
- [25] Naiel B, Fawzy M, Halmy MWA, Mahmoud AED. Green synthesis of zinc oxide nanoparticles using Sea Lavender (*Limonium pruinolum*

- L. Chaz.) extract: characterization, evaluation of anti-skin cancer, antimicrobial and antioxidant potentials. *Sci Rep.* 2022;12(1):20370.
- [26] Abdelbaky AS, Abd El-Mageed TA, Babalghith AO, Selim S, Mohamed AM. Green synthesis and characterization of ZnO nanoparticles using *Pelargonium odoratissimum* (L.) aqueous leaf extract and their antioxidant, antibacterial and anti-inflammatory activities. *Antioxidants.* 2022;11(8):1444.
- [27] Hara T, Takeda TA, Takagishi T, Fukue K, Kambe T, Fukada T. Physiological roles of zinc transporters: molecular and genetic importance in zinc homeostasis. *J Physiol Sci.* 2017;67(2):283–301.
- [28] Bisht G, Rayamajhi S. ZnO nanoparticles: a promising anticancer agent. *Nanobiomedicine.* 2016;3(9):3–9.
- [29] Tubek S. Zinc supplementation or regulation of its homeostasis: advantages and threats. *Biol Trace Elem Res.* 2007;119(1):1–9.
- [30] Lee SR. Critical role of zinc as either an antioxidant or a prooxidant in cellular systems. *Oxid Med Cell Longev.* 2018;2018:9156285.
- [31] Prasad AS. Zinc is an antioxidant and anti-inflammatory agent: its role in human health. *Front Nutr.* 2014;1:14.
- [32] Marreiro DDN, Cruz KJC, Morais JBS, Beserra JB, Severo JS, De Oliveira ARS. Zinc and oxidative stress: current mechanisms. *Antioxidants.* 2017;6(2):24.
- [33] Prasad AS. Zinc: an overview. *Nutrition* (Burbank, Los Angeles County, Calif). 1995;11(1 Suppl):93–9.
- [34] Boron B, Hupert J, Barch DH, Fox CC, Friedman H, Layden TJ, et al. Effect of zinc deficiency on hepatic enzymes regulating vitamin A status. *J Nutr.* 1988;118(8):995–1001.
- [35] Espitia P, Otoni C, Soares N. Zinc oxide nanoparticles for food packaging applications. *Antimicrobial food packaging.* Amsterdam, The Netherlands: Elsevier; 2016. p. 425–31.
- [36] Jin S-E, Jin H-E. Antimicrobial activity of zinc oxide nano/micro-particles and their combinations against pathogenic microorganisms for biomedical applications: From physicochemical characteristics to pharmacological aspects. *Nanomaterials.* 2021;11(2):263.
- [37] Miao J, Liu B. II–VI semiconductor nanowires: ZnO. *Semiconductor Nanowires.* Amsterdam, The Netherlands: Elsevier; 2015. p. 3–28.
- [38] Kołodziejczak-Radzimska A, Jesionowski T. Zinc oxide—from synthesis to application: a review. *Materials.* 2014;7(4):2833–81.
- [39] Wang L, Giles N. Temperature dependence of the free-exciton transition energy in zinc oxide by photoluminescence excitation spectroscopy. *J Appl Phys.* 2003;94(2):973–8.
- [40] Capper P, Kasap SO, Willoughby A. Zinc oxide materials for electronic and optoelectronic device applications. Hoboken, New Jersey, US: John Wiley & Sons; 2011.
- [41] Mirzaei H, Darroudi M. Zinc oxide nanoparticles: Biological synthesis and biomedical applications. *Ceram Int.* 2017;43(1):907–14.
- [42] Dai H, Sun T, Han T, Guo Z, Wang X, Chen Y. Aggregation behavior of zinc oxide nanoparticles and their biotoxicity to *Daphnia magna*: Influence of humic acid and sodium alginate. *Environ Res.* 2020;191:110086.
- [43] Pulit-Prociak J, Banach M. Silver nanoparticles—a material of the future...? *Open Chem.* 2016;14(1):76–91.
- [44] Yashni G, Al-Gheethi A, Mohamed RMSR, Dai-Viet NV, Al-Kahtani AA, Al-Sahari M, et al. Bio-inspired ZnO NPs synthesized from *Citrus sinensis* peels extract for Congo red removal from textile wastewater via photocatalysis: Optimization, mechanisms, techno-economic analysis. *Chemosphere.* 2021;281:130661.
- [45] Islam F, Shohag S, Uddin MJ, Islam MR, Nafady MH, Akter A, et al. Exploring the journey of zinc oxide nanoparticles (ZnO-NPs) toward biomedical applications. *Materials.* 2022;15(6):2160.
- [46] Sadhasivam S, Shanmugam M, Umamaheswaran PD, Venkattappan A, Shanmugam A. Zinc oxide nanoparticles: green synthesis and biomedical applications. *J Clust Sci.* 2021;32(6):1441–55.
- [47] Ismail A, Menazea A, Kabary HA, El-Sherbiny A, Samy A. The influence of calcination temperature on structural and antimicrobial characteristics of zinc oxide nanoparticles synthesized by Sol–Gel method. *J Mol Struct.* 2019;1196:332–7.
- [48] Chung YT, Ba-Abbad MM, Mohammad AW, Hairom NHH, Benamor A. Synthesis of minimal-size ZnO nanoparticles through sol–gel method: Taguchi design optimisation. *Mater Des.* 2015;87:780–7.
- [49] Ghoshal T, Biswas S, Paul M, De S. Synthesis of ZnO nanoparticles by solvothermal method and their ammonia sensing properties. *J Nanosci Nanotechnol.* 2009;9(10):5973–80.
- [50] Reuge N, Bacsar R, Serp P, Caussat B. Chemical vapor synthesis of zinc oxide nanoparticles: experimental and preliminary modeling studies. *J Phys Chem C.* 2009;113(46):19845–52.
- [51] Goorabjavari SVM, Golmohamadi F, Haririmomfared S, Ahmadi H, Golisani S, Yari H, et al. Thermodynamic and anticancer properties of inorganic zinc oxide nanoparticles synthesized through coprecipitation method. *J Mol Liq.* 2021;330:115602.
- [52] Ramimoghaddam D, Bin Hussein MZ, Taufiq-Yap YH. Hydrothermal synthesis of zinc oxide nanoparticles using rice as soft biotemplate. *Chem Cent J.* 2013;7(1):1–10.
- [53] Madathil ANP, Vanaja K, Jayaraj M, editors. Synthesis of ZnO nanoparticles by hydrothermal method. *Nanophotonic materials IV.* United States: SPIE; 2007.
- [54] Shamsipur M, Pourmortazavi SM, Hajmirsadeghi SS, Zahedi MM, Rahimi-Nasrabadi M. Facile synthesis of zinc carbonate and zinc oxide nanoparticles via direct carbonation and thermal decomposition. *Ceram Int.* 2013;39(1):819–27.
- [55] Anand V, Srivastava VC. Zinc oxide nanoparticles synthesis by electrochemical method: Optimization of parameters for maximization of productivity and characterization. *J Alloys Compd.* 2015;636:288–92.
- [56] El-Gendy AO, Nawaf KT, Ahmed E, Samir A, Hamblin MR, Hassan M, et al. Preparation of zinc oxide nanoparticles using laser-ablation technique: Retinal epithelial cell (ARPE-19) biocompatibility and antimicrobial activity when activated with femtosecond laser. *J Photochem Photobiol B, Biol.* 2022;234:112540.
- [57] Xiong HM, Shchukin DG, Möhwald H, Xu Y, Xia YY. Sonochemical synthesis of highly luminescent zinc oxide nanoparticles doped with magnesium (II). *Angew Chem Int Ed.* 2009;48(15):2727–31.
- [58] Sajjad A, Bhatti SH, Ali Z, Jaffari GH, Khan NA, Rizvi ZF, et al. Photoinduced fabrication of zinc oxide nanoparticles: Transformation of morphological and biological response on light irradiance. *ACS Omega.* 2021;6(17):11783–93.
- [59] Salah N, Habib SS, Khan ZH, Memic A, Azam A, Alarfaj E, et al. High-energy ball milling technique for ZnO nanoparticles as antibacterial material. *Int J Nanomed.* 2011;6:863.
- [60] Mandal AK, Katuwal S, Tettey F, Gupta A, Bhattarai S, Jaisi S, et al. Current research on zinc oxide nanoparticles: Synthesis, characterization, and biomedical applications. *Nanomaterials.* 2022;12(17):3066.

- [61] Köseoğlu Y. A simple microwave-assisted combustion synthesis and structural, optical and magnetic characterization of ZnO nanoplatelets. *Ceram Int.* 2014;40(3):4673–9.
- [62] Kandjani AE, Tabriz MF, Pourabbas B. Sonochemical synthesis of ZnO nanoparticles: The effect of temperature and sonication power. *Mater Res Bull.* 2008;43(3):645–54.
- [63] Tsuzuki T, McCormick PG. ZnO nanoparticles synthesised by mechanochemical processing. *Scr Mater.* 2001;44(8–9):1731–4.
- [64] Chandrasekaran R, Gnanasekar S, Seetharaman P, Keppan R, Arockiaswamy W, Sivaperumal S. Formulation of Carica papaya latex-functionalized silver nanoparticles for its improved antibacterial and anticancer applications. *J Mol Liq.* 2016;219:232–8.
- [65] Fouda A, Eid AM, Abdelkareem A, Said HA, El-Belely EF, Alkhalifah DHM, et al. Phyco-synthesized zinc oxide nanoparticles using marine macroalgae, *Ulva fasciata* Delile, characterization, antibacterial activity, photocatalysis, and tanning wastewater treatment. *Catalysts.* 2022;12(7):756.
- [66] Dhandapani P, Siddarth AS, Kamalasekaran S, Maruthamuthu S, Rajagopal G. Bio-approach: ureolytic bacteria mediated synthesis of ZnO nanocrystals on cotton fabric and evaluation of their antibacterial properties. *Carbohydr Polym.* 2014;103:448–55.
- [67] Sangeetha G, Rajeshwari S, Venkatesh R. Green synthesis of zinc oxide nanoparticles by aloe barbadensis miller leaf extract: Structure and optical properties. *Mater Res Bull.* 2011;46(12):2560–6.
- [68] Sohail MF, Rehman M, Hussain SZ, Huma Z-E, Shahnaz G, Qureshi OS, et al. Green synthesis of zinc oxide nanoparticles by Neem extract as multi-facet therapeutic agents. *J Drug Deliv Sci Technol.* 2020;59:101911.
- [69] Agarwal H, Kumar SV, Rajeshkumar S. A review on green synthesis of zinc oxide nanoparticles—An eco-friendly approach. *Resource-Efficient Technologies.* 2017;3(4):406–13.
- [70] Singh TA, Das J, Sil PC. Zinc oxide nanoparticles: A comprehensive review on its synthesis, anticancer and drug delivery applications as well as health risks. *Adv Colloid Interface Sci.* 2020;286:102317.
- [71] Abu-Huwajj R, Abbas MM, Al-Shalabi R, Almasri FN. Synthesis of transdermal patches loaded with green synthesized zinc oxide nanoparticles and their cytotoxic activity against triple negative breast cancer. *Appl Nanosci.* 2022;12(1):69–78.
- [72] Naser R, Abu-Huwajj R, Al-khateeb I, Abbas MM, Atoom AM. Green synthesis of zinc oxide nanoparticles using the root hair extract of *Phoenix dactylifera*: Antimicrobial and anticancer activity. *Appl Nanosci.* 2021;11(5):1747–57.
- [73] Fakhari S, Jamzad M, Kabiri Fard H. Green synthesis of zinc oxide nanoparticles: a comparison. *Green Chem Lett Rev.* 2019;12(1):19–24.
- [74] Salem SS, Fouda A. Green synthesis of metallic nanoparticles and their prospective biotechnological applications: an overview. *Biol Trace Elem Res.* 2021;199:344–70.
- [75] Zong Y, Li Z, Wang X, Ma J, Men Y. Synthesis and high photocatalytic activity of Eu-doped ZnO nanoparticles. *Ceram Int.* 2014;40(7):10375–82.
- [76] Ramesh M, Anbuvarannan M, Viruthagiri G. Green synthesis of ZnO nanoparticles using *Solanum nigrum* leaf extract and their antibacterial activity. *Spectrochim Acta A Mol Biomol Spectrosc.* 2015;136:864–70.
- [77] Bahrulolom H, Nooraei S, Javanshir N, Tarrahimofrad H, Mirbagheri VS, Easton AJ, et al. Green synthesis of metal nanoparticles using microorganisms and their application in the agrifood sector. *J Nanobiotechnol.* 2021;19(1):1–26.
- [78] Dhanmozhi AC, Rajeswari V, Sathyajothi S. Green synthesis of zinc oxide nanoparticle using green tea leaf extract for super-capacitor application. *Mater Today: Proc.* 2017;4(2):660–7.
- [79] Ijaz I, Gilani E, Nazir A, Bukhari A. Detail review on chemical, physical and green synthesis, classification, characterizations and applications of nanoparticles. *Green Chem Lett Rev.* 2020;13(3):223–45.
- [80] Hasnidawani J, Azlina H, Norita H, Bonnia N, Ratim S, Ali E. Synthesis of ZnO nanostructures using sol-gel method. *Procedia Chem.* 2016;19:211–6.
- [81] Naveed Ul Haq A, Nadhman A, Ullah I, Mustafa G, Yasinza M, Khan I. Synthesis approaches of zinc oxide nanoparticles: the dilemma of ecotoxicity. *J Nanomater.* 2017;2017:8510342.
- [82] Xu J, Huang Y, Zhu S, Abbas N, Jing X, Zhang L. A review of the green synthesis of ZnO nanoparticles using plant extracts and their prospects for application in antibacterial textiles. *J Eng Fibers Fabr.* 2021;16:15589250211046242.
- [83] Krstulović N, Salamon K, Budimlija O, Kovač J, Dasović J, Umek P, et al. Parameters optimization for synthesis of Al-doped ZnO nanoparticles by laser ablation in water. *Appl Surf Sci.* 2018;440:916–25.
- [84] Sundrarajan M, Ambika S, Bharathi K. Plant-extract mediated synthesis of ZnO nanoparticles using *Pongamia pinnata* and their activity against pathogenic bacteria. *Adv Powder Technol.* 2015;26(5):1294–9.
- [85] Lingaraju K, Raja Naika H, Manjunath K, Basavaraj R, Nagabhushana H, Nagaraju G, et al. Biogenic synthesis of zinc oxide nanoparticles using *Ruta graveolens* (L.) and their antibacterial and antioxidant activities. *Appl Nanosci.* 2016;6:703–10.
- [86] da Silva LAL, Pezzini BR, Soares L. Spectrophotometric determination of the total flavonoid content in *Ocimum basilicum* L.(Lamiaceae) leaves. *Pharmacogn Mag.* 2015;11(41):96.
- [87] Pillai AM, Sivasankarapillai VS, Rahdar A, Joseph J, Sadeghfard F, Rajesh K, et al. Green synthesis and characterization of zinc oxide nanoparticles with antibacterial and antifungal activity. *J Mol Struct.* 2020;1211:128107.
- [88] Rambabu K, Bharath G, Banat F, Show PL. Green synthesis of zinc oxide nanoparticles using *Phoenix dactylifera* waste as bioreductant for effective dye degradation and antibacterial performance in wastewater treatment. *J Hazard Mater.* 2021;402:123560.
- [89] Alsmadi MM, Al-Nemrawi NK, Obaidat R, Alkahi AEA, Koeshed KM, Lahlouh IK. The mapping between the green synthesis conditions of zinc oxide nanoparticles and their toxicokinetics. *Nanomedicine.* 2022;17(18):1281–303.
- [90] Soltys L, Olkhovyy O, Tatarchuk T, Naushad M. Green synthesis of metal and metal oxide nanoparticles: Principles of green chemistry and raw materials. *Magnetochemistry.* 2021;7(11):145.
- [91] Nasrollahzadeh M, Sajadi SM. Pd nanoparticles synthesized in situ with the use of *Euphorbia granulate* leaf extract: Catalytic properties of the resulting particles. *J Colloid Interface Sci.* 2016;462:243–51.
- [92] Arumugam M, Manikandan DB, Dhandapani E, Sridhar A, Balakrishnan K, Markandan M, et al. Green synthesis of zinc oxide nanoparticles (ZnO NPs) using *Syzygium cumini*: Potential multifaceted applications on antioxidants, cytotoxic and as nanonutrient for the growth of *Sesamum indicum*. *Environ Technol Innov.* 2021;23:101653.
- [93] Sharma D, Sabela MI, Kanchi S, Bisetty K, Skelton AA, Honarparvar B. Green synthesis, characterization and

- electrochemical sensing of silymarin by ZnO nanoparticles: Experimental and DFT studies. *J Electroanal Chem.* 2018;808:160–72.
- [94] Muthuchamy N, Atchudan R, Edison TNJI, Perumal S, Lee YR. High-performance glucose biosensor based on green synthesized zinc oxide nanoparticle embedded nitrogen-doped carbon sheet. *J Electroanal Chem.* 2018;816:195–204.
- [95] Shaba EY, Jacob JO, Tijani JO, Suleiman MAT. A critical review of synthesis parameters affecting the properties of zinc oxide nanoparticle and its application in wastewater treatment. *Appl Water Sci.* 2021;11:1–41.
- [96] Naseer M, Aslam U, Khalid B, Chen B. Green route to synthesize Zinc Oxide Nanoparticles using leaf extracts of *Cassia fistula* and *Melia azadarach* and their antibacterial potential. *Sci Rep.* 2020;10(1):1–10.
- [97] Jayachandran A, Aswathy T, Nair AS. Green synthesis and characterization of zinc oxide nanoparticles using *Cayratia pedata* leaf extract. *Biochem Biophys Rep.* 2021;26:100995.
- [98] Hassan Basri H, Talib RA, Sukor R, Othman SH, Ariffin H. Effect of synthesis temperature on the size of ZnO nanoparticles derived from pineapple peel extract and antibacterial activity of ZnO–starch nanocomposite films. *Nanomaterials.* 2020;10(6):1061.
- [99] Padalia H, Baluja S, Chanda S. Effect of pH on size and antibacterial activity of *Salvadora oleoides* leaf extract-mediated synthesis of zinc oxide nanoparticles. *Bionanoscience.* 2017;7:40–9.
- [100] Umamaheswari A, Prabu SL, John SA, Puratchikody A. Green synthesis of zinc oxide nanoparticles using leaf extracts of *Raphanus sativus* var. *Longipinnatus* and evaluation of their anticancer property in A549 cell lines. *Biotechnol Rep.* 2021;29:e00595.
- [101] Mohammadi FM, Ghasemi N. Influence of temperature and concentration on biosynthesis and characterization of zinc oxide nanoparticles using cherry extract. *J Nanostructure Chem.* 2018;8:93–102.
- [102] Agarwal H, Menon S, Kumar SV, Rajeshkumar S. Mechanistic study on antibacterial action of zinc oxide nanoparticles synthesized using green route. *Chem Biol Interact.* 2018;286:60–70.
- [103] Barzinjy AA, Azeez HH. Green synthesis and characterization of zinc oxide nanoparticles using *Eucalyptus globulus* Labill. leaf extract and zinc nitrate hexahydrate salt. *SN Appl Sci.* 2020;2(5):991.
- [104] Fouda A, Saied E, Eid AM, Kouadri F, Alemam AM, Hamza MF, et al. Green synthesis of zinc oxide nanoparticles using an aqueous extract of *punica granatum* for antimicrobial and catalytic activity. *J Funct Biomater.* 2023;14(4):205.
- [105] Aldeen TS, Mohamed HEA, Maaza M. ZnO nanoparticles prepared via a green synthesis approach: Physical properties, photocatalytic and antibacterial activity. *J Phys Chem Solids.* 2022;160:110313.
- [106] Happy A, Soumya M, Kumar SV, Rajeshkumar S, Sheba RD, Lakshmi T, et al. Phyto-assisted synthesis of zinc oxide nanoparticles using *Cassia alata* and its antibacterial activity against *Escherichia coli*. *Biochem Biophys Rep.* 2019;17:208–11.
- [107] Khan MF, Khan MA. Plant-derived metal nanoparticles (PDMNPs): Synthesis, characterization, and oxidative stress-mediated therapeutic actions. *Future Pharmacol.* 2023;3(1):252–95.
- [108] Vijayaraghavan K, Ashokkumar T. Plant-mediated biosynthesis of metallic nanoparticles: A review of literature, factors affecting synthesis, characterization techniques and applications. *J Environ Chem Eng.* 2017;5(5):4866–83.
- [109] Alamdari S, Sasani Ghamsari M, Lee C, Han W, Park H-H, Tafreshi MJ, et al. Preparation and characterization of zinc oxide nanoparticles using leaf extract of *Sambucus ebulus*. *Appl Sci.* 2020;10(10):3620.
- [110] Sabir S, Arshad M, Chaudhari SK. Zinc oxide nanoparticles for revolutionizing agriculture: synthesis and applications. *Sci World J.* 2014;2014:925494.
- [111] Mishra PK, Mishra H, Ekielski A, Talegaonkar S, Vaidya B. Zinc oxide nanoparticles: a promising nanomaterial for biomedical applications. *Drug Discov Today.* 2017;22(12):1825–34.
- [112] El-Belely EF, Farag MM, Said HA, Amin AS, Azab E, Gobouri AA, et al. Green synthesis of zinc oxide nanoparticles (ZnO-NPs) using *Arthrospira platensis* (Class: Cyanophyceae) and evaluation of their biomedical activities. *Nanomaterials.* 2021;11(1):95.
- [113] Pinho AR, Martins F, Costa MEV, Senos AM, da Cruz e Silva, Pereira MdL OA, et al. In vitro cytotoxicity effects of zinc oxide nanoparticles on spermatogonia cells. *Cells.* 2020;9(5):1081.
- [114] Aydin Sevinç B, Hanley L. Antibacterial activity of dental composites containing zinc oxide nanoparticles. *J Biomed Mater Res Part B Appl Biomater.* 2010;94(1):22–31.
- [115] Song W, Zhang J, Guo J, Zhang J, Ding F, Li L, et al. Role of the dissolved zinc ion and reactive oxygen species in cytotoxicity of ZnO nanoparticles. *Toxicol Lett.* 2010;199(3):389–97.
- [116] Shen C, James SA, de Jonge MD, Turney TW, Wright PF, Feltis BN. Relating cytotoxicity, zinc ions, and reactive oxygen in ZnO nanoparticle-exposed human immune cells. *Toxicol Sci.* 2013;136(1):120–30.
- [117] Liao C, Jin Y, Li Y, Tjong SC. Interactions of zinc oxide nanostructures with mammalian cells: cytotoxicity and photocatalytic toxicity. *Int J Mol Sci.* 2020;21(17):6305.
- [118] Álvarez-Chimal R, García-Pérez VI, Álvarez-Pérez MA, Tavera-Hernández R, Reyes-Carmona L, Martínez-Hernández M, et al. Influence of the particle size on the antibacterial activity of green synthesized zinc oxide nanoparticles using *Dysphania ambrosioides* extract, supported by molecular docking analysis. *Arab J Chem.* 2022;15(6):103804.
- [119] Preedia Babu E, Subastri A, Suyavaran A, Premkumar K, Sujatha V, Aristatile B, et al. Size dependent uptake and hemolytic effect of zinc oxide nanoparticles on erythrocytes and biomedical potential of ZnO-ferulic acid conjugates. *Sci Rep.* 2017;7(1):1–12.
- [120] Wahab R, Mishra A, Yun S-I, Kim Y-S, Shin H-S. Antibacterial activity of ZnO nanoparticles prepared via non-hydrolytic solution route. *Appl Microbiol Biotechnol.* 2010;87(5):1917–25.
- [121] Gupta J, Bhargava P, Bahadur D. Fluorescent ZnO for imaging and induction of DNA fragmentation and ROS-mediated apoptosis in cancer cells. *J Mater Chem B.* 2015;3(9):1968–78.
- [122] Choudhury SR, Ordaz J, Lo C-L, Damayanti NP, Zhou F, Irudayaraj J. From the cover: zinc oxide nanoparticles-induced reactive oxygen species promotes multimodal cyto-and epigenetic toxicity. *Toxicol Sci.* 2017;156(1):261–74.
- [123] Hajjo R, Sabbah DA, Al, Bawab AQ. Unlocking the Potential of the Human Microbiome for Identifying Disease Diagnostic Biomarkers. *Diagnostics.* 2022;12(7):1742.
- [124] Aljanabi R, Alsous L, Sabbah DA, Gul HI, Gul M, Bardaweel SK. Monoamine oxidase (MAO) as a potential target for anticancer drug design and development. *Molecules.* 2021;26(19):6019.
- [125] Attia H, Nounou H, Shalaby M. Zinc oxide nanoparticles induced oxidative DNA damage, inflammation and apoptosis in rat's brain after oral exposure. *Toxics.* 2018;6(2):29.

- [126] Babel PK, Thakre PK, Kumawat R, Tomar RS. Zinc oxide nanoparticles induce toxicity by affecting cell wall integrity pathway, mitochondrial function and lipid homeostasis in *Saccharomyces cerevisiae*. *Chemosphere*. 2018;213:65–75.
- [127] Yu K-N, Yoon T-J, Minai-Tehrani A, Kim J-E, Park SJ, Jeong MS, et al. Zinc oxide nanoparticle induced autophagic cell death and mitochondrial damage via reactive oxygen species generation. *Toxicol In Vitro*. 2013;27(4):1187–95.
- [128] Abomuti MA, Danish EY, Firoz A, Hasan N, Malik MA. Green synthesis of zinc oxide nanoparticles using *salvia officinalis* leaf extract and their photocatalytic and antifungal activities. *Biology*. 2021;10(11):1075.
- [129] Hameed S, Khalil AT, Ali M, Numan M, Khamlich S, Shinwari ZK, et al. Greener synthesis of ZnO and Ag–ZnO nanoparticles using *Silybum marianum* for diverse biomedical applications. *Nanomedicine*. 2019;14(6):655–73.
- [130] Datta A, Patra C, Bharadwaj H, Kaur S, Dimri N, Khajuria R. Green synthesis of zinc oxide nanoparticles using *parthenium hysterophorus* leaf extract and evaluation of their antibacterial properties. *J Biotechnol Biomater*. 2017;7(3):271–6.
- [131] Nair S, Sasidharan A, Divya Rani V, Menon D, Nair S, Manzoor K, et al. Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. *J Mater Sci Mater Med*. 2009;20(1):235–41.
- [132] Raghupathi KR, Koodali RT, Manna AC. Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. *Langmuir*. 2011;27(7):4020–8.
- [133] Gunalan S, Sivaraj R, Rajendran V. Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. *Prog Nat Sci: Mater Int*. 2012;22(6):693–700.
- [134] Vatansever F, de Melo WC, Avci P, Vecchio D, Sadasivam M, Gupta A, et al. Antimicrobial strategies centered around reactive oxygen species–bactericidal antibiotics, photodynamic therapy, and beyond. *FEMS Microbiol Rev*. 2013;37(6):955–89.
- [135] Lallo da Silva B, Abuqafy MP, Berbel Manaia E, Oshiro Junior JA, Chiari-Andréo BG, Pietro RCR, et al. Relationship between structure and antimicrobial activity of zinc oxide nanoparticles: An overview. *Int J Nanomed*. 2019;9395–410.
- [136] Slavin YN, Asnis J, Hrfeli UO, Bach H. Metal nanoparticles: understanding the mechanisms behind antibacterial activity. *J Nanobiotechnol*. 2017;15:1–20.
- [137] Shi L-E, Li Z-H, Zheng W, Zhao Y-F, Jin Y-F, Tang Z-X. Synthesis, antibacterial activity, antibacterial mechanism and food applications of ZnO nanoparticles: a review. *Food Addit Contam Part A*. 2014;31(2):173–86.
- [138] Joe A, Park S-H, Shim K-D, Kim D-J, Jhee K-H, Lee H-W, et al. Antibacterial mechanism of ZnO nanoparticles under dark conditions. *J Ind Eng Chem*. 2017;45:430–9.
- [139] Siddiqi KS, Husen A. Properties of zinc oxide nanoparticles and their activity against microbes. *Nanoscale Res Lett*. 2018;13(1):1–13.
- [140] Shinde VV, Dalavi DS, Mali SS, Hong CK, Kim JH, Patil PS. Surfactant free microwave assisted synthesis of ZnO microspheres: Study of their antibacterial activity. *Appl Surf Sci*. 2014;307:495–502.
- [141] Reddy KM, Feris K, Bell J, Wingett DG, Hanley C, Punnoose A. Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. *Appl Phys Lett*. 2007;90(21):213902.
- [142] Tayel AA, EL-TRAS WF, Moussa S, EL-BAZ AF, Mahrour H, Salem MF, et al. Antibacterial action of zinc oxide nanoparticles against foodborne pathogens. *J Food Saf*. 2011;31(2):211–8.
- [143] d'Água RB, Branquinho R, Duarte MP, Maurício E, Fernando AL, Martins R, et al. Efficient coverage of ZnO nanoparticles on cotton fibres for antibacterial finishing using a rapid and low cost in situ synthesis. *New J Chem*. 2018;42(2):1052–60.
- [144] Jiang Y, Zhang L, Wen D, Ding Y. Role of physical and chemical interactions in the antibacterial behavior of ZnO nanoparticles against *E. coli*. *Mater Sci Eng C*. 2016;69:1361–6.
- [145] Saliani M, Jalal R, Goharshadi EK. Effects of pH and temperature on antibacterial activity of zinc oxide nanofluid against *Escherichia coli* O157: H7 and *Staphylococcus aureus*. *Jundishapur J Microbiol*. 2015;8(2).
- [146] Moreau JW, Weber PK, Martin MC, Gilbert B, Hutcheon ID, Banfield JF. Extracellular proteins limit the dispersal of biogenic nanoparticles. *Science*. 2007;316(5831):1600–3.
- [147] Miao AJ, Zhang XY, Luo Z, Chen CS, Chin WC, Santschi PH, et al. Zinc oxide–engineered nanoparticles: dissolution and toxicity to marine phytoplankton. *Environ Toxicol Chem*. 2010;29(12):2814–22.
- [148] Sharma S, Kumar K, Thakur N, Chauhan S, Chauhan M. The effect of shape and size of ZnO nanoparticles on their antimicrobial and photocatalytic activities: a green approach. *Bull Mater Sci*. 2020;43:1–10.
- [149] Peng X, Palma S, Fisher NS, Wong SS. Effect of morphology of ZnO nanostructures on their toxicity to marine algae. *Aquat Toxicol*. 2011;102(3–4):186–96.
- [150] Arakha M, Saleem M, Mallick BC, Jha S. The effects of interfacial potential on antimicrobial propensity of ZnO nanoparticle. *Sci Rep*. 2015;5(1):1–10.
- [151] Tang S, Zheng J. Antibacterial activity of silver nanoparticles: structural effects. *Adv Healthc Mater*. 2018;7(13):1701503.
- [152] Mendes CR, Dilarri G, Forsan CF, Sapata VdMR, Lopes, de Moraes PRM, PB, et al. Antibacterial action and target mechanisms of zinc oxide nanoparticles against bacterial pathogens. *Sci Rep*. 2022;12(1):2658.
- [153] Thi TUD, Nguyen TT, Thi YD, Thi KHT, Phan BT, Pham KN. Green synthesis of ZnO nanoparticles using orange fruit peel extract for antibacterial activities. *RSC Adv*. 2020;10(40):23899–907.
- [154] Umavathi S, Mahboob S, Govindarajan M, Al-Ghanim KA, Ahmed Z, Virik P, et al. Green synthesis of ZnO nanoparticles for antimicrobial and vegetative growth applications: A novel approach for advancing efficient high quality health care to human wellbeing. *Saudi J Biol Sci*. 2021;28(3):1808–15.
- [155] Safavina L, Akhgarg MR, Tahamipour B, Ahmadi SA. Green synthesis of highly dispersed zinc oxide nanoparticles supported on silica gel matrix by *Daphne oleoides* extract and their antibacterial activity. *Iran J Biotechnol*. 2021;19(1):e2598.
- [156] S M NH, P.P, V. In vitro biocompatibility and antimicrobial activities of zinc oxide nanoparticles (ZnO NPs) prepared by chemical and green synthetic route— A comparative study. *BioNanoScience*. 2020;10(1):112–21.
- [157] Bekele B, Degefa A, Tesgera F, Jule LT, Shanmugam R, Priyanka Dwarampudi L, et al. Green versus chemical precipitation methods of preparing zinc oxide nanoparticles and investigation of antimicrobial properties. *J Nanomater*. 2021;2021:1–10.
- [158] Hussein BY, Mohammed AM. Green synthesis of ZnO nanoparticles in grape extract: Their application as anti-cancer and antibacterial. *Mater Today: Proc*. 2021;42:A18–26.
- [159] Nilavukkarasi M, Vijayakumar S, Prathipkumar S. Capparis zeylanica mediated bio-synthesized ZnO nanoparticles as

- antimicrobial, photocatalytic and anti-cancer applications. *Mater Sci Energy Technol.* 2020;3:335–43.
- [160] Ahamed M, Akhtar MJ, Khan MM, Alhadlaq HA. Facile green synthesis of ZnO-RGO nanocomposites with enhanced anticancer efficacy. *Methods.* 2022;199:28–36.
- [161] Ahmadi Shadmehri A, Namvar F. A review on green synthesis, cytotoxicity mechanism and antibacterial activity of ZnO-NPs. *Int J Appl Basic Med Res.* 2020;6(1):23–31.
- [162] Hanley C, Layne J, Punnoose A, Reddy K, Coombs I, Coombs A, et al. Preferential killing of cancer cells and activated human T cells using ZnO nanoparticles. *Nanotechnology.* 2008;19(29):295103.
- [163] Sharmila G, Thirumarimurugan M, Muthukumaran C. Green synthesis of ZnO nanoparticles using *Tecoma castanifolia* leaf extract: characterization and evaluation of its antioxidant, bactericidal and anticancer activities. *Microchem J.* 2019;145:578–87.
- [164] Ruangtong J, Jiraroj T, T-Thienprasert NP. Green synthesized ZnO nanosheets from banana peel extract possess anti-bacterial activity and anti-cancer activity. *Mater Today Commun.* 2020;24:101224.
- [165] Jevapatarakul D, Jiraroj T, Payungporn S, Chavalit T, Khamwut A, T-Thienprasert NP. Utilization of *Cratogeomys formosum* crude extract for synthesis of ZnO nanosheets: Characterization, biological activities and effects on gene expression of nonmelanoma skin cancer cell. *Biomed Pharmacother.* 2020;130:110552.
- [166] Duan X, Liao Y, Liu T, Yang H, Liu Y, Chen Y, et al. Zinc oxide nanoparticles synthesized from *Cardiospermum halicacabum* and its anticancer activity in human melanoma cells (A375) through the modulation of apoptosis pathway. *J Photochem Photobiol B, Biol.* 2020;202:111718.
- [167] Selim YA, Azb MA, Ragab I, Abd El-Azim HM, M. Green synthesis of zinc oxide nanoparticles using aqueous extract of *Deverra tortuosa* and their cytotoxic activities. *Sci Rep.* 2020;10(1):1–9.
- [168] Vakayil R, Muruganantham S, Kabeerdass N, Rajendran M, Ramasamy S, Alahmadi TA, et al. *Acorus calamus*-zinc oxide nanoparticle coated cotton fabrics shows antimicrobial and cytotoxic activities against skin cancer cells. *Process Biochem.* 2021;111:1–8.
- [169] Chandrasekaran S, Anusuya S, Anbazhagan V. Anticancer, anti-diabetic, antimicrobial activity of zinc oxide nanoparticles: A comparative analysis. *J Mol Struct.* 2022;1263:133139.
- [170] George D, Maheswari PU, Begum KMS. Synergic formulation of onion peel quercetin loaded chitosan-cellulose hydrogel with green zinc oxide nanoparticles towards controlled release, biocompatibility, antimicrobial and anticancer activity. *Int J Biol Macromol.* 2019;132:784–94.
- [171] Chelladurai M, Margavelu G, Vijayakumar S, González-Sánchez ZI, Vijayan K, Sahadevan R. Preparation and characterization of amine-functionalized mupirocin-loaded zinc oxide nanoparticles: A potent drug delivery agent in targeting human epidermoid carcinoma (A431) cells. *J Drug Deliv Sci Technol.* 2022;70:103244.
- [172] Batool M, Khurshid S, Daoush WM, Siddique SA, Nadeem T. Green synthesis and biomedical applications of ZnO nanoparticles: Role of PEGylated-ZnO nanoparticles as doxorubicin drug carrier against MDA-MB-231 (TNBC) cells line. *Crystals.* 2021;11(4):344.
- [173] Vimala K, Sundarraj S, Paulpandi M, Vengatesan S, Kannan S. Green synthesized doxorubicin loaded zinc oxide nanoparticles regulates the Bax and Bcl-2 expression in breast and colon carcinoma. *Process Biochem.* 2014;49(1):160–72.
- [174] Morovati MR, Ghanbari-Movahed M, Barton EM, Farzaei MH, Bishayee A. A systematic review on potential anticancer activities of *Ficus carica* L. with focus on cellular and molecular mechanisms. *Phytomedicine.* 2022;154333.
- [175] Shrihastini V, Muthuramalingam P, Adarshan S, Sujitha M, Chen J-T, Shin H, et al. Plant derived bioactive compounds, their anti-cancer effects and in silico approaches as an alternative target treatment strategy for breast cancer: An updated overview. *Cancers (Basel).* 2021;13(24):6222.
- [176] Ibrahim MH, Jaafar HZ, Karimi E, Ghasemzadeh A. Primary, secondary metabolites, photosynthetic capacity and antioxidant activity of the Malaysian Herb Kacip Fatimah (*Labisia pumila* Benth) exposed to potassium fertilization under greenhouse conditions. *Int J Mol Sci.* 2012;13(11):15321–42.
- [177] Yadav B, Bajaj A, Saxena M, Saxena A. In vitro anticancer activity of the root, stem and leaves of *Withania somnifera* against various human cancer cell lines. *Indian J Pharm Sci.* 2010;72(5):659.
- [178] Akindele AJ, Wani ZA, Sharma S, Mahajan G, Satti NK, Adeyemi OO, et al. In vitro and in vivo anticancer activity of root extracts of *Sansevieria liberica* Gerome and Labroy (Agavaceae). *Evid Based Complement Alternat Med.* 2015;2015:560404.
- [179] Ardalani H, Hejazi Amiri F, Hadippanah A, Kongstad KT. Potential antidiabetic phytochemicals in plant roots: A review of in vivo studies. *J Diabetes Metab Disord.* 2021;20:1–18.
- [180] Mutukwa D, Taziwa R, Khotseng LE. A review of the green synthesis of ZnO nanoparticles utilising Southern African Indigenous medicinal plants. *Nanomaterials.* 2022;12(19):3456.
- [181] Roszkowski S. Application of Polyphenols and Flavonoids in Oncological Therapy. *Molecules.* 2023;28(10):4080.
- [182] Koklesova L, Jakubikova J, Cholujova D, Samec M, Mazurakova A, Šudomová M, et al. Phytochemical-based nanodrugs going beyond the state-of-the-art in cancer management—Targeting cancer stem cells in the framework of predictive, preventive, personalized medicine. *Front Pharmacol.* 2023;14:1121950.
- [183] Gobinath P, Packialakshmi P, Hatamleh AA, Al-Dosary MA, Al-Wasel YA, Balasubramani R, et al. *Calotropis gigantea* assisted synthesis of zinc oxide nanoparticle catalysis: synthesis of novel 3-amino thymoquinone connected 1, 4-dihydropyridine derivatives and their cytotoxic activity. *J Nanomater.* 2022;2022:9697057.
- [184] Jansen J, Karges W, Rink L. Zinc and diabetes—clinical links and molecular mechanisms. *J Nutr Biochem.* 2009;20(6):399–417.
- [185] Egeffjord L, Petersen AB, Bak AM, Rungby J. Zinc, alpha cells and glucagon secretion. *Curr Diabetes Rev.* 2010;6(1):52–7.
- [186] Rajakumar G, Thiruvengadam M, Mydhili G, Gomathi T, Chung I-M. Green approach for synthesis of zinc oxide nanoparticles from *Andrographis paniculata* leaf extract and evaluation of their anti-oxidant, anti-diabetic, and anti-inflammatory activities. *Bioprocess Biosyst Eng.* 2018;41(1):21–30.
- [187] Argade PA, Bhutkar MA, Magdum CS. Albizzia lebeck extract mediated synthesis of Zinc Oxide Nanoparticles and study of its In-vitro Anti-diabetic and Anti-oxidant activity. *Asian J Pharm Technol.* 2019;9(2):93–8.
- [188] Rahman MM, Islam MB, Biswas M, Khurshid, Alam A. In vitro antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. *BMC Res Notes.* 2015;8(1):1–9.
- [189] Suresh D, Shobharani R, Nethravathi P, Kumar MP, Nagabhushana H, Sharma S. *Artocarpus gomezianus* aided green synthesis of ZnO nanoparticles: luminescence, photocatalytic and

- antioxidant properties. *Spectrochim Acta A Mol Biomol Spectrosc.* 2015;141:128–34.
- [190] Suresh D, Nethravathi P, Rajanaika H, Nagabhushana H, Sharma S. Green synthesis of multifunctional zinc oxide (ZnO) nanoparticles using *Cassia fistula* plant extract and their photo-degradative, antioxidant and antibacterial activities. *Mater Sci Semicond Process.* 2015;31:446–54.
- [191] Nagajyothi P, Cha SJ, Yang JJ, Sreekanth T, Kim KJ, Shin HM. Antioxidant and anti-inflammatory activities of zinc oxide nanoparticles synthesized using *Polygala tenuifolia* root extract. *J Photochem Photobiol B Biol.* 2015;146:10–7.
- [192] Siripireddy B, Mandal BK. Facile green synthesis of zinc oxide nanoparticles by *Eucalyptus globulus* and their photocatalytic and antioxidant activity. *Adv Powder Technol.* 2017;28(3):785–97.
- [193] Murali M, Mahendra C, Rajashekar N, Sudarshana M, Raveesha K, Amruthesh K. Antibacterial and antioxidant properties of bio-synthesized zinc oxide nanoparticles from *Ceropegia candellabrum* L.—an endemic species. *Spectrochim Acta A Mol Biomol Spectrosc.* 2017;179:104–9.
- [194] Khan ZUH, Sadiq HM, Shah NS, Khan AU, Muhammad N, Hassan SU, et al. Greener synthesis of zinc oxide nanoparticles using *Trianthema portulacastrum* extract and evaluation of its photocatalytic and biological applications. *J Photochem Photobiol B Biol.* 2019;192:147–57.
- [195] Faisal S, Jan H, Shah SA, Shah S, Khan A, Akbar MT, et al. Green synthesis of zinc oxide (ZnO) nanoparticles using aqueous fruit extracts of *Myristica fragrans*: their characterizations and biological and environmental applications. *ACS Omega.* 2021;6(14):9709–22.
- [196] Padalia H, Chanda S. Characterization, antifungal and cytotoxic evaluation of green synthesized zinc oxide nanoparticles using *Ziziphus nummularia* leaf extract. *Artif Cells Nanomed Biotechnol.* 2017;45(8):1751–61.
- [197] Miri A, Mahdinejad N, Ebrahimi O, Khatami M, Sarani M. Zinc oxide nanoparticles: Biosynthesis, characterization, antifungal and cytotoxic activity. *Mater Sci Eng C.* 2019;104:109981.
- [198] Yassin MT, Mostafa AA-F, Al-Askar AA, Al-Otibi FO. Facile green synthesis of zinc oxide nanoparticles with potential synergistic activity with common antifungal agents against multidrug-resistant candidal strains. *Crystals.* 2022;12(6):774.
- [199] T-Thienprasert NP, T-Thienprasert J, Ruangtong J, Jaithon T, Srifah Huehne P, Piasai O. Large scale synthesis of green synthesized zinc oxide nanoparticles from banana peel extracts and their inhibitory effects against *Colletotrichum* sp., isolate KUFC 021, causal agent of anthracnose on dendrobium orchid. *J Nanomater.* 2021;2021:1–10.
- [200] Dhiman S, Varma A, Prasad R, Goel A. Mechanistic Insight of the Antifungal Potential of Green Synthesized Zinc Oxide Nanoparticles against *Alternaria brassicae*. *J Nanomater.* 2022;2022:713884.
- [201] Jamdagni P, Khatri P, Rana J. Green synthesis of zinc oxide nanoparticles using flower extract of *Nyctanthes arbor-tristis* and their antifungal activity. *J King Saud Univ Sci.* 2018;30(2):168–75.
- [202] Zhu W, Hu C, Ren Y, Lu Y, Song Y, Ji Y, et al. Green synthesis of zinc oxide nanoparticles using *Cinnamomum camphora* (L.) Presl leaf extracts and its antifungal activity. *J Environ Chem Eng.* 2021;9(6):106659.
- [203] Pasquet J, Chevalier Y, Couval E, Bouvier D, Noizet G, Morlière C, et al. Antimicrobial activity of zinc oxide particles on five micro-organisms of the Challenge Tests related to their physicochemical properties. *Int J Pharm.* 2014;460(1–2):92–100.
- [204] Melk MM, El-Hawary SS, Melek FR, Saleh DO, Ali OM, El Raey MA, et al. Antiviral activity of zinc oxide nanoparticles mediated by plumbago indica L. Extract against herpes simplex virus type 1 (HSV-1). *Int J Nanomed.* 2021;16:8221.
- [205] Melk MM, El-Hawary SS, Melek FR, Saleh DO, Ali OM, El Raey MA, et al. Nano zinc oxide green-synthesized from plumbago auriculata lam. alcoholic extract. *Plants.* 2021;10(11):2447.
- [206] Hassan HS, Abol-Fotouh D, Salama E, Elkady MF. Assessment of antimicrobial, cytotoxicity, and antiviral impact of a green zinc oxide/activated carbon nanocomposite. *Sci Rep.* 2022;12(1):8774.
- [207] Rodelo CG, Salinas RA, JaimeArmenta EA, Armenta S, Galdámez-Martínez A, Castillo-Blum SE, et al. Zinc associated nanomaterials and their intervention in emerging respiratory viruses: Journey to the field of biomedicine and biomaterials. *Coord Chem Rev.* 2022;457:214402.
- [208] Hamdi M, Abdel-Bar HM, Elmowafy E, El-Khouly A, Mansour M, Awad GA. Investigating the internalization and COVID-19 antiviral computational analysis of optimized nanoscale zinc oxide. *ACS Omega.* 2021;6(10):6848–60.
- [209] Alrabayah IN, Elhawary SS, Kandil ZA, El-Kadder EMA, Moemen YS, Saleh AM, et al. Green Synthesized Zinc Oxide Nanoparticles Based on *Cestrum diurnum* L. of Potential Antiviral Activity against Human Corona 229-E Virus. *Molecules.* 2022;28(1):266.
- [210] Dorostkar R, Ghalavand M, Nazarizadeh A, Tat M, Hashemzadeh MS. Anthelmintic effects of zinc oxide and iron oxide nanoparticles against *Toxocara vitulorum*. *Int Nano Lett.* 2017;7:157–64.
- [211] Saleh M, Abdel-Baki A-A, Dkhil MA, El-Matbouli M, Al-Quraishy S. Antiprotozoal effects of metal nanoparticles against *Ichthyophthirius multifiliis*. *Parasitology.* 2017;144(13):1802–10.
- [212] Jan H, Shah M, Usman H, Khan MA, Zia M, Hano C, et al. Biogenic synthesis and characterization of antimicrobial and antiparasitic zinc oxide (ZnO) nanoparticles using aqueous extracts of the Himalayan Columbine (*Aquilegia pubiflora*). *Front Mater.* 2020;7:249.
- [213] Najoom S, Fozia F, Ahmad I, Wahab A, Ahmad N, Ullah R, et al. Effective antiplasmodial and cytotoxic activities of synthesized zinc oxide nanoparticles using *Rhazya stricta* leaf extract. *Evid-based Complement Altern Med.* 2021;2021:558674.
- [214] Agarwal H, Shanmugam VK. Synthesis and optimization of zinc oxide nanoparticles using *Kalanchoe pinnata* towards the evaluation of its anti-inflammatory activity. *J Drug Deliv Sci Technol.* 2019;54:101291.
- [215] Rahimi Kalateh Shah Mohammad G, Homayouni Tabrizi M, Ardalan T, Yadamani S, Safavi E. Green synthesis of zinc oxide nanoparticles and evaluation of anti-angiogenesis, anti-inflammatory and cytotoxicity properties. *J Biosci.* 2019;44(2):1–9.
- [216] Liu H, Kang P, Liu Y, An Y, Hu Y, Jin X, et al. Zinc oxide nanoparticles synthesised from the *Vernonia amygdalina* shows the anti-inflammatory and antinociceptive activities in the mice model. *Artif Cells Nanomed Biotechnol.* 2020;48(1):1068–78.
- [217] Lopez-Miranda JL, Molina GA, González-Reyna MA, España-Sánchez BL, Esparza R, Silva R, et al. Antibacterial and anti-inflammatory properties of ZnO nanoparticles synthesized by a green method using sargassum extracts. *Int J Mol Sci.* 2023;24(2):1474.
- [218] Anjum S, Hashim M, Malik SA, Khan M, Lorenzo JM, Abbasi BH, et al. Recent advances in zinc oxide nanoparticles (ZnO NPs) for

- cancer diagnosis, target drug delivery, and treatment. *Cancers* (Basel). 2021;13(18):4570.
- [219] Mahdavi B, Saneei S, Qorbani M, Zhaleh M, Zangeneh A, Zangeneh MM, et al. Ziziphora clinopodioides Lam leaves aqueous extract mediated synthesis of zinc nanoparticles and their antibacterial, antifungal, cytotoxicity, antioxidant, and cutaneous wound healing properties under in vitro and in vivo conditions. *Appl Organomet Chem*. 2019;33(11):e5164.
- [220] Metwally AA, Abdel-Hady A-NA, Haridy MA, Ebnalwaleed K, Saied AA, Soliman AS. Wound healing properties of green (using *Lawsonia inermis* leaf extract) and chemically synthesized ZnO nanoparticles in albino rats. *Environ Sci Pollut Res*. 2021;28:1–13.
- [221] Shao F, Yang A, Yu DM, Wang J, Gong X, Tian HX. Bio-synthesis of *Barleria gibsoni* leaf extract mediated zinc oxide nanoparticles and their formulation gel for wound therapy in nursing care of infants and children. *J Photochem Photobiol B: Biol*. 2018;189:267–73.
- [222] Wang P, Jiang L, Han R. Biosynthesis of zinc oxide nanoparticles and their application for antimicrobial treatment of burn wound infections. *Mater Res Express*. 2020;7(9):095010.
- [223] Batool M, Khurshid S, Qureshi Z, Daoush WM. Adsorption, antimicrobial and wound healing activities of biosynthesized zinc oxide nanoparticles. *Chem Pap*. 2021;75:893–907.
- [224] Iqbal Y, Malik AR, Iqbal T, Aziz MH, Ahmed F, Abolaban FA, et al. Green synthesis of ZnO and Ag-doped ZnO nanoparticles using *Azadirachta indica* leaves: Characterization and their potential antibacterial, antidiabetic, and wound-healing activities. *Mater Lett*. 2021;305:130671.
- [225] Kabeerdass N, Thangasamy S, Murugesan K, Arumugam N, Almansour AI, Suresh Kumar R, et al. Embedding green synthesized zinc oxide nanoparticles in cotton fabrics and assessment of their antibacterial wound healing and cytotoxic properties: An eco-friendly approach. *Green Process Synth*. 2022;11(1):875–85.
- [226] Bandeira M, Chee BS, Frassini R, Nugent M, Giovanela M, Roeschely M, et al. Antimicrobial PAA/PAH electrospun fiber containing green synthesized zinc oxide nanoparticles for wound healing. *Materials*. 2021;14(11):2889.
- [227] Kalyani R, Kumar KS, Swamy P, Sekhar KC, Manish S, Murthy KR, et al. Synergetic potential of green synthesized nano-antibiotic combinational therapy for wound healing. Durham, North Carolina: Research Square; 2021.
- [228] Umavathi S, Mahboob S, Govindarajan M, Al-Ghanim KA, Ahmed Z, Virik P, et al. Green synthesis of ZnO nanoparticles for antimicrobial and vegetative growth applications: A novel approach for advancing efficient high quality health care to human wellbeing. *Saudi J Biol Sci*. 2021;28(3):1808–15.
- [229] Bandeira M, Possan AL, Pavin SS, Raota CS, Vebber MC, Giovanela M, et al. Mechanism of formation, characterization and cytotoxicity of green synthesized zinc oxide nanoparticles obtained from *Ilex paraguariensis* leaves extract. *Nano-Struct Nano-Objects*. 2020;24:100532.
- [230] Elumalai K, Velmurugan S. Green synthesis, characterization and antimicrobial activities of zinc oxide nanoparticles from the leaf extract of *Azadirachta indica* (L.). *Appl Surf Sci*. 2015;345:329–36.
- [231] Bala N, Saha S, Chakraborty M, Maiti M, Das S, Basu R, et al. Green synthesis of zinc oxide nanoparticles using *Hibiscus subdariffa* leaf extract: effect of temperature on synthesis, anti-bacterial activity and anti-diabetic activity. *RSC Adv*. 2015;5(7):4993–5003.
- [232] Salam HA, Sivaraj R, Venckatesh R. Green synthesis and characterization of zinc oxide nanoparticles from *Ocimum basilicum* L. var. *purpurascens* Benth.-Lamiaceae leaf extract. *Mater Lett*. 2014;131:16–8.
- [233] Vijayakumar S, Mahadevan S, Arulmozhi P, Sriram S, Praseetha P. Green synthesis of zinc oxide nanoparticles using *Atalantia monophylla* leaf extracts: Characterization and antimicrobial analysis. *Mater Sci Semicond Process*. 2018;82:39–45.
- [234] Gur T, Meydan I, Seckin H, Bekmezci M, Sen F. Green synthesis, characterization and bioactivity of biogenic zinc oxide nanoparticles. *Environ Res*. 2022;204:111897.
- [235] Khatami M, Alijani HQ, Heli H, Sharifi I. Rectangular shaped zinc oxide nanoparticles: Green synthesis by *Stevia* and its biomedical efficiency. *Ceram Int*. 2018;44(13):15596–602.
- [236] Ramesh P, Saravanan K, Manogar P, Johnson J, Vinoth E, Mayakannan M. Green synthesis and characterization of biocompatible zinc oxide nanoparticles and evaluation of its antibacterial potential. *Sens Bio-Sens Res*. 2021;31:100399.
- [237] Ogunyemi SO, Abdallah Y, Zhang M, Fouad H, Hong X, Ibrahim E, et al. Green synthesis of zinc oxide nanoparticles using different plant extracts and their antibacterial activity against *Xanthomonas oryzae* pv. *oryzae*. *Artif Cells Nanomed Biotechnol*. 2019;47(1):341–52.
- [238] Patrón-Romero L, Luque P, Soto-Robles C, Nava O, Vilchis-Nestor A, Barajas-Carrillo V, et al. Synthesis, characterization and cytotoxicity of zinc oxide nanoparticles by green synthesis method. *J Drug Deliv Sci Technol*. 2020;60:101925.
- [239] Ifeanyiichukwu UL, Fayemi OE, Ateba CN. Green synthesis of zinc oxide nanoparticles from pomegranate (*Punica granatum*) extracts and characterization of their antibacterial activity. *Molecules*. 2020;25(19):4521.
- [240] Pachaiappan R, Rajendran S, Ramalingam G, Vo D-VN, Priya PM, Soto-Moscoso M. Green synthesis of zinc oxide nanoparticles by *Justicia adhatoda* leaves and their antimicrobial activity. *Chem Eng Technol*. 2021;44(3):551–8.
- [241] Jayappa MD, Ramaiah CK, Kumar MAP, Suresh D, Prabhu A, Devasya RP, et al. Green synthesis of zinc oxide nanoparticles from the leaf, stem and *in vitro* grown callus of *Mussaenda frondosa* L.: characterization and their applications. *Appl Nanosci*. 2020;10(8):3057–74.
- [242] Gao Y, Xu D, Ren D, Zeng K, Wu X. Green synthesis of zinc oxide nanoparticles using *Citrus sinensis* peel extract and application to strawberry preservation: A comparison study. *LWT*. 2020;126:109297.
- [243] Ahmad H, Venugopal K, Rajagopal K, De Britto S, Nandini B, Pushpalatha HG, et al. Green synthesis and characterization of zinc oxide nanoparticles using *Eucalyptus globules* and their fungicidal ability against pathogenic fungi of apple orchards. *Biomolecules*. 2020;10(3):425.
- [244] Sathappan S, Kirubakaran N, Gunasekaran D, Gupta PK, Verma RS, Sundaram J. Green synthesis of zinc oxide nanoparticles (ZnO NPs) using *cissus quadrangularis*: Characterization, antimicrobial and anticancer studies. *Proc Natl Acad Sci India Sect B Biol Sci*. 2021;91(2):289–96.
- [245] El-Hawwary SS, Abd Almaksoud HM, Saber FR, Elimam H, Sayed AM, El Raey MA, et al. Green-synthesized zinc oxide nanoparticles, anti-Alzheimer potential and the metabolic profiling of *Sabal blackburniana* grown in Egypt supported by molecular modelling. *RSC Adv*. 2021;11(29):18009–25.
- [246] Jobie FN, Ranjbar M, Moghaddam AH, Kiani M. Green synthesis of zinc oxide nanoparticles using *Amygdalus scoparia* Spach stem bark extract and their applications as an alternative antimicrobial,

- anticancer, and anti-diabetic agent. *Adv Powder Technol.* 2021;32(6):2043–52.
- [247] Dangana RS, George RC, Agboola FK. The biosynthesis of zinc oxide nanoparticles using aqueous leaf extracts of *Cnidioscolus aconitifolius* and their biological activities. *Green Chem Lett Rev.* 2023;16(1):2169591.
- [248] Salem NM, Awwad AM. Green synthesis and characterization of ZnO nanoparticles using *Solanum rantonnetii* leaves aqueous extract and antifungal activity evaluation. *Chem Int.* 2022;8(1):12–7.
- [249] Garg R, Rani P, Garg R, Eddy NO. Study on potential applications and toxicity analysis of green synthesized nanoparticles. *Turk J Chem.* 2021;45(6):1690–706.
- [250] Sharma R, Garg R, Kumari A. A review on biogenic synthesis, applications and toxicity aspects of zinc oxide nanoparticles. *EXCLI J.* 2020;19:1325.
- [251] Verma R, Pathak S, Srivastava AK, Prawer S, Tomljenovic-Hanic S. ZnO nanomaterials: Green synthesis, toxicity evaluation and new insights in biomedical applications. *J Alloys Compd.* 2021;876:160175.
- [252] Cheng T-M, Chu H-Y, Huang H-M, Li Z-L, Chen C-Y, Shih Y-J, et al. Toxicologic Concerns with Current Medical Nanoparticles. *Int J Mol Sci.* 2022;23(14):7597.
- [253] Moatamed ER, Hussein AA, El-Desoky MM, Khayat ZE. Comparative study of zinc oxide nanoparticles and its bulk form on liver function of Wistar rat. *Toxicol Ind Health.* 2019;35(10):627–37.
- [254] Salami M, Khosravi M, Zarei MH. Comparative toxic effect of bulk zinc oxide (ZnO) and ZnO nanoparticles on human red blood cells. *Main Group Met Chem.* 2022;45(1):219–24.
- [255] Roy R, Tripathi A, Das M, Dwivedi PD. Cytotoxicity and uptake of zinc oxide nanoparticles leading to enhanced inflammatory cytokines levels in murine macrophages: comparison with bulk zinc oxide. *J Biomed Nanotech.* 2011;7(1):110–1.
- [256] Saranya S, Vijayanarai K, Pavithra S, Raihana N, Kumanan K. In vitro cytotoxicity of zinc oxide, iron oxide and copper nanoparticles prepared by green synthesis. *Toxicol Rep.* 2017;4:427–30.
- [257] Jeyabharathi S, Kalishwaralal K, Sundar K, Muthukumaran A. Synthesis of zinc oxide nanoparticles (ZnONPs) by aqueous extract of *Amaranthus caudatus* and evaluation of their toxicity and antimicrobial activity. *Mater Lett.* 2017;209:295–8.
- [258] Najim N, Rusdi R, Hamzah AS, Shaamari Z, Zain MM, Kamarulzaman N. Effects of the absorption behaviour of ZnO nanoparticles on cytotoxicity measurements. *J Nanomater.* 2014;2014:19.
- [259] Khan M, Naqvi AH, Ahmad M. Comparative study of the cytotoxic and genotoxic potentials of zinc oxide and titanium dioxide nanoparticles. *Toxicol Rep.* 2015;2:765–74.
- [260] Dubey A, Goswami M, Yadav K, Chaudhary D. Oxidative stress and nano-toxicity induced by TiO₂ and ZnO on WAG cell line. *PLoS One.* 2015;10(5):e0127493.
- [261] Uzar NK, Abudayyak M, Akcay N, Algun G, Özhan G. Zinc oxide nanoparticles induced cyto-and genotoxicity in kidney epithelial cells. *Toxicol Mech Methods.* 2015;25(4):334–9.
- [262] Yousef MI, Mutar TF, Kamel MAE-N. Hepato-renal toxicity of oral sub-chronic exposure to aluminum oxide and/or zinc oxide nanoparticles in rats. *Toxicol Rep.* 2019;6:336–46.
- [263] Nazir S, Rabbani A, Mehmood K, Maqbool F, Shah GM, Khan MF, et al. Antileishmanial activity and cytotoxicity of ZnO-based nano-formulations. *Int J Nanomed.* 2019;14:7809–22.
- [264] Guan Z, Ying S, Ofoegbu PC, Clubb P, Rico C, He F, et al. Green synthesis of nanoparticles: Current developments and limitations. *Environ Technol Innov.* 2022;26:102336.
- [265] Sana SS, Dogiparthi LK. Green synthesis of silver nanoparticles using *Givotia moluccana* leaf extract and evaluation of their antimicrobial activity. *Mater Lett.* 2018;226:47–51.
- [266] Scherzad A, Meyer T, Kleinsasser N, Hackenberg S. Molecular mechanisms of zinc oxide nanoparticle-induced genotoxicity. *Materials.* 2017;10(12):1427.
- [267] Martínez R, Poupard MFN, Álvarez A, Soprano E, Migliavacca M, Carrillo-Carrión C, et al. Nanoparticle behavior and stability in biological environments. *Nanoparticles for Biomedical Applications.* Amsterdam, The Netherlands: Elsevier; 2020. p. 5–18.