Thamar University Journal of Natural & Applied Sciences ***8***(2) (2023) 38 – 46

DOI: https://doi.org/10.59167/tujnas.v8i2.1718

TUJNAS

Review Article

A Review on the Green Synthesis of ZnO Nanoparticles Using the Aqueous Extract of *Origanum Majorana* for Antimicrobial Applications

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Received: 13 November 2023. Revised (in revised form): 6 December 2023. Accepted: 9 December 2023. Published: 31 December 2023

Abstract:

Zinc oxide nanoparticles (ZnO-NPs) have been extensively researched for their potential applications in various fields such as pharmaceuticals, cosmetics, biotechnology, sensing, photocatalysis, and photovoltaics due to their unique nanoscale properties. However, the conventional methods for producing ZnO NPs require the use of hazardous chemicals and high energy consumption, which imposes certain limitations. In contrast, the green synthesis of ZnO-NPs using plant extracts, especially *Origanum majorana*, has gained much attention as a promising alternative approach. Plant extracts contain phytochemicals that are biologically safe and non-toxic, making them a preferred choice. In addition, the ZnO-NPs synthesized with *O. majorana* extracts exhibit higher stability and can be customized in terms of shape and size, unlike the ZnO-NPs obtained by bacterial or fungal methods. The aqueous leaf extract of *O. majorana* contains flavonoids, tannins, and phenolic derivatives, which serve as reducing and capping agents for the biosynthesis of ZnO-NPs. These extracts also contain functional groups such as -OH and -C=O, which further enhance the physicochemical properties of the resulting ZnO-NPs and influence their ability to target specific molecules. The plant-mediated synthesis of ZnO-NPs using *O. majorana* leaf extract is not only fast and straightforward but also offers a wide range of functionalized nanoparticles with specific morphologies and sizes. These ZnO-NPs prepared with *O. majorana* have been shown to have potential applications in various fields, including antimicrobial, antioxidant, and anticancer activities. This review focuses specifically on the antimicrobial applications of ZnO-NPs synthesized using *O. majorana* leaf extract.

Keywords: Zinc Oxide Nanoparticles; Green Synthesis; *Origanum Majorana*; Antimicrobial Activity.

Abbreviations: AFM, Atomic Force Microscopy; EDS, Energy-dispersive X-ray spectroscopy; FTIR, Fourier Transform Infrared Spectroscopy; MIC, Minimum Inhibitory Concentration; NPs, Nanoparticles; ROS, Reactive Oxygen Species; SEM, Scanning Electron Microscopy; TEM, Transmission Electron Microscopy; XRD, X-Ray Diffraction; ZnO-NPs, Zinc oxide nanoparticles.

1. Introduction

The field of nanotechnology is rapidly growing and focuses on the development of materials at the nanoscale, ranging from 1 to 100 nm in diameter. These materials have a wide range of applications in various sectors, including biomedical science, cancer treatment, healthcare, drug delivery, food, cosmetics, electronics, energy science, and chemical industries [1].

The synthesis and characterization of these nanomaterials have garnered significant attention due to their unique properties, such as magnetic, structural, and optical characteristics, which arise from the quantum confinement effect. Furthermore, the ability to control and manipulate the distribution, morphology, size, and interfacial effects of nanoparticles adds to their intriguing nature, resulting in a diverse range of chemical, physical, and biological properties [2].

The ZnO-NPs possess exceptional properties, including high photosensitivity, chemical and physical stability, thermal conductivity, and non-toxicity. These properties make them highly attractive for various applications, such as pharmaceuticals, perfumes, dyes, communication, petroleum, electronic sensors, optics, wastewater treatment, packaged foods, and medicine [3,4]. Additionally, ZnO-NPs find extensive use in biomedical applications, serving as antifungal and antibacterial agents, biological labels, antioxidants, anti-inflammatory agents, coatings for medical implants, facilitators of gene transfer, and promoters of wound healing [3]. Various synthesis methods for ZnO-NPs have been extensively studied and documented in the literature, including chemical, physical, and biological approaches. However, recent research has primarily focused on biosynthesis methods, aiming to eliminate the use of hazardous substances and reduce energy consumption. This shift in focus is a response to the reliance of conventional synthesis methods on hazardous chemicals and significant energy input [5]. Moreover, plants, algae, fungi, and bacteria have been successfully utilized for the synthesis of ZnO-NPs, with plants showing promising results [6,7].

2. Classification of Nanoparticles

Nanoparticles are classified into different categories based on materials used, size, characteristics, fabrication methods and dimensions Figure 1 [8,9].

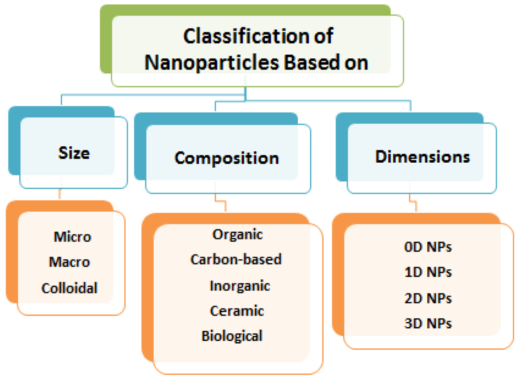

Figure 1: Classification of Nanoparticles.

2.1 Classification of nanoparticles based on their composition

Nanoparticles can be categorized into different classes based on their composition. These classes include carbon-based nanoparticles, organic nanomaterials, nonorganic nanoparticles, ceramic nanoparticles, and biological nanoparticles.

2.1.1 Carbon-based nanoparticles

Carbon-based nanoparticles are composed solely of carbon atoms. These nanoparticles possess unique properties such as electrical conductivity, optical characteristics, electron affinity, high strength, sorption properties, and thermal stability. Due to these properties, carbon-based nanoparticles find applications in a wide range of fields [9].

2.1.2 Organic nanomaterials

Organic nanomaterials are composed of carbohydrates, proteins, polymers, lipids, or other organic compounds. Examples of this class include liposomes, protein complexes, micelles, and dendrimers [10].

2.1.3 Nonorganic nanoparticles

Nonorganic nanoparticles are composed of materials other than organic or carbon, such as nickel, copper, gold, zinc, silver, and iron nanoparticles [11].

2.1.4 Ceramic nanoparticles

Ceramic nanoparticles, also known as nonmetallic solids, are synthesized through cooling or successive heating processes [12].

2.1.5 Biological nanoparticles

Biological nanoparticles are assemblies of atoms or molecules that are prepared within biological systems through methods such as green synthesis or biosynthesis. These methods involve the use of microorganisms such as algae, fungi, yeast, bacteria, and plant extracts as reducing agents [13].

3. Zinc oxide nanoparticles

Zinc oxide nanoparticles possess a range of desirable characteristics that make them an attractive option as a low-cost n-type bandgap semiconductor. One notable feature is their ability to exhibit dielectric and piezoelectric properties while still maintaining transparency.

These nanoparticles have a wide bandgap of 3.37 eV at room temperature, along with high thermal conductivity and a significant exciton binding energy of 60 meV. These unique attributes make them suitable for various applications in fields such as gas sensors, photovoltaics, optoelectronics, light-emitting diodes, aerospace, and photocatalysis [14]. Furthermore, ZnO-NPs exist in the form of white odorless solid powders, which are composed of hexagonal wurtzite crystals.

They can take on different shapes, including spheres for zero-dimensional (0D) structures, nanotubes, nanorods, needles, and dumbbells for one-dimensional (1D) structures, platelets and disks for two-dimensional (2D) structures, and flakes, stars, and flowers for three-dimensional (3D) structures [9]. These physicochemical properties have a significant impact on the ability of ZnO-NPs to effectively target pathogenic bacteria and fungi through their antimicrobial properties. The synthesis techniques used during their preparation also play a crucial role in determining these properties [15]. The superior antibacterial and fungi activity against pathogenic microorganisms is attributed to the porosity, morphology, and particle size of these nanoparticles [16].

4. Synthesis of Zinc oxide nanoparticles

An optimal method for synthesizing zinc oxide nanoparticles should possess environmentally friendly and cost-effective characteristics, while also being capable of producing high-quality ZnO-NPs with the desired morphology and size for their intended applications. The synthesis of nanoparticles has been achieved through biological, physical, and chemical methods, which can be classified into top-down and bottom-up approaches, as illustrated in Figure 2 [17,18]. The top-down approach encompasses both physical and chemical synthesis methods, which typically involve the use of chemicals or force to break down bulk materials into smaller particles.

Examples of top-down methods include laser ablation, ball milling, electrospinning, electron explosion, sputtering, solid state, and mechanochemical techniques [18,19]. On the other hand, various chemical synthesis methods fall under the bottom-up approach, such as microwave synthesis, co-precipitation, spray pyrolysis, sol-gel, hydrothermal, sonochemical, microemulsion, solvochemical, and hydrothermal methods [20].

This approach involves the formation of nanoparticles through the nucleation of molecules, ions, or atoms in a solution, followed by their aggregation. It is a widely reported approach for the preparation of nanoparticles [21]

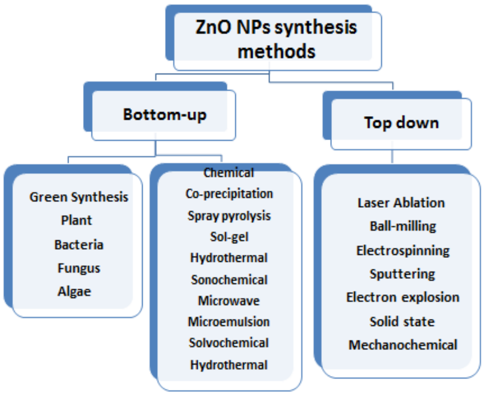

Figure 2: Zinc oxide nanoparticle synthesis methods.

While both physical and chemical conventional synthesis processes have been used for the preparation of ZnO-NPs, some of these methods are limited by high pressure and energy consumption, requiring complex equipment and resulting in high overall costs [20]. Additionally, toxic chemicals used during synthesis, such as hydrazine, polyethylene glycol, sodium borohydride, dimethylformamide, ethylene glycol, pyridine, and cetyltrimethylammonium bromide, can be harmful to the environment and the person handling the chemicals [22]. Therefore, safer and more cost-effective synthesis methods are needed for the preparation of NPs. Green synthesis using microorganisms or plant extracts can be a substitute for conventional physical and chemical synthesis methods.

5. Green Synthesis

Green synthesized nanoparticles possess a diverse array of applications owing to their distinct physical, thermal, chemical, and catalytic characteristics, alongside their substantial surface area to volume ratio, stability, biocompatibility, elevated surface energy, and exceptional adsorption phenomenon [23,24]. The technique of green synthesis for nanomaterials is based on twelve basics of green chemistry, which include pollution prevention, atom economy, designing less grave chemical syntheses, using renewable feedstocks, designing safer chemicals and products, increasing energy efficiency, using safer dissolvent and reaction conditions, using catalysts, designing chemicals and products to break down after use, avoiding chemical derivatives, minimizing the potential for accidents, and analyzing in real time to prevent pollution [25,26]. Zinc oxide nanoparticles have been synthesized using plants, algae, bacteria, and fungi, as depicted in Figure 3. Plant extracts are especially advantageous for the output of metallic nanoparticles due to their genetic variability and diverse chemical composition, which is affected by ecological and environmental factors.

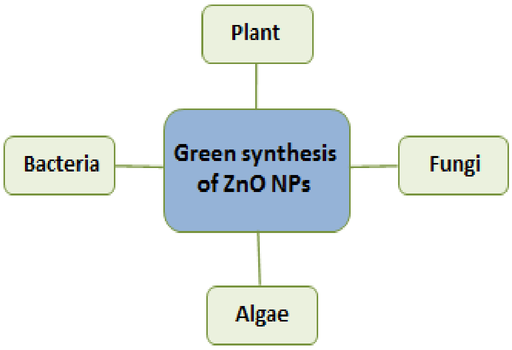

Figure 3: Green synthesis of ZnO NPs.

The presence of diverse components in plant extracts results in varying levels of natural reducing agents, ultimately impacting the properties of nanoparticles [27].

6. *Origanum majorana* L.

Origanum majorana L., a member of the Lamiaceae family, is a herb that holds significant importance in Yemen's traditional medicine and the production of essential oils. It belongs to a vast group of plants within the genus *Origanum*, which consists of 221 genera and 5600 species worldwide, many of which possess medicinal properties. Among the Lamiaceae family, there are 23 endemic species, as documented by [28,29]. Commonly known as 'sweet marjoram' or Majorana hortensis Moench, this herb can reach a height of 30-50 cm. While it is native to the Mediterranean region, *O. majorana* has been cultivated in various countries across Middle Asia, North Africa, Eastern Europe, and America, as reported by [30,31]. In North Yemen, specifically in Sanaa, Taiz, and Ibb, it can be found in the higher mountains [32]. In Islamic Arab traditional medicine, it is referred to as "Bardaqush," while its local Yemeni names are Ozzab or Lizzab [33].

This plant holds great value in Yemen's folk medicine, as it is believed to possess healing properties for a range of ailments, including kidney disease, diabetes, cough, wounds, stomachache, dysentery, and diarrhea [34]. The medicinal uses of *O. marjoram* include treating respiratory, gastrointestinal, and urinary tract disorders, as well as providing relief from spasms, rheumatism, diuretic effects, and asthma[35]. These aromatic plants are widely utilized in the food industry in various countries [36]. In traditional medicine, *O. majorana* L. is used as an antiepileptic and sedative medication [37]. *O. majorana* leaves are rich in phytochemicals such as terpenoids, flavonoid aglycons and glycosides, tannins, and phenolic acids [31,35]. These phytoconstituents, including phenolic compounds, flavonoids, amides, alkenes, and proteins, are responsible for the biofabrication of ZnO-NPs.

They have the ability to reduce or chelate metal ions and act as stabilizing and capping agents for the biogenic ZnO-NPs [38,39]. The polyphenolic ingredients of the plant extract, with their hydroxyl functional groups, also contribute to the reduction and biostabilization of ZnO-NPs [40]. Another study showed that phytochemical components donated their electrons, leading to the biostabilization of Zn^{2+} ions, which were then converted to ZnO-NPs through thermal annealing [41]. *O. majorana* has been found to have a wide range of beneficial effects, including antioxidant, antifungal, analgesic, antitumoral, antispasmodic, antibacterial, and antihyperglycemic properties. It also exhibits insecticide activity against *Anopheles labranchiae* [42,43]. Furthermore, *O. majorana* has potent antibacterial and anti-fungal activity against various pathogenic bacteria, including drug-resistant strains of *S. aureus* and *E. coli*, as reported by [44,45].

In their study, Ghazal *et al.* [46] have provided evidence of the antibacterial properties of terpenoids derived from this plant against both sensitive and drug-resistant strains of *S. aureus* and *E. coli*. Furthermore, *O. majorana* has been found to possess antioxidant and antifungal activity against *Candida albicans* and *Aspergillus niger*, as well as exhibiting antifungal effects against other pathogenic fungi, as reported by [47]. Moreover, have reported that *O. majorana* displays antiparasitic and larvicidal activities, along with anti-inflammatory, antitumor, and antimicrobial properties [48,49]. Considering its potential as a natural antioxidant in the food industry and as a potential anticancer drug, further investigation of *O. majorana* is warranted [50,51].

7. Plant Mediated Synthesis of ZnO-NPs

Plant-mediated synthesis of ZnO-NPs provides a viable and sustainable approach for the mass production of nanoparticles. This method offers several advantages over other synthesis techniques, including its cost-effectiveness, environmental friendliness, and simplicity of operation. Unlike conventional methods, plant-based routes do not necessitate intricate protocols or preparation methodologies. Moreover, the use of plant extracts as a synthetic medium eliminates the requirement for sophisticated equipment, thereby enhancing accessibility for large-scale production. Various plant parts, such as stems, leaves, barks, roots, and flowers, have demonstrated their efficacy in successfully synthesizing ZnO-NPs, as depicted in Figure 4 [7].

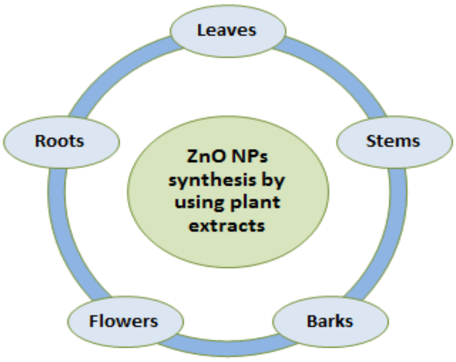

Figure 4: Zinc oxide nanoparticle synthesis by using plant extracts.

The success of synthesizing ZnO-NPs can be attributed to the presence of phytochemicals in plants, which serve as both reducing agents and stabilizing agents during the fabrication process. These phytochemicals encompass a wide range of compounds, including tannins, alkaloids, terpenoids, phenolic compounds, carbohydrates, saponins, and flavonoids. Through their interaction with Zn salt precursors, these compounds effectively reduce them and facilitate the formation of ZnO-NPs throughout the synthesis process [6,7]. Leaves, an integral part of plants, play a vital role in sustaining terrestrial animals through photosynthesis.

Numerous studies have investigated the use of various leaf extracts for the synthesis of ZnO-NPs and their photocatalytic activities, as documented in Table 1. For instance, Khaleghi *et al.* [52] successfully synthesized ZnO-NPs with a hexagonal structure and sizes ranging from 20 to 80 nm using an aqueous leaf extract from *O. majorana*. Similarly, Upadhyay *et al.* [53] conducted a comparative analysis of ZnO-NPs synthesized using leaf extracts of *Ocimum tenuiflorum* and conventional chemical methods, examining their morphological, structural, and visual properties. The results of their study revealed that the biosynthesis approach using plant extracts yielded ZnO-NPs with superior properties compared to those synthesized using chemical methods. Additionally, Yassin *et al.* [54] achieved the successful synthesis of ZnO-NPs using *O. majorana* leaves, employing various analytical techniques to characterize these nanomaterials.

The synthesized ZnO-NPs exhibited exceptional antibacterial properties and possessed a spherical shape with a hexagonal structure. In addition, Karam *et al.* [55] employed the use of thyme leaf extract to generate ZnO-NPs using an environmentally friendly technique. The resulting nanoparticles exhibited a spherical shape and had an average diameter ranging from 39 to 51 nm. Another investigation conducted by researchers utilized *Anacardium occidentale* leaf extract for the synthesis of ZnO nanoparticles. In this process, two zinc salt precursors, namely zinc acetate dihydrate and zinc chloride, were employed [56]. The antibacterial activity of ZnO-NPs, synthesized using *Rumex dentatus* leaf extract and zinc nitrate precursors, was evaluated against *Exiguobacterium aquaticum*, *Staphylococcus aureus*, *Escherichia coli*, and *Acinetobacter baumannii* by the researchers [57]. The study successfully demonstrated the effective antibacterial properties of these nanoparticles. Similarly, Aldalbahi *et al.* [58] utilized zinc nitrate hexahydrate as a precursor to produce ZnO nanoparticles from the Kalanchoe blossfeldiana plant and assessed their anticancer and cytotoxicity properties. The successful application and understanding of nanoparticles heavily rely on their characterization.

Table 1: List of Preparation extract of O.majorana, Zn salt precursors, synthesis conditions, properties, particle sizes and applications of the ZnO-NPs synthesised using *O.majorana*.

Preparation Extract of *O.majorana*	**Zn Salt Precursor**	**Synthesis condition**	**Properties and Particle Size**	**Applications**	**Ref.**
50 g, 200 mL Deionised water as a solvent, 80 C for 15 min	(Zn ($(NO_3)_2 6H_2O$)	5 mL of extract was combined with 95 mL of zinc nitrate, an hour at70 C	Hexagonal, Particle size (TEM): 12.4 nm, zeta potential be -14.8 mV, hydrodynamic size of 71.93 nm.	Antibacterial activity	[54]
50 g, 200 mL Deionised water as a solvent, 40 ^{o}C	($ZnSO_4.7H_2O$)	3 mL leaves extract of O.majorana and 50 mL (0.2 M) zinc sulfate solution	absorption band has been obtained at 379.75 nm, Particle size (SEM): 90 to 125 nm.	Antibacterial activities	[59]
20 g, 250 mL Deionised water, 50 ^{o}C for 30 min	$Zn(CH_3COO)_2.2H_2o$	5 mL extract of O.majorana and 50 mL zinc nitrate, In order to adjust the pH solution to 12,$(NaOH)_2$, for 12 h at 60°C.	Hexagonal, Particle size (TEM): 32 nm,	-	[38]
(10 g) was mixed in distilled water (100 ml) and heated up to 100°C for two hours.	zinc acetate dehydrates (0.5 mM)	added to the filtered solution at a proportion of 1:1 and stirred by magnetic stirrer at 25°C.	spherical, Particle size (TEM): 32 nm,	antioxidant and cytotoxic activities	[52]
20 g, 100 mL Deionised water, 70 °C for 60 min	(Zn(NO3)6H2O) Zinc nitrate hexahydrate	50 mL of O. vulgare extract was added to 2 M zinc nitrate, at 60 C for 2 h	Spherical, crystalline size from 19.67 to 28.78 nm. Particle size (TEM): from 20 to 30nm, zeta potential be -14.7 mV, the hydrodynamic size of 36.15 nm.	Antimicrobial and Biofilm Inhibition Activity	[60]

8. Zinc oxide nanoparticles characterization

The successful application and understanding of nanoparticles heavily rely on their characterization. Numerous studies have demonstrated that the morphology and surface chemistry of nanoparticles significantly impact their safety, biodistribution, and effectiveness in biological systems. However, accurately determining the size of nanoparticles is challenging due to the polydispersity of materials [61].

To characterize nanoparticles, various strategies can be employed, which can be categorized into biological and physicochemical methods. Biological characterization involves assessing genotoxicity, anti-microbial activity, and antibiofilm activity. On the other hand, physicochemical methods encompass a range of techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), X-Ray Diffraction (XRD), Energy-dispersive X-ray spectroscopy (EDS), Fourier Transform Infrared Spectroscopy (FTIR), and Ultraviolet-visible Spectrophotometry Figure 5 [62].

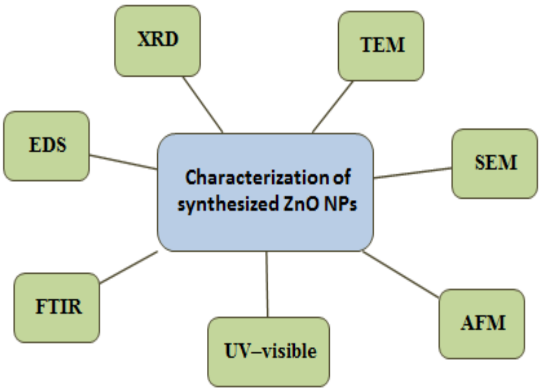

Figure 5: Characterization of synthesized ZnO-NPs.

8.1. Scanning Electron Microscopy

Scanning electron microscopy is a valuable tool for obtaining significant insights into the porosity, size, aggregation, and shape of nanoparticles through imaging[63]. SEM images are particularly effective in evaluating the flatness topography of ZnO-NPs due to their significant field depth and high magnification capabilities [64]. By allowing for the imaging of nanoparticles, SEM provides relevant information on their porosity, size, aggregation, and shape [63]. This technique enables direct visualization, which facilitates the identification of morphological characteristics [61]. When exposed to electron beams, ZnO-NPs generate and detect signals, offering valuable insights into the orientation, morphology, and structure of the crystal particles [65]. The mechanism behind SEM is based on the passage of a finely focused scanned electron beam across the sample surface. These signals are then collected by detectors and displayed as images on a cathode ray tube screen [63].

8.2. Transmission Electron Microscopy

Transmission electron microscopy is a powerful tool that enables precise analysis of nanostructures and compositions. By utilizing a high-energy electron beam to expose ultrathin sections, TEM offers high-resolution imaging. This imaging is achieved by analyzing the imaging and angular distribution of scattered electrons, as well as conducting energy analysis of the emitted X-rays. Through the application of TEM, different phases can be identified and the structure of ZnO-NPs can be characterized. The TEM machine operates by capturing images through transmitted electrons, allowing for the observation of the morphological properties of ZnO-NPs. Furthermore, it is important to note that the size and shape of ZnO-NPs are influenced by the interaction between the sample and the transmitted electron [66].

8.3. Atomic force microscopy

Atomic force microscopy is an advanced technique used to analyze and treat ZnO-NPs at the nanoscale. This powerful tool allows for the characterization of nanomaterials in three dimensions and is particularly valuable for studying the behavior of ZnO-NPs in a biological environment. One of the key advantages of AFM is its ability to acquire high-resolution images in an aqueous medium, providing valuable insights into the structure and properties of ZnO-NPs [67].

8.4. X-Ray Diffraction

X-ray diffraction is a commonly employed method for evaluating the crystallinity of ZnO-NPs. This technique involves subjecting the particles to energetic x-rays emitted by a specialized machine, which then penetrates the particles and gathers important data about their structure [68]. XRD is widely used for non-destructive characterization purposes and offers several advantages. It enables the examination of the crystallographic structure, physical properties, and chemical composition of ZnO-NPs [67]. Additionally, XRD can provide insights into various structural aspects, including defect structure, phase composition, strain, and grain size. Moreover, XRD is utilized for the analysis of atomic arrangements and the limitation of ultrathin sections, as highlighted by [69].

8.5. Energy-dispersive X-ray spectroscopy

Energy-dispersive X-ray spectroscopy is a technique employed to examine the surface of a sample and determine its elemental composition. This method focuses on the analysis of X-rays emitted by the elements of the sample when they are bombarded by an electron beam. It serves as an alternative approach for studying the surface and elemental characteristics of a sample [70]. By investigating the quantity and structure of metal NPs present on the sample's surface, EDS can be utilized to identify the elemental structure of ZnO-NPs and assess their level of purity [71]. Each element's unique atomic structure generates distinct peaks on the X-ray spectrum, enabling accurate analysis of the elemental composition [72].

8.6. Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy can be employed to identify the functional groups responsible for the reduction process in the interaction between zinc precursors and plant extracts [73]. When biosynthesized ZnO-NPs samples are exposed to infrared radiation, certain wavelengths are absorbed while others remain unabsorbed. These unabsorbed wavelengths serve as a molecular indication that characterizes the ZnO-NPs [74]. Analysis using FTIR has demonstrated that extracts containing biomolecules with functional groups such as C=O, -O-H, C-N, C=C, N-H, and C-H exhibit strong reducing properties for the biosynthesis of ZnO-NPs [68].

8.7. Ultraviolet-visible Spectrophotometry

Ultraviolet-visible spectrophotometry is a technique that allows for the analysis of the ultraviolet-visible spectral region. This method utilizes either reflectance or absorption spectroscopy to measure light in the visible and neighboring ranges, such as near-infrared and near-UV [75]. It is particularly useful in determining the chemical nature, transition metal ions, and highly conjugated chemical structure of molecules. In the context of confirming the formation of ZnO-NPs, UV-visible spectrophotometry proves to be a cost-effective approach [76]. By scanning the synthesized NPs in the UV region of the electromagnetic wave, specifically around 200-700 nm, the presence of ZnO-NPs can be verified. This interaction between light and the mobile surface electrons of ZnO-NPs leads to the phenomenon of surface plasmon resonance [77]. Previous research has demonstrated that the surface plasmon resonance band of ZnO-NPs significantly impacts their morphological properties and can be observed at wavelengths ranging from 289-385 nm [78].

9. Zinc oxide nanoparticles bio-medical applications

The utilization of natural raw materials and living organisms as capping and reducing agents in the biosynthesis of ZnO-NPs has greatly advanced the field of nanomaterials for bio-medical purposes. This novel approach has yielded ZnO-NPs that possess remarkable efficacy in delivering various compounds to diseased tissue, targeting bacterial infections, and quantifying concentrations of specific biomarkers within the body [79]. These nanoparticles demonstrate enhanced biocompatibility and exhibit favorable responses when interacting with biological tissue, resulting in improved performance. Consequently, there is an abundance of literature available that extensively explores the diverse bio-medical applications of ZnO-NPs, as depicted in Figure 6 [3,4,52,59].

9.1. Antifungal action of ZnO-NPs

Numerous studies have been conducted to explore the antifungal properties of ZnO-NPs in the treatment of yeasts and fungi. These nanoparticles are commonly used as antifungal additives in the food industry [80]. ZnO-NPs have shown efficacy against various pathogens, including *Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus* sp, and *Penicillium* sp [81,82]. In a study by Nasiri *et al.* [83], the antifungal effects of ZnO-NPs synthesized using *Lavandula angustifolia* extract were compared to nystatin. The ZnO-NPs group exhibited significantly better antifungal activity against *C. albicans*. Another study by Shobha *et al.* [84] reported that ZnO-NPs synthesized using *Ricinus communis* extract showed antifungal activity against *Aspergillus* and *Penicillium*, effectively inhibiting their growth. Zhu *et al.* [81] successfully synthesized ZnO-NPs using *Cinnamomum camphora* leaf extract and investigated their antifungal properties. The study revealed that ZnO-NPs treatments at concentrations ranging from 20 to 160 mg/L significantly inhibited the growth of *A. alternata*.

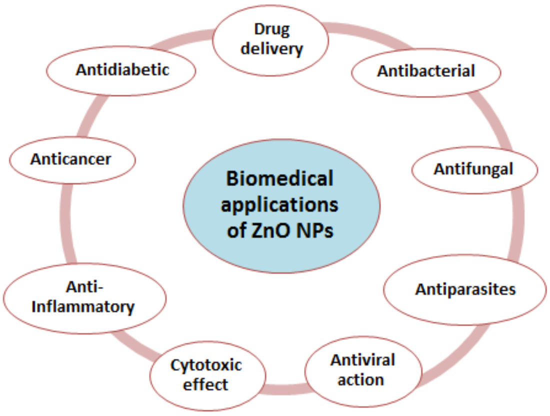

Figure 6: Potential biomedical applications of green synthesized ZnO-NPs.

Zinc oxide nanoparticles synthesized at pH 7 demonstrated the most effective anti-fungal properties, as evidenced by a minimum inhibitory concentration (MIC) value of 20 mg/L. These nanomaterials also exhibited significant inhibition of spore emergence and germ tube protraction of *A. alternata* at a concentration of 20 mg/L. In a study conducted by Krola *et al.* [82], ZnO-NPs were synthesized using *Medicago sativa* L. extract and their antimicrobial potential was evaluated against yeast, specifically Saccharomyces cerevisiae and *Candida albicans*. The MIC values reported for these microorganisms were 9.31 mg/mL and 0.58 mg/mL, respectively [85]. Furthermore, ZnO-NPs synthesized from *Carissa opac*a were found to have the highest zone of inhibition against *Pseudomonas aeruginosa* (a bacterial strain), while exhibiting a greater zone of inhibition against *Candida albican* in the case of fungi [86]. The growth of *Botrytis cinerea* was inhibited by the ZnO-NPs, which caused disfigurement in fungal hyphae and disrupted cellular functions. Similarly, in the case of *P. expansum*, the formation of conidiophores and conidia was prevented, leading to the death of fungal hyphae. This difference in sensitivity between *P. expansum* and *B. cinerea* can be attributed to the microbe-dependent nature of their sensitivity [87]. According to Pasquet *et al.* [88], the antimicrobial activity of ZnO-NPs is influenced by their physicochemical characteristics, such as morphology and size of rods and platelets. The mechanism of action of ZnO-NPs against fungi involves various processes, including protein and DNA binding, disruption of fungal DNA amplification, increased production of reactive oxygen species (ROS), disruption of cell membrane, and alteration of gene expression. Research conducted by [89] observed oxidative damage and changes in mitochondrial function in fungi exposed to ZnO-NPs. These effects may be attributed to modifications in the membrane potential of mitochondria and the expression of antioxidant enzymes, such as superoxide dismutase. Furthermore, Sun *et al.* [90] performed a high-throughput transcriptome sequencing analysis on mycelial cells treated with ZnO-NPs, revealing changes in gene expression levels in *Aspergillus flavus*. These changes involved genes related to oxidative stress, transmembrane transport, zinc ion binding, and oxidative phosphorylation processes [91].

9.2. Antibacterial action of ZnO-NPs

Bacterial diseases pose a significant threat to the global human population. In recent years, individual cells within pathogenic bacterial communities have shown a decrease in susceptibility to antibiotics, leading to a decline in metabolic rates [92]. Consequently, the emergence of antibiotic resistance has become one of the most pressing health concerns of the 21st century. Therefore, it is crucial to evaluate an antibiotic agent that can effectively eliminate pathogenic bacteria that have developed resistance to medication.

Nanoparticles, due to their small size and large surface area compared to larger molecules, possess potent antibacterial properties. They have the ability to penetrate the bacterial membrane at various levels, disrupting it, inhibiting bacterial protein production, and even infiltrating the cells themselves [93].

Given the growing resistance of traditional antibiotics to microbial growth, numerous experiments have been conducted to enhance antimicrobial activity. In vitro tests on antimicrobial efficacy have consistently shown that metallic nanoparticles effectively inhibit a wide range of bacterial species [94]. Moreover, it is widely acknowledged that ZnO-NPs exhibit antibacterial effects by penetrating the cell membrane and inhibiting the growth of microorganisms. Extensive research has been conducted by various scientists to biosynthesize ZnO-NPs against different bacterial strains, resulting in significant antimicrobial efficacy [54]. Additionally, studies have demonstrated that ZnO-NPs synthesized by *O. majorana* extract display antibacterial and antifungal activity against *E.coli*, *K.pneumoniae*, *Salmonella typhimurium*, *Enterobacter cloacae*, and *P. aeruginosa.* Saini et al. [59] conducted a study to investigate the antibacterial and fungi activity of ZnO-NPs using *O. majorana* leaf aqueous extract. The results showed that these ZnO-NPs exhibited strong bactericidal activity against *S. aureus*, *P. aeruginosa*, *E. coli*, and *S. pneumoniae*. The minimum inhibitory concentration (MIC) for *E. coli* was found to be 100-125 mg/mL, while for P. aeruginosa, it ranged from 150-175 mg/mL. Both *S. aureus* and *S. pneumoniae* showed MIC values of 76-100 mg/mL. Additionally, the biosynthesis of ZnO-NPs using *S. aromaticum* extracts in water and ethanol resulted in the inhibition of *K. pneumonia*, *P. aeruginosa*, and *E. coli* [95]. The study also demonstrated the formation of an inhibition zone against *B. subtills*, *S. aureus*, *K. pneumonia*, and *E. coli* at a concentration of 100 µg/mL of ZnO-NPs synthesized using *Brassica oleracea* var. botrytis leaf extract. Therefore, these findings suggest that the eco-friendly biosynthesized ZnO-NPs can be utilized for various environmental and antipathogenic applications [96]. In another study, ZnO-NPs were fabricated using *P. granatum* peel and coffee ground extracts, showing antibacterial effects against *S. aureus*, *E. aerogenes*, *P. aeruginosa*, and *K. pneumoniae*. Similarly, green synthesized ZnO-NPs using *Bauhinia tomentosa* leaf extract exhibited bactericidal effects against *S. aureus*, *E. coli*, *B. subtilis*, and *P. aeruginosa*, with higher efficiency against gram-negative bacteria [97,98].

9.3. Antiviral action of ZnO-NPs

Zinc oxide nanoparticles have been found to possess notable antiviral properties against various viruses, such as hepatitis C virus, human papillomavirus, human immunodeficiency virus, and herpes simplex virus [99]. The antiviral efficacy of ZnO-NPs is attributed to their ability to activate both the adaptive and innate immune responses through toll-like receptor signaling pathways and downstream proteins. This activation leads to the release of pro-inflammatory cytokines, which impede viral activity [100]. Moreover, ZnO-NPs exhibit antiviral effects by generating ROS, inhibiting the activity of viral RNA-dependent RNA polymerase, and preventing viral infection. They also hinder virus adsorption, block viral coating, and impede replication, assembly, and release throughout the virus's life cycle. Notably, ZnO-NPs synthesized using Plumbago indica extract have shown promising activity against Herpes Simplex Virus Type 1 [101].

9.4. Antiparasites action of ZnO-NPs

Zinc oxide nanoparticles demonstrate a potent anthelminthic effect by inducing the production of ROS and hydroxyl ions, resulting in oxidative stress. This oxidative stress leads to the electrostatic binding and subsequent damage of the helminth membrane [102]. In a study conducted by Kalpana et al. [103], zinc nitrate was utilized as a precursor for the eco-friendly synthesis of ZnO-NPs using an aqueous peel extract of *Lagenaria siceraria*. This approach provides a significant environmentally friendly alternative for combating malaria parasites and vectors.

9.5. Anticancer activity of ZnO-NPs

Cancer is a group of diseases characterized by abnormal tissue growth, leading to the formation of tumors that can metastasize to other tissues and have severe consequences for patients, potentially resulting in fatality [104]. Current treatment modalities for cancer encompass surgical intervention, chemotherapy, and radiotherapy. However, while these treatments are theoretically effective in eradicating cancer cells, they also come with significant adverse effects [105]. In a study conducted by Rafique et al. [106], it was demonstrated that the green synthesis approach for producing ZnO-NPs using *Moringa oleifera*, *Mentha piperita*, and *Citrus lemon* exhibited potent anticancer properties, offering a wide range of potential applications, particularly in the field of biomedicine. Despite their beneficial effects, these non-selective treatment methods have notable drawbacks such as immunosuppression, anemia, nausea, and even mortality. Furthermore, literature suggests that certain cancer cells have developed resistance to these therapies, leading to the emergence of chemotherapy-resistant tumors and rendering these treatments ineffective for specific patients. Consequently, extensive efforts have been dedicated to the development of novel approaches in cancer treatment, with nanotechnology gaining prominence [107]. The remarkable potential of ZnO-NPs lies in their ability to induce apoptosis in leukemic cells without exerting cytotoxic effects on healthy cells. Additionally, ZnO-NPs have been reported to exhibit significant selective toxicity against tumor T cells while sparing normal cells from harm. Moreover, research has elucidated that ZnO-NPs selectively target brain tumor cells without causing damage to normal human astrocytes [7]. The interaction between ZnO-NPs and cells leads to the generation of ROS, resulting in mitochondrial damage and triggering cell death in cancerous tissues. The effectiveness of ZnO-NPs in combating cancer has been confirmed through experiments conducted on various cancer cell lines, utilizing a green synthesis method [108]. A549 lung cancer cells were subjected to a comprehensive investigation in a particular study, which aimed to assess the anticancer characteristics of the NPs that were biosynthesized. The inhibitory concentration of 50 of these ZnO-NPs, which were synthesized using the leaves of *Artocarpus heterophyllus*, was found to be 15.6 mg/ml. The primary objective of this study was to ensure the safety and stability of the biosynthesized NPs [109].

9.6. Anti-Inflammatory activity of ZnO-NPs

Inflammation refers to an exaggerated response of living tissue to injury, which is characterized by pain, redness, swelling, and heat. It plays a vital role in the complex reaction of body tissues to harmful stimuli such as irritants, damaged cells, or pathogens [92]. When a specific area of the body is injured, the arterioles in the surrounding tissue widen, leading to increased blood flow and resulting in redness [110]. The resulting ZnO-NPs biosynthesis have been utilized for their potential antioxidant, antimicrobial, antidiabetic, cytotoxic, anti-inflammatory, and anti-aging properties. Additionally, ZnO-NPs have demonstrated a dose-dependent antidiabetic and cytotoxic effect. Similarly, ZnO-NPs have also shown significant anti-aging properties [111]. ZnO-NPs have been found to possess anti-inflammatory properties by inhibiting the release of pro-inflammatory cytokines, mast cell degranulation, myeloperoxidase, and inducible nitric oxide synthase expression. The mRNA expression of pro-inflammatory cytokines was effectively suppressed in a dose-dependent manner by the ZnO-NPs synthesized using Polygala tenuifolia [112]. A comparison between ZnO-NPs and the standard form of ZnO revealed that ZnO-NPs reduced carrageenan-induced paw edema and enhanced the anti-inflammatory activity of the nonsteroidal anti-inflammatory drug, ketoprofen [113].

10. Conclusion

Zinc oxide nanoparticles have demonstrated great potential in the field of medicine due to their biocompatibility and their ability to combat fungal and bacterial infections, treat cancer, deliver drugs, reduce inflammation, and fight viruses. The use of plant extracts for the biosynthesis of these nanoparticles offers several advantages, such as cost-effectiveness, energy efficiency, and the ability to protect human health and the environment by reducing waste and producing safer products. This approach also encompasses the significant aspects of nanotechnology in various applications. Therefore, the utilization of plant extracts for synthesis has the potential to significantly impact the future diagnosis and treatment of various diseases. However, there is still a need to explore commercially viable and eco-friendly methods to determine the effectiveness of natural reducing agents in forming ZnO-NPs, which requires further investigation.

Conflict of interest

The authors declare that they have no conflict of interest.

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