

Review Article

Bio-inspired fabrication of zinc oxide nanoparticles: Insight into biomedical applications

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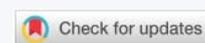
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Abstract

Nanotechnology is starting the characterization, fabrication, and possible applications of numerous materials at the Nano-scale. Over the last few eras, nanomaterials provide a platform for researchers from diverse arenas due to the high surface-to-volume ratio and other novelties, and new significant belongings. Zinc oxide nanoparticles are receiving diverse biomedical applications because of their distinctive antimicrobial, antioxidant, anticancer, antifungal, antileishmanial, anti-larvicidal, wound healing, anticholinergic, and anti-diabetic properties. Different physical and chemical approaches have been used to synthesize zinc oxide nanoparticles, but these methods cause ecotoxicity and are time-consuming and costly. Therefore, there is a need for more eco-friendly, cost-effective, and safe methods. Such biogenic Zinc oxide nanoparticles offer more advantages over other physiochemically synthesized methods. In this review, we have summarized the recent literature for the understanding of the green synthesis of Zinc oxide nanoparticles, their characterization, and their various biomedical applications.

Introduction

In the United States, the National Nanotechnology Initiative started at the commencement of 2000, had systematized the exploration and to develop nanotechnology. Nanomaterials are commonly stated that minor entities having single or many exterior sizes in size range from 1-100 nm. By these sizes, constituents reveal a unique performance in contrast to greater atoms of a similar conformation [1]. Due to the smaller size, they reveal phenomenal unique features and uses in the field of biotechnology, medicine, drug delivery, sensors, and DNA labeling [2,3]. Which are treated as a bridge between bulk material, atomic, and molecular structures [4]. Different routes are used to synthesize metal nanoparticles [5]. They can be synthesized biologically and chemically. Most frequently the chemical methods are used to synthesize metal nanoparticles [6,7] but the ratio of production of toxic

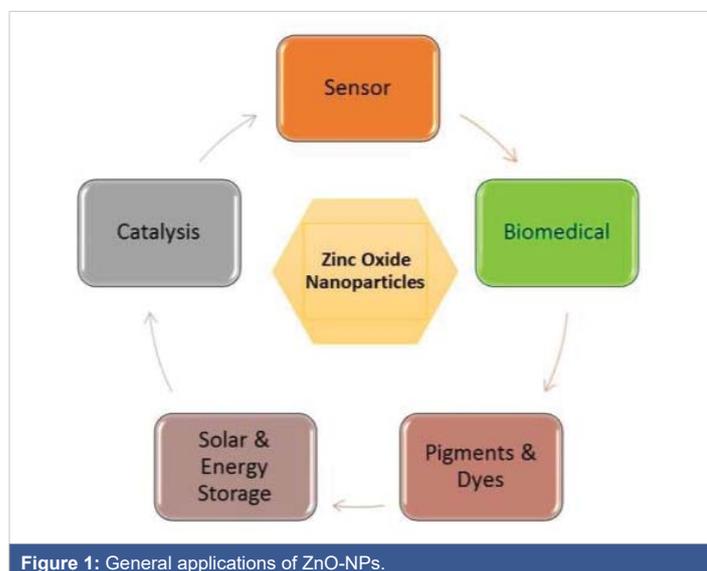
chemicals and byproducts is high or requires high pressure and temperatures. This is also the problem with the physical process. Biosynthesis of nanoparticles using plant extracts provides a facile and 'green' method of synthesis [8,9].

The characteristics of these nanostructures were the growth mechanisms, fabrication methods, and potential application. The factors affecting the growth mechanisms, the crystallographic natures, growth models of anisotropic nanostructures, and growth of nanocrystals. The differential thermal expansion, the existence of lattice mismatch, and high deposition temperature have affected the uniform deposition of nanoparticles on substrates and caused heteroepitaxy, which can lead to forming a defective nanostructure [10]. The presence of neutral stance levels of apparent state acceptor and donor field makes the characterization of surface state, constructed on photoluminescence and cathode luminescence

likely, henceforward, the ease of starting electrons collecting and hole collecting nano interactions for nanostructures short of p-n junctions [11]. The growth of nanostructures throughout fabrications is determined by altered situations which may affect the structural growth along a given direction and result in, unlike growth characteristics and morphologies. The growth way may depend on the surface energy, which tends to direct growth more, along with the facets with higher energy to the detriment of surfaces with lower energy [10].

Among various nanoparticles, zinc oxide nanoparticles (ZnO-NPs) have received more importance [12,13]. Zinc oxide performs an active role in various regulatory functions and is involved in the identification of and response in plants to abiotic stresses. It has been identified that zinc has a vital role in the protection of plant cells against oxidative stresses and the management of reactive oxygen species [14]. Applications of these nanostructures are seen in sensors and antibacterial properties (Figure 1) [8]. ZnO-NPs are inorganic nanoparticles that offer larger material properties with functional flexibility [15]. The nature of ZnO-NPs, they are white crystalline powders which insoluble in water. Additionally, the significant feature of ZnO is due to its wide bandgap energy and it belongs to the II-IV group of n-type semiconductors. ZnO has a high excitation binding energy of 60 meV and a wide semiconductor bandgap of 3.37 eV [10].

In recent decades ZnO-NPs as an imperative ceramic material that is used in entirely diverse industrial areas such as cosmetic materials medication, concrete, different microorganism, textile, and automotive industries. The ZnO-NPs are widely wont to treat a range of different skin conditions and have anti-cancer properties. However, ZnO-NPs have arisen as an appropriate utensil in drug delivery and sensing horizons [16]. The review aims to summarize the green and biological synthesis methods, characterization and growth mechanism of ZnO-NPs, and investigation of their biomedical applications.



Nanoparticles synthesis

Different approaches can be employed for the synthesis of nanoparticles. These approaches are divided into two classes' i.e. top-down approach and the bottom-up approach. A top-down approach is a destructive approach in which larger molecules are decomposed into smaller ones, and these smaller molecules are then converted into suitable nanoparticles. While the bottom up is a building approach that involves the assembly of atomic size to form nano-sized particles [17]. These two approaches are further divided into different classes. The Top-down synthesis process includes Chemical etching, Mechanical milling, Laser ablation, Sputtering, and Electro-explosion. While the Top- bottom approach is divided further, such as Biological, and non-biological synthesis. The biological synthesis of NPs from Plant, Microorganisms, and other biological sources while the non-biological method includes the Template support synthesis, Flame spraying synthesis, Chemical vapor deposition, and Spinning Atomic condensation. While in this review, we will only focus on the green synthesis of ZnO-NPs nanoparticles and their biological applications.

Green synthesis of ZnO-NPs (Biological methods)

Nanoparticles are fabricated by physical, chemical, and biological methods. Conventionally, ZnO-NPs are fabricated by physical and chemical methods, which produce a higher production rate and better size control of nanoparticles. However, these fabrication methods are considered to be discouraging due to high capital cost, energy-intensive, timing consumption, and use of toxic and hazardous chemicals [18,19]. Moreover, earlier studies show that the chemical synthesis of nanoparticles is toxic and less biocompatible [20]. Hence physical and chemical approaches have limited nanoparticles' clinical and biomedical applications. To synthesize NPs, there is a need to explore and grow safer, environmentally friendly, economical, and biocompatible alternatives. The green process of NPs has emerged in recent years through the use of biologically mediated approaches as an alternative to traditional physical and chemical processes [21]. As safe alternatives to chemical methods, biological approaches using microorganisms and plants or plant extracts for the synthesis of metal nanoparticles have been proposed. Several biological systems, including plants, bacteria, fungi, and yeast, have been safely used in biogenic nanoparticle synthesis [22].

Plant-mediated synthesis of zinc oxide nanoparticles: Plants have rich genetic diversity in terms of biomolecules and metabolites like carbohydrates proteins, vitamins, phenols, coenzymes, and flavonoids. These plant metabolites have functional groups like carbonyl, hydroxyl, and amine. Metal ions react with functional groups and reduce their size into the Nano range [23,24]. More specifically, flavonoids contain many functional groups, and the -OH group of flavonoids is thought to be primarily responsible for the reduction of NPs metal ions [25]. These molecules not only assist in the creation

Figure 1: General applications of ZnO-NPs.



of nano-scale ions but also play a key role in the capping of nanoparticles which is required for biocompatibility and stability. Phenolic compounds, sterols, and alkaloids act as a reducing agent in a single reaction and reduce metal ions to NPs [26].

Plants/plant extracts have been widely used for the fabrication of biogenic nanoparticles and are considered the most suitable source due to their easy availability, safety, and cost-effectiveness as compared to bacteria, algae, and other biomolecules. A broad range of biomolecules such as proteins, carbohydrates, enzymes, alkaloids, flavonoids, phenolic acids, and various other molecules in plants plays an important role in the reduction, capping, and stabilization of synthesis of nanoparticles. These molecules react with various zinc salts which leads to the production of ZnO NPs. To date, various plant species have been used for the synthesis of ZnO NPs [27]. The general protocol for the green synthesis of ZnO NPs has various steps. The NPs synthesis mechanism comprises three principal phases: (a) activation phase: the phase of activation comprising the reduction of metal ions and subsequent nucleation of the reduced atom; (b) production phase: the production phase involving spontaneous assemblies and further reducing metal particles by small, adjacent nanoparticles; a process called the Ostwald ripening, which increases thermodynamic stability; and (c) termination phase: in termination phase the final size of Nanoparticles are determined. Primarily, the bio-reducing agents present in the plant extract bind to the metal ions forming metal ions β metabolites complexes. The resulting complexes allow the metal ions to be decreased to metal atoms and then the reduced metal nanoparticles to be nucleated. The individual small nanoparticles merge into large particles to take on a stable size and shape through a coarsening process. When the growth phase extends, nanoparticles continue adding and attain different sizes and shapes including nanorods, nanotubes, hexahedrons, nanoprisms, and various other nanotubes [28]. In the final phase, which is heavily influenced by the stabilization capability of plant extract, the final confirmation has been acquired. If the nanoparticles' surface energy is very high, which makes them less stable, they will change their shape to obtain a more stable morphology [29]. Table 1 represents various plants/plant extracts used for the synthesis of ZnO NPs. ZnO-NPs have been produced using *Trifolium pratense* flower extract which contains various metabolites such as anthocyanin, phenolic acids and small amounts of tannins, carotene, essential oils and vitamin C. ZnO NPs that are synthesized from *T. Pratense* demonstrates effective antibacterial activity against strains of *S. aureus*, *E. coli* and *P. aeruginosa* [30]. The synthesis of ZnO-NPs was also investigated using *Aloe barbadensis* leaf extract with variable shapes in the 8-18nm dimension range. The synthesized NPs have been investigated to check antibacterial activity against *E.coli* and *S. aureus* bacteria and it was concluded that cellular and tissue damage was caused by these biosynthesized ZnO-NPs in both bacteria and thus ZnO-

NPs with these properties are considered nano-antibiotics and are more effective than conventional antibiotics against the bacterial strain [31].

Euphorbia prolifera leaf extract has also been used to form ZnO-NPs as a reduction and stabilization agent. The deterioration of harmful and toxic organic dyes in wastewater is a major concern and has always been the subject of chemists' attention over the past few years. Because of their chemical and biological stability, it is very hard to extract dyes by natural processes. NPs synthesized with *Euphorbia prolifera* leaf extract demonstrated strong catalytic activity for Methylene blue and Congo red degradation in the presence of NaBH₄ at room temperature in water [32]. Spherical ZnO-NPs synthesized from 25-40 nm rambutan (*Nephelium lappaceum* L) peel extract showed photocatalytic activity against methyl orange dye and this dye degradation showed that bio-synthesized ZnO-NPs can effectively degrade 83.99 percent under UV light within 120 min [33]. Sharma et al. synthesized ZnO-NPs using the biological method of using *Carica papaya* milk latex and ZnO variable-shaped nano-flowers. Due to some favorable properties, such as the ability to minimize recombination of the electron-hole pair, surface defect, crystallite size, texture, and large energy gap, it was found. Excellent photocatalytic activity and exceptional antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* were found in these ZnO-NPs [34]. Table 1 contained various examples of plant-mediated synthesis of ZnO-NPs.

Fungal mediated synthesis of zinc oxide nanoparticles: ZnO-NPs are fabricated by using fungi as the living system. Fungi can be utilized under controlled environments and have huge potential for intracellular and extracellular synthesis of metallic NPs. Due to large-scale production, convenient downstream processing, and cost-effectiveness, extracellular synthesis of NP from fungi is of major use due to its better tolerance and the metal bioaccumulation properties, fungal strains are selected over bacteria. Various fungal species such as *Fusarium semi-tectum*, *Fusarium oxysporum*, *Pleurotus sojarcaju*, *Aspergillus fumigates*, *Penicillium brevicompactum*, *Clostridium Versicolor*, and *Alternaria alternate* are being used for the synthesis of nanoparticles; thus an attempt to synthesize ZnO-NPs with *Aspergillus niger* is being made. Using UV-vis absorption, EDX, and SEM analyses, confirmed the ZnO-NPs particles synthesis. These nanoparticles also report antimicrobial and cytotoxic effects [78].

In the extracellular synthesis of ZnO-NPs, the synthesized nanoparticles were stabilized by fungus-released protein. The process is simple, cheap, and safe to use, produces large amounts of stable nanoparticles, and has no contaminants or pollution. This method was useful for zinc oxide extracellular synthesis but is slow and requires specific conditions for successful nanoparticle synthesis. After a period of time, the synthesized NPs decompose, and the solution to this problem is a challenge for scientists [79].

Table 1: Plant-mediated synthesis of zinc oxide nanoparticles.

Specie name	Family	Part use	Phytochemical Constituent	Shape	Size nm	Characterization	Ref
<i>Trifolium Pratense</i>	<i>Fabaceae</i>	Flower	Flavonoids, Phenolic Acids, Tannins	Spherical	60–70 nm	XRD, SEM, EDX, UV–Vis, FT-IR	[32]
<i>Azadirachta indica</i>	<i>Meliaceae</i>	leaves	Flavonoids, Steroids, Carbohydrates, Glycosides, Antiquinone, Terpenoids And Alkaloids,	Hexagona, Buds, Cones, Bundles, And Closed Pine Cone	28 nm	PXRD, FTIR, XPS, UV–Vis	[35]
<i>Aloe vera</i>	<i>Liliaceae</i>	Leaves	Glycosides, Antiquinones, Phlobatannins, Carbohydrates, Alkaloids, Terpenes, Saponins, Tannins, Steroids, Flavonoids	Spherical, Oval And Hexagonal	8–18 nm	XRD, UV vis, SEM, FTIR HRTEM,	[36]
<i>Polygala tenuifolia</i>	<i>Polygalaceae</i>	Roots	Saponins, Oligosaccharide Esters, Xanthones, Oleic Acid	Spherical	33.03–73.48 nm	UV–Vis, FT-IR, TEM, SEM,AAS	[37]
<i>Ficus religiosa</i>	<i>Moraceae</i>	Leaf extract	Alkaloids, Flavonoids, And Terpenoids	Spherical	76.21 nm	FE-SEM, DLS, XRD, UV–vis, FTIR, EDX,	[38]
<i>Pongamia pinnata</i>	<i>Legumes</i>	Fresh Leaf extract		Spherical, Hexagonal, Nano Rod	32 nm	UV–vis, SEM XRD, TEM, EDX	[39]
<i>Carissa EDULIS</i>	<i>Apocynaceae</i>	Fruits	Acids, Saponin Glycosides, Alkaloids, Tannins, And Terpenoids	Flower Shaped	50-55 nm	UV-Vis, SEM FT-IR, HR-TEM, and XRD	[40]
<i>Camellia sinensis</i>	<i>Theaceae</i>	waste	Steroids, Terpenoids, Carotenoids, Flavonoids, Alkaloids, Tannins, And Glycosides	Spherical	88 nm	XRD, UV-Vis, FT-IR, AFM	[41]
<i>Citrus aurantifolia</i>	<i>Rutaceae</i>	Fruits and peel	Flavonoids, Flavones, Limonoids, Triterpenoid, And Naringenin	Spherical And Pyramids	50 – 200 nm	XRD, FE-SEM,	[42]
<i>Anisochilus carnosus</i>	<i>Lamiaceae</i>	leaves	Alkaloids, Steroids, Glycosides, Flavonoids, And Tannins	Spherical	20-40 nm	UV–vis, TEM, FTIR, XDR	[43]
<i>Rosa canina</i>	<i>Rosaceae</i>	Fruit extract	Polyphenols, Vitamin C, Flavonoids	Spherical	25-204 nm	DLS, XRD, SEM	[44]
<i>Cocus Nucifera</i>	<i>Arecaceae</i>	fresh flower extract	Terpenoids, Resins, Alkaloids, Steroids And Glyco-Sides	Spherical And Predominantly Hexagonal Without Any Agglomeration	30–50 nm	FT-IR, SEM, UV-vis	[45]
<i>Calatropis Gigantea</i>	<i>Apocynaceae</i>	Fresh leaves	Alkaloids, Flavonoids, Tannins, Glycosides, Terpenoids, And Saponins,	Spherical Shaped Forming Agglomerates	30–35 nm	SEM, XRD,	[46]
<i>Plectranthus amboinicus</i>	<i>Lamiaceae</i>	Leaf extract	Alkaloids, Glycosides, Carbohydrates, Proteins, Flavonoids, Amino Acids, Quinone, Tannins, Terpenoids, And Phenolic Compounds	Rod Shape Nanoparticle With Agglomerate	50–180 nm	SEM, TEM, XRD, FTIR, UV-vis	[47]
<i>Gossypium</i>	<i>Malvaceae</i>	Cellulosic fiber	Alkaloid, Flavonoid, Saponins, And Cyanogenic Glycosides	Wurtzite, Nano Rod, Spherical,	13 nm	XRD, SEM, EDX, FT-IR	[48]
<i>Solanum lycopersicum</i>	<i>Solaneseae</i>	Leaf extract	Amino Acid, Terpenoids Steroids, Saponins, Flavonoids, Phenols, Carbohydrates, And Essential Oil,	Spherical	20–40 nm	UV–vis, DLS FT-IR, HR-TEM, XRD, FE-SEM	[49]
<i>Caralluma Fimbriata</i>	<i>Apocynaceae</i>	Aerial part	Glycosides, Polyphenols	Hexagonal	30 nm	UV-vis, XRD, FTIR	[50]
<i>Vitex negundo</i>	<i>Lamiaceae</i>	Leaf extract	Carbohydrates, Anthraquinones, Amino Acids, Saponins, Tannins, Flavonoids And Phenolic Compounds.	Spherical	75-80 nm	SEM, EDX, XRD	[39]
<i>E. crassipes</i>	<i>Pontederiaceae</i>	Leaf extract	Phenols, Alkaloids Protein, Amino Acids, Flavonoids, Carbohydrate, Glycosides, Terpenoids, And Tannins	Spherical	32 nm	UV-vis, TEM, XRD, SEM, EDX	[51]
<i>Nephelium lappaceum L.</i>	<i>Sapindaceae)</i>	Fruit peels	Acids, Flavonoids, Carbohydrates, Protein	Needle-Shaped Forming Agglomerate	50.95 nm	XRD, SEM, TEM	[52]
<i>Coccinia indica</i>	<i>Cucurbitaceae</i>	Leaves, stem, roots, fruits	Phenolic Compounds Carbohydrate, Terpenoids Oil And Fats, Flavonoids, Saponins, Alkaloids, Tannins And Proteins	Hexagonal	10.4 nm	XRD, FESEM, FTIR, TEM, SEM,	[53]
<i>Passiflora caerulea</i>	<i>Passifloraceae</i>	Leaf extract	Flavonoids, Indole Alkaloids, And Maltol,	-	30–50 nm	EDAX, FTIR, TEM,SEM, UV-vis, AFM	[54]
<i>Artocarpus gomezianus</i>	<i>Moraceae</i>	Fruit	Secondary Metabolites Of Tannins Flavonoids And Steroids.	Hexagonal Wurtzite	30–40 nm	PXRD, SEM,TEM, FTIR, UV–Vis	[55]
<i>Rhamnus virgata</i>	<i>Rhamnaceae</i>	Leaf extract	Anthraquinone, Lactone, Phenanthrene, Flavonol, And Xanthone And	Hexagonal, Triangle	~20 nm	TEM, SEM,FTIR,UV-vis	[56]
<i>Tecoma castanifolia</i>	<i>Bignoniaceae</i>	Leaf	Alkaloids, Phenols, Flavonoids, And Quinones Saponins	Spherical	70–75 nm	UV–Vis, EDX TEM, FTIR and XRD	[57]
<i>Tamarindus indica</i>	<i>Fabaceae</i>	Pulp extract	Alkaloid, Reducing Sugar, Tannin, Flavonoid, Saponin, Glycoside, Anthraquinone, Phenols And Terpenoid	Hetero Structures	19-37 nm	XRD, FTIR, TEM, SEM,	[58]
<i>Cassia densitipulata taub</i>	<i>Fabaceae</i>	Flower	Aglycone, Sitosterol, Glycoside Derivative	Spherical	30 nm	FT-IR, SEM, UV-Vis,	[59]



<i>Calliandra haematocephala</i>	<i>Fabaceae</i>	Leaves	Alkaloids, Tannins, Cardiac Glycosides, Saponins, And Flavonoids	Hexagonal Wurtzite Structure	19.45 nm	XRD, UV-Vis SEM, TEM,	[60]
<i>Moringa oleifera</i>	<i>Moringaceae</i>	Leaves	Alkaloids, Glucosinolates, Saponins, Steroids, Phenolic Acids, Flavonoids Tannins, And Terpenes.	Spherical	6–20 nm	UV-Vis, XRD, FE-SEM, EDX, FT-IR, PL	[61]
<i>Aloe vera</i>	<i>Liliaceae</i>	Freeze-dried	Glycosides, Carbohydrates, Alkaloids, Terpenes, Saponins, Tannins, Steroids, Flavonoids	Spherical, Hexagonal	25–65 nm	SEM & TEM	[62]
<i>Catharanthus roseus</i>	<i>Apocynaceae</i>	Leaves	Alkaloids, Flavonoids	Spherical	23-27 nm	XRD, EDAX, FT-Raman Spectroscopy, SEM,	[63]
<i>Agathosma betulina</i>	<i>Rutaceae</i>	Leaf	Limonene, Psi-Diosphenol Menthone, And Pulegone	Quasi-Spherical	15 nm	FITR, SEM, TEM, XRS,	[64]
<i>Olea Europea</i>	<i>Oleaceae</i>	Leaf	Flavonoids, Biophenols, Secoiridoids, Flavanones, Iridoids, Triterpenes, Isochromans, Benzoic Acid Derivatives,	Zinc Nano Sheets	18–30 nm	FITR, SEM, XRD	[65]
<i>Hibiscus rosa-Sinensis</i>	<i>Malvaceae</i>	Leaf	Tannins, Phenols, Anthraquinones, Quinines, Alkaloids, Flavonoids, Saponins, Terpenoids, Protein, Cardiac Glycosides, Carbohydrates, Mucilage, Reducing Sugars, Essential Oils And Steroid	Spherical Aggregates	> 70 nm	XRD, SEM	[66]
<i>Calotropis procera</i>	<i>Apocynaceae</i>	Milky latex	Flavonoids, Cardenolides, And Saponins	Spherical, Granular	5-40 nm	XRD, PL, SEM, TEM, UV-Vis	[67]
<i>Phaseolus vulgaris</i>	<i>Fabaceae</i>	seed	Alkaloids, Carbohydrates, Anthocyanins, Catechins, Fiber, And Flavonoids Phytic Acid, Saponins, Quercetin, Steroids, Tannins, And Terpenoids	Spherical	20 nm	EDXRF, SEM, XRD,	[68]
<i>Corriandrum sativum</i>	<i>Apiaceae</i>	Leaves	Alkaloids, Terpenoids, Flavanoids, Saponin, Carbohydrates, Tannins, And Sterols	Wurtzite Hexagonal	66 nm	XRD, FTIR SEM, EDAX	[69]
<i>Coffee</i>	<i>Rubiaceae</i>	Powder	Phenolics, Terpenoids Flavonoids, Saponins, And Steroids	Hexagonal	4.6 nm	DRUV-Vis, XRD, TGA	[70]
<i>Nephelium lappaceum</i>	<i>Sapindaceae</i>	Peel	Flavonoids, Terpenes, Tannins, And Steroids	Needle Like	50 nm	XRD, FTIR, SEM, AFM	[52]
<i>Anisochilus carnosus</i>	<i>Lamiaceae</i>	Leaves	Steroids, Alkaloids, Flavonoids, Glycosides, Tannins, Carbohydrates, Oils, And Fats	Spherical	20-40 nm	UV-vis-DRS, SEM, TEM, FT-IR, XRD, FE-SEM	[43]
<i>Aspalathus Linearis</i>	<i>Fabaceae</i>	Flowers	Aspalathin	Spherical	1-8 nm	EDS, XPS, PL	[71]
<i>S. album</i>	<i>Santalaceae</i>	Leaves	Terpenoids, Phenolics Saponin, And Tannins	Nano Rods	70–140 nm	DLS, SEM, TEM,	[72]
<i>Saraca asoca</i>	<i>Fabaceae</i>	Flower extract	Flavonoids, Lignin, Terpenoid, Cardiac Glycosides, Phenolic Compounds, And Tannins	Roughly Spherical	7–19 nm	XRD, TEM, SEM, UV vis, EDS	[73]
<i>Sedum alfredii</i>	<i>Crassulaceae</i>	Leaf	Flavonoid, Phenol, And Flavonol	Hexagonal Wurtzite, Pseudo-Spherical	53.7 nm	XRD, TEM, SEM, EDS	[74]
<i>Boswellia ovalifoliolata</i>	<i>Burseraceae</i>	Stem bark	Boswellic Acid, Terpenoids I.E. Tetracyclic Triterpenes And Pentacyclic Triterpenes	Spherical	20 nm	TEM, UV-vis, LSPR, FTIR, XRD	[75]
<i>Physalis alkekengi L.</i>	<i>Solanaceae</i>	Seeds	Flavonoids, Withanolides, Phenylpropanoids, Physalins And Alkaloids,	Triangular And Elongated	50–200 nm	XRD, SEM, EDS, EDX, TEM	[76]
<i>Mimosa pudica</i>	<i>Fabaceae</i>	Leaves	Alkaloids, Tannin And Proteins	Wurtzite	2.71 nm	XRD, DRUV-Vis	[70]
<i>Jacaranda mimosifolia</i>	<i>Bignoniaceae</i>	Fruit	Tannins, Oleanolic, Flavonoids, Coumarins Terpenes, Ursolic Acid And Steroids	Spherical	2-4 nm	XRD, TEM, HRTEM, GC-MS, UV-vis, FITR	[77]

The analytical techniques like XRD, SEM, and EDX characterized the synthesized nanoparticles. In the XRD study, zinc oxide NPs are 40 nm in size. The SEM study shows that the ZnO-NPs have a hexagonal shape of 66 nm average size, which deviates from the agglomerated XRD results. The polarity and electrostatic interactions between zinc oxide NPs are the reason for this agglomeration. The EDX confirmed that the synthesized nanoparticles are pure, and the impurities in the sample are only traced. ZnO is used in agriculture, pharma, drugs, ceramics, glass, cement, oil, paint and lubricant, glue, plastic, battery, and fire retardants. In the fields of nanotechnology, UV detectors, nanoscale detectors, and actuators, zinc oxide is showing promising signs. In chip production, it would replace silicon. Due to its dual

semiconductor and piezoelectric properties, the ZnO has a bright future. There are many aspects of biological approaches which still need to be found and manipulated in the field of biogenic synthesis of microbial metallic NP.

Another study reported *Aspergillus fumigatus* mycelia synthesized ZnO-NPs. DLS analyzes have revealed an average size range of NPs of 1.2 to 6.8. The average NP height confirmed by AFM was 8.56 nm. For 90 days it was a particle size of >100 nm but after 90 days they were formed into a medium size 100 nm agglomerate, which suggested 90 days of stability to formed NPs [21]. SEM was confirmed in the size between 54.8 – 82.6 nm for the NPs synthesized in the *Aspergillus terreus* family of *Trichocomaceae* and the average size of 29 nm was calculated with the Debye-Sherrer equation



using XRD results. FTIR studies confirmed the presence of primary alcohol, primary or secondary amine, amide, and aromatic nitro compounds in NPs [80]. A similar range of 15 to 25 nm, as confirmed by SEM, TEM, and XRD analysis, was presented in NPs synthesized by *Candida albicans* [81]. *Aspergillus* species were widely used for the synthesis of ZnO NPs and in most cases, spherical NPs synthesized by fungal strains. Table 2 provides a brief account of commonly used ZnO-NPs synthesis funguses.

Kalpana, et al. 2018 evaluate the extracellular summary of ZnO-NPs using *Aspergillus niger* culture filtrate. There was a maximum absorption rate of 320 nm for the obtained UV visible spectrum of the culture filters. In the FTIR strip, the carboxylic acid appearance and the strong aromatic ring could lead to a ZnO NPs synthesis. Studies of the antimicrobial activity of ZnO-NPs coated fabric showed a reduction of viable *E. coli* and *S. aureus* have it. The treatment with 100 μ l ZnO-NPs resulted in 90 percent discolors of Bismarck brown dye. In comparison to dye with ZnO-NPs, the germination (percent) of the seeds of plants was lower with the raw dye. This study shows that ZnO-NPs have been found to be mediated in *A. niger* through a synthesis in degrading coloration which can be incorporated into cotton textiles since the antibacterial activity is revealed [82]. Table 2 contained the example of fungus-mediated synthesized ZnO-NPs.

Bacterial mediated synthesis of zinc oxide nanoparticles: Synthesis of NP using the bacteria is a green approach, but has many drawbacks such as microbial screening is a time-consuming procedure, diligent control for the cultivation of bacteria, and a complete procedure to protect against contamination. The biological synthesis of micro-organism NP seems to be more environmentally friendly and has gained a lot of interest in comparison with plants, provided that without seasonal or geographical constraints micronutrients can be easily cultivated. In addition, the use of bacteria for ZnO-NPs biosynthesis has important advantages because of the fabrication of supernatant functional biomolecules, which can reduce metal ions to metal NPs [85,86]. In addition, bacteria's cell biomass can act as a nano-factory in ZnO-NPs due to the existence of a functional bacterial cell group, which reduces the metal ions to metal NPs [87,88].

Prokaryotes and eukaryotes, including bacteria, fungi, and yeast, are used to synthesize ZnO-NPs through the use of intracellular or extra-cellular microbial cells or enzymes, proteins, and other biomolecules compounds. However, the

properties of nanoparticles (NPs) rely on their size and type which make them unique to different applications. ZnO-NPs have antimicrobial properties. However, by controlling their reaction conditions the necessary size and shape of NPs can be obtained by optimizing the synthesis mediated by microbes. The synthesis of ZnO-NPs with different chemical and physical techniques should be noted. These approaches, however, are costly and unfriendly to the environment. The microbes that mediate ZnO-NP synthesis have therefore evolved rapidly, as alternatives to chemical and physical methods have become safer, environmentally friendly, non-toxic, and biocompatible. In addition, zinc is more efficient in the form of NPs than its bulk equivalents and has been studied in a variety of possible applications including in the animal industry. In particular, ZnO-NPs have grown as potential antimicrobial agents with the advent of multidrug-resistant strains. This is primarily because of their superior properties in the fight against a wide variety of pathogens [21].

Shabib, et al. 2016 investigated that Probiotic lactic acid bacteria (LAB) are of great interest among bacteria used for the synthesis of ZnO-NPs because of their non-pathogenic and beneficial properties. LAB is a gram-positive bacterium that consists of several biostructures and functional groups with a thick cell wall. The metal ion ligands act as functional groups and promote ZnO-NP formation. LAB also secretes multiple enzymes acting as a ZnO-NPs reducer and stabilizer. Several studies have therefore been performed to evaluate the effectiveness of probiotic LAB in mediating the biosynthesis of ZnO-NPs by cell-biomass or cell-free supernatant. Nevertheless, it remains uncharged to date the potential to use both routes to mediate ZnO-NP biosynthesis [89].

ZnO nanoflowers were synthesized by *B. licheniformis* through an eco-friendly approach which showed photocatalytic activity and degraded Methylene blue dye. These nanoflowers showed enhanced photocatalytic activity as compared to already present photocatalytic substances and it has been presumed that larger oxygen vacancy in the synthesized nanoparticles imparts the property of enhanced photocatalytic activity. Photocatalysis generates active species by absorption of light which degrades the organic waste material and thus can be used as an effective bioremediation tool. Nanoflowers synthesized using *B. licheniformis* were 40 nm in width and 400 nm in height [83]. *Rhodococcus* is able to survive in adverse conditions and it has the ability to metabolize hydrophobic compounds thus, can help in biodegradation [90]. Spherical-shaped NPs had been synthesized using *Rhodococcus pyridinivorans* and

Table 2: Fungus-mediated synthesis of zinc oxide nanoparticles.

Fungal stain	Family	Characterization	Size nm	Shape	Ref
<i>Aspergillus fumigatus TFR-8</i>	<i>Trichocomaceae</i>	DLS, SEM	1.2–6.8 nm (DLS), 100 (agglomerate)	Oblate spherical and hexagonal forms aggregate	[83]
<i>Aspergillus aeneus</i>	<i>Trichocomaceae</i>	SEM,	100~140 nm	Spherical	[82]
<i>Fusarium spp.</i>	<i>Nectriaceae</i>	TEM, XRD, FTIS, EDS	> 100m,	Triangle	[84]
<i>Candida albicans</i>	<i>Saccharomycetaceae</i>	XRD, SEM, TEM	25nm	Quasi-spherical	[81]
<i>Aspergillus fumigatus JCF</i>	<i>Trichocomaceae</i>	UV–Vi, SEM, FTIS	60–80 nm	Spherical	[21]



Zinc Sulphate as a substrate which showed a size range of 100–130 nm confirmed through FE-SEM and XRD Analysis. It also demonstrated the presence of Phosphorus compound, secondary sulphonamide, monosubstituted alkyne, β -lactone, amine salt, amide II stretching band, enol of 1-3-di ketone, hydroxy aryl ketone, amide I bending band, alkane, and mononuclear benzene band confirmed through FTIR analysis [91]. ZnO was used as a substrate to synthesize ZnO-NPs through *A. hydrophilla*. NPs synthesized showed a size range of 42–64 nm, confirmed through AFM and XRD analysis with varied shapes like oval and spherical [92]. Singh, et al. compared the antioxidant activity of bare ZnO-NPs and *Pseudomonas aeruginosa* rhamnolipid stabilized NPs and it had been found that rhamnolipid stabilizes the ZnO-NP because it is tough to form micelle aggregates on the surface of carboxymethyl cellulose [93] and it acts as a better capping agent because of its long carbon chain. It showed the formation of spherical-shaped NP with a nano-size of 27–81 nm confirmed through TEM, XRD, and DLS analysis [94]. Tables 3, 4, and 5 contained the Bacterial; actinomycetes, and Algae mediated synthesized ZnO-NPs.

Biomedical applications

Antibacterial activity: The misuse and unregulated use of antibiotics have developed bacterial resistance which became

a challenge for the treatment of several infectious diseases. In this regard, nanotechnology offers a new therapeutic strategy to treat diseases that developed from antibiotic-resistant pathogenic bacteria [114]. Zinc oxide (ZnO) is a bio-safe material that has photo-oxidizing and photocatalysis properties that makes them effective on biological species. Furthermore, Zinc oxide has been investigated as an associate degree agent in medication when used in each microscale and nanoscale formulations [115]. Researchers studied the photoactivation of ZnO-NPs while exposed to UV light at 254 nm for 30 min in a UV transilluminator, and the growth analysis was performed with 2 and 5 mM concentrations using similar conditions. The results showed that the antibacterial activity of ZnO-NPs in the dark is less than that in ambient laboratory conditions [116]. In most of the studies, it was found that the mechanism of Zinc oxide nanoparticles could be due to the formation of reactive oxygen species (ROS), Figure 2. Researchers study the antibacterial effect of zinc oxide nanoparticles against *Staphylococcus aureus* (*S. aureus*) bacteria and they proved that small-sized zinc oxide nanoparticles showed a better antibacterial effect. The mechanism was explained by the generation of H_2O_2 in *S. aureus* cells which leads to cell death [117]. Similarly, Zinc oxide nanoparticles exhibited antibacterial activity against *E.coli* 0157:H7 and the data showed complete inhibition

Table 3: Bacterial mediated synthesis of zinc oxide nanoparticles.

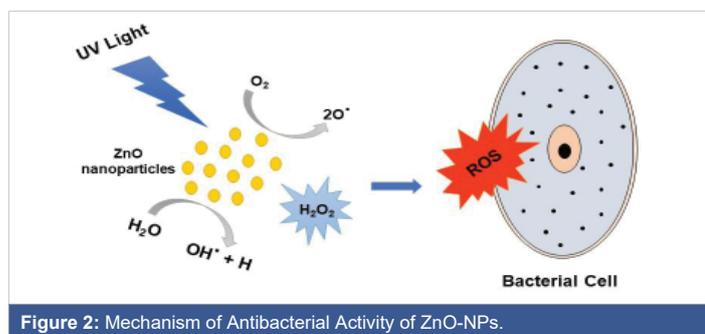
Bacterial stain	Family	Gram +/Gram -	Size nm	Shape	Characterization	Ref
<i>Bacillus licheniformis</i>	Bacillaceae	Gram +	200 with nano pedals 40 in width and 400 in length	Nano-flowers	TEM, XRD, TGA	[91]
<i>Lactobacillus casei</i>	Lactobacillaceae	Gram +	20–50	spherical	FT-IR, EDX, XRD, FESEM, TEM	[95]
<i>Staphylococcus aureus</i>	Staphylococcaceae	Gram +	10-50	Acicular	XRD, FTIR, TGA, UV-vis, EDX, FESEM	[96]
<i>Pseudomonas aeruginosa</i>	Pseudomonadaceae	Gram -	35–80 (TEM), 27 (XRD), 81 (DLS)	Spherical	TEM, XRD, DLS	[94]
<i>Lactobacillus Sporogenous</i>	Bacillaceae	Gram +	5–15 (TEM), 11 (XRD)	Hexagonal unit cell	TEM, XRD SEM, UV-vis	[97]
<i>Rhodococcus pyridinivorans</i>	Nocardiaceae	Gram +	100–120 (FE-SEM), 120–130 (XRD)	Hexagonal phase, roughly spherical	FE-SEM, XRD	[93]
<i>Serratia ureilytica</i>	Enterobacteriaceae	Gram -	170–250 (30 min), 300–600 (60 min), 185–365 (90 min) [SEM]	Spherical to nanoflower shaped	SEM, TEM	[98]
<i>Halomonas elongate</i>	Halomonadaceae	Gram -	10-61	Multiform	FTIR, SEM, TEM, XRD, UV-Vis	[99]
<i>Lactobacillus johnsonii</i>	Lactobacillaceae	Gram +	4–9	Spherical	SEM, TEM, UV-Vis	[100]
<i>Bacillus megaterium</i>	Bacillaceae	Gram +	45–95	Rod and cubic	UV-Vis, FTIR, XRD, FESEM	[101]
<i>Sphingobacterium thalophilum</i>	Sphingobacteriaceae	Gram -	40	Triangle	FTIR, TEM, UV-Vis	[102]
<i>Aeromonas hydrophila</i>	Aeromonadaceae	Gram -	57.7	Spherical	TEM, XRD, SEM	[103]

Table 4: Synthesis of Zinc oxide nanoparticles from actinomycetes.

Actinomycetes	Characterization	Size	Shape	Ref
<i>Streptomyces sps</i>	UV-VIS, Scanning Electron Microscope (SEM), FTIR spectroscopy	15-24	Spherical	[104]
<i>Nocardopsis sp. GRG1 (KT23540)</i>	UV-vis absorption spectra, Fourier transform infrared (FTIR), X-ray diffraction (XRD) pattern, Raman spectra, energy-dispersive X-ray spectroscopy (EDS) and morphologically using scanning electron microscopy (SEM).	10-14	Hexagonal phase, roughly spherical	[105]
<i>Streptomyces sp. HBUM171191</i>	UV-Vis Spectrophotometer	10-20	Spherical	[106]
<i>Sphingobacterium thalophilum</i>	X-ray diffractometer (model: X'Pert Pro Panalytical), Field emission scanning electron microscope (FESEM; model: Supra 55) with EDAX (model: Ultra 55), y Ultraviolet visible spectrophotometer (UV-Vis; model: Lambda 35).	40	Triangle	[107]
<i>Staphylococcus aureus</i>	UV-visible spectroscopy, transmission and scanning electron microscopy, X-ray diffraction analysis	10-50	Acicular	[108]
<i>Rhodococcus pyridinivorans NT2</i>	(FE-SEM), (XRD)	100-120	Hexagonal phase, roughly spherical	[109]

Table 5: Algal-Mediated synthesis using Zinc Oxide Nano Particle.

Algae Strains	Size (nm)	Functional Group	Shape	Ref
<i>Chlamydomonas reinhardtii</i> (<i>Chlamydomonaceae</i>)	21 (XRD), 55–80 (HR-SEM)	C = O stretching, N-H bending band of amide I and amide II, C = O stretch of zinc acetate, C-O-C of polysaccharide	Nanorod, porous nanosheet	[110]
<i>Sargassum muticum</i> (<i>Sargassaceae</i>)	42 (XRD), 30–57 (FE-SEM)	Asymmetric stretching band of the sulfate group, an asymmetric C-O band associated with C-O-SO ₃ & -OH group, sulfated polysaccharides	Hexagonal	[111]
<i>S. myriocystum</i> (<i>Sargassaceae</i>)	20–36 (AFM), 46.6 (DLS)	O-H and C = O stretching band, carboxylic acid	Spherical, triangle, hexagonal	[112]
<i>Anabaena cylindrica</i> (<i>Nostocaceae</i>)	40-60nm (FESEM and TEM)	stretching vibration of bonded and non-bonded O-H groups, asymmetric –CH ₂ , symmetric –CH ₃ , and CH ₂ - stretching vibrations, C-O-C stretching, stretching C-N bond	Road Like	[113]



opened the avenue for exploring the potential of zinc oxide nanoparticles as effective anticancer material. The following reports present the possible therapeutic approaches brought by zinc oxide nanoparticles. The genotoxicity of zinc oxide nanoparticles was evaluated in a previous study while DNA damage occur causing reduction in cell viability upon exposure to ZnO-NPs. Furthermore, ZnO-NPs were found to induce oxidative stress which was indicated by a decrease in glutathione (59% and 51%); catalase (64% and 55%), and superoxide dismutase (72% and 75%) at a concentration of 0.8 and 0.08 g/ml respectively [124]. Further *in vivo* study in mice investigated the oral toxicity of ZnO-NPs while the results came up with a cellular injury after oral demonstration of ZnO-NPs (300 mg/kg) for 14 days due to the accumulation of nanoparticles in the liver. This liver injury was assessed by the increased serum levels of both alanine aminotransferase (ALT) and alkaline phosphatase (ALP) liver enzymes besides pathological lesions in the liver. Furthermore, ZnO-NPs were found to increase oxidative stress inside liver cells and subsequently cause DNA damage and apoptosis [125]. Earlier in 2016, Pandurangan, et al. [126] studied the anticancer effect of zinc oxide nanoparticles (10 nm) in human cervical carcinoma cells (Hela) with different concentrations ranging from 0.001 to 0.06 mg/mL for 48 hrs. Results showed a reduction in cell viability in a range from 5% to 50% in HeLa cells while they did not cause a great effect on normal Madin-Darby Canine Kidney (MDCK) cells as their cell viability results at 0.06 mg/mL was 95%. Another study in 2017, Manshian, et al. [127] assessed the anticancer activities of free zinc oxide nanoparticles and Fe-doped zinc oxide nanoparticles (10 - 20 nm), and the results revealed that doping zinc oxide nanoparticles with iron (2 - 10%) diminished the ionization of Zn into Zn²⁺ ions and reduced ZnO cytotoxicity towards normal cells (murine mesenchymal stem cells and human bronchial epithelial cells) and cancer cells (HeLa and murine lung squamous carcinoma cells). Later in 2018, Heng and Zhang [128] studied the anticancer effects of both Chitosan coated and non-coated zinc oxide nanoparticles in HeLa cells while the concentration of ZnO ranged from 0.1 to 75 µg/mL for 24 hrs. From the results, both coated and uncoated ZnO-NPs exhibited low cytotoxicity as they reduced cell viability by a percentage less than 10% when using a concentration of 1 µg/mL. Thereby, chitosan-coated zinc oxide nanoparticles which are positively charged caused elevated cytotoxicity via easier cellular internalization. Subsequently, they increased reactive oxygen species formation and apoptosis whereas

from a concentration of 12 mmol/ L. This inhibition occurred due to cellular disruption which lead to cell death [118]. A further study explained that the antibacterial properties of Zinc oxide nanoparticles against *S. aureus* were developed due to the formation of intracellular ROS which was found to be the reason behind the bacterial cell death [119]. Another study revealed that zinc oxide nanoparticles have antibacterial activity against *Campylobacter jejuni*. From the results, scanning electron microscopy confirmed that, after exposure to 0.5 mg/ mL of zinc oxide nanoparticles for 16 h, morphological changes were developed which caused a transformation from spiral shapes into coccoid forms in *C. jejuni* [120]. Recently, Hameed, et al. [121] studied the effect of zinc oxide nanoparticles immobilized on fabricated neodymium which exhibited unique antibacterial activity against extended-spectrum β-lactamases (ESBLs)-generating *E. coli* and *Klebsiella pneumoniae* bacteria. In this study, it was noticed that the cell membrane developed more distortion, besides bacterial cell shrinkage when compared to free zinc oxide nanoparticles.

Anticancer activity: Cancer results from unrestricted proliferation and immortality of cells due to impairment in the uncontrolled program of cell death (apoptosis) [122]. The elimination of cancer cells inside the human body ever requires searching for effective scopes to bring up new therapeutic strategies. As known, present chemotherapy agents are not effectively treating cancer and fail to bring a complete anticancer effect because these chemotherapeutic substances cannot differentiate between cancerous and normal cells. Thereby, they may lead to normal cell toxicity and dangerous side effects such as; suppression of bone marrow function, damage to hair and nails, neurotoxicity, and cardiomyopathy [123]. Therefore, searching for alternative therapeutic agents that have a higher degree of selectivity and affinity toward cancer cells is highly required. This belief



complete cell death occurred at a concentration of 75 $\mu\text{g}/\text{mL}$. Recently in 2019 [128], a concentration of ZnO-NPs ranging from (20 - 200 $\mu\text{g}/\text{mL}$) was used for the treatment in SiHa cells for 24 hrs and showed cytotoxicity with an IC₅₀ value of 35 $\mu\text{g}/\text{mL}$. Complete cell death was obtained at 200 $\mu\text{g}/\text{mL}$ which was the maximum concentration. However, ZnO-NPs did not show exhibit any toxicity towards normal peripheral blood mononuclear cells at a concentration up to 200 $\mu\text{g}/\text{mL}$. Another study on HeLa cells evaluated the anticancer activity of green synthesized ZnO-NPs in normal human embryonic kidney (HEK-293) cells. Herein, cell toxicity was observed when treated with ZnO-NPs with a concentration ranging from 6.25-200 $\mu\text{g}/\text{mL}$ for 24 hrs due to the production of ROS [129]. Another study showed the anticancer effect of ZnO-NPs and 55 % of cell death happened while applying the maximum concentration of 20 $\mu\text{M}/\text{mL}$. They revealed no cytotoxicity toward normal MCF-10A cells [130]. In 2020, Zinc oxide nanoparticles' anticancer mechanism against A549 cells was studied while ZnO-NPs showed cytotoxicity in A549 cells at a concentration ranging from 1 to 25 $\mu\text{g}/\text{mL}$ for 24 hrs. Thereby, the mechanism was by induction of apoptosis and depressing the migration rate in MCF-7 cells [131].

Antifungal activity: A prior study was conducted to test the viability of the pathogenic yeast, *Candida albicans* (*C. Albicans*) after exposure to ZnO-NPs. Here, inhibition of over 95% in the growth of *C. Albicans* was obtained at the minimal fungicidal concentration of ZnO which is 0.1 mg/ ml. Furthermore, visible light excited ZnO-NPs and enhanced yeast cell death. It was suggested that cell death was due to the involvement of reactive oxygen species; including hydroxyl radicals and singlet oxygen [132]. Further study in 2017, green synthesized zinc oxide nanoparticles from *Ziziphus nummularia* leaf extract were characterized by various spectral analyses, and their antifungal (anticandidal) activity was evaluated against multidrug-resistant isolates. The results were better than four standard azole antibiotics and ZnO-NPs further exhibited a cytotoxic effect against HeLa cells [133]. In 2018 Sardella et al., developed a rapid and accurate technique to assess the efficacy of novel antifungal agents such as zinc oxide nanoparticles against three isolates. *A. alternate*, *B. cinerea*, and *R. stolonifer*. Results did not achieve significant growth inhibition in *A. alternate* and *B. cinerea* at 15 mM of ZnO-NPs concentration while in the case of *R. stolonifer* the maximum inhibition occurred at that concentration [134]. Another study was performed on metal oxide nanomaterials (zinc oxide, magnesium oxide, and ZnO: MgO and ZnO: Mg(OH)₂ composites) and explored their antifungal activities against the fungal strain of *C. gloeosporioides* obtained from papaya and avocado fruits. The results showed that all nanoparticles significantly inhibited the germination of conidia and caused structural damage to the fungus cells at the tested concentrations [135]. Later in 2019, Miri, et al. studied the antifungal activity of ZnO which has been synthesized greenly by *Prosopis fractal* aqueous extract, and the characterization have been done by UV-Vis

absorption, Fourier-Transform Infrared spectroscopy (FT-IR), Raman, Powder X-ray Diffraction (PXRD), Transmission Electron Microscopy (TEM), Field Emission Scanning Electron Microscopy (FESEM) and Energy Dispersive X-ray analysis (EDX). Herein, results have shown the antifungal activity of ZnO-NPs against *Candida albicans* and the minimum fungicidal concentration (MFC) was 256 $\mu\text{g}/\text{ml}$ [136]. Extra studies focused on three-particle morphologies of Zinc oxide and in vitro study of their antifungal activity against three phytopathogenic fungi species; *Fusarium oxysporum lycopersici*, *Fusarium solani*, and *Colletotrichum gloeosporioides*. Herein, ZnO particle shapes were nanoparticles, lamellar platelets, and hexagonal rods. Furthermore, they were obtained by colloidal and hydrothermal synthesis techniques, the synthesis parameters, and methodology. Electron microscopy imaging techniques were used for the determination of ZnO-NPs size and morphology. Results came up with that ZnO with platelets shaped particles have better antifungal inhibition activity than rods and nanoparticles and it inhibited the growth of *Fusarium solani* by a maximum percentage of 65% [137]. Reactive DC magnetron was used for the production of thin films of zinc oxide and tested against the pathogenic fungus *Candida albicans*. The results revealed the successful formation of stoichiometric ZnO thin films and their significant antifungal activity with inhibition of cell growth by 68%. From this paper, it was suggested that the formation of mesoporous films of ZnO enhanced its antifungal activity against *Candida albicans* when compared with ZnO-NPs [138]. A recent study in 2020 focused on the antifungal effect of ZnO-NPs and Nystatin on gene expression of SAP1-3 in *Candida albicans* (*C. Albicans*) and the results showed a significant reduction in gene expression for both ZnO-NPs and Nystatin [139]. Another study was performed against *Penicillium chrysogenum* to exploit the antifungal activity of ZnO-NPs. In that study, biogenesis and capping processes of the synthesized nanoparticles were performed using the functional fungal extracellular enzymes and proteins. The ability of the bio-secreted proteins cape ZnO have been demonstrated to change their particle shape to hexagonal and spherical ZnO-NPs with particle size at 9.0 - 35.0 nm which exhibited good antimicrobial activities against some phytopathogenic fungal strains and some bacterial species. Highlighted from that study, the green synthesized ZnO-NPs have been considered as smart nano-material for the application in the medical field and treatment of some pathogenic microbes [140].

Antiviral activity: Zinc oxide nanoparticles have been used for developing effective treatment of several viruses and here previous studies will be demonstrated. Kumar et al., investigated the use of ZnO-NPs which were synthesized by precipitation method and characterized by Transmission Electron Microscope (TEM), Fourier transform infrared (FTIR), and zeta dynamic light scattering for treatment of Chikungunya (CHIKV). After treatment of cells with zinc oxide nanoparticles, results revealed that ZnO-NPs could

interact with viruses, and this caused inhibition of viral growth and cell death. Therefore, ZnO could be considered a new effective antiviral drug against the chikungunya virus though further tests on animals are still required for approval [141]. In another study, Hydroxyl group rich ZNPs (H-ZNPs), oleic acid modified ZNPs (OA-ZNPs) and chitosan-Zinc nanoparticles (C-ZNPs) were chemically synthesized and their characterization was performed by ultra-violet (UV) and Fourier transforms infrared (FTIR) spectroscopy, transmission electron microscope (TEM) and selected area electron diffraction (SAED). Moreover, their antiviral activity was assessed and the results suggested that surface-modified ZnO-NPs could obstruct the virus infectivity potential by the virus neutralization rather than interfering with cellular targets. In this regard, the electrostatic interference of H-ZNPs and physical entrapment exhibited by C-ZNPs is more significant than the hydrophobic interaction with OA-ZNPs [142]. The further study focused on evaluating the antiviral activity of zinc oxide nanoparticles and PEGylated zinc oxide nanoparticles against the H1N1 influenza virus. Herein, results showed that the PEGylated and unPEGylated ZnO-NPs both have antiviral activity against the H1N1 virus at the highest non-toxic concentrations with inhibition percentages of 94.6% and 52.2%, respectively [143]. In a 2020 recent study, green synthesized zinc oxide nanoparticles from aqueous leaf extract of *Mentha spicata* were tested against Tobacco mosaic virus (TMV) and the results confirmed the antiviral effect of ZnO-NPs with reduction of viral accumulation reached 90.21% [144]. The biomedical applications are shown in Figure 3.

Other biological applications: Zinc oxide nanoparticles have other biological applications including bioimaging where ZnO-NPs have many desirable optical properties which make them suitable for application for optical imaging [145]. Furthermore, ZnO-NPs are unique nanomaterials for application in drug delivery due to their high surface area, versatile surface chemistry, phototoxic effect, and others [146]. Moreover, ZnO-NPs also exhibit semiconducting

properties, high catalytic efficiency, and strong adsorption capability which enables them to be reliable for biosensors applications. Besides they have high isoelectric point ~ 9.5 which are versatile for some proteins adsorption for instance; certain enzymes and antibodies by electrostatic interaction [147].

Conclusion and future prospects

The green synthesized ZnO-NPs have diverse biological and biomedical applications. Traditionally, nanoparticles are synthesized by either physical or chemical methods, which not only leads to environmental toxicity but also costly and energy-intensive labor. ZnO-NPs are synthesized by a green route using the extracts of different plants/parts of plants, microorganisms, and other biological molecules such as gelatin, oleic acid, and starch. The bio-mediated ZnO-NPs are environmentally friendly, facile in terms of synthesis, cost-effective, most fascinating, and biocompatible. The FDA has ZnO-NPs on its list of safe substances. However, additional research is still needed on a few important ZnO-NPs-related concerns, such as the following: The limitations of ZnO-NPs toxicity toward biological systems remain a contentious issue in recent research, and there is a lack of evidence-based randomized studies specifically examining therapeutic roles in improving anticancer, antibacterial, anti-inflammatory, and antidiabetic activities. Additionally, there is a lack of understanding of corresponding animal studies about its anticancer, antibacterial, anti-inflammatory, and antidiabetic activities. The usage of ZnO nanoparticles in biomedical diagnostic and therapeutic sectors may be better clarified and understood through the conduct of further studies centered on the aforementioned problems. We think that nanomaterials would significantly advance the ZnO nanoparticles and are anticipated to make more intriguing contributions in various domains of medicine.

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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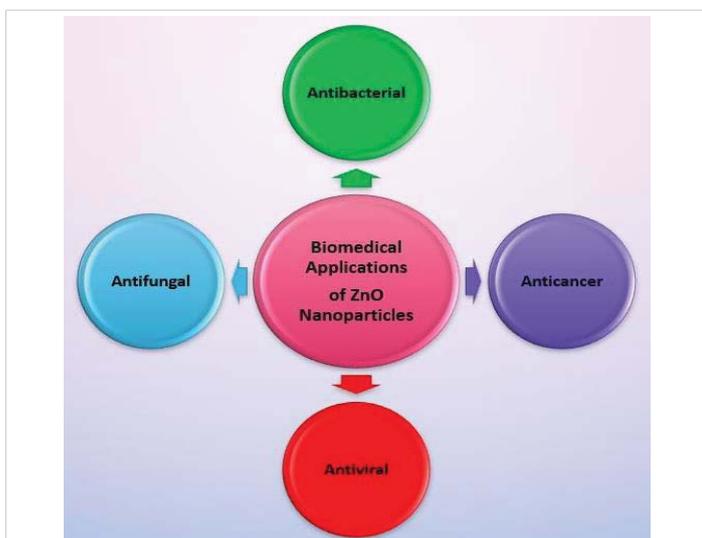


Figure 3: Various Biomedical applications of ZnO-NPs.



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