

GREEN SYNTHESIS OF NANOPARTICLES USING RHIZOSPHERE SOIL CONSORTIA AND THEIR ANTIMICROBIAL PROPERTIES

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Abstract

One of the fastest evolving areas of science and technology is nanotechnology which provides novel ways of solving problems in medicine, agriculture, pharmacy, and environmental management. Zinc oxide nanoparticles (ZnO NPs) are one of the types of metal oxides that have attracted much interest owing to their physicochemical properties such as large surface area, biocompatibility, antimicrobial activity, and photocatalytic efficiency. The traditional techniques for production of ZnO NPs make use of toxic reagents and have high energy consumption and produce hazardous waste products. ZnO nanoparticle synthesis using rhizosphere bacteria as biological agents has been gaining considerable popularity in recent years because of their ability to produce enzymes, proteins, and metabolites for the reduction and stabilization of metal ions. Biological ZnO nanoparticles have improved biological activity and lower toxicity than chemically synthesized ZnO nanoparticles. It has been observed in recent researches that rhizosphere bacteria-assisted ZnO nanoparticles are effective against various pathogenic bacteria and fungi. Moreover, these nanoparticles have significant antioxidant properties owing to which they can be used in biomedical and pharmaceutical applications. This article is an overview of the literature on ZnO nanoparticle synthesis using rhizosphere bacteria and their antimicrobial, antifungal, and antioxidant properties.

Key words: Zinc oxide Nanoparticles, Rhizosphere bacteria, Green synthesis, Antibacterial activity, Antifungal activity, Antioxidant activity, Nanotechnology.

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1. INTRODUCTION

Nanoengineering is one of the most rapidly advancing interdisciplinary branches of science and technology involved in designing, manufacturing, characterizing, and applications of materials having dimensions of 1 to 100 nm (Wang, 2004). The novel materials have novel physical, chemical, optical, electrical, and biological properties different from their bulk counterparts owing to the higher surface area and quantum effects (Khan et al., 2019). Unique properties have led to the widespread use of nanoparticles in medicine, agriculture, environmental science, food science, electronics, and biotechnology (Kumar et al., 2020). Various nanoparticles have been studied; however, metal oxide nanoparticles have gained immense importance owing to their stability, versatile nature, and extensive biological activities (Siddiqi et al., 2018). Among metal oxide nanoparticles, zinc oxide nanoparticles (ZnO NPs) stand out as one of the most promising nanomaterials on account of their remarkable physical and chemical properties, as well as versatile uses (Moezzi et al., 2012). The notable properties of ZnO nanoparticles include high thermal stability, good ultraviolet light absorbance, photocatalytic activities, and semiconducting abilities that are useful in multiple industrial and biomedical applications (Sirelkhatim et al., 2015). Besides, zinc is an essential micronutrient necessary in different biological processes, hence making ZnO nanoparticles less toxic and highly biocompatible than most other metal nanoparticles (Rasmussen et al., 2010). Their applications include wound dressings, antibacterial coatings, sunscreens, drug delivery carriers, and cancer treatments (Gupta et al., 2019).

ZnO nanoparticles have been extensively utilized in the pharmaceutical industry for their broad-spectrum antibacterial, antifungal, antiviral, and antioxidative effects (Jones et al., 2008). It has been found that the antibacterial effect of ZnO nanoparticles mainly results from the generation of reactive oxygen species, release of zinc ions, and disruption of microorganism membrane (Raghupathi et al., 2011). Antibacterial activity of ZnO nanoparticles against drug-resistant microorganisms makes their use even more important in contemporary medicine (Premanathan et al., 2011). On the other hand, increased production and application of these nanomaterials pose environmental risks because of their possible accumulation in the environment and adverse impact on ecology (Rajput et al., 2018).

Traditional nanoparticle synthesis methods usually use physical and chemical methods such as laser ablation, chemical reduction, sol-gel method, and hydrothermal technique (Sharma et al., 2016). Despite the ability of these techniques to create size and shape-controlled nanoparticles, they usually demand expensive instruments, energy-intensive processes, and toxic chemicals which could potentially be harmful to human health and the environment (Iravani, 2011). The creation of toxic waste during the chemical process has inspired scientists to look for alternative and environmentally safe methods of nanoparticle synthesis (Singh et al., 2018). Green synthesis, therefore, has become an attractive option for creating nanoparticles.

Green synthesis involves using biological systems, such as plants, algae, fungi, yeast, and bacteria, as reducing and stabilizing agents for the synthesis of nanoparticles (Narayanan & Sakthivel, 2010). Methods involving biological synthesis are

comparatively safer and economical than traditional methods (Mittal et al., 2013). Bioparticles, which include enzymes, proteins, polysaccharides, and secondary metabolites, aid in the reduction process of metals and stabilization of synthesized nanoparticles (Yusof et al., 2019). Additionally, biologically synthesized nanoparticles are biocompatible due to capping bioparticles present on the surface of nanoparticles (Singh et al., 2020).

Among many other biological resources, rhizosphere microbes have been considered to be quite an effective candidate for nanoparticle synthesis due to their great metabolic diversity and versatility (Philippot et al., 2013). Rhizosphere refers to the region of soil affected by plant roots and their secretions and it has one of the most diverse microbial communities existing on Earth (Hiltner, 1904). Plant root exudates such as sugars, amino acids and organic acids form a very rich substrate for microbial growth and activities (Bais et al., 2006). Rhizosphere microorganisms are known to play key roles in the functioning of many ecosystems as they are involved in nutrient recycling, nitrogen fixation, phosphorus mobilization and inhibition of pathogens (Berendsen et al., 2012).

The rhizospheric bacteria have received special importance in nanobiotechnology owing to their fast growth rates, easy culture conditions, and the capability of producing nanoparticles in very moderate environmental conditions (Rajeshkumar & Bharath, 2017). Enzymes produced by the bacteria, such as nitrate reductase and oxidoreductases, are involved in the reduction of metal ions that are required in the production of nanoparticles (Ahmad et al., 2003). Proteins and polysaccharides produced extracellularly by the bacteria can serve as capping agents to avoid nanoparticle agglomeration (Mohanpuria et al., 2008). The bacterial species like *Bacillus*, *Pseudomonas*, *Rhizobium*, and *Streptomyces* have been used effectively to produce zinc oxide nanoparticles (Shankar et al., 2016).

In spite of the great pharmacological advantages provided by ZnO nanoparticles, there is growing scientific evidence about their possible impacts on soil microorganisms and ecology (Brayner, 2008). ZnO nanoparticles can contaminate agricultural soils due to wastewater irrigation, sewage sludge application, pharmaceutical waste disposal, and industrial effluent (Auffan et al., 2009). Having been added to the soil system, the particles will be able to affect the microorganisms inhabiting the rhizosphere and change microbial diversity and activity (Mahmood et al., 2020). In several researches, the presence of an excess of ZnO nanoparticles has been identified as the factor affecting oxidative stress, decreasing microbial populations and disrupting nutrient cycles (Dimkpa & Bindraban, 2018).

For this reason, it is necessary to understand the behavior of ZnO nanoparticles when interacting with rhizosphere microorganisms in order to guarantee the safe use of nanotechnology in agricultural and medicinal sciences (Saxena et al., 2020). Development of the bacterial green synthesis methods for nanoparticles could offer an environmentally friendly alternative to the traditional method of nanoparticle manufacturing (Salem et al., 2021). Also, research in the mechanisms of nanoparticle biosynthesis and its biological activities could lead to the development of new drug formulations (Jha et al., 2023). In this review, emphasis is placed on the biosynthesis of ZnO nanoparticles by rhizosphere bacteria, characterization of their biological activities and pharmaceutical applications.

2. RHIZOSPHERE MICROORGANISMS

2.1 Rhizosphere and its Importance

Rhizosphere is described as the small region of soil that is affected by the presence of the plant roots and the exudation process (Hiltner, 1904). The microbe population in the rhizosphere region is significantly higher compared to the soil in general due to the constant secretion of sugars, amino acids, vitamins, and organic acids from the plant roots (Bais et al., 2006). Exudates act as nutrients for the microorganisms and cause a high level of microbial activity around the roots of plants (Compant et al., 2010). Rhizosphere microorganisms have the ability to facilitate nitrogen fixation, phosphate solubilization, siderophore formation, and phytohormone production (Bhattacharyya & Jha, 2012). These processes increase nutrient availability and stimulate the growth of plants in normal and stressed environments (Glick, 2012). The beneficial rhizosphere microorganisms are capable of inhibiting pathogens present in the soil by producing antibiotics and lytic enzymes (Lugtenberg & Kamilova, 2009). As such, the rhizosphere environment has been described as one of the most biologically active soil environments (Berendsen et al., 2012).

2.2 Rhizosphere Bacterial Diversity

Rhizosphere harbors a large microbial population that includes beneficial, neutral and pathogenic microbes (Bulgarelli et al., 2013). Some common bacterial genera present in the rhizosphere include *Bacillus*, *Pseudomonas*, *Rhizobium*, *Azotobacter*, *Enterobacter* and *Streptomyces* (Mendes et al., 2013). Such microorganisms help in transforming nutrients and maintain soil fertility (Vessey, 2003). Some rhizosphere bacteria are known as plant growth promoting rhizobacteria due to their contribution towards improving the development of plants in many ways (Glick, 2012). There is variation in the nature of rhizosphere bacterial community composition due to the variability in plant species, soil type, environment, and agricultural methods (Philippot et al., 2013). There is selective enrichment of microorganisms by root exudates which are useful for the plants in stress and growth (Bais et al., 2006). The development of molecular tools such as 16S rRNA sequencing has provided significant progress in studying rhizosphere microbial diversity and community (Bulgarelli et al., 2013).

2.3 Role of Rhizosphere Bacteria in Nanoparticle Synthesis

Rhizosphere bacteria have shown potential for use as biological factories in the fabrication of nanoparticles due to their capability to generate a diverse array of enzymes and metabolites (Narayanan & Sakthivel, 2010). Rhizosphere bacteria can synthesize nanoparticles via the reduction of metal ions through enzymatic processes that take place either within or outside the bacterial cells (Ahmad et al., 2003). Proteins and polysaccharides released from rhizosphere bacteria can serve as stabilizing and capping agents during the synthesis of nanoparticles (Mittal et al., 2013). The approach minimizes the use of dangerous chemicals often employed in conventional nanoparticle fabrication approaches (Iravani, 2011). There are many benefits that accrue from bacterial synthesis such as low cost, environmental sustainability, quick synthesis, and enhanced biocompatibility of nanoparticles (Singh et al., 2018). Various rhizosphere bacteria, such as *Bacillus spp.*, *Pseudomonas spp.*, and *Rhizobium spp.*, have been used in the production of ZnO nanoparticles (Rajeshkumar & Bharath, 2017). The nanoparticles produced using the biological process possess enhanced antimicrobial and antioxidative characteristics owing to microbial molecules attached on the surface of the nanoparticles (Sharma et al., 2016). Consequently, rhizosphere bacteria-based synthesis of nanoparticles is an essential field in green nanotechnology and pharmaceutical microbiology (Yusof et al., 2019). (Table.1).

Table 1. Rhizosphere Bacteria Reported for The Biosynthesis of Zinc Oxide Nanoparticles

Bacterial Species	Gram Reaction	Morphology	ZnO NP Characteristics
<i>Bacillus subtilis</i>	Positive	Rod	Spherical, 20-40 nm
<i>Bacillus megaterium</i>	Positive	Rod	Hexagonal, 30-60 nm
<i>Pseudomonas aeruginosa</i>	Negative	Rod	Spherical, 10-30 nm
<i>Pseudomonas fluorescens</i>	Negative	Rod	Spherical, 15-35 nm
<i>Rhizobium leguminosarum</i>	Negative	Rod	Spherical, 25-35 nm
<i>Streptomyces griseus</i>	Positive	Filamentous	Spherical, 20-50 nm
<i>Bacillus cereus</i>	Positive	Rod	Spherical, 10-20 nm
<i>Enterobacter cloacae</i>	Negative	Rod	Hexagonal, 20-40 nm
<i>Azotobacter chroococcum</i>	Negative	Coccoid	Spherical, 30-60 nm
<i>Klebsiella pneumoniae</i>	Negative	Rod	Spherical, 15-30 nm

3. ZINC OXIDE NANOPARTICLES

3.1 Overview of Zinc Oxide Nanoparticles

ZnO NPs refer to metal oxide nanoparticles that exhibit an average size of 1–100 nm (Wang, 2004). Due to their high surface-to-volume ratio, they have unique optical, catalytic, and antimicrobial properties (Moezzi et al., 2012). One of the most vital semiconductor nanomaterials is zinc oxide nanoparticles since they contain a wide band gap energy of 3.37 eV (Sirelkhatim et al., 2015). Their good biocompatibility and lower toxicity have led to their utilization in pharmaceuticals and biomedical science (Rasmussen et al., 2010). At present, ZnO nanoparticles are added to cosmetic products, wound dressings, sunscreens, and antimicrobial formulations (Khan et al., 2019). The rising need for ZnO nanoparticles has promoted

studies on eco-friendly synthesis processes involving biological systems (Singh et al., 2020).

3.2 Physicochemical Properties of Zinc Oxide Nanoparticles

The physicochemical properties of zinc oxide nanoparticles depend significantly on their size and morphology (Moezzi et al., 2012). Due to their tiny size, the specific surface area is big, which makes zinc oxide nanoparticles highly chemically reactive (Khan et al., 2019). Zinc oxide nanoparticles show excellent photocatalytic properties at ultraviolet radiation due to the creation of the electron-hole pair (Wang et al., 2004). They have good thermal and chemical stability that allows using them in industries and pharmaceuticals (Sirelkhatim et al., 2015). Zinc oxide nanoparticles are characterized by strong ultraviolet absorbent properties that provide the possibility of their application in sunscreens (Rasmussen et al., 2010). (Table.2).

Table 2. Physicochemical Properties of Zinc Oxide Nanoparticles and their Relevance to Pharmaceutical Applications

Property	Description	Relevance to Pharmaceutical Applications
Particles size (10-100 nm)	Nanoscale dimension with high surface area	Enhances bioavailability, cellular uptake and antimicrobial activity

High surface area	Large surface area to volume ratio	Improves interaction with microbial cells and drug molecules
Semiconducting nature	Wide band gap (3.2 eV)	Useful in drug delivery, biosensors and photocatalytic applications
Antimicrobial Activity	Generates ROS and release Zn^{2+} ions	Effective against Gram-positive, Gram-negative bacteria and fungi
UV absorption	Strong absorption in UV region	Used in sunscreens and protective pharmaceutical formulations
Biocompatibility	Low toxicity and good biocompatibility	Suitable for biomedical and drug delivery applications
Chemical stability	Resistant to oxidation and degradation	Ensures longer shelf life in pharmaceutical products
Photocatalytic Activity	Generates electron – hole pairs under light	Useful in wound healing and anticancer therapies

3.3 Pharmaceutical Applications of ZnO Nanoparticles

The reason why the ZnO nanoparticles have been extensively investigated by pharmaceutical industries is their broad spectrum of antimicrobial activity (Jones et al., 2008). The capacity of ZnO nanoparticles to inhibit the growth of bacteria has made them useful in wound healing and topical cream formulations (Premanathan et al., 2011). In addition to this, ZnO nanoparticles are used in sunscreen preparations as they effectively block the ultraviolet light (Sirelkhatim et al., 2015). They are biocompatible and thus can be considered promising carriers for the development of drug delivery systems (Rasmussen et al., 2010). Moreover, their selectivity for cancer cells has enabled scientists to explore their use in anticancer therapy (Gupta et al., 2019).

4. GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES

Green synthesis is regarded as a greener technique than traditional methods used for nanoparticle synthesis (Iravani, 2011). Biological agents like plants, fungi, algae, and bacteria are capable of serving as both reducing and stabilizing agents in the process of nanoparticle production (Narayanan & Sakthivel, 2010). The process of green synthesis does not require the use of any toxic materials and is associated with less environmental pollution (Mittal et al., 2013). It is cost-effective and energy-efficient compared with other physical and chemical techniques

(Singh et al., 2018).

4.1 Biological or Green Synthesis

The use of biological synthesis involves the use of biological molecules for the reduction of metallic ions and stabilization of nanoparticles (Narayanan & Sakthivel, 2010). Protein, enzymes, amino acid, and polysaccharide molecules facilitate nanoparticle formation (Mittal et al., 2013). It is environmentally friendly compared to chemical synthesis as no toxic reducing agents are used (Agarwal et al., 2017). Biological synthesis is deemed sustainable and environmentally friendly (Singh et al., 2020). Therefore, biological synthesis is a popular area of study in nanobiotechnology.

4.2 Bacteria-Mediated Synthesis of Zinc Oxide Nanoparticles

Bacteria are efficient nanomaterials producers owing to their fast growth rate and metabolic abilities (Ahmad et al., 2003). Zinc salt reduction to ZnO nanoparticles can be accomplished using rhizospheric bacteria enzymatically (Rajeshkumar & Bharath, 2017). Bacteria excrete extracellular proteins that serve as reducers in the synthesis process (Sharma et al., 2016). The metabolism of bacteria plays an important role in stabilizing nanoparticles due to the ability to inhibit aggregation (Mittal et al., 2013). Some species of the following genera – *Bacillus*, *Pseudomonas*, and *Rhizobium* – have been used to synthesize ZnO nanoparticles.(Figure.1).

Bacteria-Mediated Biosynthesis of Zinc Oxide Nanoparticles

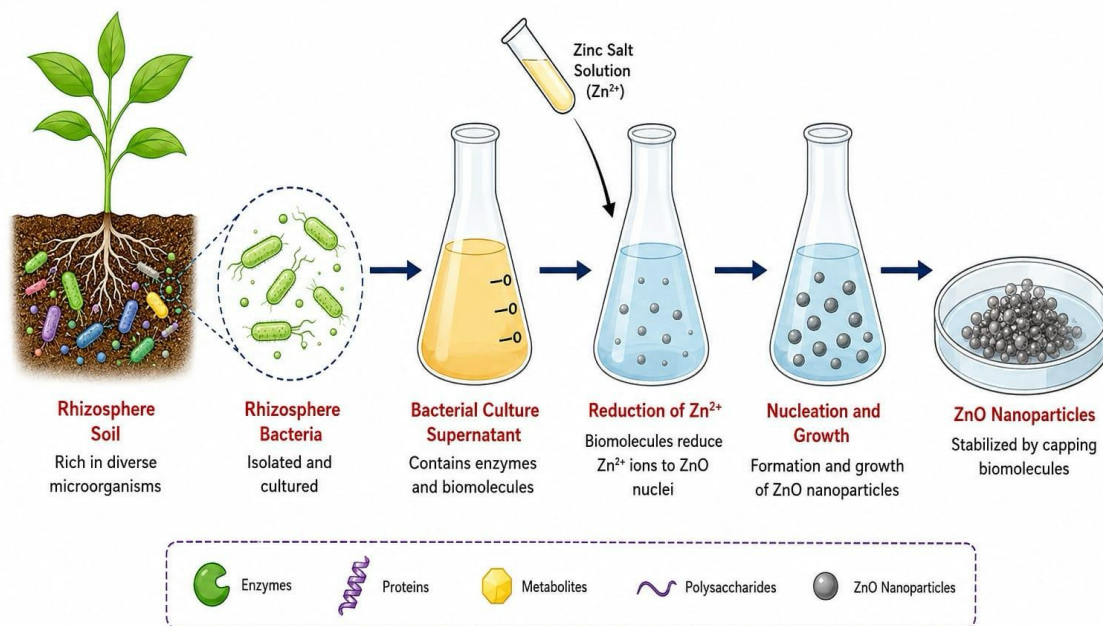


Figure.1. Bacteria-Mediated Biosynthesis of Zinc Oxide Nanoparticles.

5. MECHANISM OF BIOSYNTHESIS OF ZINC OXIDE NANOPARTICLES

The production of ZnO nanoparticles is comprised of four steps, which are reduction, nucleation, growth, and stabilization of nanoparticles (Narayanan & Sakthivel, 2010). The enzymes produced by microorganisms aid in the process of electron transfer, which is required to reduce zinc ions (Ahmad et al., 2003). After the reduction stage, zinc ions come together to form nuclei (Mittal et al., 2013). Further growth leads to the creation of nanoparticles having a definite size and shape (Rajeshkumar & Bharath, 2017).

5.1 Extracellular Biosynthesis

Extracellular synthesis involves the production of enzymes and other metabolites that act on the zinc ions present outside the bacterial cell through release into the culture medium (Ahmad et al., 2003). The process of nanoparticle synthesis happens in the surrounding solution and not within the bacterial cell (Jayaseelan et al., 2012). Nanoparticle recovery and purification is relatively easier using extracellular synthesis systems (Singh et al., 2020).

5.2 Intracellular Biosynthesis

The intracellular synthesis process takes place after the zinc ions have been taken into the microorganisms' cells through the membrane transport systems (Narayanan & Sakthivel, 2010). The enzymes present in the intracellular compartment convert the ions into nanoparticles in the cytoplasm (Rajeshkumar & Bharath, 2017). The nanoparticle formation takes place inside the cell prior to their isolation and purification processes (Ahmad et al., 2003).

5.3 Role of Enzymes and Metabolites

The nitrate reductase enzyme is regarded as one of the key

enzymes that play a significant role in the biosynthesis of nanoparticles (Ahmad et al., 2003). The oxidoreductase enzymes help in transferring electrons during the process of reduction (Sharma et al., 2016). The proteins produced by microorganisms provide surface stabilization for nanoparticles (Mittal et al., 2013). The polysaccharides and amino acids act as capping agents (Yusof et al., 2019).

6. CHARACTERIZATION TECHNIQUES OF ZINC OXIDE NANOPARTICLES

6.1 UV-Visible Spectroscopy

Ultraviolet-visible spectroscopy is usually employed for verifying the successful synthesis of nanoparticles (Suresh et al., 2015). It is known that ZnO nanoparticles have a characteristic absorption peak ranging from 320 to 380 nm (Agarwal et al., 2017). Absorption behavior gives information about nanoparticle synthesis (Sharma et al., 2016).

6.2 Fourier Transform Infrared Spectroscopy

FTIR spectroscopy characterizes functional groups that play a vital role in nanoparticles' preparation and stabilization (Sangeetha et al., 2011). It helps to identify biomolecules that interact with the surface of the nanoparticles (Mittal et al., 2013). Thus, FTIR spectroscopy is used to determine the involvement of metabolites produced by bacteria in biosynthesis processes (Sharma et al., 2016).

6.3 Scanning Electron Microscopy

SEM can be used to study nanoparticle surface morphologies and shapes (Moezzi et al., 2012). The technique gives information about aggregation and particle distribution (Khan et al., 2019). SEM pictures are helpful to ensure the creation of nanoparticles (Kumar et al., 2020).

6.4 Transmission Electron Microscopy

TEM is able to produce high-resolution images of nanoparticles

(Wang, 2004). This method enables accurate measurement of the particle size and shape (Sirelkhatim et al., 2015). TEM is recognized as one of the most essential characterization methods in nanotechnology (Kumar et al., 2020).

6.5 X-ray Diffraction

The crystalline nature of the nanocrystals is determined using XRD (Wang, 2004). The method is used to confirm that the ZnO nanoparticles have hexagonal wurtzite crystalline structure (Sirelkhatim et al., 2015). The crystal size may be determined from the Debye-Scherrer equation (Moezzi et al., 2012).

6.6 Dynamic Light Scattering

DLS is used to analyze size distributions of nanoparticles in liquids (Rajput et al., 2018). This method gives hydrodynamic diameters of colloidal nanoparticles (Khan et al., 2019). Moreover, DLS can be applied to examine nanoparticle stability in liquid media (Kumar et al., 2020).

7. ANTIBACTERIAL ACTIVITY OF ZINC OXIDE NANOPARTICLES

ZnO nanoparticles display antibacterial property against both Gram-positive and Gram-negative bacteria (Jones et al., 2008). Reactive oxygen species produced by nanoparticles result in injury to the bacterial membrane (Raghupathi et al., 2011). Zinc ion generation is involved in metabolic disruption (Premanathan et al., 2011). Ultimately, altered membrane permeability results in the death of bacteria (Sirelkhatim et al., 2015). Examples of bacteria targeted by ZnO nanoparticles include *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Gupta et al., 2019).

8. ANTIFUNGAL ACTIVITY OF ZINC OXIDE NANOPARTICLES

ZnO nanoparticles have been found to show high antifungal efficacy against multiple pathogenic fungi (Jayaseelan et al., 2012). Nanoparticles inhibit germination of fungal spores as well as the growth of fungal hyphae (Khan et al., 2019). Damage to fungal cell membrane through oxidative stress caused by reactive oxygen species is another way that nanoparticles show their efficacy against pathogens (Sirelkhatim et al., 2015).

9. ANTIOXIDANT ACTIVITY OF ZINC OXIDE NANOPARTICLES

Antioxidant nature of biosynthesized zinc oxide nanoparticles is associated with the ability of biomolecules present on the surface to scavenge free radicals (Suresh et al., 2015). Assessment of the antioxidant nature is carried out using DPPH assay (Patel & Prajapati, 2022). ABTS assay is another test that is used to measure the ability to scavenge free radicals (Kumar et al., 2020).

10. PHARMACEUTICAL AND BIOMEDICAL APPLICATIONS

Zinc oxide nanoparticles are known to be employed in wound dressings due to their antimicrobial characteristics (Rasmussen et al., 2010). The biocompatibility of ZnO nanoparticles has made it a feasible material to use in drug delivery (Kumar et al., 2020). Zinc oxide nanoparticles are also utilized in cancer therapy and targeted drug delivery (Gupta et al., 2019). The capacity of ZnO nanoparticles to enhance cell growth is another application area (Sirelkhatim et al., 2015). (Figure.2).

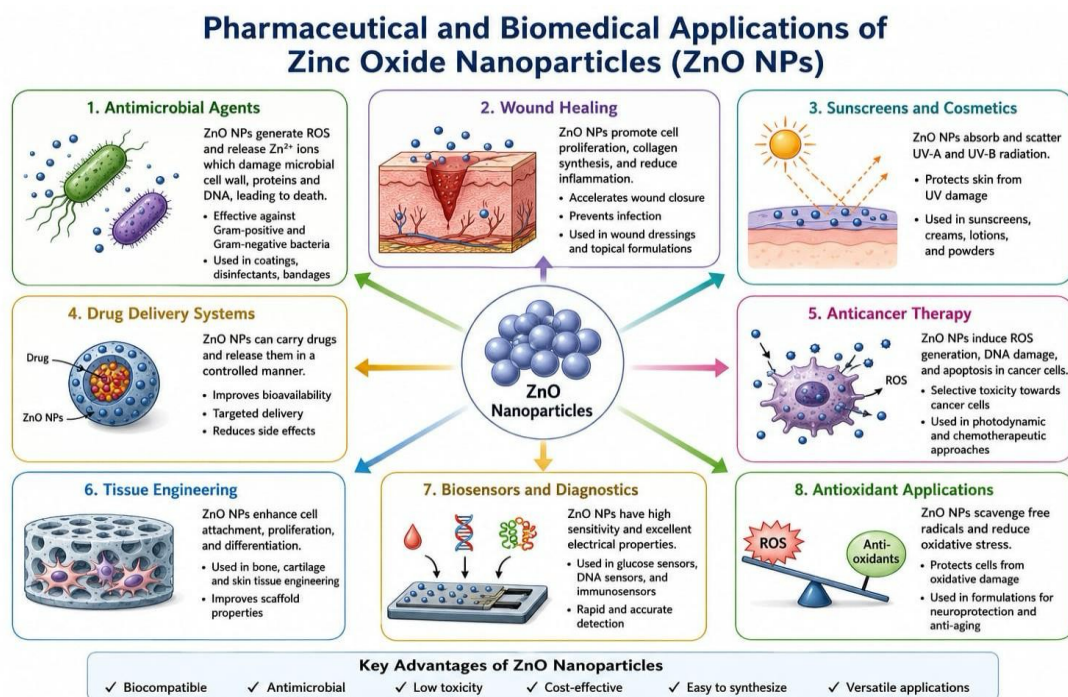


Figure.2. Pharmaceutical and Biomedical Application of Zinc Oxide Nanoparticles(ZnO NPs)

11. CHALLENGES AND FUTURE PROSPECTS

The large-scale manufacture of ZnO nanoparticles by biosynthesis technique is one of the greatest challenges (Singh

et al., 2018). There is a need to establish standardized synthesis procedures for consistency in results (Sharma et al., 2016). The toxic effects of nanoparticles against the good bacteria remain one of the main issues to be addressed (Dimkpa & Bindraban, 2018). The effect of nanoparticles on the rhizosphere

microbiome is not well studied (Rajput et al., 2018).

12. CONCLUSION

The synthesis of zinc oxide nanoparticles through the use of rhizosphere bacteria as green technology offers an eco-friendly approach compared to other traditional methods involving physical and chemical approaches. The various metabolisms of rhizosphere bacteria help in producing nanoparticles efficiently without relying heavily on toxic substances. Zinc oxide nanoparticles produced via biological process have outstanding antibacterial, antifungal, and antioxidant activities making them useful in various pharmaceutical, biomedical, agricultural and environmental sectors. Characterization tools such as UV-Visible spectroscopy, FTIR, SEM, TEM, and XRD are vital in proving the formation of nanoparticles. Despite great strides having been made, there are still several problems regarding standardization, scaling and safety. Future studies should concentrate on increasing efficiencies, molecular mechanisms and various medical uses.

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