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Zinc Oxide Nanoparticles Bio-Synthesis: An Eco-Friendly Approach And Its Application

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Abstract
Nanotechnology gives a clear-cut view of the production and utilization of materials with nanoscale dimensions. Due to the very high surface area to volume ratio that nanoscale dimension offers them an extremely particular feature. Recent research has focused on zinc oxide nanoparticles (ZnO NPs) because of their broad bandwidth and strong excitation binding energy. Antibacterial, antifungal, diabetic, wound-healing, anti-inflammatory, antioxidant, and optical materials could all be made from these particles. Due to the substantial amount of harmful chemicals and harsh environment used in the chemical and physical synthesis of these NPs, green manufacturing techniques utilizing plants, fungi, algae, and bacteria have been adopted. This study carefully examines synthesis and characterization methods applied for the green production of ZnO NPs from various bio physiological sources. It includes an introduction, an overview, a discussion of the nanoparticles' characterization, and a concluding section with an eye toward the future.

Keywords: Zinc oxide nanoparticle, Biosynthesis, Plant, Microbes, Fungus.

INTRODUCTION

Nanoparticles are made of materials with a minimum dimension between one and one hundred nanometers, and the term "nanus" in Greek means "dwarf." ZnONPs have unusual optical, magnetic, and catalytic properties in addition to having low melting points. The plant produces a wide variety of secondary metabolites that act as capping and reducing agents and aid in the formation of nanoparticles. The capping agents employ a variety of stabilizing processes, such as van der Waals, steric, electrostatic, etc. Today's use of nanomaterials can provide answers to numerous technological and environmental problems in the areas of wastewater treatment, medicine, and solar energy conversion (Azzazy *et al*., 2012). As a result of globalization, efforts must be made to reduce hazardous waste, and it is always essential to develop a synthesis method that is affordable, cost-effective, non-toxic, and—this is a very, very important point—productive for the benefit of humanity. This method should follow the so-called "Green synthesis ," which is a technique for the meticulously controlled production of nanoparticles with precise size and shape. In addition, it has been noted that during the past decades, it has been a greater emphasis on the creation of metal nanoparticles and quantum dots (Mehta *et al*., 2010; Mehta *et al*., 2015). There are two methods for carrying out the synthesis. i.e.,
(1) Physicochemical techniques
(2) Biological or biogenic process
Nowadays, only a single step reduces metal ions to nanoparticles. Because of this, the biogenic reduction of metal ions to base metal can be done quickly, conveniently, and at room temperature and pressure. It is also environmentally friendly for plant extracts to facilitate synthesis. This review paper will go on to cover the ecologically friendly production of nanoparticles using just fungus and plant extracts. Some reducing agents include terpenoids, alkaloids, and other water-soluble metabolites. Zinc (Zn) nanoparticles with silver (Ag) nanoparticles have garnered particular attention in plant-based synthesis. This area of science has converged, which means it now encompasses a variety of disciplines and technologies. There are a lot of active areas of nanotechnology research. As a result of the expanding demand for knowledge in a number of scientific fields (chemistry, physics, material science, electrical/mechanical/chemical engineering, biology), many active domains in nanotechnology research are in reality becoming more informed these days. Nanoparticles are categorized into three groups because they can be made from a wide range of materials:
1) metallic nanoparticles (such as Ag,Au, Fe, Cu and Zn);
2) non - metal and metal oxides (such as ZnO, VO, AlO, and FeO); and

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3) semiconductor nanoparticles (such as CdSe,ZnS , CdS and ZnSe).
Zn has an atomic weight of 65.38 and is located in position 30 in the periodic table. Our bodies cannot produce zinc, so we must obtain it from food sources like red meat, chicken, and fish. A lot of macromolecules and enzymes are needed for effective operation. It is particularly important for growth, DNA synthesis, and immunological response. Since zinc serves both structural and catalytic functions in enzymes, it is well-known to be important. According to (Klug *et al*., 1987), zinc finger motifs give protein subdomains a special way to interact with either DNA or other proteins. There is growing evidence that free zinc ions may have negative consequences, including the degeneration of neurons, despite the fact that zinc always lacks redox activity and is frequently thought to be non-toxic (Frederickson *et al.,* 2005). Metalloproteins require zinc to function properly. Therefore, to lessen its negative effects, zinc cationic activity is bound with bioactive ligands (such as proteins), and ZnO nanoparticles are produced (Pomastowski *et al*., 2017). ZnO has unique chemical sensing, optical, semiconducting, piezoelectric properties, and electrical conductivity according to (Fan *et al*., 2005).

METHOD FOR SYNTHESIZING NANOPARTICLES

This method implies that the synthesis method for creating nanoparticles normally requires either a "top-down" method or a "bottom-up" method, the bottom-up strategy was largely used in the synthesis of silver nanoparticles relative to the top-down methodology (4% of evaluated studies) (Sepeur *et al*., 2008). Due to the presence of the organic contents, the current study utilizes cell-free enzymatic activity of protein solution made by the secretion of a fungal ball of the mycelia for the recovery of higher protein contents used for the nanoparticle biosynthesis procedure. Zn and Ag nanoparticles are created using various precursor salt compounds. The creation of metal nanoparticles from fungus has various advantage over different bacteria, plants, and different techniques such as chemical,physical and aerosol because the rhizosphere region displays a more diversified niche for microbial population.Two processes are used to create nanoparticles:
(1) the top-down method (size reduction);
(2) The bottom-up method (growth from smaller entities)

PLANT EXTRACT-BASED PRODUCTION OF ZNO NANOPARTICLES

ZnO nanoparticles have attracted a lot of attentions from researchers and scientists over the past four to five years due to its numerous applications in the medicinal area as well as in optics and electronics. It is simple to create ZnO nanoparticles by combining plant extract clear solution with a 0.5 Mm solutions of zinc sulphate hydrated (ZnS), zinc oxide (ZnO), and zinc nitrate $(ZnNO_3)_2$, and the boiling the resulting combination at the desirable time and temperatures. And at this stage, it is possible to tune a number of parameters, including time, temperature, pH, and others, to achieve the required and efficient nanorange.
The green approach of ZnO nanoparticles using *Murraya koenigii* (curry leaf)by leaf extract was reported by (T. Lakshmikandhan *et al*., 2020). The produced nano crystallines of ZnO are in the ranges of 19.53 nm. Only when there are potent oxidizers present can zinc nanoparticles exist as ions. ZnO nanoparticle synthesis is still in its infancy, and additional study of the mechanism of nanoparticle production is needed. This could help to fine-tune the process and eventually produce nanoparticles with the strict control over the different size and the shape characteristics (Paul *et al.*, 2019) recently reported using biogenic pathway "One-pot green synthesis" method from the seeds extract of the Parkia roxburghii synthesized ZnO nanoparticles,in order to create a variety of 2-substituted benzimidazoles for use as a sonocatalysts for the degradation of the organic dye, researchers used these ZnO-nanoparticles as a catalyst in the reaction between the o-phenylenediamine and different aldehydes. After R. Sharmila (Devi *et al.,* 2014) published "Green Synthesis of the Zinc Oxide Nanoparticles by using *Hibiscus rosa-sinensis,*"(Gurhal) the powered samples was employed by the Cu K - X Ray Diffractometer to verify the existence of ZnO and study the structures. The various peak appeared at 2 values ranging from the 31.73°, 34.38°, 36.22°, 56.56°, 62 values corresponds to pure ZnO. *Azadirachta indica* was used in the production of zinc oxide nanoparticles by (Bhuyan *et al.*, 2015) for photocatalytic and antibacterial purposes. Pure and primarily spherical in shape, the produced ZnO nanoparticles had a size range of 9.6 to 25.5 nm. Using different concentrations of biosynthesized ZnO nanoparticles, the antibacterial activity of the characterized samples was assessed against Gram-negative and Gram-positive bacteria, including *Streptococcus pyogenes*, *Staphylococcus aureus, and Escherichia coli*, using the flask shaker method. The results showed that growth of bacteria decreases with increasing the concentration of biosynthesized ZnO nanoparticle. ZnO nanoparticles appeared to be most toxic to gram-positive bacteria than gram-negative bacteria. When made from *Utrica diocia* leaf extracts for treating with the alloxan caused by diabetic rats, ZnO nanoparticles were tested for their anti-diabetic efficiency. These nanoparticles function as an very active phyto-nanotherapeutic agent for reducing the complications in diabetes (A. Bayrami *et al.,* 2020).

ZNO NANOPARTICLE PRODUCTION THROUGH FUNGAL EXTRACT

Basically, the invention of targeted drug delivery using *Aspergillus terreus* and biosynthesis of ZnO nanoparticles has proved helpful in effective cancer treatment. A fungus was used to produce metalloproteins because it can produces both the L-asparaginase and proteins needed to reduce the large metal ions to nanoparticles as shown in fig. 1.Various biomolecules like therapeutic proteins, miRNAs, and different enzymes are extremely conjugated to the nanoparticles instead of xenobiotics drug molecules because they have a very fast turnover rate without any side type of effects (Kocbek *et al*., 2007; Cho *et al.*, 2008). After 48 hours of incubation, the colour of the culture filtrate was changed from a pale yellow solution to

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an opaque, colourless liquid due to the high excitation of surface plasmon resonance (SPM). This colour changes indicates that the proteins in the fungal culture filtrate reduced the zinc sulphate ions, causing the formation of the ZnO nanoparticles. According to (Martin Sebasta *et al.,* 2020), the fungus Aspergillus niger was transformed into a biogenic oxalate mineral by exogenous zinc nanoparticles.

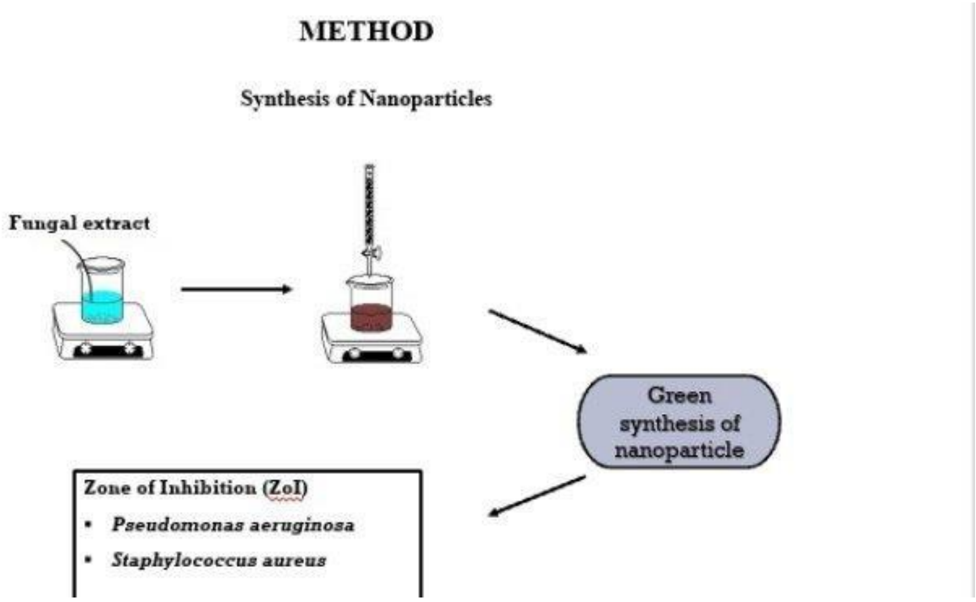

Fig. 1.Method for synthesis of Nanoparticles

Table 1. Zinc oxide nanoparticles extracted from different plants

S.No.	Plant extract	Type	Shape and Size	References
1.	*Hibiscus rosa-sinensis*	Zno	Spongy shape; 31.73°- 76.90	R. Sharmila Devi *et al*., (2014)
2.	*Azadirachta indica*	ZnO	530–600 nm	Bhuyan *et al*., (2015).
3.	*Prosopis juliflora*	ZnO	Irregular; 31.80–32.39	Sheik Mydeen *et al*., (2020)
4.	*Vitex negundo*	ZnO	38.17 nm	Ambika and Sundrarajan *et al*., (2015)
5.	*Acalypha fruticosa*	ZnO	Spherical, hexagonal; 50 nm	Vijayakumar *et al.,* (2020)
6.	*Calotropis gigantea*	ZnO	Hexagonal and pyramidal; 31 nm	Kumar *et al.,* (2020)
7.	*Urtica dioica*	ZnO	Spherical; 20–22 nm	Bayrami *et al.,* (2020)
8.	*Poncirus trifoliate*	ZnO	*Nearly spherical, 8.48–32.5 nm*	B. Balusamy *et al*., (2012)
9.	*Physalis alkekengi L.*	ZnO	Triangular, elongated, 72.5 nm	J. Qu *et al*., (2011)

APPLICATIONS OF NANOPARTICLES IN DIFFERENT FIELDS

ZnO nanoparticles are employed in different variety of disciplines, which includes agriculture, cancer treatment, viral treatment, antimicrobial (antibacterial and antifungal), and are also used on a bigger scale in cosmetics and coating applications.

(1) Antimicrobial properties of ZnO:

ZnO nanoparticles have been employed in sunscreens, and paints because of their excellent UV absorption efficiency to visible light (Franklin *et al*., 2007). The growth of a wide range of microorganisms is considerably inhibited by them, and they display a variety of morphologies (Yamamoto *et al.,* 2001).

(2) Antibacterial Activity of ZnO:

According to (Harish Chandra *et al*., 2019), The synthesized zinc oxide-based nanoparticles from Berberis aristata were tested for the antibacterial activity using the Agar well diffusion methods against pathogens, including *Klebsiella pneumoniae, B. subtilis, E. coli,* although no activity was seen against the two pathogenic bacteria, *Proteus spp. and S. typhi*. (Dobrucka and Dugaszewska *et al*., 2016) demonstrated that the antibacterial activity of NPs against Gram-positive and the Gram-negative bacteria *S. aureus and the E. coli* using Trifolium pretense water extract and the diffusion method.

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The report on the biosynthesis of the ZnO nanoparticles using Prosopis juliflora by the plant leaf extract given by (S. Sheik Mydeen *et al.*, 2020) . They also investigated antibacterial activities and rust-induced photocatalysis using a novel method using the hydrothermal method at 170^{o}C, characterization by various methods including DRS, XRD , FT-IR, TEM, SEM PL spectra and EDAX. *Rhodococcus rhodochrous (R. rhodochrous), Escherichia coli (E. coli), Vibrio cholera (V. cholera) and Bacillus subtilis (B. subtilis),* were used as pathogens in in vitro experiments to measure the antibacterial activity against a standard (streptomycin sulfate). Two different ZCA and ZLE nanoparticles were tested for their antibacterial effects on *R. rhodochrous, E.coli* ,and *V. cholera and B.subtilis* . The nanoparticle concentrations ranged from 50 to 100 g/ml, and a highest zone of inhibition of 25 mm was noticed in the gram negative bacteria *V. cholera* compared to thd other gram-positive bacterial strains.

(3) Antifungal Activity of ZnO:
Most of the evidence supporting nanosized drugs' strong antifungal activity is convincing. According to (M. Hasan *et al*., 2019)" The role of the Green approach of ZnO Nanoparticles as the antifungals against Post-Harvest Gray and the Black Mold of the Sweet Bell Pepper", marjoram (*Origanum majorana*) and olive leaf extract (GS ZnO NPs) can both stop *Botrytis cinerea* and *Alternaria alternata* mycelium growth. When it came to preventing gray and black mold in pepper fruits, ZnO nanoparticles made from olive leaf powder at the concentration of 400 g/ml were superior to metal ZnO nanoparticles. The highest level of linear growth inhibition for both fungi was produced by marjoram leaf powder at 400 g/ml. According to (Chishti *et al*., 2016), *Origanum vulgare* growing in the wild in the Kashmir Himalaya has demonstrated a significant inhibitory effect against four different types of fungi, including *Aspergillus fumigatus, Candida albicans, Pencillium cryogeneum, and Saccharomyces cereviceae.* ZnS:Mn Nanoparticles were examined for their antifungal properties against a few identified pathogenic fungi by (Dheeb *et al*., 2019).

(4) Antiviral Activity of ZnO:
Padryk Merkl *et al.*, 2021) , examined the SARS-CoV-2 virus, which killed millions of people globally, and the antiviral capacities of Ag, CuO, and ZnO-NPs. These nanocoatings were created via aerosol nanoparticle self-assembly during flame synthesis. The activity of the antiviral AgNPs as a coating is the most potent of the three, but CuO has a more mild effect and ZnO does not appear to appreciably lessen the virus' ability to infect cells.
Results showed that the (ZnO NPs) has very potent antiviral activity at the very low concentration (IC_{50} = 526 ng/mL), but with the some of the cytotoxic effect to the cell host by (CC_{50} = 292.2 ng/mL). Therefore, use of (ZnO NPs) as high potent of the disinfectant against (SARS-Cov-2) with minimal side effects on the cell (Samy M. El-Megharbel *et al.*, 2021) reports revealed .

(5) Anticancer Activity of ZnO:
It has been shown that the manufactured nanobiocomposites of zinc oxide linked L-asparaginase have the intended effects since they lowered the viability of the MCF-7 cells to 35.02% when given the treatment. According to (G. Baskar *et al*., 2015), a zinc oxide nanoparticle-L-asparaginase linked nanobiocomposite ranges in size from 28 to 63 nm.

(6) Agricultural Activities Using ZnO:
J. C. Tarafdar *et al.*, 2014) reported on development of the Zn nanofertilizer that improves crop productions in the Pearl Millet (*Pennisetum americanum*). These nanoparticles, which ranged in size from 15 to 25 nm, were produced, and their size, shape, surface structure, and crystallinity were all examined. Six-week-old plants significantly outperformed controls in terms of root length (4.2%), shoot length (15.1%), root area (24.2%), total soluble leafs protein (38.7%),and chlorophyll content (24.4%), plant dry biomass (12.5%), phytase (322.2%), alkaline phosphatase (61.7%), and the enzyme activities of the acid phosphatase (76.9%), and dehydrogenase (21%). Zinc nanofertilizer was therefore used, which results in a 37.7% increased in grains yield at crop maturity.
According to (Mohammad Azam *et al*., 2022) research, a maize cultivar's growth and the extract yield were enhanced by ZnO, a nano-fertilizer. It boosted the crop's quantity and quality and has a strong potential for usage as a nano-fertilizer for the crops produced in Zn-deficient soils. They applied four different concentrations of the nanofertilizer (80, 120, and 160 mg/kg) as well as control, salt, and six different soil treatments. Plant growth increased by 61.1% in comparison to control plants, while photosynthetic pigments increased by 51.8% and antioxidant activity increased by 49.25%. Five treatments were employed for the foliar application, including the control and four different dosages of nanofertilizer (10, 20, 30 and 40 ppm).

(7) ZnO in various fields:
To maximize the different antidiabetic properties of ZnO synthesis process and minimize chemical pollution, (Abolfazl Bayrami *et al*., 2020) reported using the leaf extract of the *Urtica dioica* plant, ZnO nanoparticles exhibit synergistic anti-diabetic efficacy when used to treat diabetic rats brought on by alloxan. Several methods were used to verify the sample, including GC-MS, FT-IR, and TGA studies. ZnO-extract outperformed the other treatments when it came to controlling the normal problems related to diabetes. In the diabetic rats, this biologically produced sample considerably increased the only levels of HDLC and insulin while the significantly decreasing the levels of FBS, TG, and TC.

T. Bhuyan *et al.,* 2015) state that to test and assess the photocatalytic activity of the biosynthesized ZnO nanoparticles, (Kuriakose *et al*., 2013) documented the breakdown of MB (the test pollutant) under UV irradiation.

Three phases make up the photocatalytic process:
(a) producing electron pairs,
(b) separating those electron pairs, and
(c) performing a surface redox reaction to produce highly active hydroxyl (OH) radicals (Yu *et al.*, 2013).
The characteristic of Methylene Blue absorption peak at 664 nm has been found to significantly decline after the first 30 min, followed by a progressive decline over time (30–180 min), and Methylene Blue was deteriorated to 50% of its original value during the first 30 min of exposure. While these work supports the use of ZnOs as an effective photocatalyst in applications for environmental cleanup.

CONCLUSION

ZnO is a reliable and secure source of the zinc supplements and has a wide range of uses, including piezoelectricity, optics,gas sensing, solar cells, and the photocatalysts, to name a few. Because of its large bandgap, zinc oxide nanoparticles (ZnONPs) function as semiconductors. ZnONPs stand out among the many metallic nanoparticles due to their advantageous qualities, including affordability, biocompatibility, and biodegradability. Additionally, ZnONPs have strong antibacterial properties (100 nm) as a result of their high surface to the volume ratio, which makes it easier for bacteria to dissolve and penetrate them. ZnONPs are also used in waste water treatment as cleaning agents to get rid of hazardous compounds like arsenic and sulfur. ZnONPs can be used to alternatively decompose airborne pollutants. They are also used as insecticides and fertilizers to improve the properties of the soil. They are also highly UV absorbent and translucent, making them ideal for usage in paints, varnishes, and sunscreens. Due to their antimicrobial qualities, they are often used as components in creams and lotions that fight bacteria. The field of nanobiotechnology is currently in its infancy. Chemical-based synthesis, which is used to create it, uses toxic chemicals that are very destructive to nature. Green synthesis, on the other hand, uses environmentally friendly processes that are less toxic or non-toxic than chemical ones. However, more experimental work was required for the synthesis of ZnNPs from fungi, which can lead to increased literature and reader interest in the original paper.
The "green method" or "biological synthesis" is a less harmful or non-toxic way to create nanomaterials; this review article also discusses the synthesis of microbes and plant parts from living plants. The frequency and number of plants that fall under the category of endangered species are not affected by the nanocomposites that are produced since only parts of the plant, such as the peel, bark , callus, flower, leaves , fruit, seed,stem and rhizome, are employed. From a future perspective, NPs can be used to coat insecticides that can be produced from various plant parts or microbes, to make plants resistant to infections, and to produce essential oils that are not produced by human bodies, among other things. These nanoparticles have application potential in biomedical equipment,polymers , hydrogels, printed electronics, glass composites, and materials with extremely low water absorption (Shrestha *et al*., 2023) .

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