GREEN SYNTHESIS OF ZnO NANOPARTICLES FROM ONION ROOT, ONION PEEL AND POTATO PEEL

A Research Project

Submitted by

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VIVEKANAND COLLEGE, KOLHAPUR

(AN EMPOWERED AUTONOMOUS INSTITUTE)

YEAR 2025-2026

"Dissemination of education for Knowledge, Science and culture"

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DEPARTMENT OF MICROBIOLOGY (PG)

CERTIFICATE

OF

RESEARCH PROJECT COMPLETION

This is to certify that Ms. **SAKSHI P. GHODASARA** studying in M. Sc. part II, Sem-IV at Vivekanand College, Kolhapur (An Empowered Autonomous Institute) has sincerely completed research project work entitled **"GREEN SYNTHESIS OF ZnO NANOPARTICLES FROM ONION ROOT, ONION PEEL AND POTATO PEEL"** during academic year 2025-26.

Dr. Savita D. Mali

Research Project Guide

24.4.26

Examiner

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ACKNOWLEDGEMENT

I wish to express my deep sense of appreciation to Dr. Savita D. Mali, Assistant Professor, Department of Microbiology, Vivekanand College, Kolhapur (An Empowered Autonomous Institute) for her valuable support and expert guidance during the course of this study. She has been extremely understanding and cooperative and has always taken great interest in this work.

I wish to express my sincere thanks to Dr. C. B. Patil, Principal, Vivekanand College, Kolhapur (An Empowered Autonomous Institute) and Dr. T. C. Gaupale, Head, Department of Microbiology for providing the laboratory facilities in the department to carry out the experimental work.

I express my thanks to Ms. V. V. Misal, Coordinator of PG Department of Microbiology and faculty members, Dr. K. K. Bhise, Ms. M. M. Nadkarni, Ms. S. S. Shaikh, Ms. A. T. Patil, and Ms. P. P. Patil for their valuable suggestions and help during the work.

I convey my gratitude to Mrs. S. N. Salokhe (Laboratory Assistant), Mr. S. K. Maskar and Mr. D. R. Patil (Laboratory Staff) of the department for their kind help in the laboratory.

I am thankful to the librarian and library staff for providing facilities of computer and reference books. My special thanks and gratitude to my entire classmates who have been constant source of inspiration and help during entire project work. I am highly obliged to authors past and present whose literature has been cited.

Finally, I thank my family members for their blessings and support because of which this work has proved satisfactory to me.

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1.0
INTRODUCTION

Nanoparticles are tiny particles that have at least one dimension (length, width, or height) between 1 and 100 nanometers (1nanometer = one-billionth of a meter 10^{-9} m). A nanoparticle is so small that thousands could fit across the width of a single hair. Because of this extremely small size, nanoparticles show special physical and chemical properties that are very different from the same material in bulk form. The use of very fine particles actually started long ago even before modern science recognized them. In ancient times, craftsmen unknowingly used nanoparticles in Roman glassware (like the famous "Lycurgus Cup") and medieval stained-glass windows, which showed beautiful colours due to tiny gold or silver nanoparticles. In modern discovery, the scientific study of nanoparticles began in the 20th century. In 1959, physicist Richard Feynman gave a famous talk called "There's Plenty of Room at the Bottom", predicting that scientists could one day manipulate atoms and molecules directly. In the 1980s–1990s, with the invention of powerful microscopes like the Scanning Tunnelling Microscope (STM) and Atomic Force Microscope (AFM), scientists could actually see and build structures at the nanoscale. This led to the rise of nanotechnology, the science of creating and using materials at the nanometre level.

Nanoparticles behave differently from bulk materials because of two main reasons which include large surface area to volume ratio and smaller particles have more atoms on their surface, making them more reactive. At nanoscale, electrons behave differently, giving nanoparticles unique optical, electrical, magnetic and mechanical properties such as gold nanoparticles appear red or purple, not gold. Silver nanoparticles show strong antimicrobial properties. Carbon nanotubes are stronger than steel but very lightweight. There are various types of nanoparticles such as metal nanoparticles [gold (Au), silver (Ag), iron (Fe)}, metal oxide nanoparticles {titanium dioxide (TiO_2), zinc oxide (ZnO)], carbon-based nanoparticles (fullerenes, carbon nanotubes, graphene), polymeric nanoparticles [Poly lactic co glycolic acid], lipid-based 2 nanoparticles (liposomes, solid lipid nanoparticles).

Nanoparticles are used in many fields such as Medicine, Vaccines, Cosmetics, Agriculture, Electronics, Environment, etc. In Medicine, lipid-based nanoparticles like liposomes are used in mRNA vaccines production, polymeric nanoparticles are used for targeted drug delivery in cancer therapy, diagnostic imaging and biosensors also involve the use of nanoparticle. ZnO and TiO_2 nanoparticles are used in cosmetics

like sunscreen to protect skin. In agriculture nanoparticles like ZnO, AgO, TiO_2 are used in nano fertilizers and Nano pesticides to improve plant growth, soil health and to avoid pest infection respectively. In electronics, carbon nanotubes nanoparticles are used in aerospace and automotive industries. Nanoparticles like LiFePO4 are used in nanochips, transistors, and batteries. In environment, nanoparticles like Fe2O3, ZnO, TiO_2, etc are used for water purification, pollution control, and waste treatment.

Nanoparticles of different shapes and sizes are synthesized by physical, chemical, and biological methods. Nanoparticles are produced through nanotechnology development by reducing the metal to its nuclear size. Physical methods avoid nanoparticles solvent contamination but it consumes a large quantity of energy for condensation and evaporation of particles. In the chemical technique, reducing agents and protective agents are used to synthesize nanoparticles and prevent agglomeration in order to synthesize high purity and stable nanoparticles. Example of physical method includes nanoparticle synthesis through colloidal dispersion method. It also includes basic techniques like vapor condensation, amorphous crystallization, physical fragmentation and many others (Agarwal, 2017).

Conventional physical and chemical methods utilize less time for synthesizing large quantities of nanoparticles but they require toxic chemicals as capping agents to maintain stability, thus leading to toxicity in the environment. Keeping this in consideration, green nanotechnology is emerging as an eco-friendly alternative (Salam et al. 2014). Green synthesis is also called biosynthesis. It uses biological materials like plants, microbes, biomolecules, instead of harsh chemicals to make nanoparticles. It's a bottom-up, eco-friendly approach that's low-cost, scalable and often produces biocompatible nanoparticles suitable for biomedical, environmental and agricultural uses. The green synthesis method, which is included in the biological methods, allows the easy, inexpensive, and environmentally friendly synthesis of nanoparticles without the need to use high pressure, high temperature, energy, and hazardous chemicals (Fakhari et al. 2019). More environmentally friendly processes have been developed in recent years.

Green nanotechnology has achieved great interest in synthesis of functional nanoparticles from iron, zinc, copper and gold without the use of dangerous toxic products. Techniques for obtaining nanoparticles by green synthesis have been

oriented towards the use of naturally occurring reagents such as vitamins, sugars, proteins, biodegradable polymers, microorganisms and plant extracts as reducing and stabilizing agents. Sugars such as glucose and fructose, water-soluble vitamins such as C and B12, contain radicals such as hydroxyls or amino in their chemical structure that give them the characteristic of natural antioxidant agents that can effectively reduce metal ions in an aqueous solution to produce metallic nanoparticles and of metal oxides (Kharissova et al. 2013). Green nanotechnology replaces toxic reducing or stabilizing chemicals with natural reducing agents (plant phytochemicals, microbial enzymes, polysaccharides, proteins). It can operate under mild conditions (room temperature or slightly elevated, atmospheric pressure). It lowers energy use and avoids production of hazardous byproducts. It often gives stable, capped nanoparticles that are more biocompatible and less likely to agglomerate. It is cheaper and easier to scale in low-resource settings compared with some physical or chemical methods. In biological method of nanoparticle synthesis, we use biological sources like plants & microorganism. Plants & plant extracts (leaves, fruits, seeds, bark) which are rich in polyphenols, flavonoids, terpenoids, alkaloids and act as both reducing and capping or 4 stabilizing agents. Fungi and yeasts secrete enzymes and metabolites that efficiently reduce metal ions and are good for producing large amounts extracellularly. Bacteria and actinomycetes can synthesize nanoparticles intracellularly or extracellularly, sometimes with unique shapes. Algae and cyanobacteria produce polysaccharides and pigments useful for reduction or stabilization.

In nanoparticle synthesis metal precursor [Ag^{+} from $AgNO_3$, Au^{3+} from $AuCl_4$, Zn^{2+} from $Zn(NO_3)_2$] is mixed with a biological extract or culture. During production of nanoparticles, reducing biomolecules (polyphenols, sugars, terpenoids, proteins, enzymes) donate electrons to metal ions reduction to zero-valent metal atoms. These atoms nucleate and grow into nanoparticles. Proteins, polysaccharides, flavonoids etc. adsorb to the nanoparticles surface and prevent aggregation and they also influence shape and size of nanoparticles.

Zinc is an essential micronutrient that is absorbed in the form of divalent cations. It is required for the growth of the plant, protein synthesis, maintenance of membrane integrity and energy production. Zinc is also required for the activation of various enzymes which are used for the formation of chlorophyll as well as auxin

synthesis. In addition to this, it also has a role in the activation of enzyme-like dehydrogenase, phosphoryl hydrolase, peptide, and proteases (Bhardwaj et al. 2022).

Zinc on reaction with oxygen forms Zinc Oxide. Zinc oxide nanoparticles belong to II- VI semiconductor family which reports a broad energy gap spectrum at 3.2 eV and greater excitonic bond energy at 60 meV (Matinise et al. 2017). The most significant properties enable them to be utilized for varying applications. Zinc oxide nanoparticles are meant to possess potential applications in medicine such as antimicrobial, antioxidant and anti-diabetic functions etc. Previously many researchers reported that ZnO nanoparticle have been synthesized Zno 5 nanoparticles from different plants like *Zingiber offcinale* (Ginger), *Moringa oleifera* (Drumstick), *Azadirachta indica* (Neem), *Cinnamomum verum* (True Cinnamomum), *Ixora coccinea* (Flame of the woods), *Punica granatum* (Pomegranate), *Abutilon indicum* (Indian mallow) with various antimicrobial and anticancer activities (Vijayakumar, 2020).

Onion peels and roots, often discarded as agro-industrial or kitchen waste, represent valuable and underutilized bio resources with significant potential for sustainable nanomaterial synthesis. These plant-derived wastes are rich in bioactive compounds such as flavonoids, phenolic, alkaloids, and sulphur-containing compounds, which can act as natural reducing, capping, and stabilizing agents during nanoparticle formation. In the present study, an eco- friendly and cost-effective approach is proposed for the green synthesis of zinc oxide (ZnO) nanoparticles utilizing extracts derived from onion peels and roots. ZnO nanoparticles have gained considerable attention due to their multifunctional properties, including high photo stability, chemical stability, non-toxicity, and biocompatibility. They find extensive applications in diverse fields such as agriculture (as antimicrobial agents and nan fertilizers), cosmetics (in sunscreens and skin-protective formulations), electronics (in sensors and nano chips), and biomedicine (in drug delivery and wound healing). Despite substantial research on the synthesis of ZnO nanoparticles using onion peels, studies focusing on the utilization of onion roots remain scarce. The root extracts, however, are known to contain distinct phytochemical profiles that may influence nanoparticle morphology, size distribution, and functional properties. Therefore, the present work aims to explore and optimize the synthesis of ZnO nanoparticles using onion root extracts through a green synthesis route, thereby promoting waste

valorization and contributing to sustainable nanotechnology. The green-synthesized ZnO nanoparticles from onion waste hold immense potential for applications in medicine, pharmaceuticals, water purification, and environmental remediation. This study also seeks to study antimicrobial activity of Zno nanoparticles and characteristics of ZnO nanoparticles synthesized from both onion peels and roots, providing insights into the role of different plant parts in nanoparticle formation mechanisms.

2.0 Review Of Literature

2.1 Classification of Nanoparticles

Nanotechnology deals with particles having dimensions between 1-100 nm, known as nanoparticles. Based on their dimensional structure, nanoparticles are classified as zero-dimensional (0D), one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D). Compared with bulk materials, nanoparticles exhibit unique properties such as increased chemical reactivity, mobility, energy absorption, and antimicrobial activity, often referred to as nano-antibiotics. Because of these properties, nanoparticles are widely used in industries such as manufacturing, sanitation, food, cosmetics, chemicals, and space technology. According to Madhumitha et al. (2016), nanomaterials can be broadly categorized into organic nanoparticles and inorganic nanoparticles.

2.1.1 Organic Nanoparticles

Organic nanoparticles are widely used in medicinal and pharmaceutical applications, including household cleaning products, antimicrobial agents, and anticancer drugs (Sadiku et al., 2019). Many pharmaceutical products contain organic nanoparticles because they enhance the formulation and effectiveness of bioactive compounds. One major challenge in drug development is the insolubility of many pharmacological substances in water, which reduces their bioavailability and therapeutic effectiveness. Organic nanoparticles help overcome this problem by improving solubility and delivery efficiency.

Organic nanoparticles are solid particles with diameters between 10 nm and 1 µm, mainly composed of lipids or polymeric substances. The pharmaceutical industry has led research on these nanoparticles in recent years. Although they dissolve slowly in aqueous environments compared with inorganic nanoparticles, organic nanoparticles eventually degrade and therefore do not persist long in the environment, making them relatively eco-friendly.

2.1.2 Inorganic Nanoparticles

Inorganic nanoparticles have been extensively studied in the medical and biomedical fields. They show significant potential in the diagnosis and treatment of diseases such as cancer, diabetes, and microbial infections. Recent research has also led to their application in surface modification and fabrication of medical devices.

Inorganic nanoparticles exhibit unique biological activities. They can participate in responsive imaging and targeted therapy by interacting with specific biochemical features in biological systems. Some inorganic nanomaterials demonstrate enzyme-like catalytic activity, similar to enzymes such as peroxidase, oxidase, catalase, and superoxide dismutase. Through interactions with cellular proteins, inorganic nanoparticles can regulate cellular processes including ferroptosis, proptosis, autophagy, and necroptosis, thereby influencing immune responses and tumour microenvironments.

Furthermore, certain inorganic nanoparticles or metal ions (such as Mg, Ca, Ag, V_2O_5, and ZnO) can interact with the immune system and trigger specific immune responses including T-cell activation.

Among inorganic nanoparticles, zinc oxide nanoparticles (ZnO NPs) have attracted considerable attention due to their biocompatibility, safety, low production cost, and ease of synthesis. ZnO nanoparticles possess several useful properties including UV-filtering capability, high photocatalytic activity, optical properties, and antimicrobial activity. Because of these features, ZnO nanoparticles are widely used in cosmetics (especially sunscreens), medicine, agriculture, rubber, paint, and drug delivery systems.

ZnO nanoparticles exhibit antibacterial, antifungal, anti-inflammatory, and wound-healing properties. However, their use in drug delivery is sometimes limited due to cytotoxic effects associated with the release of zinc ions. Despite this limitation, ZnO nanoparticles demonstrate broad-spectrum antibacterial activity even at low concentrations.

Zinc oxide is a semiconductor nanoparticle with a large band gap (3.37 eV) and strong excitation binding energy (60 meV) at room temperature. It usually appears as a white powder, is chemically stable, and is resistant to UV radiation and high temperatures. The physical and chemical properties of ZnO nanoparticles depend on factors such as crystal phase, particle size, and morphology. Due to their antibacterial, antioxidant, and antidiabetic properties, ZnO nanoparticles are widely applied in biosensing, drug delivery, nanomedicine, and biological labelling.

Green synthesis of ZnO nanoparticles

2.2 Nanoparticle Synthesis

Nanoparticles can be synthesized using physical, chemical, and green synthesis methods (Abid et al., 2021). These methods follow either top-down or bottom-up approaches (Goswami, 2021). Research on nanoparticle synthesis involves multiple disciplines including physics, chemistry, biology, engineering, and material science.

Traditional physical and chemical synthesis methods often involve hazardous chemicals, complex procedures, and toxic by-products. In contrast, green synthesis methods are gaining popularity because they are cost-effective, environmentally friendly, and suitable for large-scale production.

2.2.1 Physical Methods

Physical methods produce nanomaterials through processes such as sputtering, evaporation, microwave-assisted synthesis, pulsed laser deposition, ultrasonication, spray pyrolysis, and ball milling (Abid et al., 2021). However, these techniques often require high energy, high temperature, vacuum conditions, expensive equipment, and complex procedures. Additionally, they may cause environmental damage and difficulties in product collection (Saleh, 2022).

2.2.2 Chemical Methods

Chemical synthesis methods include co-precipitation, sol-gel process, chemical reduction, polyol process, and hydrothermal techniques (Hachem et al., 2022). These methods are widely used for producing nanoparticles with controlled size and shape. However, they often require toxic surfactants and stabilizing agents, which pose environmental risks. Because of these drawbacks, researchers increasingly prefer plant- and microorganism-based green synthesis methods.

An example reaction for ZnO nanoparticle formation from zinc acetate is:

$$Zn(CH_3COO)_2 \cdot 2H_2O + 2NaOH \rightarrow ZnO + 2CH_3COONa + H_2O$$

2.2.3 Green Synthesis of ZnO Nanoparticles

Green synthesis is an eco-friendly method that utilizes biological materials such as plant extracts, microorganisms, or agricultural waste to produce nanoparticles.

Modi et al. (2022) reported the synthesis of ZnO nanoparticles using onion (*Allium cepa*) peel waste as a biogenic source. In their study, $ZnCl_2$ was used as a precursor, and synthesis was performed at pH 8 with 4-hour stirring followed by calcination. Characterization techniques including FTIR, DLS, and FESEM confirmed the formation of spherical nanoparticles with sizes ranging from 20–80 nm. The nanoparticles enhanced the germination and growth of *Vigna radiata* (mung bean) and *Triticum aestivum* (wheat), demonstrating their potential as nano-fertilizers.

Similarly, Islam et al. (2024) used aqueous onion peel extract and zinc nitrate to synthesize ZnO nanoparticles via a one-pot green synthesis method. Characterization using FTIR, XRD, SEM, EDX, and UV-Vis spectroscopy confirmed hexagonal nanoparticles with an average crystallite size of 57.38 nm. The synthesized nanoparticles exhibited antibacterial activity against *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*.

Other plant sources have also been used for green synthesis. For example, *Vinca* rosea leaf extract was used to synthesize ZnO nanoparticles with spherical and hexagonal morphology (Remya et al., 2022). Characterization by XRD and TEM confirmed crystalline nanoparticles with an average size of about 20 nm (Balaji et al., 2022).

Similarly, *Senna auriculata* flower extract has been used to synthesize stable spherical ZnO nanoparticles with an average size of 25 nm (Padalia et al., 2018). These nanoparticles demonstrated significant antidiabetic and anticancer activity in biological studies.

Green synthesis follows the principles of green chemistry, which aim to minimize hazardous substances and environmental damage during chemical production (Ahmed et al., 2020). Plant extracts are particularly suitable for nanoparticle synthesis because they contain bioactive metabolites such as flavonoids, alkaloids, phenolic compounds, and proteins, which act as reducing and stabilizing agents.

Because plants are widely available, inexpensive, and safe to handle, plant-mediated synthesis is considered one of the most effective and sustainable methods for nanoparticle production (Mandhata, 2011).

3.0

OBJECTIVES

1) Collection and processing of potato peel, onion peel and onion roots to make its extract.

2) Synthesis of ZnO nanoparticles from extract.

3) Study of antimicrobial activity of Zno nanoparticles.

4) Characterization of ZnO nanoparticles.

4.0 MATERIAL AND METHODS

4.1 Synthesis of ZnO nanoparticle using potato peel

4.1.1 Sample collection

Fresh potato peels were collected from household kitchen waste. The peels were selected to ensure they were free from fungal contamination and excessive spoilage that might affect the nanoparticle synthesis process. The peels were further washed with tap water to remove any dirt or debris and then washed with distilled water.

4.1.2 Preparation of potato peel extract

The washed peels were dried in a hot air oven at 40-50°C for 2-3 days (Fig. 1). Once it was completely dried, it was converted into powder by using a home grinder.

Fig 1. Dried potato peel

Further, 10gm of dried potato peel powder was weighed and soaked in 100ml of distilled water. The mixture was further heated at 60°C for 20 min along with continuous stirring. After heating, the mixture was cooled to room temperature followed by passing it through Whatman filter paper to obtain filtrate which is potato peel extract (Fig. 2). The potato peel extract obtained was then stored at 4°C for further use.

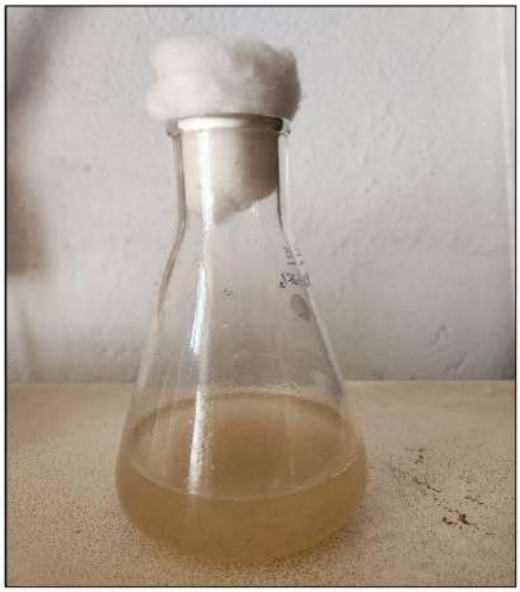


Fig 2. Potato Peel Extract

4.1.3 Green synthesis of Zinc Oxide Nanoparticles (ZnO NPs)

4.1.3.1 Preparation of reaction mixture

For green synthesis of ZnO NPs, 100 ml of potato peel extract was added to 2mM $ZnCl_2$ solution slowly under continuous stirring. The pH of the mixture was adjusted to 8 by adding 0.1 N NaOH followed by continuous stirring for 20-24 hours using a shaker at room temperature. The reaction begins within 10 mins leading to colour change from pale brown to pale yellow colour. Colour change occurred because of the phytochemicals present in the potato peel extract which acted as reducing and stabilizing agents, leading to the formation of zinc oxide nanoparticles.

4.1.3.2 Preparation of ZnO nanoparticle powder

The reaction mixture containing ZnO nanoparticles was transferred to centrifuge tubes and centrifuged at 8000–10000 rpm for 10–15 minutes. The supernatant obtained after centrifugation was discarded and the pellet containing nanoparticles was collected. To get rid of any unreacted zinc salts, soluble impurities and plant extract residues, the pellet was washed using distilled water and ethanol (Fig. 3).

Fig 3. Pellet of potato peel extract after centrifugation

Furthermore, to obtain a dry powder of ZnO NPs, the precipitate was dried in a hot air oven at 70-80°C for 24-48 hours.

4.1.4 Antibacterial activity

The agar well diffusion method was used for evaluating antibacterial activity of nanoparticles synthesized using potato peel. Antimicrobial activity was performed against different bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Salmonella typhi* on nutrient agar and Cow Urine Distillate (CUD) agar as well. 0.1gm of nanoparticle powder was suspended in 1 ml of sterile distilled water. Further, the sterile agar plates were prepared and the fresh suspension of 24 hours old culture was spread on it. Wells were formed on it and the nanoparticle solution was dropped in the well. Control wells were also prepared containing alcohol and streptomycin. The plates were further kept in a refrigerator for 30 mins for diffusion process and then incubated at 37°C for 24 hrs. After incubation, the plates were observed for zone of inhibition around the wells and the diameter of zone was measured.

4.2 Synthesis of ZnO nanoparticle using onion peel

4.2.1 Sample collection

Fresh onion peels were collected from household kitchen waste. The peels were selected to ensure they were free from fungal contamination and excessive spoilage that might affect the nanoparticle synthesis process. The peels were further washed with tap water to remove any dirt or debris and then washed with distilled water.

4.2.2 Preparation of onion peel extract

The washed peels were dried in a hot air oven at 70- 80°C for 2-3 days (Fig.4). Once it was completely dried, it was converted into powder by using a home grinder.

Fig 4. Dried onion peel

Further, 10gm of dried onion peel powder was weighed and soaked in 100ml of distilled water. The mixture was further heated at 60°C for 20 min along with continuous stirring. After heating, the mixture was cooled to room temperature followed by passing it through Whatman filter paper to obtain filtrate which is onion peel extract (Fig. 5). The onion peel extract obtained was then stored at 4°C for further use.

Fig 5. Onion peel Extract

4.2.3 Green synthesis of Zinc Oxide Nanoparticles (ZnO NPs)

4.2.3.1 Preparation of reaction mixture

For green synthesis of ZnO NPs, 100 ml of onion peel extract was added to an 2mM $ZnCl_2$ solution slowly under continuous stirring. The pH of the mixture was adjusted to 8 by adding 0.1 N NaOH followed by continuous stirring for 20-24 hours using a shaker at room temperature. The reaction begins within 10 mins leading to colour change to brown. Colour change occurred because of the phytochemicals present in the onion peel extract which acted as reducing and stabilizing agents, leading to the formation of zinc oxide nanoparticles.

4.2.3.2 Preparation of ZnO nanoparticle powder

The reaction mixture containing ZnO nanoparticles was transferred to centrifuge tubes and centrifuged at 8000–10000 rpm for 10–15 minutes. The supernatant obtained after centrifugation was discarded and the pellet containing nanoparticles was collected. To get rid of any unreacted zinc salts, soluble impurities and plant extract residues, the pellet was washed using distilled water and ethanol (Fig. 7).

Fig 6. Nanoparticle containing solution

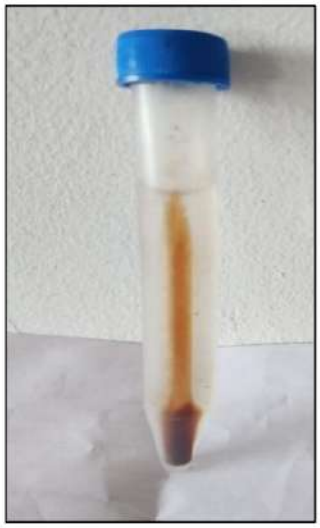


Fig 7. Pellet of onion peel extract after centrifugation

Furthermore, to obtain a dry powder of ZnO NPs, the precipitate was dried in a hot air oven at 70-80°C for 24-48 hours.

Fig 8. Dried powder of ZnO NPs obtained from onion extract

4.2.4 Antibacterial activity

The agar well diffusion method was used for evaluating antibacterial activity of nanoparticles synthesized using potato peel. Antimicrobial activity was performed against different bacterial strains such as *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Salmonella typhi* on nutrient agar and Cow Urine Distillate (CUD) agar as well. 0.1gm of nanoparticle powder was suspended in 1 ml of sterile distilled water. Further, the sterile agar plates were prepared and the fresh suspension of 24 hours old culture was spread on it. Wells were formed on it and the nanoparticle solution was dropped in the well. Control wells were also prepared containing alcohol and streptomycin. The plates were further kept in a refrigerator for 30 mins for diffusion process and then incubated at 37°C for 24 hrs. After incubation, the plates were observed for zone of inhibition around the wells and the diameter of zone was measured.

4.3 Synthesis of ZnO nanoparticle using onion root

4.3.1 Sample collection

Fresh onion root was collected from household kitchen waste. The roots were selected to ensure they were free from fungal contamination and excessive spoilage that might affect the nanoparticle synthesis process. The roots were further washed with tap water to remove any dirt or debris and then washed with distilled water.

4.3.2 Preparation of onion root extract

The washed roots were dried in a hot air oven at 40-50°C for 2-3 days (Fig. 9). Once it was completely dried, it was converted into powder by using a home grinder.

Fig 9. Dried onion roots

Further, 10gm of dried onion root powder was weighed and soaked in 100ml of distilled water. The mixture was further heated at 60°C for 20 min along with continuous stirring. After heating, the mixture was cooled to room temperature followed by passing it through Whatman filter paper to obtain filtrate which is onion root extract (Fig. 10). The onion root extract obtained was then stored at 4°C for further use.

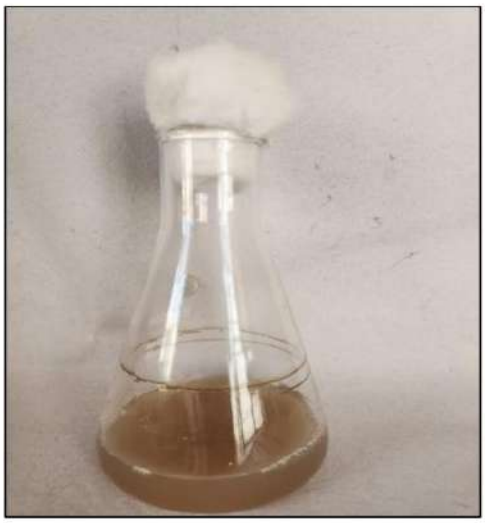


Fig 10. Onion root Extract

4.3.3 Green synthesis of Zinc Oxide Nanoparticles (ZnO NPs)

4.3.3.1 Preparation of reaction mixture

For green synthesis of ZnO NPs, 100 ml of onion root extract was added to an 2mM $ZnCl_2$ solution slowly under continuous stirring. The pH of the mixture was adjusted to 8 by adding 0.1 N NaOH followed by continuous stirring for 20-24 hours using a shaker at room temperature. The reaction begins within 10 mins leading to colour change to pale brown colour. Colour change occurred because of the phytochemicals present in the onion root extract which acted as reducing and stabilizing agents, leading to the formation of zinc oxide nanoparticles.

4.3.3.2 Preparation of ZnO nanoparticle powder

The reaction mixture containing ZnO nanoparticles was transferred to centrifuge tubes and centrifuged at 8000–10000 rpm for 10–15 minutes. The supernatant obtained after centrifugation was discarded and the pellet containing nanoparticles was collected. To get rid of any unreacted zinc salts, soluble impurities and plant extract residues, the pellet was washed using distilled water and ethanol (Fig. 11).

Fig11. Pellet of onion root extract after centrifugation

Furthermore, to obtain a dry powder of ZnO NPs, the precipitate was dried in a hot air oven at 70-80°C for 24-48 hours (Fig 12).

Fig12. Powder of ZnO NPs obtained from onion root

4.3.4 Characterization of ZnO nanoparticles synthesized

The synthesized nanoparticles were further characterized using Dynamic Light Scattering (DLS) technique.

4.3.5 Antibacterial activity

The agar well diffusion method was used for evaluating antibacterial activity of nanoparticles synthesized using onion root. Antimicrobial activity was performed

against different bacterial strains such as *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, Proteus vulgaris* on nutrient agar. 0.1gm of nanoparticle powder was suspended in 1 ml of sterile distilled water. Further, the sterile agar plates were prepared and the fresh suspension of 24 hours old culture was spread on it. Wells were formed on it and the nanoparticle solution was dropped in the well. Control wells were also prepared containing alcohol and streptomycin. The plates were further kept in a refrigerator for 30 mins for diffusion process and then incubated at 37°C for 24 hrs. After incubation, the plates were observed for zone of inhibition around the wells and the diameter of zone was measured.

4.3.6 Antifungal activity

The agar well diffusion method was used to evaluate the antifungal activity of nanoparticles synthesized using onion root. The antifungal activity was tested against fungal strains such as *Candida albicans*. Potato Dextrose Agar (PDA) was used as the culture medium for fungal growth. About 0.1 g of nanoparticle powder was suspended in 1 ml of sterile distilled water to prepare the nanoparticle solution. Sterile PDA plates were prepared, and a fresh suspension of a 24-hour-old fungal culture was evenly spread over the surface of the agar plate. Wells were then made in the agar using a sterile cork borer. The prepared nanoparticle solution was carefully added into the wells. Control wells were also prepared containing alcohol and streptomycin. The plates were kept in a refrigerator for 30 minutes to allow proper diffusion of the nanoparticles into the agar medium. After diffusion, the plates were incubated at 37°C for 24–48 hours.

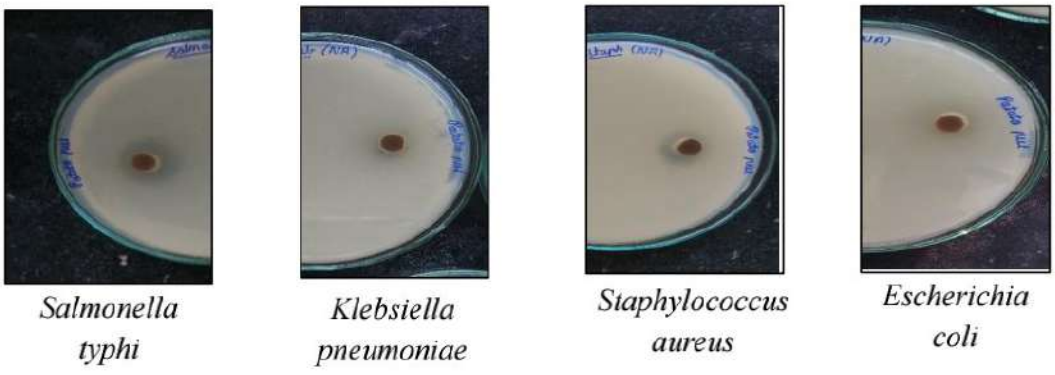
After incubation, the plates were examined for the presence of a clear zone of inhibition around the wells, which indicates antifungal activity. The diameter of the inhibition zone was measured in millimetres (mm) to determine the effectiveness of the nanoparticles against the fungal strain.

5.0 RESULT AND DISCUSSION

5.1 ZnO Nanoparticles synthesized using potato peel

5.1.1 Antibacterial activity

In this investigation, we examined the effectiveness of nanoparticles synthesized using potato peel against different bacterial strains. Clear zone was observed around the wells indicating the synthesized ZnO nanoparticles exhibited antibacterial activity against all the tested bacterial strains (Fig. 12 and 13). The clear zones were measured to compare the antibacterial activity against different bacterial strains (Table 1 and 2).



Salmonella typhi | *Klebsiella pneumoniae* | *Staphylococcus aureus* | *Escherichia coli*

Fig 12. Antibacterial activity on nutrient agar plates

Escherichia coli | *Staphylococcus aureus*

Fig 13. Antibacterial activity on CUD agar plates

Table 1. Diameter of clear zones against ZnO nanoparticles synthesized using potato peel on nutrient agar plates

Organism	Diameter of clear zone against ZnO nanoparticles (mm)	Diameter of clear zone of Control 1 (Alcohol) (mm)	Diameter of clear zone of Control 2 (Streptomycin) (mm)
Staphylococcus aureus	12	18	21.5
Escherichia coli	8	14	18
Salmonella typhi	17	18	22
Klebsiella pneumoniae	9	19.5	18

Table 2. Diameter of clear zones against ZnO nanoparticles synthesized using potato peel on CUD (Cow Urine Distillate) agar plates

Organism	Diameter of clear zone against ZnO nanoparticles (mm)	Diameter of clear zone of Control 1 (Alcohol) (mm)	Diameter of clear zone of Control 2 (Streptomycin) (mm)
Staphylococcus aureus	10	18	21.5
Escherichia coli	17	14	18

The results indicated that the ZnO nanoparticles synthesized using potato peel extract exhibit noticeable antimicrobial activity against the tested bacterial strains. The diameter of the inhibition zones varied among organisms and culture media. Among the tested organisms, *Salmonella typhi* showed the highest inhibition on nutrient agar plates (17 mm), indicating strong sensitivity to the synthesized nanoparticles.

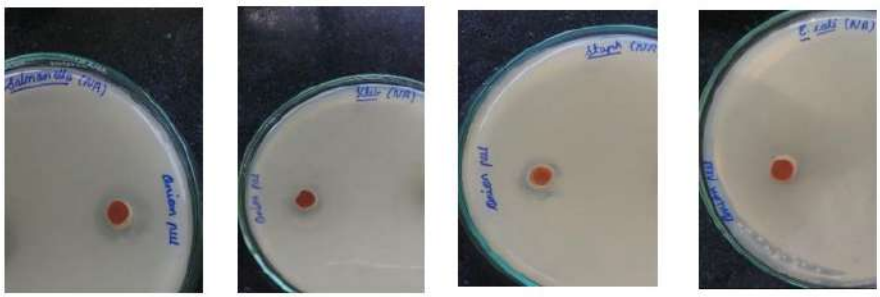
Staphylococcus aureus showed moderate inhibition (12 mm) while *Escherichia coli* (8 mm) and *Klebsiella pneumoniae* (9 mm) showed comparatively lower inhibition on nutrient agar plates. On CUD agar plates, *Escherichia coli* demonstrated a significant inhibition zone (17 mm) whereas *Staphylococcus aureus* showed a moderate inhibition (10 mm) by potato peel ZnO nanoparticles.

When compared with the controls, streptomycin showed the highest inhibition zones (18–22 mm), confirming its strong antibacterial activity, while alcohol showed moderate inhibitory effects. Although the inhibition zones produced by the ZnO nanoparticles were smaller than those of streptomycin, the results clearly demonstrate that potato peel–mediated ZnO nanoparticles possess effective antibacterial properties. Overall, the study suggests that potato peel extract can be successfully used as a green reducing and stabilizing agent for the synthesis of ZnO nanoparticles with promising antimicrobial potential.

5.2 ZnO Nanoparticles synthesized using onion peel

5.2.1 Antibacterial activity

In this investigation, we examined the effectiveness of nanoparticle synthesized using onion peel against different bacterial strains. Clear zone was observed around the wells indicating the synthesized ZnO nanoparticles exhibited antibacterial activity against all the tested bacterial strains (Fig. 14 and 15). The clear zones were measured to compare the antibacterial activity demonstrated against different bacterial strains (Table 3 and 4).



Salmonella typhi | *Klebsiella pneumoniae* | *Staphylococcus aureus* | *Escherichia coli*

Fig. 14 Antibacterial activity on nutrient agar plate

Staphylococcus aureus | *Escherichia coli*

Fig 15. Antibacterial activity on CUD agar plates

Table 3. Diameter of clear zones against ZnO nanoparticles synthesized using onion peel on nutrient agar plates

Organism	Diameter of clear zone against ZnO nanoparticles (mm)	Diameter of clear zone of Control 1 (Alcohol) (mm)	Diameter of clear zone of Control 2 (Streptomycin) (mm)
Staphylococcus aureus	9	18	21.5
Escherichia coli	8	14	18
Salmonella typhi	15	18	22
Klebsiella pneumoniae	9	19.5	18

Table 4. Diameter of clear zones against ZnO nanoparticles synthesized using onion peel on CUD (Cow Urine Distillate) agar plates

Organism	Diameter of clear zone against ZnO nanoparticles (mm)	Diameter of clear zone of Control 1 (Alcohol) (mm)	Diameter of clear zone of Control 2 (Streptomycin) (mm)
Staphylococcus aureus	14	18	21.5
Escherichia coli	10	14	18

The results indicated that the ZnO nanoparticles synthesized using onion peel extract exhibit noticeable antimicrobial activity against the tested bacterial strains. The diameter of the inhibition zones varied among organisms and culture media. Among

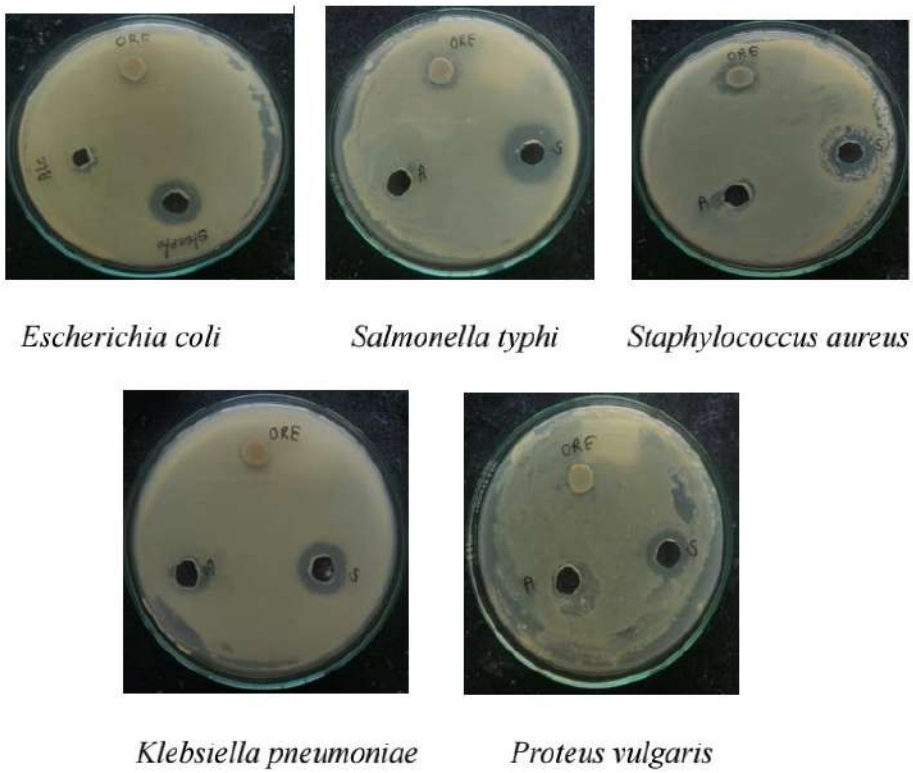
the tested organisms, *Salmonella typhi* showed the highest inhibition on nutrient agar plates (15 mm), indicating strong sensitivity to the synthesized nanoparticles. *Staphylococcus aureus* and *Klebsiella pneumoniae* showed moderate inhibition (9 mm) while *Escherichia coli* (8 mm). On CUD agar plates, *Staphylococcus aureus* showed a moderate inhibition zone (14 mm) and *Escherichia coli* comparatively showed least inhibition zone (10 mm).

When compared with the controls, streptomycin showed the highest inhibition zones (18–22 mm), confirming its strong antibacterial activity, while alcohol showed moderate inhibitory effects. Although the inhibition zones produced by the ZnO nanoparticles were smaller than those of streptomycin, the results clearly demonstrate that onion peel–mediated ZnO nanoparticles possess effective antibacterial properties. Overall, the study suggests that onion peel extract can be successfully used as a green reducing and stabilizing agent for the synthesis of ZnO nanoparticles with promising antimicrobial potential.

5.3 ZnO Nanoparticles synthesized using Onion Roots

5.3.1 Antibacterial activity

In this investigation, we examined the effectiveness of nanoparticle synthesized using onion root against different bacterial strains. Clear zone was observed around the wells indicating the synthesized ZnO nanoparticles exhibited antibacterial activity against all the tested bacterial strains (Fig. 16). The clear zones were measured to compare the antibacterial activity demonstrated against different bacterial strains (Table 5 and 6).



Escherichia coli *Salmonella typhi* *Staphylococcus aureus*

Klebsiella pneumoniae *Proteus vulgaris*

Fig 16. Antibacterial activity on nutrient agar plates

Table 5. Diameter of clear zones against ZnO nanoparticles synthesized using onion root on nutrient agar plates

Organism	Diameter of clear zone against ZnO nanoparticles (mm)	Diameter of clear zone of Control 1 (Alcohol) (mm)	Diameter of clear zone of Control 2 (Streptomycin) (mm)
Staphylococcus aureus	21.5	18	23
Escherichia coli	11	14	18
Salmonella typhi	15	18	22
Klebsiella pneumoniae	17	19.5	18
Proteus vulgaris	11	24.5	16

5.3.2 Antifungal activity

The antifungal activity of onion root–mediated nanoparticles was evaluated by the agar well diffusion method against *Candida albicans*. After incubation, a clear zone of inhibition was observed around the wells containing the nanoparticle solution, indicating that the synthesized nanoparticles possess antifungal activity. The diameter of the inhibition zone was measured in millimetres (mm) (Fig 17). The presence of this inhibition zone confirms that the nanoparticles were effective in suppressing the growth of *Candida albicans*.

Candida albicans

Fig 17. Antifungal activity on PDA agar plates

Table 6. Diameter of clear zones against ZnO nanoparticles synthesized using onion root on PDA agar plates

Organism	Diameter of clear zone against ZnO nanoparticles (mm)	Diameter of clear zone of Control 1 (Alcohol) (mm)	Diameter of clear zone of Control 2 (Streptomycin) (mm)
Candida albicans	26	21.5	0

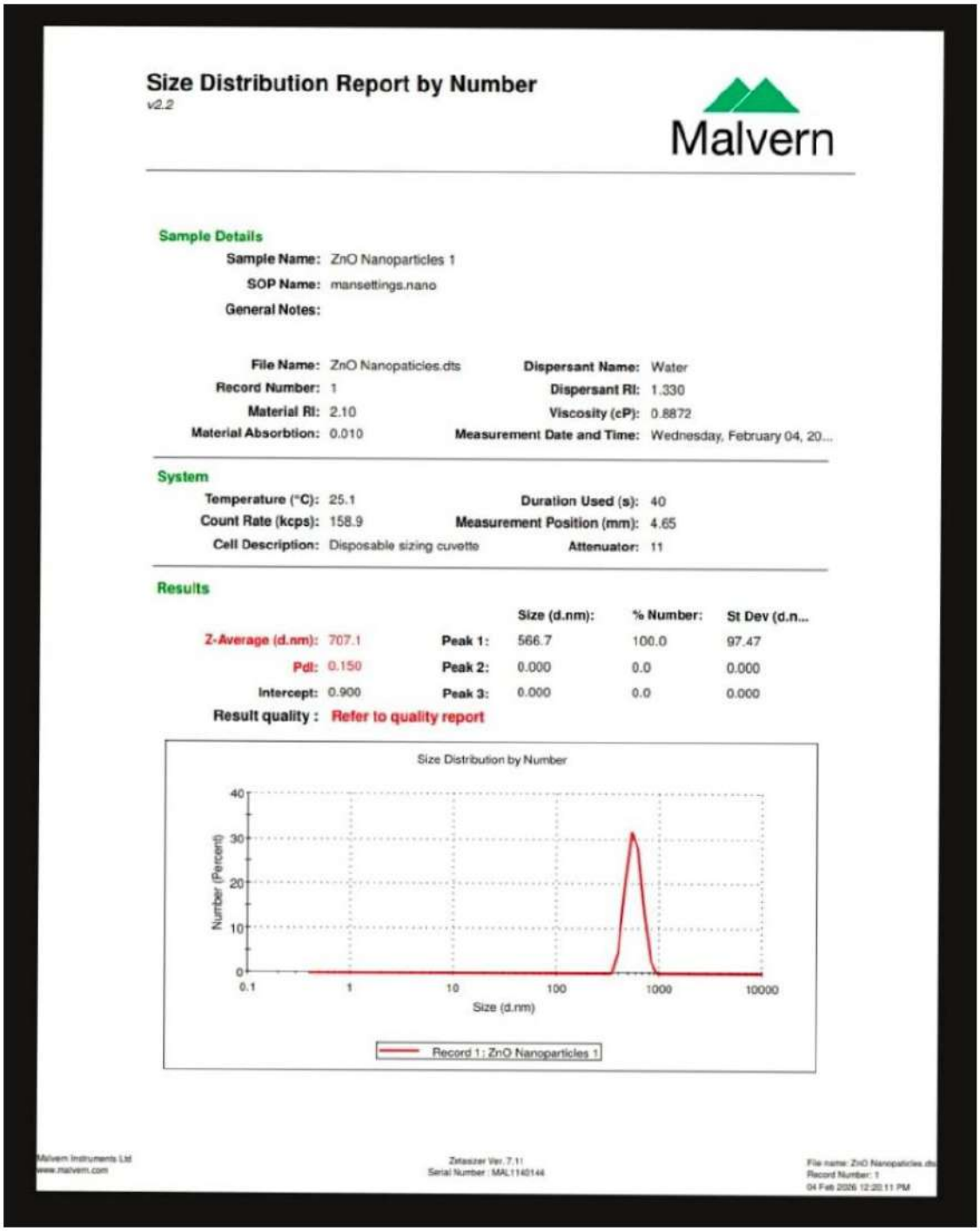
The results indicate that the ZnO nanoparticles synthesized using onion root extract exhibit noticeable antimicrobial activity and antifungal activity against the tested bacterial and fungal strains. The diameter of the inhibition zones varied among organisms and culture media. Among the tested organisms, *Staphylococcus aureus* showed the highest inhibition on nutrient agar plates (21.5 mm). In antifungal activity, *Candida albicans* exhibited a higher inhibition zone on PDA plates (26 mm) than the bacteria, indicating strong sensitivity to the synthesized nanoparticles. *Klebsiella pneumoniae* showed moderate inhibition (17 mm), while Escherichia coli (11 mm)

and *Salmonella typhi* (15 mm) showed comparatively lower inhibition on nutrient agar plates. When compared with the controls, streptomycin showed the highest inhibition zones (18–23 mm), confirming its strong antibacterial activity, while alcohol showed moderate inhibitory effects. Although the inhibition zones produced by the ZnO nanoparticles were smaller than those of streptomycin, the results clearly demonstrate that onion peel-mediated ZnO nanoparticles possess effective antibacterial properties. However, onion root-mediated ZnO nanoparticles exhibit more effective antifungal activity than antibacterial activity. Overall, the study suggests that onion root extract can be successfully used as a green reducing and stabilizing agent for the synthesis of ZnO nanoparticles with promising antibacterial and antifungal potential (Table 5 and 6).

5.3.2 Characterization of onion root nanoparticles

The DLS (Dynamic Light Scattering) analysis showed that the synthesized ZnO nanoparticles had an average particle size of 566 nm (Fig. 18).

The particle size analysis of ZnO nanoparticles was carried out using the Malvern Zetasizer based on the DLS technique. The results show that the synthesized ZnO nanoparticles have a Z-average size of 707.1 nm, indicating that the particles are in the submicron range rather than true nanoscale (<100 nm). The polydispersity index (PDI) is 0.150, which suggests a moderately uniform (monodisperse) particle distribution. The size distribution graph shows a single prominent peak at 566.7 nm with 100% intensity, confirming that the sample contains one dominant particle population without significant aggregation peaks or multiple size groups. The standard deviation of 97.47 nm indicates some variation in particle size, but overall, the distribution is relatively narrow. The intercept value of 0.900 reflects good signal quality, although the report suggests referring to the quality report for confirmation.



Size Distribution Report by Number
v2.2

Malvern

Sample Details

Sample Name:	ZnO Nanoparticles 1		
SOP Name:	mansettings.nano		
General Notes:			
File Name:	ZnO Nanopaticles.dts	Dispersant Name:	Water
Record Number:	1	Dispersant RI:	1.330
Material RI:	2.10	Viscosity (cP):	0.8872
Material Absorbtion:	0.010	Measurement Date and Time:	Wednesday, February 04, 20...

System

Temperature (°C):	25.1	Duration Used (s):	40
Count Rate (kcps):	158.9	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	11

Results

		Size (d.nm):	% Number:	St Dev (d.n...
Z-Average (d.nm):	707.1	Peak 1:	566.7	100.0	97.47
PdI:	0.150	Peak 2:	0.000	0.0	0.000
Intercept:	0.900	Peak 3:	0.000	0.0	0.000

Result quality : Refer to quality report

Malvern Instruments Ltd
www.malvern.com
Zetasizer Ver. 7.11
Serial Number : MAL1140144
File name: ZnO Nanopaticles.dts
Record Number: 1
04 Feb 2026 12:20:11 PM

Fig. 18 Dynamic Light Scattering (DLS) analysis of onion root nanoparticles

6.0

Summary

and

Conclusion

The present study demonstrated the green synthesis of ZnO nanoparticles using potato peel, onion peel and onion root extracts. These plant-based materials acted as natural reducing and stabilizing agents due to the presence of phytochemicals such as phenols, flavonoids and proteins. The synthesized ZnO nanoparticles showed significant antibacterial activity against various bacterial strains and antifungal activity confirming their potential for biomedical and environmental applications. However, the confirmation result obtained from DLS (Dynamic Light Scattering) analysis indicated that the particle size is 566 nm, which is higher than the expected nanoscale range.

This might be due to ZnO nanoparticles that tend to aggregate due to high surface energy and in solution, multiple small nanoparticles combine to form larger clusters, which are detected by DLS as bigger particles; plant extracts contain biomolecules that bind to the nanoparticle surface which increase the hydrodynamic diameter measured by DLS making particles appear larger than their actual core size; lack of proper sonication or mixing before DLS analysis can lead to poor dispersion, resulting in artificially increased particle size; during drying and high-temperature calcination (350–400°C), particles may fuse together, leading to formation of larger aggregates; DLS measures the size of particles along with surrounding solvent layers and attached molecules, so the reported size is always larger than the real nanoparticle size.

Proper synthesis of nanoparticles requires controlled preparation and processing conditions to obtain particles with desired size, purity, and stability. One of the important steps in nanoparticle synthesis is the calcination process, which involves heating the precursor material at high temperatures in the presence of air or oxygen. Fourier Transform Infrared (FTIR) spectroscopy is commonly used to identify the functional groups and chemical bonds present on the nanoparticle surface. This technique helps in understanding the interaction between nanoparticles and stabilizing agents. In addition, UV–Visible spectroscopy is used to study the optical properties of nanoparticles and to confirm their formation. The absorption peaks obtained in the UV–Visible spectrum provide information about particle size and electronic transitions. Thus, proper calcination followed by characterization using FTIR and UV–Visible spectroscopy plays a crucial role in confirming the successful synthesis and properties of nanoparticles.

Green synthesis of ZnO nanoparticles

7.0
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