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# Phyto-synthesis of zinc oxide nanoparticle using *Murraya koenigii* extract

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**Abstract-** This study explores the green synthesis of zinc oxide nanoparticles (ZnO NPs) using *Murraya koenigii* (curry leaf) extract as a reducing agent. Nanotechnology, operating at the nanometer scale, leverages unique properties of materials at these dimensions, offering potential for innovations across various fields. ZnO NPs are notable for their biocompatibility, minimal cytotoxicity, and cost-efficiency, making them valuable in optics, electronics, medicine, and more. Traditional chemical synthesis methods often involve toxic reagents, prompting interest in eco-friendly alternatives. The green synthesis method presented here utilized the phytochemicals in *Murraya koenigii* to produce ZnO NPs, which were subsequently characterized using UV-visible spectrophotometry, Dynamic Light Scattering (DLS), X-ray Diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), and Energy-Dispersive X-ray Spectroscopy (EDS). The synthesized nanoparticles exhibited a characteristic absorption peak at 374 nm, an average hydrodynamic size of 70 nm, and a crystalline size of approximately 38.40 nm. TEM and SEM analyses revealed spherical particles averaging 30 nm in size, while EDS confirmed their purity. This green synthesis approach demonstrates a sustainable, cost-effective, and biocompatible method for producing ZnO NPs, with promising applications in various scientific and industrial fields.

**Key words: Green synthesis, zinc oxide nanoparticles, *Murraya koenigii*, nanotechnology, eco-friendly**

## INTRODUCTION

Nanotechnology encompasses technologies operating at the nanometer scale, ranging from approximately 1 to 100 nanometers, and finds practical applications across various fields. This domain involves manipulating matter at the atomic and molecular levels to harness the unique properties that emerge at such minuscule dimensions. As materials are reduced to the nanoscale, their properties initially remain consistent with their larger counterparts. However, as the size decreases further, subtle changes become evident, leading to significant alterations in properties under the 100 nm threshold.[2] These distinctive physical and chemical characteristics of nanomaterials offer tremendous potential for commercial innovation and enhanced functionalities that can significantly benefit society. The late 20th century marked a pivotal era with the discovery of new materials, processes, and phenomena at the nanoscale, alongside the advent of novel experimental and theoretical approaches for nanoscale research. Particularly, nanobiotechnology has revolutionized the synthesis of metal oxide nanoparticles, showcasing remarkable advantages over traditional physical and chemical methods.[3]

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Zinc oxide nanoparticles (ZnO NPs) have attracted significant interest in various domains, including optics, electronics, packaged foods, and medicine, attributed to their biocompatibility, minimal cytotoxicity, and cost-efficiency. A key aspect of ZnO NPs is their ability to trigger cellular apoptosis through the generation of reactive oxygen species (ROS) and the release of zinc ions ($Zn^{2+}$), known for their cytotoxic effects.[4] Additionally, zinc is an essential trace element in human physiology, known for its biocompatibility due to low toxicity and high biodegradability, linked to the solubility of $Zn^{2+}$ ions.[5] However, the therapeutic application of chemically synthesized ZnO nanoparticles is limited due to the toxic nature of the chemicals used in their production.[6,7] Consequently, there's a growing interest in exploring eco-friendly production methods for ZnO NPs. Recent advancements highlight the use of plant components as reducing agents in the green synthesis of ZnO nanoparticles[8,9], offering several advantages over traditional methods.[10]

The green synthesis of ZnO NPs using plant mediators has shown promising potential in developing novel therapeutics for diseases such as malaria and urinary tract infections.[11,12] Additionally, green-synthesized ZnO nanoparticles have proven to be highly effective in wound healing, primarily due to their antibacterial properties, particularly when formulated at nano and micro scales.[13]

In the present study, ZnO nanoparticles were synthesized using green synthesis methods with *Murraya koenigii* leaf extract. The resulting ZnO nanoparticles were characterized using scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and X-ray diffraction spectroscopy (XRD).

## MATERIALS & METHODS

### Plant Material

In the framework of our investigation, the *Murraya koenigii* (Curry Leaf) plant has been meticulously selected as the biological precursor for the fabrication of Zinc Oxide (ZnO) nanoparticles. This selection is predicated on the plant's profusion of phytochemicals, which encompass a diverse array of secondary metabolites such as alkaloids, flavonoids, and terpenoids. *Murraya koenigii*, commonly known as curry leaf, is a plant deeply intertwined with Indian culture, revered for its culinary and medicinal significance. Originating from India, this botanical treasure has been cherished for centuries for its aromatic flavour and potent healing properties.

### Preparation of plant extract

Leaves of *Murraya koenigii,* sourced from the premises of Govt. College, Banswara, Rajasthan, India, were authenticated by the Botany Department at the University of Rajasthan, Jaipur, Rajasthan, India. These leaves underwent an initial cleansing process involving 2-3 washes under tap water, followed by a sterilization step with double distilled water. Post-sterilization, the leaves were left to air dry at an ambient temperature of 32°C. A 20g sample of the dried leaves was then used for the extraction process. This involved boiling the measured leaves in 100 mL of double distilled water at 60°C for half an hour, which resulted in the formation of a light yellow solution. Once cooled to room temperature, this solution was filtered through Whatman No.1 filter paper to remove particulate matter. The resultant yellow extract was then refrigerated for preservation. This purified extract was earmarked for further experimental procedures, specifically for the reduction of zinc ions ($Zn^{2+}$) to form zinc oxide (ZnO) nanoparticles. This methodology aligns with established protocols for plant-based nanoparticle synthesis, offering a sustainable alternative to conventional chemical methods.

### Synthesis of ZnO nanoparticles

A 10 mL aliquot of *Murraya koenigii* leaf extract was incrementally introduced into 90 mL of a 1mM zinc nitrate ($Zn(NO_3)_2 .6H_2O$, Sigma-Aldrich, St. Louis, USA.) aqueous solution, followed by a gradual addition of few drops of sodium hydroxide (NaOH) solution, while continuously stirring at 60°C for an hour. Upon reaction completion, a dirty yellow precipitate formed, which was left undisturbed for 24 hours to settle. This precipitate was then isolated from the mixture through centrifugation at 10,000 rpm for 15 minutes, thoroughly washed with deionised water to eliminate contaminants, and subsequently dried in an oven set at 80°C. The dry powder obtained from this process was subjected to calcination in a muffle furnace at 350°C for 2 hours. The resulting pellet was further dried in a hot air oven at 80°C for 2 hours and stored in airtight bottles for future analysis.

### Characterization of the ZnO nanoparticles

The characterization process employed a multifaceted approach to thoroughly investigate the properties of the zinc oxide nanoparticles. X-Ray Diffraction (XRD)

provided insights into the crystalline structure of the nanoparticles, while Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) offered detailed visualization of their morphology and size distribution. Energy-Dispersive X-ray spectroscopy (EDX) was utilized to analyse the elemental composition of the nanoparticles. Additionally, Fourier-transform infrared spectroscopy (FTIR) was employed to identify functional groups present on the nanoparticle surface, while Dynamic Light Scattering (DLS) facilitated the measurement of their hydrodynamic size distribution in solution. This comprehensive suite of characterization techniques enabled a thorough understanding of the structure, composition, and behaviour of the zinc oxide nanoparticles.[14]

## RESULTS & DISCUSSION

The synthesis of zinc oxide nanoparticles was achieved by integrating the aqueous extract of *Murraya koenigii* with a 1mM aqueous zinc nitrate solution. This process resulted in a colour transformation of the reaction solution from light yellow to dirty yellow after a 2-hour incubation at 50°C, signifying the formation of nanoparticles. The identification of zinc oxide nanoparticles was facilitated by observing this colour shift. Subsequently, the solution underwent scanning within the 200-800 nm range using a UV-visible spectrophotometer. The UV-visible spectra, indicative of the nanoparticles synthesized through the reduction by the extract, displayed a characteristic absorption around 374 nm. This absorption band, associated with surface plasmon resonance (SPR), arises from the collective oscillation of free electrons in metal nanoparticles when in resonance with light waves. Zinc colloids demonstrated distinct SPR bands in the visible spectrum, specifically between 350-380 nm, aligning with the band gap energy of ZnO and hinting at electronic transitions within the ZnO crystal lattice. These bands widened over time, reflecting the stability and uniform size distribution of the nanoparticles, which remained in solution without agglomeration for two months. The peak's precise location can shift slightly due to factors such as particle size, shape, and the presence of dopants or defects, with smaller particles often showing a blue shift due to quantum confinement effects.

Dynamic Light Scattering (DLS) analysis was employed to determine the hydrodynamic size, polydispersity index (PDI), and surface zeta potential of zinc oxide nanoparticles synthesized in a colloidal aqueous solution. The size distribution spectrum, reveals that the green-synthesized zinc oxide nanoparticles ranged between 20 and 9. 0 nm, with a PDI value of 0.403. A PDI value of '0' signifies a monodisperse distribution, whereas a value of '1' indicates a polydisperse distribution. This suggests that the nanoparticles exhibit a moderate level of dispersity. Additionally, the DLS technique measures the hydrodynamic diameter, which represents the theoretical sphere size that diffuses at the same velocity as the nanoparticle. This measurement accounts not only for the metallic core but also for any substances adsorbed on the nanoparticle's surface, such as stabilizers, and the solvation shell, resulting in a size measurement often larger than what microscopic methods would indicate. The average hydrodynamic size reported was 70 nm.

To unveil the crystalline nature of the synthesized zinc oxide nanoparticles (ZnONPs), XRD crystallography was conducted the X-ray diffractogram obtained from the Murraya koenigii plant leaf extract-mediated synthesis of zinc oxide nanoparticles. Within the 2θ range spanning from 20 to 90°, the XRD pattern exhibited distinctive Bragg's reflection planes. The discernible diffraction peaks at 2θ= 31.74°, 34.47°, 36.22°, 47.50°, 56.54°, 62.82°, and 69.02° corresponded to the lattice planes (Miller indices) of 100, 002, 101, 102, 110, 103, and 201 of ZnONPs, respectively. These peaks were indicative of the face-centered cubic (fcc) structure of the nanoparticles, as corroborated by the Joint Committee on Powder Diffraction Standards (JCPDS) database reference.[15]

The presence of broad peaks in the XRD pattern suggested a small crystalline size of the nanoparticles. The observed Bragg's diffraction peaks were matched with the JCPDS card No. 89-1379 of ZnO, indicating a multi-phase nature of the particles. Sharp peaks observed in the figure may be attributed to the capping agent stabilizing the nanoparticles, while unassigned peaks (*) could potentially arise from biomolecule crystallization on the surface of the nanoparticles. Similar findings of Bragg's reflection for ZnNPs have been reported elsewhere.[16] The lattice constant was calculated from the diffraction pattern (α = 0.78 Å), and the d-spacing values are listed in the table 1. Utilizing the Scherrer equation:

$$D = \frac{k\lambda}{\beta \cos\theta}$$

Where, D represents the average crystalline size (Å), k is a constant set to 1, λ is the X-ray radiation wavelength (λ = 1.54 Å), β is the angular line full width at half

maximum (FWHM), and θ is the Bragg angle. The average size of ZnONPs was determined to fall within the range of 25-50 nm, as presented in the table 1.

**Table 1- Parameter calculation for average size calculation for nanoparticle**

| 2 θ | FWHM (*β*) | d-spacing | (*hkl*) | crystalline size (nm) |
|---|---|---|---|---|
| 31.74 | 0.158 | 2.82 | 100 | 46.71 |
| 34.47 | 0.16 | 2.6 | 002 | 48.62 |
| 36.22 | 0.182 | 2.48 | 101 | 40.31 |
| 47.5 | 0.228 | 1.91 | 102 | 34.2 |
| 56.54 | 0.254 | 1.63 | 110 | 25.6 |
| 62.82 | 0.232 | 1.48 | 103 | 32.33 |
| 69.02 | 0.192 | 1.36 | 201 | 38.98 |
| | | | | Average **38.40** |

The FTIR spectrum of the Zinc Oxide (ZnO) nanoparticles synthesized using plant extracts reveals several significant peaks and their potential origins (Figure 1). At 571.83 $cm^{-1}$, we observe a distinct peak, suggesting the stretching vibrations characteristic of the Zn-O bonds inherent to ZnO nanoparticles. A peak at 1134.68 cm-1 may denote the C-O stretching vibrations, pointing towards the presence of organic substances such as alcohols, esters, or ethers, likely serving as organic residues or capping agents attached to the nanoparticle surfaces. The peak occurring at 1411.27 $cm^{-1}$ is possibly linked to the O-H bending vibrations of carboxylate ions (COO-) and may also reflect symmetric stretching vibrations of these ions, hinting at carboxyl groups from organic acids in the plant extract, which could be adsorbed onto the nanoparticles. An absorption peak at 1697.36 $cm^{-1}$ aligns with C=O stretching vibrations, typically associated with carbonyl functional groups like those found in ketones, aldehydes, or carboxylic acids, substances that are part of plant-derived metabolites. At 2341.59 $cm^{-1}$, the detected peak is not usually associated with ZnO nanoparticles and could likely be an artifact from atmospheric $CO_2$ rather than the material being analyzed. Finally, the broad peak at 3321.43 $cm^{-1}$ is indicative of O-H stretching vibrations, consistent with hydroxyl groups, which suggests the involvement of hydrogen-bonded alcohols or water molecules; these may either be adsorbed onto the nanoparticles or integral to the plant phytochemicals used during synthesis.[17]

The synthesis of nanocrystalline zinc oxide particles was elucidated through Transmission Electron Microscopy (TEM) experiments. The nanoparticles predominantly exhibit a spherical morphology and are often observed clustered into small aggregates (Figure 2a and b). Notably, the nanoparticles exhibit a high degree of uniformity in shape, with an average size of 30 nm. Occasional occurrences of larger particles were noted in the sample, albeit in low numbers. The TEM images further reveal the presence of a thin organic film, acting as a capping agent, enveloping the small particle aggregates. This observation underscores the excellent dispersion of nanoparticles even on a macroscopic scale within the bio-reduced aqueous solution. Additionally, the figure 2c illustrates the Selected Area Electron Diffraction (SAED) pattern of ZnO nanoparticles, depicting point diffraction spots arranged in inner to outer ring configurations, consistent with ZnONPs (100, 002, 101, 102, 110, 103, and 201) lattice reflections. This corroborates the highly crystalline nature of the prepared ZnO nanoparticles. The histogram presented in the figure 2d indicates that a significant number of particles fall within the size range of 20-30 nm.

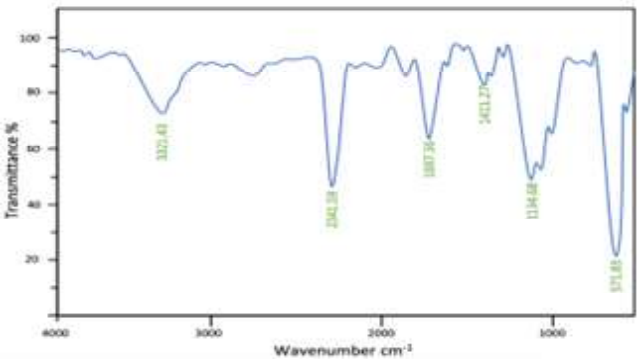


**Figure 1- FT-IR spectra of synthesized nanoparticles**

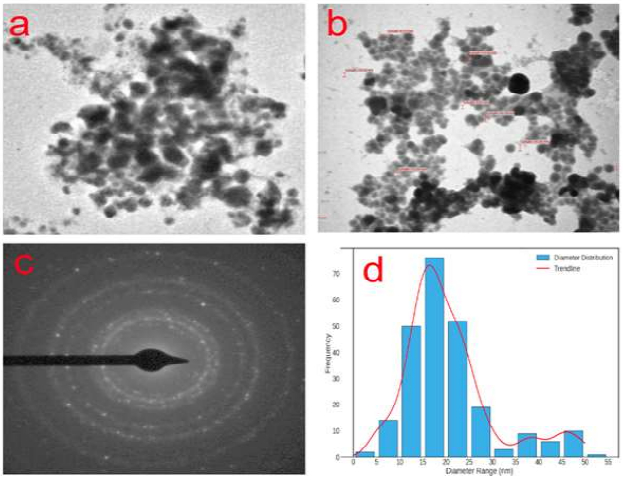


**Figure 2- TEM micrograph of ZnONPs synthesized from leaf extract (a-b), SAED pattern (c) and particle size range (d).**

The surface morphology of nanoparticles synthesized via *Murraya koenigii* leaf extract mediation was scrutinized using field emission scanning electron microscopy (FE-SEM), as depicted in the accompanying. The SEM images distinctly reveal uniformly spherical particles, indicative of a well-controlled synthesis process. Analysis of the particle size distribution reveals an average diameter of approximately 29.30 nm for the zinc oxide nanoparticles, while it also indicates the formation of larger particles of zinc oxide nanoparticles (ZnONPs) during sample preparation, likely due to nanoparticle aggregation.

The FE-SEM micrographs, captured at both 1 mm (low resolution) and 100 nm (high resolution), provide detailed insights into the surface characteristics of the spherical zinc oxide nanoparticles (Figure 3a and b). Notably, the rough surface morphology of these nanoparticles is clearly elucidated. Furthermore, the chemical composition of the synthesized nanoparticles was interrogated via energy-dispersive X-ray spectroscopy (EDS). The EDS spectrum reveals the presence of zinc (Zn) and organic compounds enveloping the zinc oxide aggregates, including oxygen (O), carbon (C), chlorine (Cl), potassium (K), and calcium (Ca) atoms. Quantitative analysis demonstrates that zinc constitutes the predominant element at 73.43%, followed by oxygen at 22.27%, with smaller percentages attributed to carbon, chlorine, potassium, and calcium. The presence of zinc is attributed to the formation of ZnO nanoparticles, while the presence of carbon, oxygen, and chlorine atoms is ascribed to constituents of the plant extract, as evidenced in Figure 3c. Importantly, the EDS spectrum confirms that the ZnONPs are predominantly metallic and devoid of impurities.

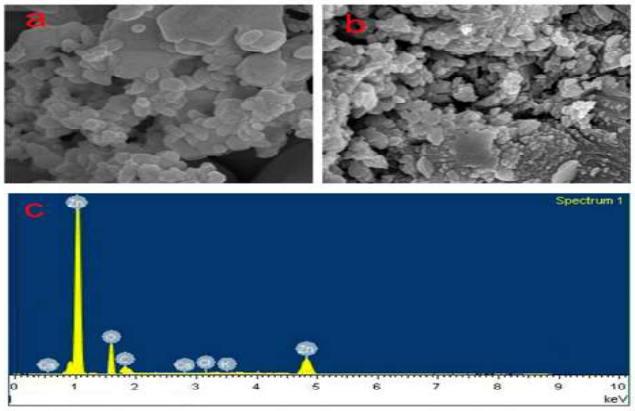


**Figure 3- SEM micrograph of ZnONPs synthesized from *Murraya koenigii* leaf extract (a-b) and dispersive spectroscopy (c)**

## CONCLUSION

This study successfully demonstrated the eco-friendly synthesis of zinc oxide nanoparticles (ZnO NPs) using *Murraya koenigii* (curry leaf) extract. Various characterization techniques confirmed the formation and properties of the ZnO NPs, including UV-visible spectrophotometry, Dynamic Light Scattering (DLS), X-ray Diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), and Energy-Dispersive X-ray Spectroscopy (EDS). The synthesized ZnO NPs showed a characteristic absorption peak at 374 nm, had an average hydrodynamic size of 70 nm, and a crystalline size of approximately 38.40 nm. The FTIR spectrum indicated the presence of functional groups from the plant extract. TEM and SEM analyses revealed spherical nanoparticles with an average size of around 30 nm, and EDS confirmed the purity and composition of ZnO NPs. This green synthesis method offers a sustainable alternative to traditional chemical methods, producing biocompatible ZnO NPs suitable for applications in medicine, optics, electronics, packaging and photocatalytic treatment of waste water. The study highlights the advantages of using plant extracts for nanoparticle synthesis, emphasizing their cost-effectiveness, environmental friendliness, and biocompatibility.

## REFERENCES

1. **Samuel M. S., Ravikumar M., John J. A., Selvarajan E., Patel H., Chander P. S., Soundarya J., Vuppala S., Balaji R. and Chandrasekar N. 2022.** A review on green synthesis of nanoparticles and their diverse biomedical and environmental applications. *Catalysts*, **12(5):** 459.

2. **Bahrulolum H., Nooraei S., Javanshir N., Tarrahimofrad H., Mirbagheri V. S., Easton A. J. and Ahmadian G. 2021.** Green synthesis of metal nanoparticles using microorganisms and their application in the agrifood sector. *Journal of Nanobiotechnology*, **19(1):** 1–26.

3. **Sabir S., Arshad M. and Chaudhari S. K., 2014.** Zinc oxide nanoparticles for revolutionizing agriculture: synthesis and applications. *The Scientific World Journal,* **2014(1)**: 925494.

4. **Alhujaily M., Albukhaty S., Yusuf M., Mohammed M. K., Sulaiman G. M., Al-Karagoly H., Alyamani A. A., Albaqami J. and AlMalki F. A. 2022.** Recent advances in plant-mediated zinc oxide nanoparticles with their significant biomedical properties. *Bioengineering*, **9(10):** 541.

5. **Asif N., Amir M., & Fatma T. 2023.** Recent advances in the synthesis, characterization and biomedical applications of zinc oxide nanoparticles. *Bioprocess and Biosystems Engineering*.

6. **Naseer M., Aslam U., Khalid B., Chen B. 2020.** Green route to synthesize zinc oxide nanoparticles using leaf extracts of *Cassia fistula* and *Melia azadarach* and their antibacterial potential. *Scientific Reports*, **10:** 9055.

7. **Chan Y. Y., Pang Y. L., Lim S., & Chong W. C. 2021.** Facile green synthesis of ZnO nanoparticles using natural-based materials: Properties, mechanism, surface modification and application. *Journal of Environmental Chemical Engineering*, **9(4):** 105417.

8. **Batra V., Kaur I., Pathania D., & Chaudhary V. 2022.** Efficient dye degradation strategies using green synthesized ZnO-based nanoplatforms: *A review. Applied Surface Science Advances*, **11:** 100314.

9. **Peralta-Videa J. R., Huang Y., Parsons J. G., Zhao L., Lopez-Moreno L., Hernandez-Viezcas J. A. and Gardea-Torresdey J. L. 2016.** Plant-based green synthesis of metallic nanoparticles: scientific curiosity or a realistic alternative to chemical synthesis? *Nanotechnology for Environmental Engineering*, **1:** 1–29.

10. **Thirugnanasambandan T., & Gopinath S. C. B. 2022.** Nanoparticles from plant-based materials: a promising value-added green synthesis for antidiabetic. *Biomass Conversion and Biorefinery*. 1–11.

11. **Aboyewa J. A., Sibuyi N. R. S., Meyer M., & Oguntibeju O. O. 2021.** Green synthesis of metallic nanoparticles using some selected medicinal plants from Southern Africa and their biological applications. *Plants*, **10(9):** 1929.

12. **Barathikannan K., Chelliah R., Selvakumar V., Elahi F., Rubab M., Sanyal S., Yeon S. J. and Oh D. H. 2023.** Plant-based metabolites and their uses in nanomaterials synthesis: an overview. Secondary Metabolites Based Green Synthesis of *Nanomaterials and Their Applications*, 1–22.

13. **Islam F., Shohag S., Uddin M. J., Islam M. R., Nafady M. H., Akter A., Mitra S., Roy A., Emran T. B. and Cavalu S. 2022.** Exploring the journey of zinc oxide nanoparticles (ZnO NPs) toward biomedical applications. *Materials* (Basel), **15(6):** 2160.

14. **Velmurugan P., Shim J., You Y., Choi S., Kamala-Kannan S., Lee K. J. & Oh B. T. 2010.** Removal of zinc by live, dead, and dried biomass of Fusarium spp. isolated from the abandoned-metal mine in South Korea and its perspective of producing nanocrystals. *Journal of hazardous materials*, **182(1-3):** 317-324.

15. **Nayak N. B., & Nayak B. B. 2016**. Temperature-mediated phase transformation, pore geometry and pore hysteresis transformation of borohydride derived in-born porous zirconium hydroxide nanopowders. *Scientific Reports*, **6(1):** 26404.

16. **Ajitha B., Reddy Y. A. K., & Reddy P. S. 2015.** Biosynthesis of silver nanoparticles using Momordica charantia leaf broth: evaluation of their innate antimicrobial and catalytic activities. *Journal of Photochemistry and Photobiology B: Biology*, **146:** 1-9.

17. **Sundrarajan, M., Ambika, S., & Bharathi, K. 2015.** Plant-extract mediated synthesis of ZnO nanoparticles using *Pongamia pinnata* and their activity against pathogenic bacteria. *Advanced Powder Technology*, **26:** 1294–1299.

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