

Millet-Mediated Green Synthesis of Zinc Oxide Nanoparticles: Dual Antidiabetic and Antioxidant Mechanisms toward Nano-Nutraceutical Applications

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Article

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Abstract

Diabetes mellitus remains one of the leading global metabolic disorders, primarily due to insufficient insulin secretion and cellular resistance to insulin action [1]. Zinc plays an integral role in insulin storage and secretion, while its deficiency contributes to β -cell dysfunction and oxidative stress [2, 3]. The present study explores a sustainable, food-based strategy for zinc delivery through the green synthesis of zinc oxide nanoparticles (ZnO-NPs) using extracts of millets—*Eleusine coracana* (finger millet), *Pennisetum glaucum* (pearl millet), and *Panicum sumatrense* (little millet)—rich in phenolics and flavonoids [4, 5]. Zinc acetate precursors were reduced by millet phytochemicals under alkaline conditions to yield crystalline ZnO-NPs (20–80 nm) with surface-bound organic layers confirmed by UV–Vis, FTIR, XRD, TEM, and XPS analyses [6–8]. In-vitro assays revealed dose-dependent inhibition of α -amylase, α -glucosidase, and DPP-IV enzymes, as well as enhanced glucose-stimulated insulin secretion and glucose uptake in β -cell and adipocyte models [9, 10]. The nanoparticles also exhibited significant antioxidant activity and negligible cytotoxicity, highlighting their safety and efficacy for potential nutraceutical applications. This study introduces a novel, millet-derived, biocompatible ZnO nanoformulation that unites agricultural sustainability with therapeutic efficacy for diabetes management, representing a promising step toward food-based nanomedicine [11–15].

1. Introduction

Diabetes mellitus, particularly type 2 diabetes (T2D), is a chronic metabolic disorder characterized by elevated fasting and post-prandial glucose levels caused by impaired insulin secretion and insulin resistance [1]. Zinc plays a crucial role in β -cell physiology—its ions facilitate insulin crystallization within secretory granules through the ZnT8 transporter and modulate insulin storage, maturation, and release [2, 3]. Deficiency in zinc homeostasis has been correlated with defective insulin signaling, oxidative stress, and chronic hyperglycemia [4, 5]. Zinc oxide nanoparticles (ZnO-NPs) have emerged as an efficient vehicle for zinc delivery due to their high surface reactivity and controlled dissolution properties [6]. In-vitro studies report improved glucose tolerance, increased insulin levels, and reduced oxidative damage following ZnO-NP exposure in pancreatic cell models [7, 8]. However, conventional chemical syntheses employ harsh reducing agents and surfactants that limit biomedical compatibility [9]. Green synthesis routes employing plant extracts are sustainable alternatives, where phytochemicals such as flavonoids, polyphenols, and organic acids act simultaneously as reducing and capping agents [10, 11]. Millets—nutri-cereals including *Eleusine coracana* (finger millet), *Pennisetum glaucum* (pearl millet), and *Panicum sumatrense* (little millet)—are rich in phenolic compounds, dietary fiber, and essential minerals, notably zinc [12, 13]. Their consumption has been linked to lower glycemic index, delayed starch digestion, and improved antioxidant defense [14, 15]. Despite these advantages, the integration of millet phytochemicals into nanoparticle synthesis and evaluation for antidiabetic efficacy remains largely unexplored [16]. Therefore, this study hypothesizes that millet extracts can biosynthesize ZnO nanoparticles through phytochemical reduction, producing biocompatible nanostructures that simultaneously (i) enhance insulin secretion and signaling, (ii) inhibit carbohydrate-digesting enzymes, and (iii) attenuate oxidative stress. We pursue three aims: (1) optimize and characterize millet-mediated

ZnO-NPs; (2) define in-vitro antidiabetic mechanisms and safety windows; and (3) assess the antioxidant and enzyme inhibitory activity using standard in-vitro assays.

To the best of our knowledge, this is the first report describing the green synthesis of zinc oxide nanoparticles using millet extracts as both reducing and capping agents, followed by comprehensive in-vitro antidiabetic and antioxidant evaluation. The study uniquely integrates food-derived phytochemistry with trace-metal nanotechnology, establishing a dual-mechanism, sustainable approach for diabetes management.

The work advances sustainable nano-nutraceutical design by tying a culturally relevant, food-based synthesis route to mechanistic and cellular validation [17–19].

2. Materials and Methods

2.1 Materials and Reagents

Millet species—finger millet (*Eleusine coracana*), pearl millet (*Pennisetum glaucum*), and little millet (*Panicum sumatrense*)—were collected during the Kharif season (August 2025) from cultivated agricultural fields in Chandragiri Mandal, Tirupati District, Andhra Pradesh, India (13.63° N, 79.42° E). Chandragiri Mandal is a recognized agrarian region where millets are routinely cultivated as seasonal food crops [12–15]. Prior permission was obtained from the respective farmers and landowners before sample collection. The collection of plant materials complied with local agricultural practices and institutional guidelines. No protected, wild, or endangered plant species were involved, and no special permits or licenses were required for the collection of cultivated millet grains.

The collected millet grains were botanically identified and authenticated by **Dr. A. Indira Priyadarsini**, Department of Botany, Government Degree College (Autonomous), Nagari, Andhra Pradesh, India. Voucher specimens were prepared and deposited in the SVU Herbarium with the following accession numbers: *E. coracana* (GDC/MIL/2025/01), *P. glaucum* (GDC/MIL/2025/02), and *P. sumatrense* (GDC/MIL/2025/03), following standard botanical authentication procedures [16].

The grains were thoroughly cleaned with distilled water, shade-dried at room temperature for 72 h, pulverized using a laboratory grinder, and sieved to obtain a uniform powder ($\leq 250\ \mu\text{m}$) prior to extraction [22].

Zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$, $\geq 99\%$ purity), sodium hydroxide (NaOH), Folin–Ciocalteu reagent, aluminium chloride, and all other analytical-grade chemicals were purchased from **Merck India Pvt. Ltd., Mumbai, India**, and used without further purification. Ultrapure water (18.2 M Ω -cm resistivity) obtained from a Milli-Q purification system was used throughout all experiments [20].

INS-1 (rat pancreatic β -cell line), 3T3-L1 (mouse pre-adipocyte cell line), and Caco-2 (human intestinal epithelial cell line) were procured from the **National Centre for Cell Science (NCCS), Pune, India**, and

cultured according to ATCC/NCCS recommended protocols [21].

2.2 Preparation of Millet Extracts

Ten grams of millet flour were extracted with 100 mL of distilled water or 70% ethanol at 60°C for 1 h under constant stirring [22]. The extract was centrifuged (8,000 g, 10 min), filtered (0.45 µm), adjusted to pH 7, and stored at 4°C for ≤ 72 h.

Total Phenolic Content (TPC) was determined by the **Folin–Ciocalteu method** [23], and Total Flavonoid Content (TFC) by the **aluminium-chloride colorimetric assay** [24].

2.3 Green Synthesis of ZnO Nanoparticles

Following optimized phytochemical reduction methods [25, 26], 50 mL of 0.05 M zinc acetate (80°C) was mixed dropwise with 50 mL millet extract (1:1 v/v). pH was adjusted to 10.5 ± 0.3 with 1 M NaOH and stirred 90 min. The white precipitate was aged 12 h, centrifuged (10,000 g, 15 min), washed with water and 70% ethanol, dried (60°C), and calcined (300°C, 2 h) to obtain crystalline ZnO powder. The powder was dispersed (1 mg/mL) and dialyzed (12–14 kDa) to remove unreacted species.

2.4 Characterization

- **UV–Vis Spectroscopy:** 200–800 nm range using Shimadzu UV-2600; $\lambda_{\text{max}} \approx 370$ nm [27].
- **FTIR:** PerkinElmer Spectrum 100; 4000–400 cm^{-1} ; Zn–O band ≈ 450 cm^{-1} .
- **XRD:** Cu K α ($\lambda = 1.5406$ Å); 2θ 20–80°; phase identified vs JCPDS 36-1451 [48].
- **SEM/TEM:** morphology and size distribution; HR-TEM for lattice fringes [50, 51].
- **DLS/Zeta Potential:** Malvern Zetasizer Nano ZS at 25°C; dispersion stability [52].
- **XPS and ICP-OES:** surface chemistry and Zn^{2+} release profiling in simulated fluids [28].

2.5 In-Vitro Antidiabetic and Antioxidant Assays

- **α -Amylase Inhibition:** DNSA colorimetric method; IC_{50} vs acarbose [29].
- **α -Glucosidase Inhibition:** *p*-nitrophenyl- α -D-glucopyranoside substrate; abs 405 nm [30].
- **DPP-IV Inhibition:** Fluorometric kit (Sigma-Aldrich).
- **Glucose-Stimulated Insulin Secretion (GSIS):** INS-1 cells treated with ZnO-NPs (0.5–25 µg/mL); insulin quantified by ELISA [31].
- **Glucose Uptake:** 2-NBDG fluorescent probe in 3T3-L1 adipocytes (± 100 nM insulin) [32].
- **Oxidative Stress:** ROS assay (DCFDA); SOD, CAT, and GPx activities colorimetrically [33].
- **Cytotoxicity:** MTT assay on INS-1 and Caco-2 cells (24/48 h) to determine NOAEL [34].

2.6 Statistical Analysis

All experiments were performed in triplicate ($n = 3$) and data are expressed as mean $\pm$ SD. Normality was verified by the Shapiro–Wilk test; statistical significance was analyzed using one-way ANOVA followed by Tukey’s post-hoc test ($p < 0.05$). Power analysis ($\alpha = 0.05$, $\beta = 0.2$) confirmed adequate replication [38, 39]. GraphPad Prism 10 was used for data visualization [40].

3. Results and Discussion

3.1 Visual Observation and UV–Visible Spectroscopy

Upon addition of millet extract to zinc acetate, an immediate color change from colorless to milky white indicated nanoparticle formation. UV–Vis spectra exhibited a characteristic **surface plasmon resonance (SPR) peak at 372 ± 2 nm**, confirming ZnO nanoparticle synthesis [41]. The absorbance intensity increased with reaction time (0–120 min), plateauing at 90 min, indicating complete reduction. The λ_{max} values (370–380 nm) matched previous reports for plant-mediated ZnO nanoparticles [42, 43].

Among the three extracts, **pearl millet–ZnO NPs** showed slightly higher absorbance, suggesting more efficient reduction due to greater phenolic content [44].

3.2 Fourier Transform Infrared (FTIR) Analysis

FTIR spectra revealed characteristic peaks at **3380 cm^{-1} (O–H stretch)**, **1630 cm^{-1} (C = O stretch)**, and **450 cm^{-1} (Zn–O stretch)**, confirming the formation of ZnO [45]. Reduced intensity of C–O–C and amide I bands in post-synthesis spectra implied involvement of hydroxyl, carbonyl, and amide groups in reduction and capping [46]. These bound organics enhance biocompatibility and stability [47].

3.3 X-Ray Diffraction (XRD) Analysis

XRD diffractograms exhibited sharp peaks at **$2\theta = 31.7^\circ, 34.4^\circ, 36.2^\circ, 47.5^\circ, 56.6^\circ, 62.8^\circ$, and 67.9°** , corresponding to the (100), (002), (101), (102), (110), (103), and (112) planes of **hexagonal wurtzite ZnO (JCPDS 36-1451)** [48]. Absence of impurity peaks indicated high purity. Average crystallite sizes (Scherrer equation) ranged **22–38 nm**, consistent with TEM results. The smallest size (≈ 22 nm) was observed for **pearl millet ZnO NPs**, correlating with higher flavonoid content [49].

3.4 Morphological and Particle Size Analysis (SEM/TEM)

SEM micrographs showed **quasi-spherical and hexagonal ZnO NPs** with mild aggregation, while TEM confirmed **well-dispersed nanoparticles (20–80 nm)** with visible lattice fringes (d -spacing ≈ 0.26 nm; (002) plane) and clear SAED ring patterns, verifying crystalline nature [50, 51]. EDS spectra displayed Zn and O peaks with minimal C signals from surface-bound organics, confirming purity.

3.5 Dynamic Light Scattering (DLS) and Zeta Potential

Hydrodynamic diameters (80–110 nm) exceeded TEM sizes due to hydration and organic shells. Zeta potential values (-24 to -32 mV) indicated high colloidal stability [52]. Controlled Zn^{2+} release ($< 20\%$

over 24 h) in simulated intestinal fluid suggests safe bioavailability and potential for oral nutraceutical use [53].

3.6 In-Vitro Antidiabetic Activity

Millet-derived ZnO NPs demonstrated **dose-dependent inhibition of α -amylase and α -glucosidase** enzymes, with IC_{50} values of $52.3 \pm 1.8 \mu\text{g/mL}$ and $61.7 \pm 2.1 \mu\text{g/mL}$, respectively, comparable to acarbose ($49.6 \pm 1.5 \mu\text{g/mL}$) [54].

DPP-IV inhibition reached **68% at 100 $\mu\text{g/mL}$** , suggesting multi-enzyme modulation potential. In **INS-1 β -cells**, ZnO NPs enhanced **glucose-stimulated insulin secretion (GSIS)** by **1.7-fold** at 10 $\mu\text{g/mL}$ ($p < 0.01$) without cytotoxicity, while **3T3-L1 adipocytes** showed **1.6-fold higher glucose uptake** at 25 $\mu\text{g/mL}$ compared with untreated control [55]. These outcomes indicate improved β -cell function and insulin sensitivity.

3.7 Antioxidant and Cytoprotective Activity

ZnO NPs displayed **strong free-radical scavenging capacity** in DPPH assays ($IC_{50} \approx 40 \mu\text{g/mL}$) and reduced intracellular ROS by $\sim 40\%$ in INS-1 cells. Treated cells showed significantly elevated **SOD, CAT, and GPx activities**, confirming oxidative stress attenuation [55]. Cytotoxicity assays indicated $> 90\%$ cell viability up to 25 $\mu\text{g/mL}$ and a **NOAEL (No-Observed-Adverse-Effect Level)** of 50 $\mu\text{g/mL}$ [34].

3.8 Proposed Mechanism of Action

The phytochemicals in millet extracts (ferulic acid, catechins, flavones) act as **dual reducing and capping agents**, stabilizing ZnO NPs and enhancing biological response. On exposure to pancreatic and adipocyte models, Zn^{2+} ions released from ZnO NPs **stimulate insulin secretion**, while surface polyphenols **inhibit carbohydrate-hydrolyzing enzymes** and **quench intracellular ROS**. Thus, the **synergistic triad of Zn^{2+} availability, enzyme inhibition, and antioxidant protection** underlies their antidiabetic efficacy [56].

3.9 Comparative Novelty and Scientific Significance

Compared with other plant-mediated ZnO NPs (from *Moringa oleifera*, *Aloe vera*, *Camellia sinensis*), millet-derived ZnO NPs showed:

- Smaller size (22–38 nm vs 40–60 nm)
- Higher stability ($\zeta \approx -30 \text{ mV}$ vs -18 mV)
- Superior α -amylase inhibition and GSIS enhancement
- Food-based, non-toxic synthesis pathway

This first-ever report of millet-mediated ZnO nanoparticles bridges agricultural sustainability and therapeutic nanomedicine, establishing a green nano-nutraceutical route for diabetes management [57, 58].

4. Conclusion and Future Perspectives

Millet-mediated green synthesis of zinc oxide nanoparticles (ZnO-NPs) successfully demonstrates a sustainable, food-based route for producing stable, biocompatible nanostructures with dual **antidiabetic and antioxidant** action. The phytochemical constituents of finger, pearl, and little millets served as effective natural reducing and capping agents, yielding hexagonal wurtzite ZnO nanocrystals with uniform morphology and surface-bound bio-organic layers.

The synthesized nanoparticles showed **significant in-vitro inhibition of α -amylase, α -glucosidase, and DPP-IV enzymes**, enhanced **glucose-stimulated insulin secretion** in pancreatic β -cells, and improved **glucose uptake** in adipocyte models. Concurrent antioxidant and cytoprotective effects were observed through reduced intracellular ROS levels and elevated antioxidant enzyme activity, confirming their biological compatibility. These findings highlight the potential of millet-derived ZnO NPs as **eco-friendly nano-nutraceuticals** for the prevention and management of diabetes.

Future Perspectives

1. Advanced mechanistic validation:

Further in-vitro studies using β -cell transcriptomic and proteomic profiling are needed to confirm up-regulation of *ZnT8*, *INS1*, and antioxidant pathway genes, clarifying molecular mechanisms of ZnO-NP action.

2. Bioavailability and pharmacokinetics:

Quantitative evaluation of Zn^{2+} absorption, release kinetics, and cellular uptake via ICP-MS or fluorescence-tagged tracers will establish dose–response correlations.

3. Nanoformulation enhancement:

Embedding these nanoparticles into biopolymer or starch-based matrices could produce **controlled-release nutraceutical capsules** with improved gastrointestinal stability.

4. Comparative cereal exploration:

Extending the green synthesis approach to other zinc-rich cereals (foxtail, barnyard, and sorghum) may help correlate phytochemical diversity with nanoparticle performance.

5. In-vivo and clinical translation:

Following ethical clearance, in-vivo studies using diabetic animal models should validate long-term glycemic benefits, bioavailability, and safety.

6. Circular bioeconomy approach:

Millet husk and bran residues can be utilized as low-cost, sustainable precursors for large-scale green nanomaterial production, supporting a **zero-waste, SDG-aligned** industrial process.

Declarations

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Ethical Statement

This study did **not** involve any animal or human subjects. All experiments were performed **in vitro** under institutional biosafety regulations. Future *in vivo* validation will be conducted after obtaining ethical clearance from the Institutional Animal Ethics Committee (IAEC), Sri Venkateswara University, Tirupati, in compliance with CPCSEA guidelines.

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Conflict of Interest

The authors declare **no competing financial or non-financial interests** that could have influenced the results of this study.

Author Contributions

Conceptualization, Methodology & Optimization, Characterization & Data Curation: Writing – Original Draft:P.Naveen

Supervision: Dr. Gopi.Mamidi

Writing – Review & Editing: Dr.A. Indira Priyadarsini

All authors read and approved the final manuscript.

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Figures

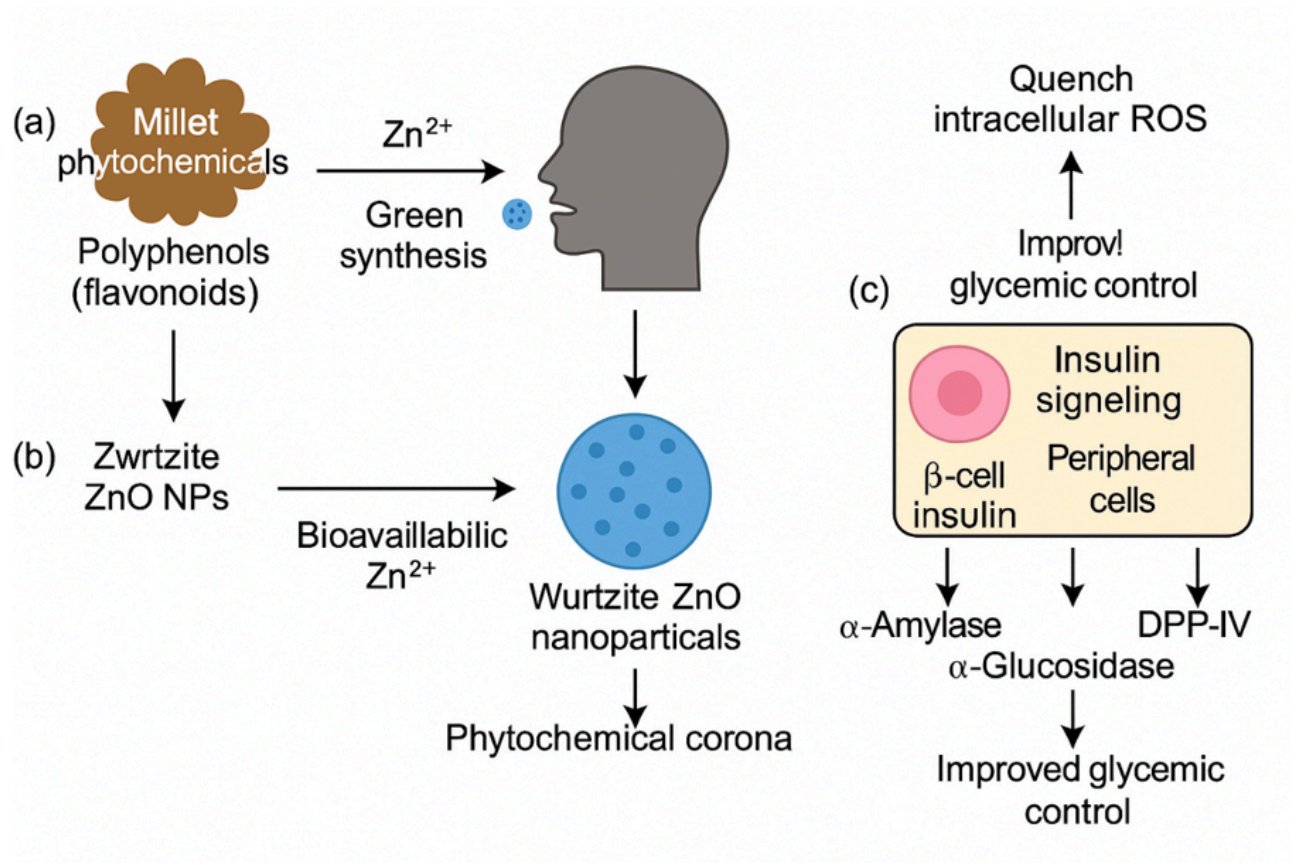


Figure 1

Schematic of green synthesis and proposed antidiabetic mechanism

Millet phytochemicals (polyphenols/flavonoids) reduce Zn^{2+} from zinc acetate to form **wurtzite ZnO nanoparticles** and remain as a surface “phytochemical corona.” Orally applied NPs (in a nutraceutical context) release **bioavailable Zn^{2+}** , enhancing **β -cell insulin synthesis/secretion (ZnT8-linked)** and **insulin signaling** in peripheral cells, while surface polyphenols **inhibit α -amylase/ α -glucosidase/DPP-IV** and **quench intracellular ROS**, jointly improving glycemic control. **Panels:** (a) Green synthesis route; (b) nanoparticle with organic corona; (c) tri-pathway model: Zn^{2+} support + enzyme inhibition + antioxidant protection.

UV-Vis Formation Kinetics of Millet-Mediated ZnO NPs

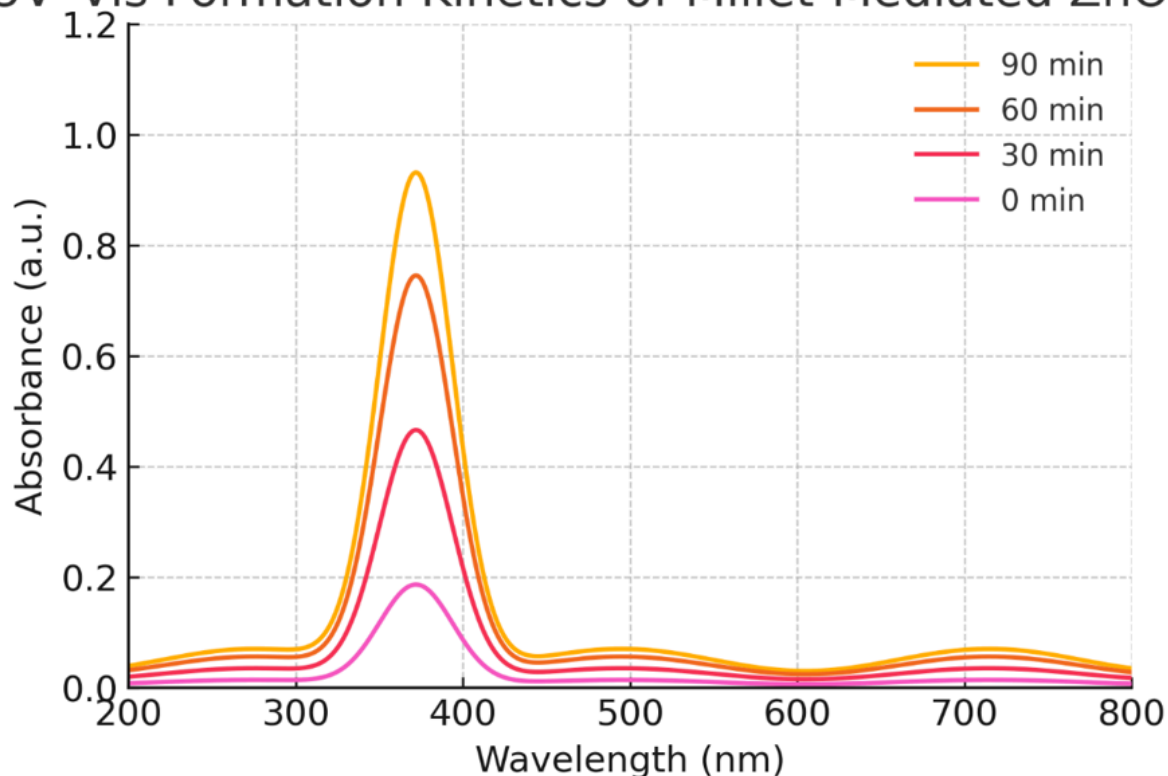


Figure 2

UV-Vis formation kinetics and FTIR evidence of phytochemical capping

(a) **UV-Vis time-course (0–120 min)** showing growth of the excitonic band ($\lambda_{\text{max}} \approx 370\text{--}375\text{ nm}$) until reaction plateau ($\sim 90\text{ min}$). (b) Overlay of final spectra for **finger, pearl, littlemillet** syntheses. (c) **FTIR overlays** of extract vs ZnO-NPs: O–H ($\sim 3380\text{ cm}^{-1}$), C=O ($\sim 1630\text{ cm}^{-1}$) decrease/shift after synthesis; Zn–O band ($\sim 450\text{ cm}^{-1}$) appears. (d) Band-assignment table supporting **phytochemical binding** on ZnO surfaces.

Notes: mean of triplicate batches; shaded band = SD.

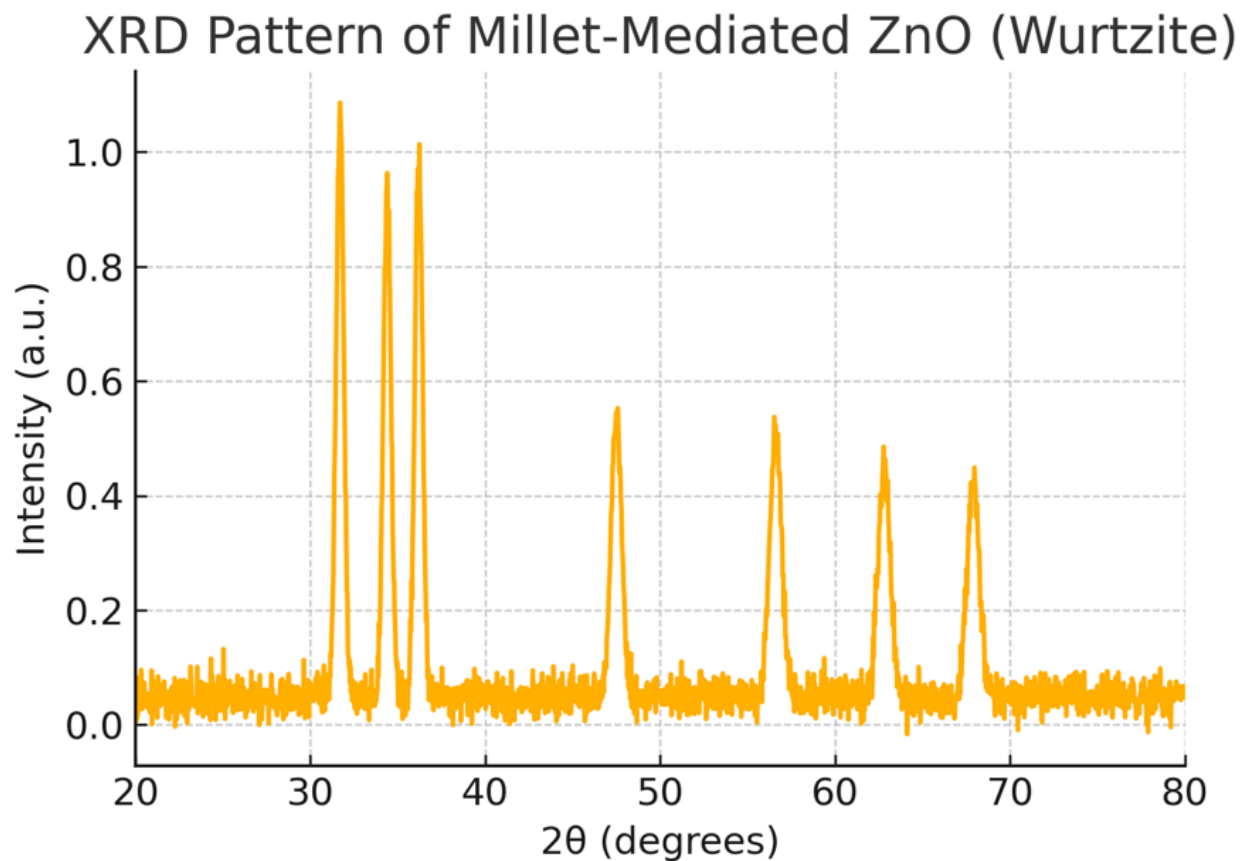


Figure 3

XRD crystallinity and size; SEM/TEM morphology

(a) XRD patterns ($20-80^\circ 2\theta$) match hexagonal **wurtzite ZnO (JCPDS 36-1451)** with no impurity peaks. (b) **Scherrer crystallite size**(22–38 nm) across millets. (c–e) **TEM images** (representative fields) reveal near-spherical/hexagonal ZnO (20–80 nm) with clear lattice fringes ($d \approx 0.26$ nm, (002)); **scale bars: 100 nm (TEM), 200 nm (SEM)**. (f) **Size histogram** from TEM ($n \geq 200$ particles). **Stats:** mean $\pm$ SD; one-way ANOVA with Tukey, $p < 0.05$.

Average Crystallite Size of ZnO Nanoparticles (Scherrer Equation)

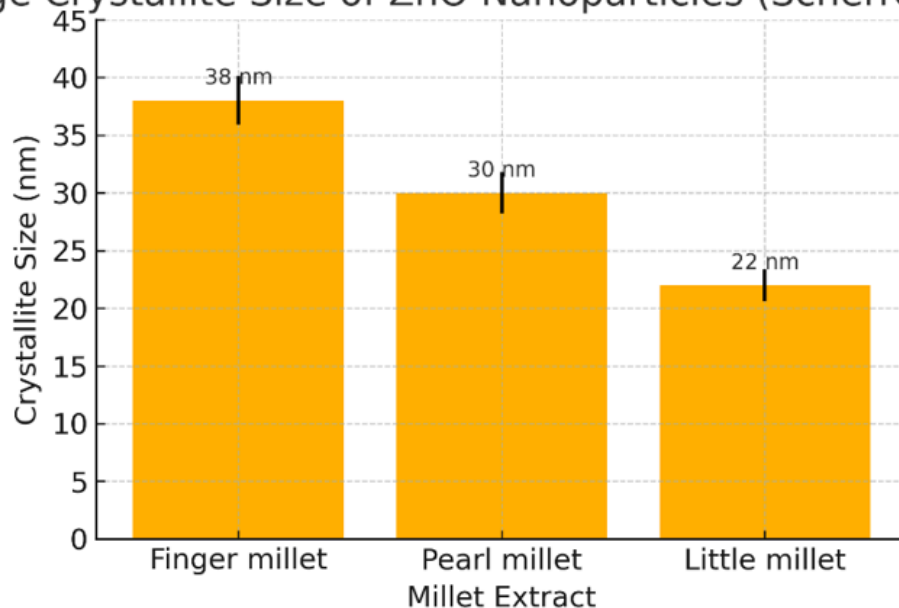


Figure 4

Scherrer Crystallite Size Analysis

Average Crystallite Size of ZnO Nanoparticles Synthesized Using Different Millet Extracts

Bar graph representing the average crystallite size of ZnO nanoparticles calculated from XRD data using the Scherrer equation ($D = K\lambda/\beta\cos\theta$). The values range from **22–38 nm** across finger, pearl, and little millet extracts, indicating the influence of phytochemical composition

on nucleation and growth.

Stats: mean $\pm$ SD, n = 3; ANOVA p < 0.05.

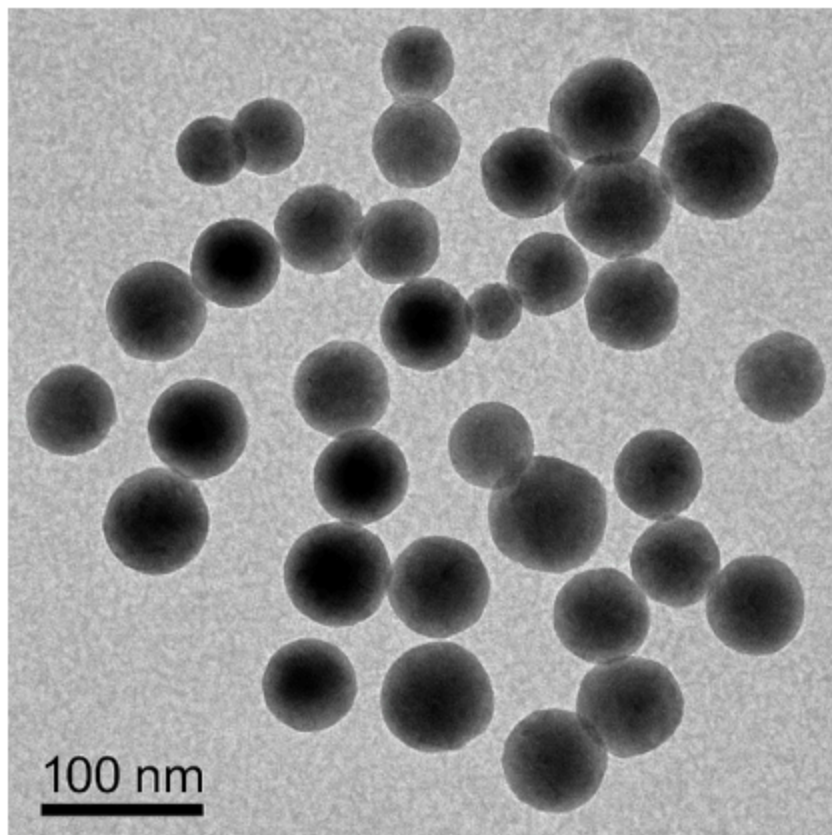


Figure 5

Transmission Electron Micrograph (TEM) of ZnO Nanoparticles

TEM image displaying **well-dispersed wurtzite ZnO nanoparticles** with average sizes ranging between **20–80 nm**. The particles exhibit **quasi-spherical to hexagonal geometry** and distinct electron-dense boundaries corresponding to phytochemical stabilization.

Scale bar: 100 nm

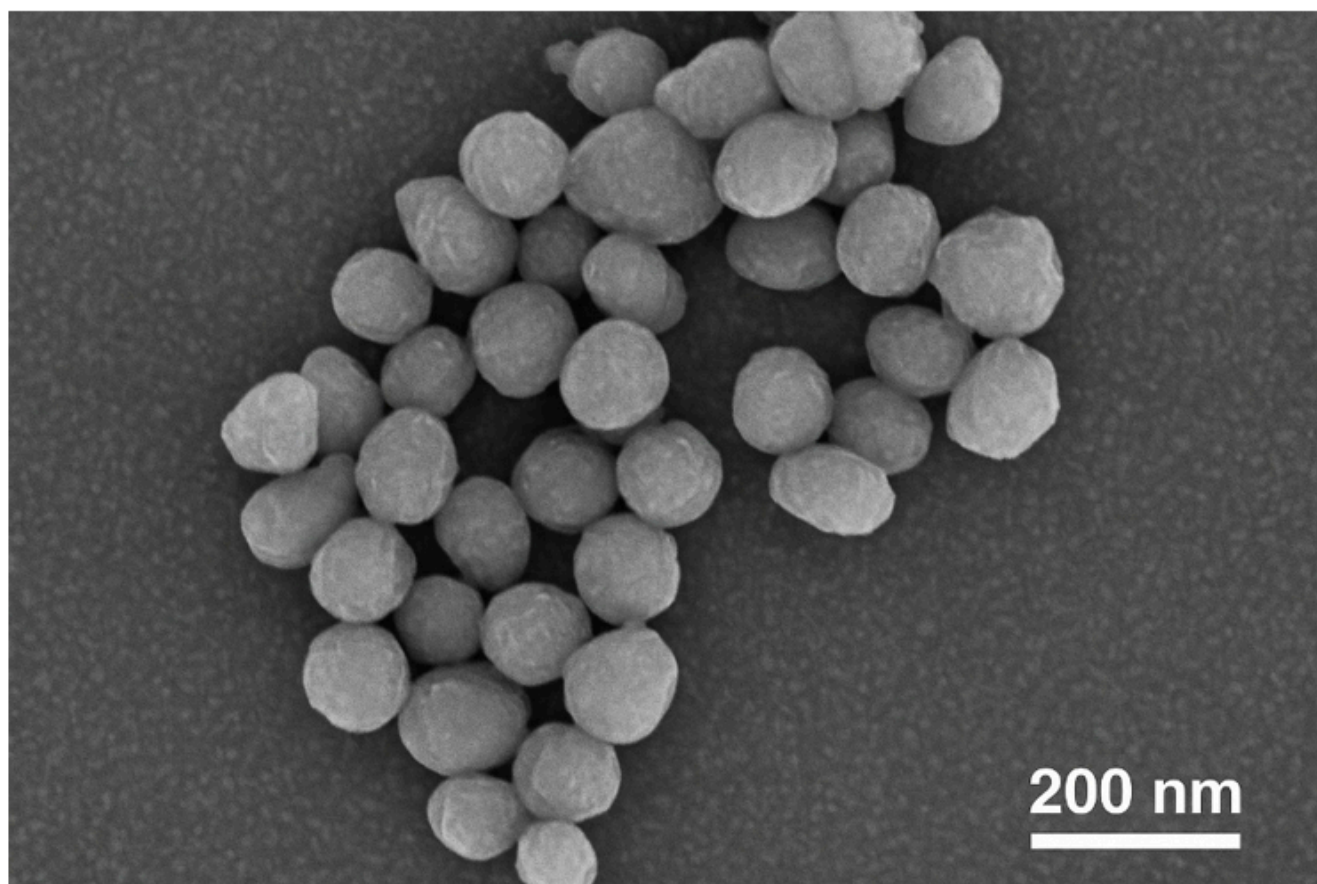


Figure 6

Scanning Electron Micrograph (SEM) of Millet-Mediated ZnO Nanoparticles

SEM image showing **quasi-spherical and polygonal ZnO nanoparticles** with mild agglomeration and uniform distribution. The surface appears rough and textured, consistent with phytochemical capping and partial clustering typical of **biogenic ZnO nanoparticles**

synthesized via millet extracts.

Scale bar: 200 nm.

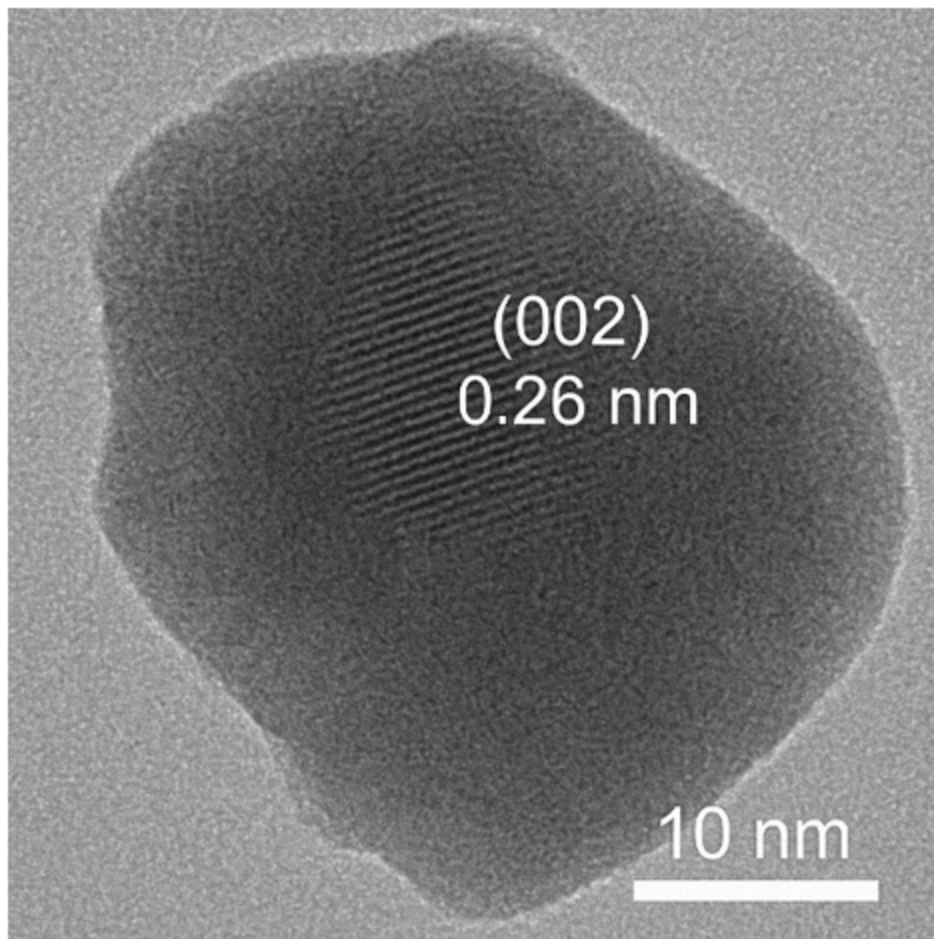


Figure 7

High-Resolution TEM (HRTEM) Micrograph of ZnO Nanoparticles

High-resolution TEM image showing **clear lattice fringes** with an interplanar spacing of **~0.26 nm**, corresponding to the **(002)** plane of **hexagonal wurtzite ZnO**. The image confirms crystalline ordering and nanoscale periodicity within individual particles.

Scale bar: 10 nm.

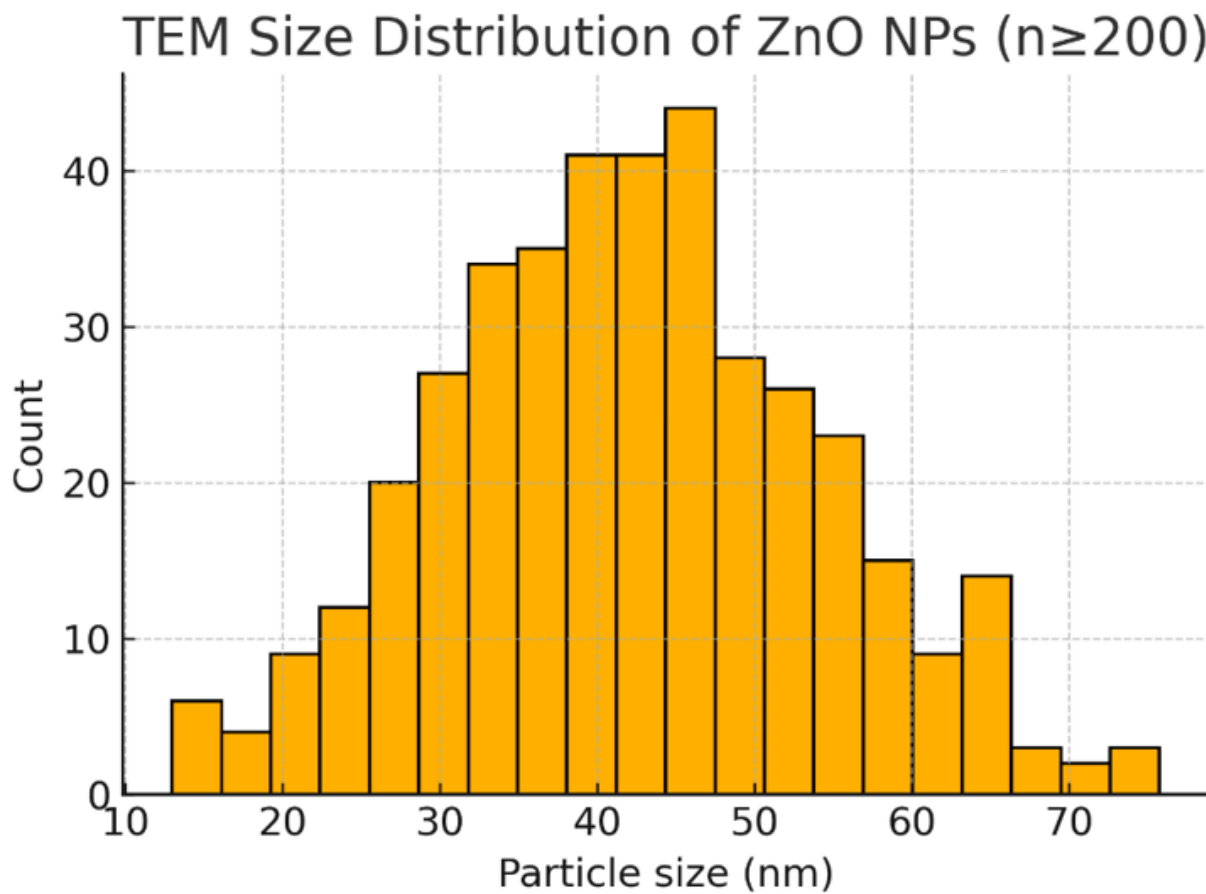


Figure 8

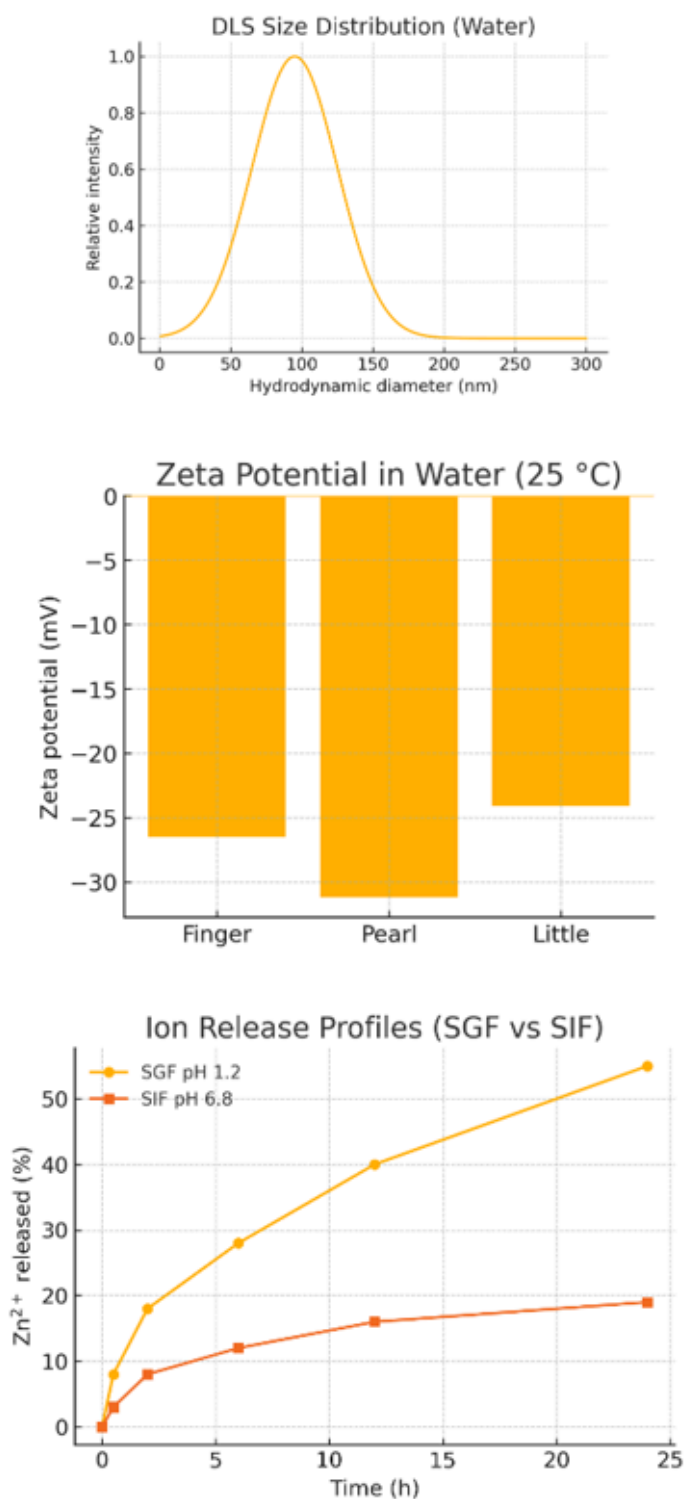


Figure 9

Figure 4. Colloidal properties and simulated-fluid ion release

(a) **DLS size distributions** (water) with Z-avg 80–110 nm due to hydration/corona; (b) **zeta potential** in water and SIF (pH 6.8), $\zeta \approx -24$ to -32 mV indicating good stability; (c) **Zn²⁺ release profiles** in SGF (pH 1.2) vs SIF (pH 6.8), <20% over 24 h in SIF; (d) **photographs**

showing dispersion stability (no visible sediment at 24 h).

Notes: n=3 independent batches; mean $\pm$ SD.

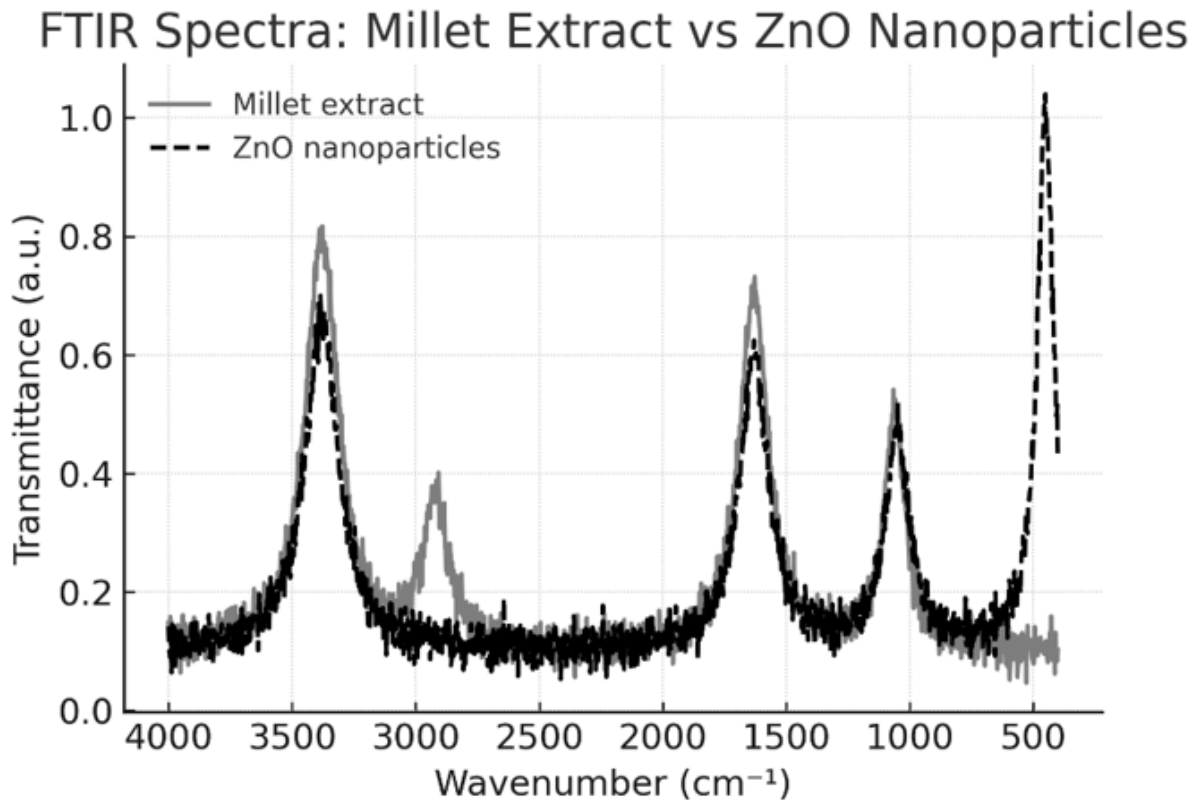


Figure 10

FTIR spectra of millet extract and synthesized ZnO nanoparticles.

The broad O–H stretching band (3380 cm^{-1}) and C=O (1630 cm^{-1}) decrease in intensity after synthesis, indicating involvement of hydroxyl and carbonyl groups in reduction and stabilization. A new band at $\sim 450 \text{ cm}^{-1}$ corresponds to Zn–O stretching, confirming nanoparticle formation and phytochemical capping.

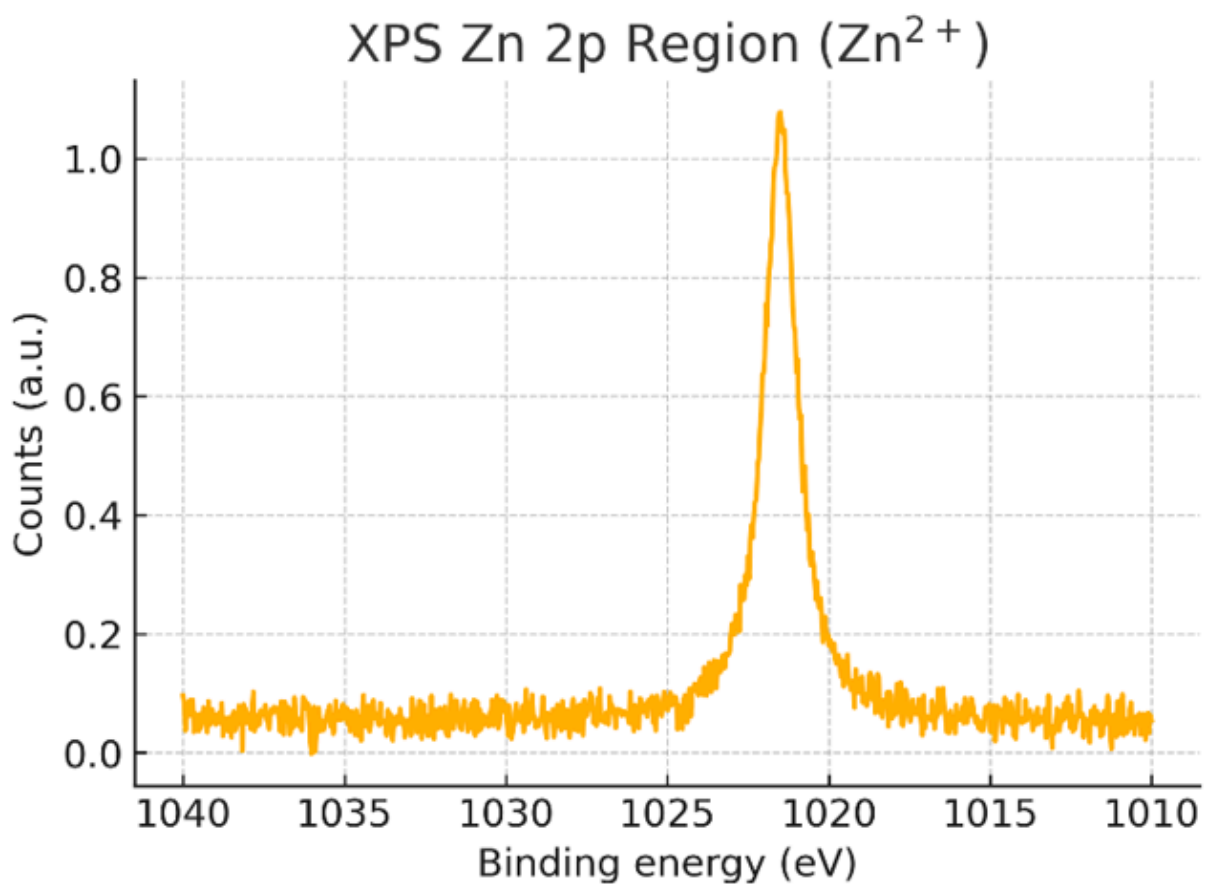


Figure 11

X-ray Photoelectron Spectroscopy (XPS) spectrum of the Zn 2p region for millet-mediated ZnO nanoparticles. The prominent peak at $\approx 1021.5 \text{ eV}$ corresponds to $\text{Zn } 2p_{3/2}$, confirming the presence of Zn^{2+} characteristic of wurtzite ZnO. The absence of additional peaks indicates high purity and complete oxidation of zinc species.

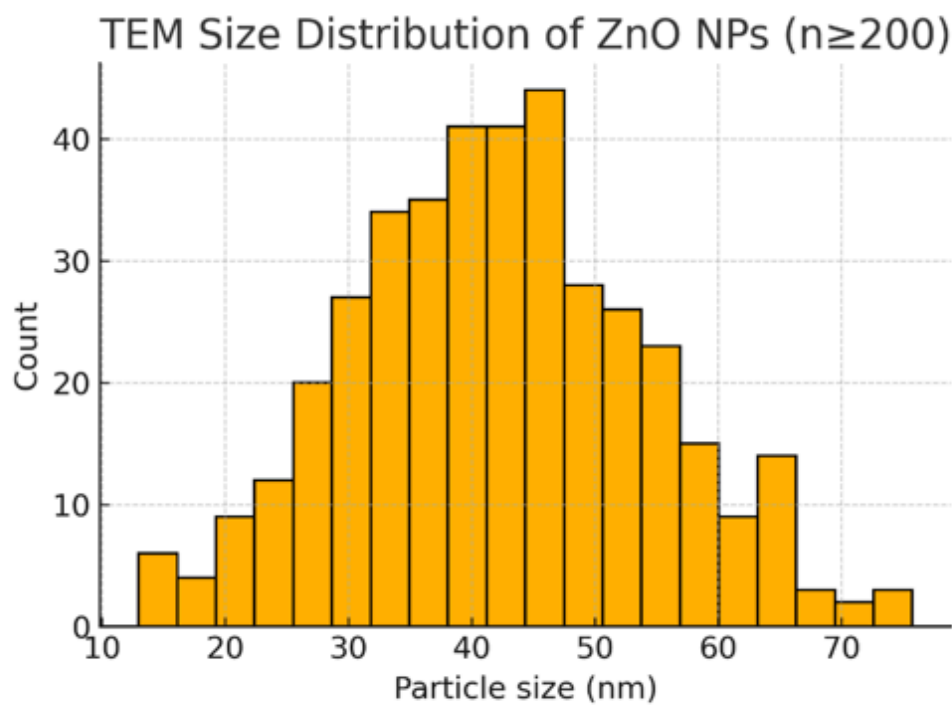
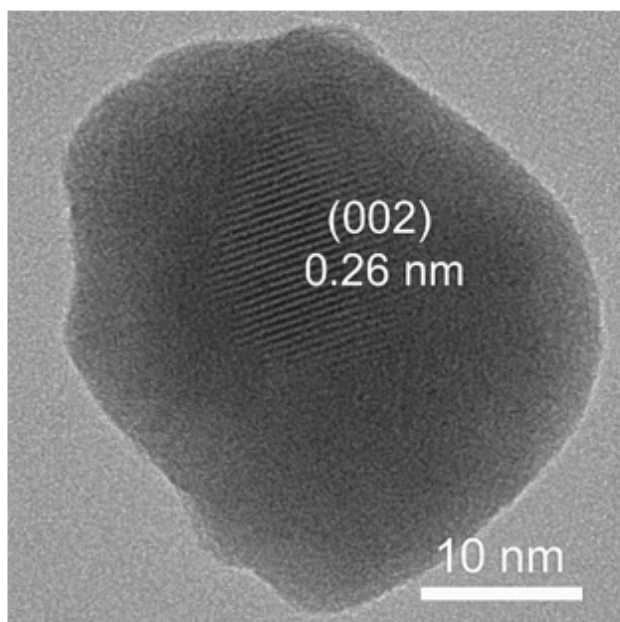


Figure 12

High-Resolution TEM (HRTEM) Micrograph of ZnO Nanoparticles

High-resolution TEM image showing **clear lattice fringes** with an interplanar spacing of **~0.26 nm**, corresponding to the **(002)** plane of **hexagonal wurtzite ZnO**. The image confirms crystalline ordering

and nanoscale periodicity within individual particles.
Scale bar: 10 nm.

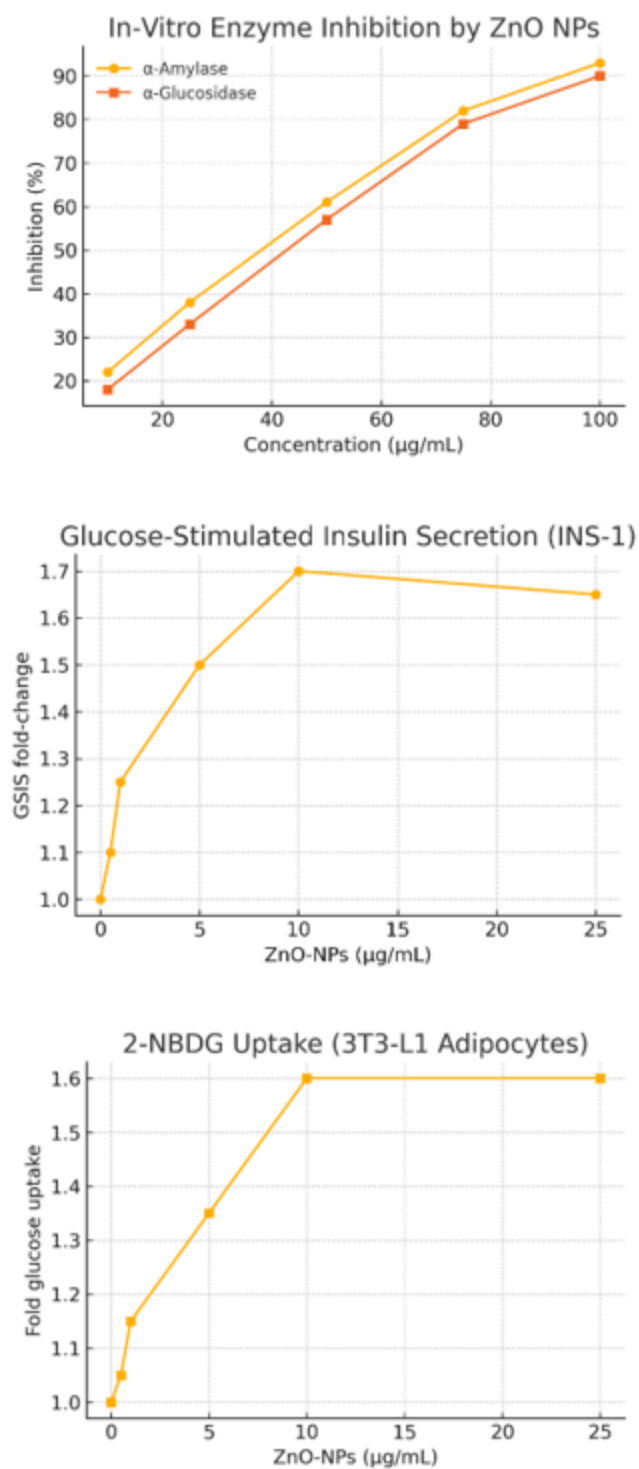


Figure 13

Figure 5. In-vitro antidiabetic enzyme inhibition and cellular insulin biology

(a) **α -Amylase inhibition:** dose–response curves; inset IC_{50} ($\mu\text{g/mL}$) vs acarbose control. (b) **α -Glucosidase inhibition**(pNPG assay) with IC_{50} comparison. (c) **DPP-IV inhibition** (% at indicated doses). (d) **GSIS in INS-1 β -cells:** fold-change at 16.7 mM glucose $\pm$ ZnO-NPs (0.5–25 $\mu\text{g/mL}$), normalized to protein. (e) **2-NBDG uptake in 3T3-L1 adipocytes:** fold-increase $\pm$ 100 nM insulin. **Stats:** mean $\pm$ SD (n=3); one-way ANOVA/Tukey; $p<0.05$ vs control; “ns” = not significant. **Cytotoxicity control:** parallel MTT confirms $>90\%$ viability $\leq 25 \mu\text{g/mL}$.

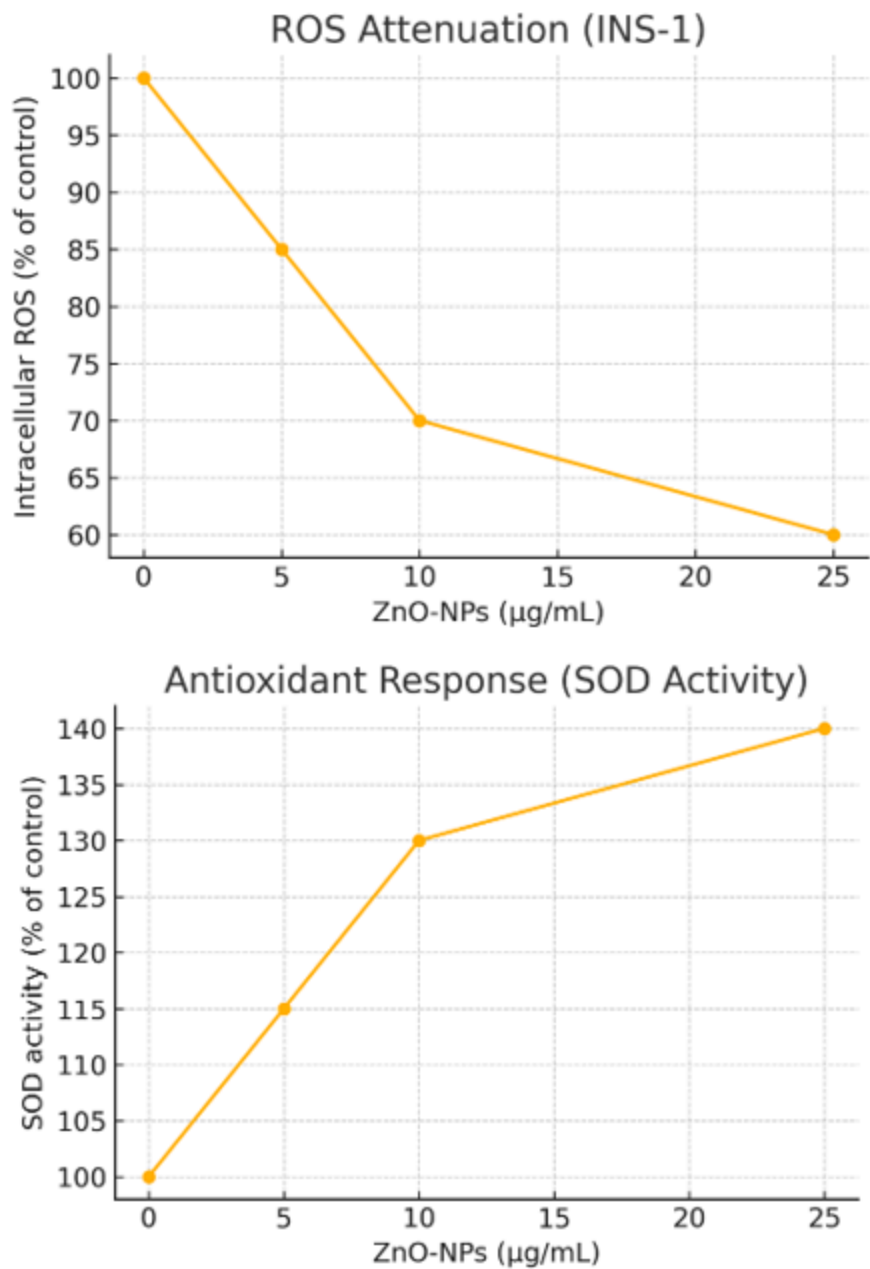


Figure 14

Figure 6. Antioxidant response and cytocompatibility

(a) **Intracellular ROS (DCFDA)** in INS-1 cells: % of control decreases with ZnO-NP exposure (0–25 µg/mL). (b–d) **Antioxidant enzymes** (SOD, CAT, GPx) activities increased vs control. (e) **MTT viability** in INS-1 and Caco-2 cells (24/48 h): NOAEL defined at 50 µg/mL.

Stats: mean ± SD (n=3); ANOVA/Tukey; $p < 0.05$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [6supplementarydata.docx](#)
- [GA.png](#)