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MORPHOLOGICAL AND SIZE CHARACTERIZATION OF ZINC OXIDE NANOPARTICLES AND EVALUATION OF THEIR CYTOTOXICITY ON THE MCF-7 CELL LINE

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The aim. *This study aimed to synthesize zinc oxide nanoparticles (ZnO NPs) using a green method based on Scutellaria Iscanderi L. extract and to evaluate their physicochemical properties and in vitro cytotoxic effects on MCF-7 human breast cancer cells.*

Methods. *ZnO nanoparticles were obtained via green synthesis using the aqueous extract of Scutellaria Iscanderi L. as a reducing and stabilizing agent. The morphology, size, and distribution of the nanoparticles were analyzed by atomic force microscopy (AFM), scanning electron microscopy (SEM), and dynamic light scattering (DLS). Elemental composition was determined by SEM-EDX. Cytotoxic activity was assessed using the CCK-8 assay on MCF-7 breast adenocarcinoma cells.*

Results. *The synthesized ZnO NPs exhibited predominantly spherical morphology with a size range of 40–120 nm. DLS measurements showed a mean particle diameter of ~40 nm and a polydispersity index of 0.3, indicating good colloidal stability. EDX confirmed the presence of zinc with a content of 6.87% by mass. Cytotoxicity analysis revealed a dose-dependent reduction in cell viability, with an IC_{50} value of 126.4 µg/mL.*

Conclusion. *Green-synthesized ZnO nanoparticles demonstrated favorable structural characteristics and moderate cytotoxic effects against MCF-7 cells. These findings suggest their potential application as a basis for further development of anticancer nanotherapeutics*

Keywords: *zinc oxide nanoparticles, green synthesis, Scutellaria Iscanderi, breast cancer, MCF-7, cytotoxicity, atomic force microscopy (AFM), scanning electron microscopy (SEM), dynamic light scattering (DLS)*

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1. Introduction

In recent years, the scope of nanoparticle application in clinical practice has significantly expanded. Nanoparticles are being developed to overcome the limitations associated with free therapeutic agents and to effectively traverse biological barriers-systemic, cellular, and microenvironmental – which vary depending on the disease and individual patient characteristics [1, 2]. Nanotechnology has attracted increasing attention due to its ability to produce materials with nanoscale dimensions (1–100 nm), offering improved physicochemical and biological properties compared to conventional systems.

Among various types of nanomaterials, zinc oxide nanoparticles (ZnO NPs) have garnered considerable interest due to their broad potential in biomedicine, environmental applications, and electronics [3, 4]. According to [5, 6], ZnO nanoparticles demonstrate high biocompatibility, low toxicity, and cost-effectiveness, making them promising candidates for biological applications. The physicochemical properties of nanoparticles – including size, morphology, size distribution, surface charge, aggregation state, hydrophilicity, and surface functionalization – play a critical role in determining their behavior in biological systems. These parameters directly influence cellular uptake kinetics, biodistribution, permeability through physiological barriers, as well as cytotoxicity and biocompatibility profiles. Therefore, comprehensive analysis of these characteristics is essential for predicting interactions with biological targets, optimizing dosage, and designing safe and effective nanomaterials for medical use. Furthermore, in-depth surface characterization enables precise functionalization and the development of targeted drug delivery systems [7, 8].

To thoroughly evaluate the physicochemical characteristics of the ZnO nanoparticles, this study employed advanced analytical techniques. The morphology and surface features of the nanoparticles were assessed using atomic force microscopy (AFM) and scanning electron microscopy (SEM), providing detailed visualization of shape and topography. Dynamic light scattering (DLS) was used to determine the hydrodynamic size and polydispersity of the nanoparticles in suspension. Together, these techniques offered a comprehensive understanding of the structural and dimensional properties of the tested material [9, 10].

Breast cancer (BC) is the most commonly diagnosed cancer and remains the leading cause of cancer-related mortality among women worldwide [11]. Its treat-

ment requires a multidisciplinary approach involving surgical intervention, radiotherapy, and both neoadjuvant and adjuvant therapies. Effective management of breast cancer aims to achieve maximum therapeutic efficacy with minimal adverse effects, thereby maintaining the patient's quality of life. A carefully selected combination of therapeutic modalities allows patients to benefit from reduced recurrence, minimized resistance and toxicity, and improved long-term outcomes [12, 13]. To evaluate the biological activity of the ZnO nanoparticles, the Michigan Cancer Foundation-7 (MCF-7) cell line was employed [14, 15]. This human breast adenocarcinoma cell model is one of the most validated in vitro systems for screening anticancer agents, owing to its stable proliferative capacity, reproducibility of results, and high sensitivity to cytotoxic exposure. The use of MCF-7 cells enabled a quantitative assessment of the cytotoxicity of the test material, as well as observation of dose-dependent changes in cancer cell viability. The findings provide a solid foundation for further preclinical validation of ZnO nanoparticles as potential anticancer agents [16, 17].

The aim of the research. This study investigates the morphology and size of zinc oxide nanoparticles synthesized using *Scutellaria Iscanderi L.* extract, and evaluates their cytotoxicity on MCF-7 human breast cancer cells.

2. Planning (methodology) of research

1. Preparation of plant extract.
2. Green synthesis of ZnO nanoparticles.
3. Physicochemical characterization.
4. In vitro cytotoxicity assessment.

3. Materials and methods

The study utilized an extract of *Scutellaria Iscanderi L.* for the green synthesis of zinc oxide nanoparticles synthesized via a green synthesis approach using the aforementioned plant extract.

The reducing and stabilizing role of the extract is attributed to its high content of phytochemicals, mainly flavonoids and phenolic acids, which act as reducing agents by converting Zn^{2+} ions into ZnO nanoparticles, while simultaneously adsorbing on the nanoparticle surface and preventing agglomeration, thereby serving as natural stabilizers.

For atomic force microscopy (AFM), nanoparticles were immobilized on atomically smooth substrates. Depending on the analysis objective, mica, graphite, and polymer films were primarily used. These materials are traditionally preferred in AFM studies due to their flat surface topography and compatibility with high-resolution imaging.

To obtain detailed information about the surface characteristics of the nanoparticle samples, scanning electron microscopy (SEM) was employed. The JSM-IT210 model from JEOL (Japan) is one of the modern SEM systems widely used for the analysis of nanoscale materials, including nanoparticles. This instrument allows for high-resolution imaging of nanoparticle morphology and their distribution within various matrices or on material surfaces. For SEM analysis, samples were prepared by depositing a thin conductive layer onto a substrate, followed by moisture removal and fixation of the sample on the specimen holder [2, 18].

To determine surface charge, nanoparticle distribution in solution, and colloidal stability, dynamic light scattering (DLS) was used. This technique is based on the scattering of a laser beam by particles or macromolecules in a liquid medium due to their Brownian motion.

An initial stock solution of the ZnO nanoparticles was prepared by dissolving 10 mg of powder in 10 mL of distilled water, with continuous stirring until a homogeneous solution was achieved. The resulting dispersion was filtered through a 0.45 μm membrane to remove any insoluble particles or aggregates. The filtered samples were then transferred into specialized plastic cuvettes for DLS analysis, ensuring minimal air bubble formation. Prior to measurement, the samples were incubated at room temperature for 30 minutes to allow stabilization. Measurements were performed using a Potocor Compact DLS analyzer, calibrated in accordance with the manufacturer's guidelines. Each sample was analyzed at a fixed temperature (typically 25°C), with a minimum of three replicates to ensure statistical relevance. Particle size and size distribution were determined based on the analysis of the recorded light scattering data [19, 20].

In this study, the cytotoxicity experiment was performed only once using technical replicates, and therefore no formal statistical analysis (such as ANOVA or Student's *t*-test) was carried out. The results were presented as preliminary findings to demonstrate the dose-dependent reduction in cell viability. Future work will include additional experiments with biological replicates and comprehensive statistical evaluation to provide more robust and comparable results.

4. Results

To further investigate our zinc oxide nanoparticle-containing substance, it was appropriate to study the shape and size of the formed nanoparticles. As part of this study, microscopic images of the substance containing zinc oxide nanoparticles were obtained using atomic force microscopy (AFM). The resulting images are presented in Fig. 1.

Fig. 1 shows a 3D image of the surface ultrastructure with molecular resolution, captured in real time under physiological conditions.

The scan profile, 49.8 nm in length with a resolution of 144 points, revealed nanoparticles with variable height ranging from approximately 40 to 120 nm, indicating significant surface topography fluctuations. The maximum peak height reached ~225 nm, as indicated by the color scale in the 3D visualization (Fig. 2).

Such morphological heterogeneity may reflect a high specific surface area and reactivity, suggesting a strong potential for interactions with biomolecules and other substances – features that are highly desirable in the development of nanomaterials for biomedical, catalytic, and sensor applications.

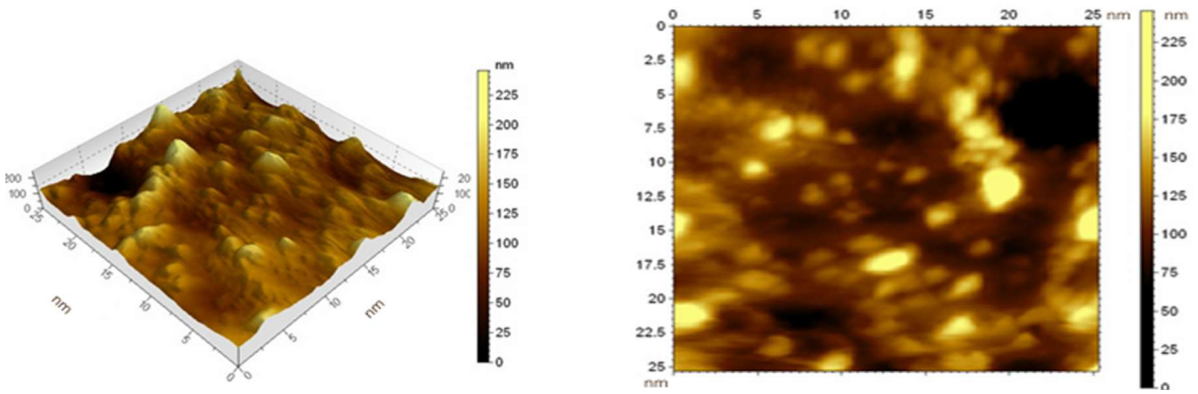


Fig. 1. Microscopic analysis of zinc oxide nanoparticles synthesized using *Scutellaria Iscanderi* L. extract by atomic force microscopy (AFM)

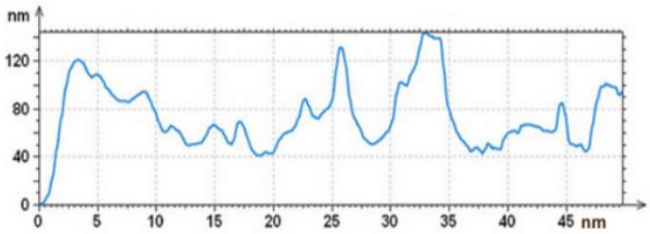


Fig. 2. AFM image of zinc oxide nanoparticles. Analysis of particle morphology, length, and width within the tested substance

According to the analysis, the predominant size of zinc oxide nanoparticles synthesized via green synthesis using *Scutellaria Iscanderi* L. extract was 25 nm (representing 75% of the particles), while the main size distribution ranged from 25 to 120 nm (25%).

Subsequently, the substance was analyzed using dynamic light scattering (DLS) with a Potocor Compact analyzer.

To characterize particle size variability, the polydispersity index (PdI) was calculated. This parameter reflects the width of the particle size distribution:

PdI < 0.1 – narrow distribution, high monodispersity;

0.1 < PdI < 0.3 – moderate polydispersity;

PdI > 0.3 – broad distribution, high polydispersity (Fig. 3).

The histogram shows that ZnO nanoparticles ranged in size from 25 to 150 nm, with an average diameter of approximately 40 nm. The even distribution pattern suggests good dispersion and minimal aggregation. This effect is likely due to the stabilizing role of phytochemicals present in the *Scutellaria Iscanderi* L. extract used during green synthesis. The X-axis represents particle size in nanometers (nm), while the Y-axis reflects the intensity of scattered light or the relative particle count. Low intensity in the region below 50 nm suggests the presence of minor small-size fractions. A PdI value of 0.3 indicates a relatively narrow and well-controlled size distribution.

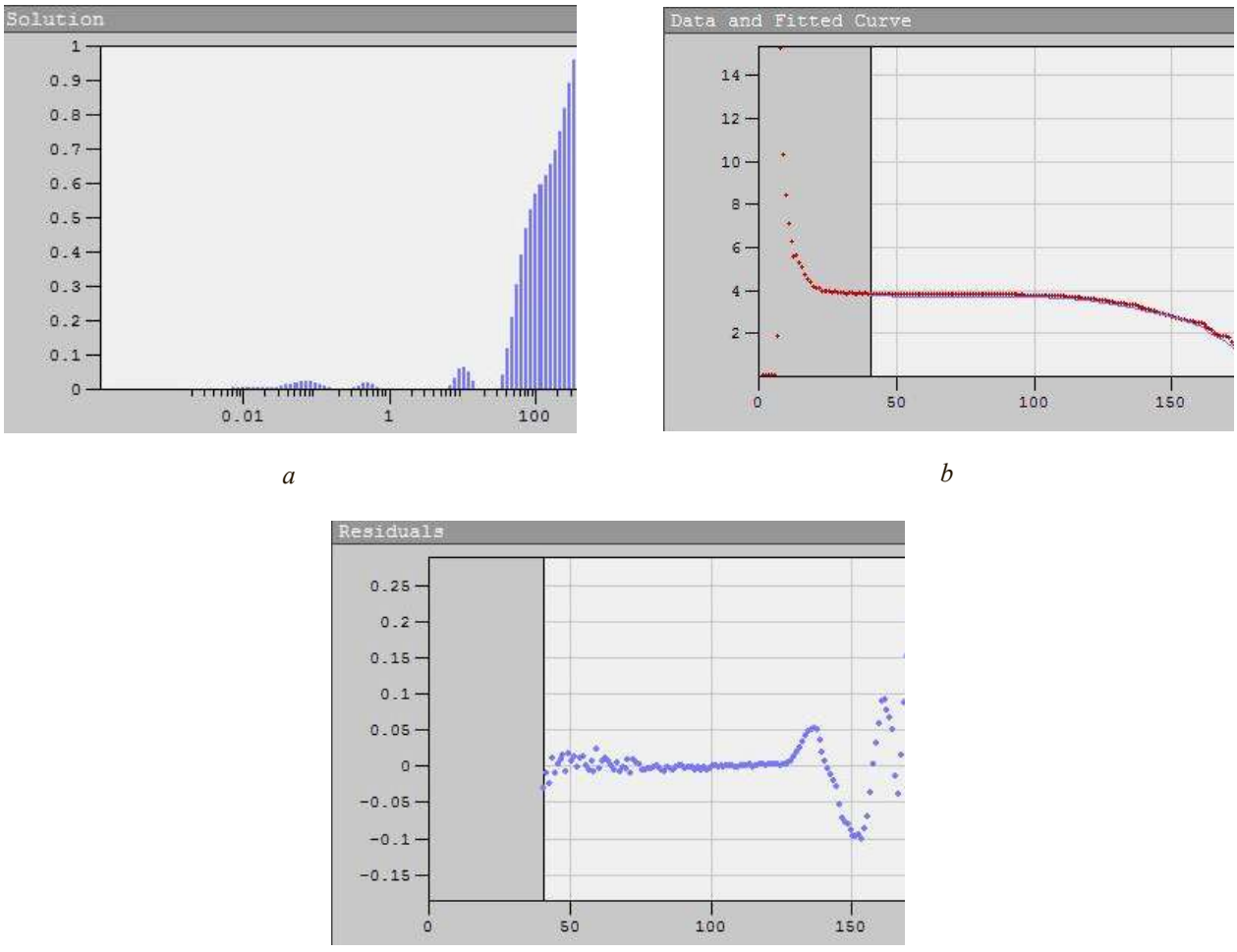
To further assess particle morphology, scanning electron microscopy (SEM) was employed. This high-resolution technique enables detailed visualization of particle shape and distribution on the sample surface, providing critical insights into the physicochemical properties of the nanoparticles.

The image clearly reveals nanoparticles with a predominantly spherical morphology, indicating uniform shape distribution and characteristic structural features of ZnO synthesized via green methods (Fig. 4).

The spectrum reveals distinct peaks corresponding to zinc, confirming the successful formation of zinc oxide nanoparticles. Minor elements such as C, Na, Mg, Al, Si, Cl, K, and Ca were also detected, most likely due to residual phytochemicals from the *Scutellaria Iscanderi* L. aqueous extract used during green synthesis. Since the nanoparticles were not purified prior to analysis, these trace elements remained present in the sample (Fig. 5).

The findings confirm the feasibility of using *Scutellaria Iscanderi* L. extract in the biosynthesis of ZnO nanoparticles. Based on EDX analysis, the zinc content in the substance was determined to be 6.87% by mass, which is consistent with the expected composition of biosynthesized ZnO nanomaterials.

In vitro evaluation of the cytotoxicity of the zinc oxide nanoparticle-containing substance. In vitro studies were carried out to assess the acute toxicity of the substance containing zinc oxide nanoparticles using the MCF-7 cell line (human breast adenocarcinoma). MCF-7 breast adenocarcinoma cells were selected because breast cancer is the most prevalent malignancy in women worldwide, and this cell line is one of the most validated and reproducible in vitro models for evaluating anticancer activity of nanoparticles. The experiments were conducted at the “Scientific Laboratory of Innovative Pharmaceutical Compounds” of the Tashkent Pharmaceutical Institute. At the initial stage, the MCF-7 cells were cultured for 2–3 days until the required confluency was achieved. Subsequently, the cells were seeded into 96-well plates at a density of 10,000 cells per well and incubated for 24 hours to allow for proper adhesion and recovery under standard incubator conditions (Fig. 6).



a *b*

c

Fig. 3. DLS histograms of ZnO nanoparticle size distribution: *a* – full size range from 25 to 150 nm; *b* – predominant fraction centered at ~40 nm; *c* – distribution curve demonstrating PdI = 0.3, indicating moderate monodispersity and good colloidal stability

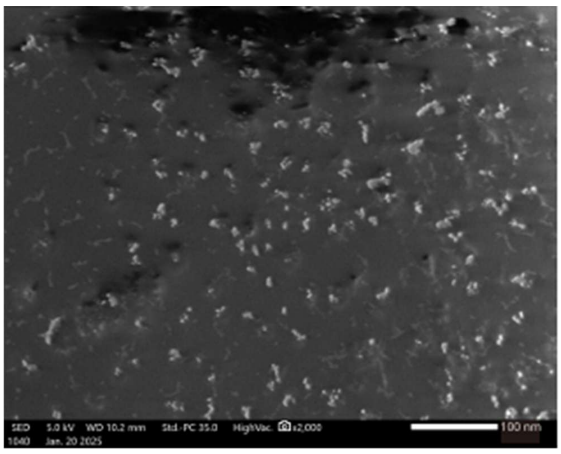
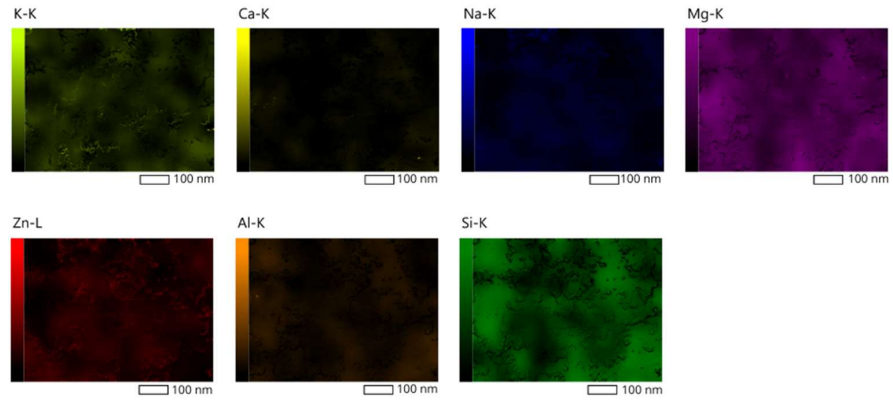


Fig. 4. SEM micrograph of zinc oxide nanoparticles

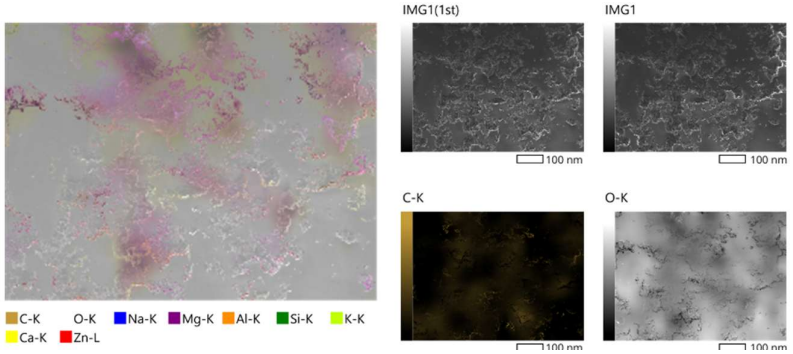
To evaluate the cytotoxic effect of the ZnO nanoparticles, a series of test solutions was prepared. The stock solution (5 mg/mL) was initially dissolved in distilled water. Serial dilutions were then performed to obtain intermediate concentrations of 2.5 mg/mL, 1.0 mg/mL, 0.5 mg/mL, and 0.25 mg/mL. Each solution was subsequently diluted 1:100 in culture medium (DMEM supplemented with 10% FBS and 1% antibiotics) to achieve final working concentrations of 500, 250, 100, 50, and 10 µg/mL, respectively (Fig. 7).

After a 24-hour preincubation period, the culture medium was replaced with fresh medium containing the corresponding concentrations of the test substance. The plates were then incubated for an additional 24 hours to allow the compound's effects to manifest. Following incubation, the medium was removed and CCK-8 reagent diluted in PBS was added to each well. The plates were further incubated for 3 hours under standard conditions (37 °C, 5% CO_2) to assess cell viability (Fig. 8).

After incubation with the CCK-8 reagent, optical density was measured using an ELISA microplate reader (Model: ELMR-112) at the wavelength recommended by the kit manufacturer. The experimental data were analyzed to determine the IC_{50} value – the concentration at which cell viability is reduced by 50%. The selected concentration range was based on data from previous studies of similar substances. The IC_{50} value for MCF-7 cells treated with the zinc oxide nanoparticle-containing substance was determined to be 126.4 µg/mL. A significant dose-dependent reduction in cell viability was observed: at 25 µg/mL, viability was reduced by approximately 25%, and at 50 µg/mL – by around 30% (Fig. 9).



a



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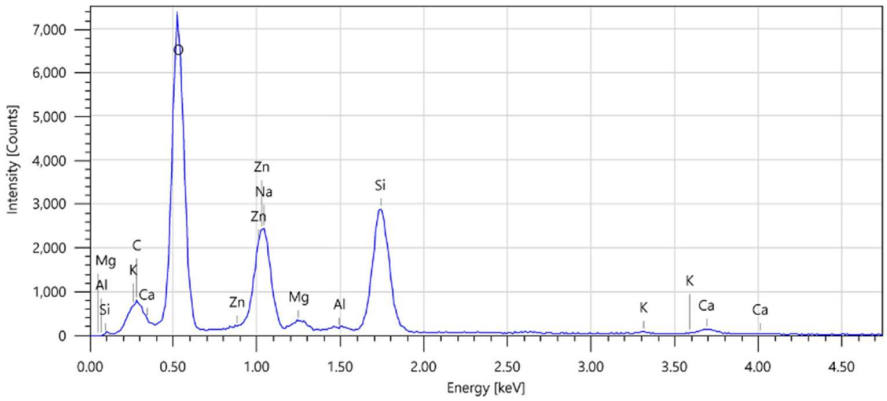
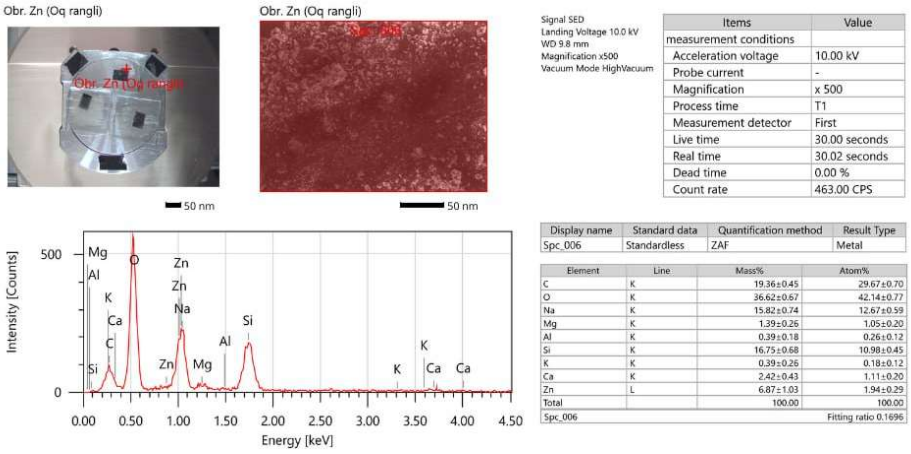


c



Signal SED
Landing Voltage 10.0 kV
WD 9.8 mm
Magnification x500
Vacuum Mode HighVacuum

Items	Value
measurement conditions	
Acceleration voltage	10.00 kV
Probe current	-
Magnification	x 500
Process time	T1
Measurement detector	First
Live time	30.00 seconds
Real time	30.02 seconds
Dead time	0.00 %
Count rate	463.00 CPS

Display name	Standard data	Quantification method	Result Type
Spc_006	Standardless	ZAF	Metal

Element	Line	Mass%	Atom%
C	K	19.36±0.45	29.67±0.70
O	K	36.62±0.67	42.14±0.77
Na	K	15.82±0.74	12.67±0.59
Mg	K	1.39±0.26	1.05±0.20
Al	K	0.39±0.18	0.26±0.12
Si	K	16.75±0.68	10.98±0.45
K	K	0.39±0.26	0.18±0.12
Ca	K	2.42±0.43	1.11±0.20
Zn	L	6.87±1.03	1.94±0.29
Total		100.00	100.00
Spc_006			Fitting ratio 0.1696

d

Fig. 5. SEM-EDX spectra of ZnO nanoparticles: *a* – overall spectrum highlighting Zn peaks; *b* – spectrum region for light elements (C, Na, Mg); *c* – spectrum region for intermediate elements (Al, Si, Cl); *d* – spectrum region showing K and Ca, likely from residual plant-derived compounds

Fig. 6. Cultivation and seeding of cells into 96-well plates

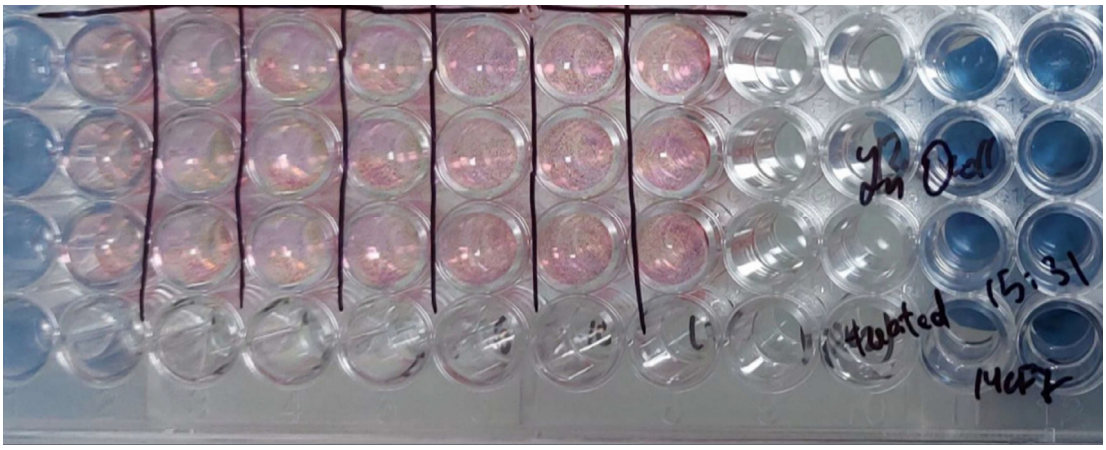


Fig. 7. Effect of the zinc oxide nanoparticle-containing substance on MCF-7 cancer cells

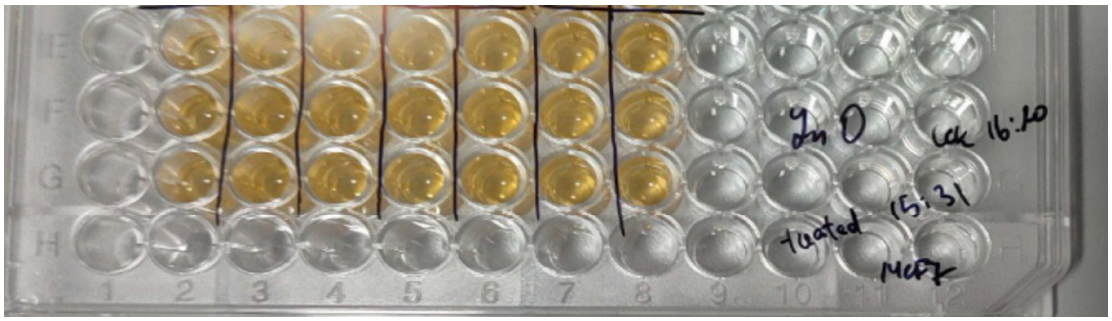


Fig. 8. Results of cell viability assessment of MCF-7 cells using the CCK-8 assay

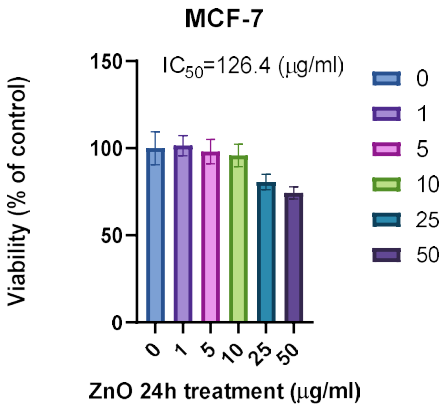


Fig. 9. Cytotoxicity analysis of the zinc oxide nanoparticle-containing substance on the human breast cancer cell line MCF-7

The IC_{50} value for the zinc oxide nanoparticle-based substance was determined to be 126.4 μg/mL, with a clear dose-dependent decrease in cell viability observed as the concentration increased.

5. Discussion

In the present study, zinc oxide nanoparticles (ZnO NPs) were synthesized via a green route employing an aqueous extract of *Scutellaria Iscanderi* L., yielding predominantly spherical particles with nanoscale dispersion. Atomic force microscopy (AFM) and scanning electron microscopy (SEM) revealed spherical morphologies with a dominant fraction of ~ 25–40 nm, while dynamic light scattering (DLS) indicated a mean hydrodynamic diameter of approximately 40 nm and an overall distribution range of 25–150 nm. The close agreement

between the microscopic (AFM/SEM) and hydrodynamic (DLS) size values is characteristic of plant-mediated synthesis protocols, in which phytochemical constituents facilitate the formation of stable, well-dispersed spherical nanoparticles on the scale of several tens of nanometers [2]. The polydispersity index (PdI ≈ 0.3) observed in this work reflects moderate size uniformity and satisfactory colloidal stability of the suspension. In the context of green synthesis, such PdI values are frequently associated with phytomolecule capping, wherein plant-derived biomolecules function simultaneously as reducing and stabilizing agents, suppressing aggregation and improving dispersion stability in aqueous media. This stabilization mechanism likely underlies the morphological reproducibility achieved here despite the simplicity of the synthesis protocol [21]. SEM–EDX elemental mapping confirmed zinc as the primary constituent, accompanied by trace elements (C, Na, Mg, Al, Si, Cl, K, Ca). These minor components are most plausibly residual bioorganic and mineral compounds originating from the *S. Iscanderi* extract – such as loosely bound polyphenols, sugars, and ions – which may alter surface properties, including protein adsorption capacity and zeta potential, and therefore influence biological interactions. Similar elemental profiles have been reported for other unpurified plant-mediated ZnO NPs [22].

In vitro testing on the MCF-7 human breast adenocarcinoma cell line demonstrated a clear dose-dependent cytotoxic effect, with an IC_{50} value of 126.4 μg/mL. The literature consistently indicates that ZnO NP-induced cytotoxicity is primarily mediated through overproduction of reactive oxygen species (ROS), disruption of mitochondrial function, activation of caspases, and induction of apoptotic pathways – mechanisms well-documented for both chemically and biogenically synthesized ZnO NPs. In some reports, phytochemical surface coatings attenuate direct nanoparticle–membrane interactions, shifting IC_{50} values towards higher concentrations relative to chemically synthesized analogues [19].

The degree of consistency between our results and those reported in similar international studies is substantial. For instance, Mongy and Shalaby (National Research Centre, Cairo, Egypt) reported biosynthesized ZnO NPs using *Rhus coriaria* fruit extract with an average size of approximately 20.5 nm and a clear dose-dependent cytotoxicity toward both MCF-7 and MDA-MB-231 cells, with IC_{50} values in the range of 35.0–44.9 μg/mL for MCF-7 and 55.5–63.7 μg/mL for MDA-MB-231 [20]. Comparable systems have also been developed internationally, such as ZnO NPs synthesized from *Camellia sinensis* extracts by Mandal and colleagues (Tribhuvan University, Nepal, in collaboration with Indian research centers) [23], and from *Pelargonium odoratissimum* by Abdelbaky et al. (Assiut University, Egypt) [21]. In addition, Anjum et al. (Quaid-i-Azam University, Islamabad, in collaboration with Indian institutions) reviewed the applications of ZnO NPs for cancer diagnosis and therapy [19]. These studies highlight the ongoing exploration of topical oncological, antimicrobial, wound-healing, and anticancer applications of green-synthesized ZnO nanoparticles across different scientific centers. Nevertheless, reported IC_{50} values vary widely, which may be explained by differences in phytochemical composition of the plant extract, post-synthesis purification, nanoparticle aggregation behavior, and methodological variations in cytotoxicity assays. Such discrepancies emphasize the urgent need for standardized synthesis protocols, purification steps, and biological testing methods to enable reproducible and comparable results across laboratories.

Practical relevance. The practical relevance of this work lies in the potential translation of *S. Iscanderi*-derived ZnO NPs into topical or localized anticancer therapies. Their demonstrated nanoscale size, stability, and cytotoxicity profile make them promising candidates for incorporation into gels, ointments, or wound dressings designed for the management of superficial malignant lesions, where controlled local release can minimize systemic exposure and reduce side effects. Importantly, the potential scope of application is not limited to oncology; these nanoparticles could also be adapted for antimicrobial coatings, wound-healing formulations, and dermatological treatments, expanding their pharmaceutical utility. The inherent phytochemical coating from *S. Iscanderi* may confer additional bioactivity and biocompatibility, offering an advantage over purely chemically synthesized ZnO NPs. The environmentally benign nature of the synthesis further enhances its appeal for pharmaceutical manufacturing, aligning with sustainable production principles and reducing regulatory concerns associated with toxic reagents.

Research limitations. Nevertheless, several limitations should be acknowledged. The cytotoxicity evaluation was restricted to a single cancer cell line (MCF-7), without inclusion of normal, non-malignant cells, thereby precluding selectivity analysis and therapeutic index determination. The nanoparticles were not subjected to rigorous post-synthetic purification, leaving residual organic and mineral components that could influence both physicochemical properties and biological effects. Mechanistic pathways underlying cytotoxicity – such as reactive oxygen species (ROS) generation, mitochondrial membrane depolarization, and caspase-mediated apoptosis – were not directly assessed, limiting mechanistic interpretation. Finally, the absence of in vivo pharmacokinetic, biodistribution, and toxicity studies constrains the translational scope of these findings.

Prospects for further research. Future research should address these gaps by performing parallel testing on malignant and non-malignant cell lines to determine selectivity; conducting mechanistic assays to quantify ROS levels, mitochondrial potential changes, and apoptosis-related protein expression; optimizing synthesis parameters to improve uniformity and reproducibility; and implementing preclinical in vivo studies to evaluate safety, biodistribution, and therapeutic efficacy. Such efforts will be essential to move this promising green-synthesized nanomaterial toward clinical applicability and integration into modern pharmaceutical systems.

6. Conclusion

Based on a comprehensive physicochemical analysis, the zinc oxide nanoparticle-containing substance synthesized using the extract of *Scutellaria Iscanderi* L. exhibited stable spherical morphology and nanoscale dimensions. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) revealed that the particle sizes predominantly ranged from 40 to 120 nm, with a homogeneous structure and well-defined spherical shape. Dynamic light scattering (DLS) further confirmed the colloidal stability of the nanoparticles in suspension, showing a size distribution from 25 to 150 nm with a mean diameter of approximately 40 nm, consistent with SEM data. These characteristics indicate that the material is highly reproducible and suitable for biomedical applications. The cytotoxic activity was evaluated in vitro using the MCF-7 breast cancer cell line, revealing a significant dose-dependent decrease in cell viability, with an IC_{50} value of 126.4 μg/mL.

The observed inhibitory effect of ZnO nanoparticles may involve multiple mechanisms, including oxidative stress, ROS generation, disruption of membrane integrity, and imbalance of intracellular homeostasis, which together can trigger apoptosis in cancer cells. However, ROS induction is not the only mechanism of anticancer action and excessive ROS may also affect healthy tissues. Therefore, further studies – including ROS quantification, antioxidant rescue assays, and selectivity testing on non-malignant cells – are necessary to clarify the therapeutic window [26–28].

Thus, the findings indicate the promising potential of the tested substance as a candidate for further preclinical evaluation in the field of anticancer nanotherapeutics.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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Data availability

Manuscript has no associated data.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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