



Structure-Property-Activity Relationships of ZnO Nanoparticles Phytochemically Synthesized from Pumpkin Pulp and Peels

Bouzidi Ali^{1,2*}, Zerrouki Hayat¹, Rebiai Abdelkarim², Belfar Mohamed Lakhder¹, Bellila Abdelkader³, Mokadem Khadra¹ and Ghiaba Zineb¹

¹VPRS Laboratory, Faculty of Mathematics & Matter Sciences, University of Kasdi Merbah, B.P. 511, 30000, Ouargla, Algeria.

²ACE Laboratory, Faculty of Exact Sciences, University of Echahid Hamma Lakhdar, 39000 El-Oued, Algeria.

³LABTHOP Laboratory, Faculty of Exact Sciences, University of Echahid Hamma Lakhdar, 3900, El Oued, Algeria.

*Corresponding Author E-mail: alibouzidi1994@gmail.com

Received : 23/06/2026 | Accepted : 01/09/2026 | Published: 01/09/2026

Abstract

The development of functional nanomaterials through environmentally sustainable approaches has attracted growing interest, particularly in the use of plant-derived resources as alternatives to conventional synthesis methods. In this study, zinc oxide nanoparticles (ZnO NPs) were synthesized using two pumpkin-derived extracts, obtained from the pulp and peel, to investigate how the nature of the biological medium influences their physicochemical characteristics and biological performance. The resulting nanoparticles were characterized using ultraviolet–visible spectroscopy (UV–Vis), X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM–EDX), and Brunauer–Emmett–Teller (BET) surface area analysis. XRD measurements confirmed the formation of crystalline ZnO with a hexagonal wurtzite structure and average crystallite sizes of approximately 29–30 nm. Surface area and porosity analyses further indicated mesoporous features that may contribute to the accessibility of reactive surface sites. Biological investigations revealed distinct responses depending on the extract used during synthesis. ZnO NPs prepared with pumpkin pulp juice exhibited appreciable antibacterial activity against *Escherichia coli*, together with an anti-inflammatory effect that showed no clear concentration dependence and a moderate level of cytotoxicity. Conversely, peel-mediated ZnO NPs displayed comparatively weaker antibacterial activity but showed improved cellular compatibility and greater inhibition of protein denaturation at intermediate concentrations. Overall, the findings suggest that the choice of pumpkin-derived extract plays an important role in determining the structural characteristics and biological behavior of ZnO nanoparticles. The observed differences provide a basis for selecting suitable biomass sources according to the intended application and support further exploration of pumpkin-derived materials as accessible resources for the sustainable production of ZnO nanomaterials.

Keywords: Green synthesis; Pumpkin biomass; Zinc oxide nanoparticles; Physicochemical characterization; Antibacterial activity; Anti-inflammatory activity.

Highlights

- ZnO nanoparticles were bio-inspired green-synthesized using pumpkin pulp juice and peel extracts.
- Physicochemical analyses (UV–Vis spectroscopy, XRD, FTIR, SEM–EDX, and BET analysis) confirmed hexagonal wurtzite ZnO nanoparticles with active surface properties.
- Minor differences in structural and surface characteristics were observed between pulp- and peel-derived ZnO nanoparticles.
- Biological assays demonstrated that the green-derived ZnO nanoparticles were active against *Escherichia coli* and exhibited measurable anti-inflammatory potential.
- Cytotoxicity evaluation indicated acceptable biological safety, supporting potential biomedical and environmental applications.

1. Introduction

Nanoscience and nanotechnology have emerged as key drivers in materials science, focusing on the rational design, development, characterization, and the utilization of nanoscale materials [1]. Nanomaterials, commonly described as materials exhibiting nanoscale features (1–100 nm) along one or more spatial dimensions, demonstrate physicochemical

behavior that deviates significantly from bulk-phase properties [1, 2]. These size-dependent characteristics have enabled a wide range of applications in chemistry, biotechnology, medicine, microbiology, catalysis, and environmental engineering [3–5]. On the basis of composition and structural features, nanoparticles can be broadly classified into carbon-based materials, quantum dots, metallic and metal oxide nanoparticles [6].

Conventional synthesis strategies for nanomaterials are generally categorized into physical and chemical methods. Despite their effectiveness, such methods are typically energy-intensive and involve toxic reagents or hazardous by-products, limiting their environmental sustainability and biological compatibility. Consequently, green synthesis has gained recognition as a sustainable approach enabling environmentally compatible and scalable nanoparticle production [7, 8].

Growing environmental concerns have intensified interest in biologically assisted nanoparticle synthesis as a greener alternative to conventional methods. This strategy relies on natural matrices, including microorganisms and plant-derived extracts, whose phytochemical constituents facilitate metal ion reduction and contribute to surface stabilization during particle formation [9].

The plant-mediated production of metal oxide nanoparticles has garnered considerable attention owing to the availability of bioactive phytochemicals, such as phenolic compounds, which are essential for metal ion reduction and surface stabilization. [10]. This rapidly growing research domain involves the production of diverse metal and metal oxide nanoparticles, including calcium, copper, gold, iron, silver, and zinc-based nanostructures. [11].

Among these materials, ZnO nanoparticles have emerged as a versatile class of multifunctional nanomaterials owing to their remarkable catalytic, electrical, optical, and antimicrobial properties. Consequently, ZnO nanoparticles have been widely investigated for potential uses in antimicrobial formulations, restoration of the environment, and biomedical fields [12–14]. Several studies have reported the eco-friendly synthesis of ZnO NPs using different plant extracts as reducing and stabilizing agents. For instance, ZnO nanoparticles synthesized using pumpkin seed extract exhibited moderate antimicrobial activity [15], while *Spondias mombin* leaf extract has been employed for the biosynthesis of ZnO NPs with notable antibacterial properties [16].

Despite these advances, comparative investigations focusing on the effect of different biomass fractions derived from the same agricultural source on the physicochemical and biological properties of ZnO nanoparticles remain scarce. Understanding how distinct plant-derived components influence structure–property–bioactivity relationships is essential for the rational design of bio-inspired nanomaterials with targeted functionalities.

In this context, the present study explores the bio-inspired green synthesis of ZnO nanoparticles using two pumpkin-derived biomass extracts, namely pulp juice and peel extract, mediating precursor conversion while regulating nucleation, growth, and aggregation of the resulting nanostructures. Pumpkin biomass represents an abundant agricultural resource, and its valorization aligns with sustainable development and circular economy principles. A systematic physicochemical investigation was conducted to elucidate the optical, crystallographic, morphological, elemental, and surface textural characteristics of the synthesized ZnO nanoparticles using UV–Vis, XRD, FTIR, SEM–EDX, and BET techniques. Furthermore, their biological performance was evaluated through antibacterial and anti-inflammatory assays, along with cytotoxicity assessments, to elucidate structure–property–bioactivity relationships and assess their potential for biomedical and environmental applications.

1. Experimental

1.1. Materials and Methods

1.1.1. Chemical Materials

Zinc chloride (ZnCl_2) of analytical grade was obtained from Sigma-Aldrich, and sodium hydroxide (NaOH) of analytical grade was purchased from SpeciLab. Absolute ethanol ($\text{C}_2\text{H}_5\text{OH}$) of analytical grade was supplied by Honeywell – Riedel-de Haën, while distilled water produced by ENPEC (Algeria) with an electrical conductivity below $20\ \mu\text{S}/\text{cm}$ was used.

1.1.2. Collection of Plant Samples

The pumpkin fruit shown in **Fig. 1** was harvested on Saturday, January 27, 2024, from the orchard of a farmer located east of the village of Bellila, in El Oued Province, Algeria. The fruit was cleaned under running tap water and subsequently rinsed with distilled water. After drying, it was cut into pieces, the seeds were removed, and the peel was separated to obtain the pumpkin pulp and the peels individually. The peels were rinsed with distilled water, then left to air-dry at room temperature, cut into small fragments, and stored for subsequent use.



Fig. 1. Pumpkin fruit used as the natural precursor for ZnO nanoparticle synthesis.

1.1.3. Preparation of Plant Extracts

Fresh pumpkin pulp was cut into small portions and then homogenized using an electric grinder. The resulting juice was extracted through a clean cloth, stored at low temperature, and centrifuged prior to each use to remove suspended solids, as depicted in **Fig.2a**.

The dried pumpkin peels were mechanically pulverized, and 50 g of the obtained powder was immersed in 1500 mL of distilled water, followed by boiling for 60 min. The suspension was then allowed to cool to ambient temperature and subsequently filtered to remove coarse residues. The resulting extract was kept under refrigerated conditions and centrifuged before each use, as illustrated in the inset of **Fig.2b**

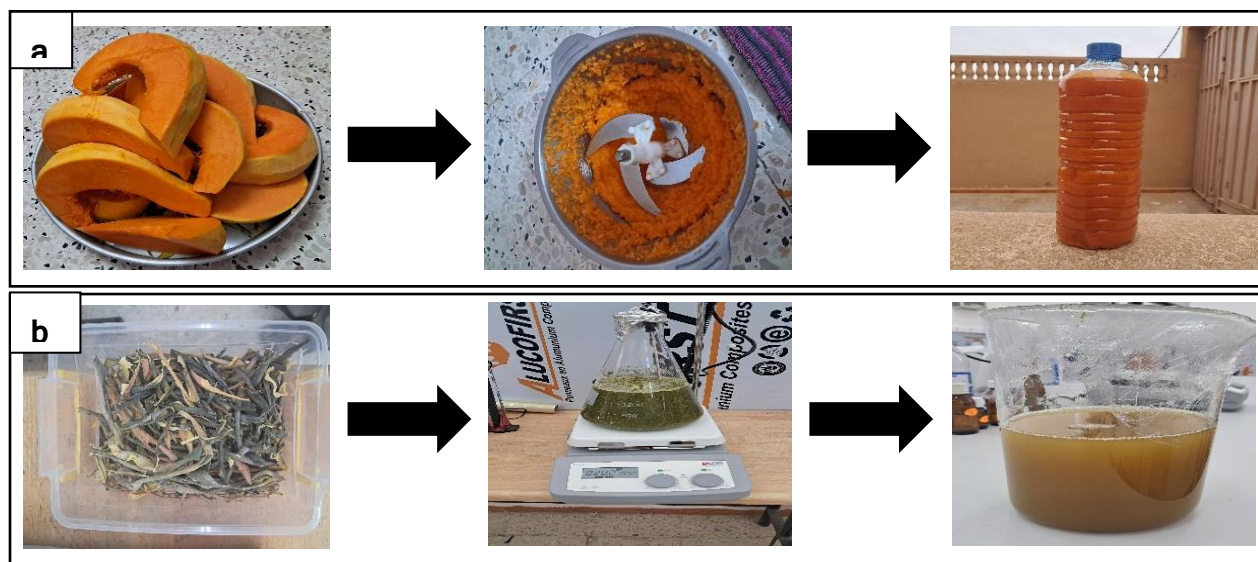


Fig. 2 Preparation of pumpkin biomass extracts used for the green synthesis of ZnO nanoparticles: (a) preparation of pumpkin pulp juice, and (b) preparation of pumpkin peel extract.

1.1.4. Synthesis of Zinc Oxide Nanoparticles (ZnO NPs)

A 0.5 M zinc chloride solution was prepared by dissolving 6.81 g of zinc chloride in distilled water in a beaker, followed by adjusting the final volume to 100 mL with continuous stirring. Subsequently, the temperature was raised to 40 °C, and a pre-centrifuged pumpkin pulp juice (centrifuged at 4010 rpm for 5 minutes) was gradually added dropwise using a burette. Following the addition, the pH of the solution was adjusted to 10 by gradually adding sodium hydroxide drop by drop. The reaction mixture was heated to 80 °C and maintained under continuous stirring for 2 h. Upon completion of the reaction, the suspension was cooled to ambient temperature, and the precipitate was recovered by centrifugation (4010 rpm, 10 min), and the collected precipitate was washed thoroughly—first with distilled water and then with 99% ethanol (C_2H_5OH). The recovered precipitate was dried at 60 °C for 24 h, followed by calcination at 600 °C for 2 h in a muffle furnace. The final product was a fine white powder of zinc oxide nanoparticles, which was stored in glass tubes for further applications.

An analogous synthesis was performed using pumpkin peel extract instead of pulp juice, as schematically illustrated in **Fig. 3**

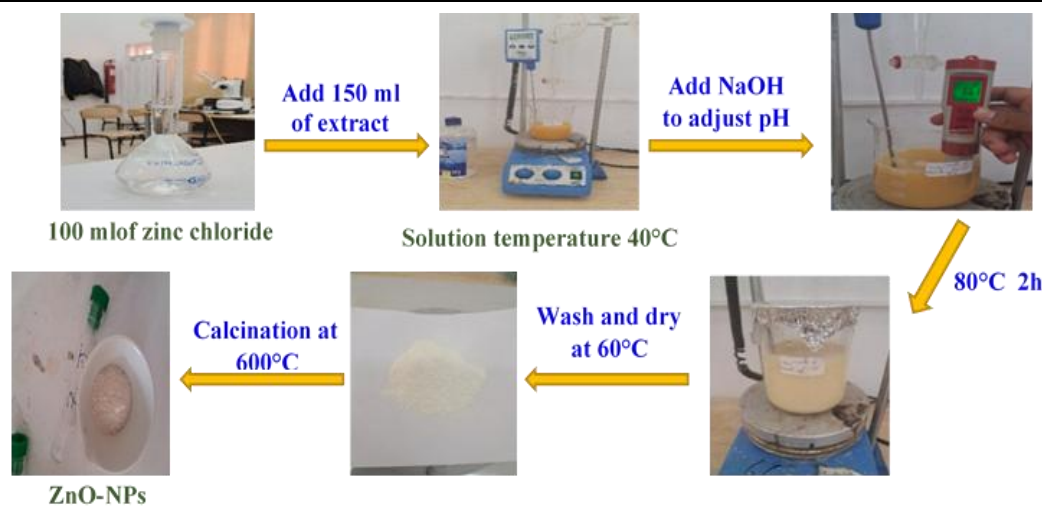


Fig. 3 Schematic illustration of ZnO nanoparticle synthesis via pumpkin-derived extracts.

1.1.5. Characterization methods

A systematic physicochemical evaluation was carried out to confirm nanoparticle formation and to elucidate their optical, crystallographic, morphological, compositional, and surface characteristics. Optical properties were analyzed using UV–Vis spectroscopy to assess absorption behavior and estimate the band gap energy. Surface functional groups and metal–oxygen interactions were examined by FTIR spectroscopy, providing insight into the contribution of biomass-derived organic constituents during nanoparticle formation. Phase composition and crystal structure were evaluated through XRD analysis, and crystallite size was derived from diffraction peak broadening using the Scherrer relation. Morphological features and particle dispersion were observed via SEM, while elemental composition was verified by EDX analysis. In addition, nitrogen adsorption–desorption measurements based on the Brunauer–Emmett–Teller (BET) method were carried out to determine the specific surface area and pore structure of the nanoparticles.

1.1.6. In Vitro Antimicrobial Evaluation

In vitro antimicrobial activity was assessed using an agar well diffusion assay, following standard protocols for microbial susceptibility testing. The study included previously prepared nanoparticle samples, which were tested against standard microbial strains representing Gram-negative and Gram-positive bacteria, namely *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, in addition to the fungal strain *Candida albicans* ATCC 10231 to assess antifungal activity. Antibacterial assays were performed using Mueller–Hinton agar (MHA), while Sabouraud dextrose agar (SDA) supplemented with 2% glucose was used for testing antifungal activity against *Candida albicans*.

Microbial inocula were prepared from fresh 24-hour cultures, and the cell density was standardized to approximately 10^6 CFU/mL. The agar plates were uniformly inoculated using sterile cotton swabs to ensure homogeneous distribution of the microorganisms. After drying of the agar surface, circular wells were created using a sterile Pasteur pipette. Each well received 50 μ L of the nanoparticle suspension at concentrations of 40, 20, 10, and 5 mg/mL. Incubation was conducted at 37 °C for 24 h for bacterial strains and for 48 h in the case of *Candida albicans*.

Once incubation was completed, the diameters of the inhibition zones (mm) formed around the wells were measured to evaluate antimicrobial efficacy. Inhibition zones exceeding 6 mm in diameter were considered indicative of significant antimicrobial activity. All experiments were conducted in three independent replicates to enhance the reliability of the findings. [17,18].

1.1.7. BSA Denaturation-Based Anti-Inflammatory Evaluation

Anti-inflammatory activity was assessed through inhibition of bovine serum albumin (BSA) denaturation. The experiment was conducted in test tubes with a final reaction volume not exceeding 3.0 mL. A 0.4% (w/v) BSA solution was prepared in TBS, with the pH adjusted to 6.4 by controlled addition of glacial acetic acid when necessary.

The tested samples were dissolved in distilled water and evaluated across a concentration range of 40, 20, 10, 5, 2.5, and 1.25 mg/mL. For each reaction, 1.0 mL of the BSA solution was added to a test tube, followed by an appropriate volume of the sample solution to obtain the desired concentration. The total volume was brought to 2.5 mL with distilled water. BSA mixed with distilled water without any sample served as the negative control, while aspirin at the same concentrations was used as a positive control and reference standard.

Incubation was performed at 37 °C for 15 min, followed by controlled thermal denaturation at 55 °C for 10 min in a water bath, after which the samples were cooled to room temperature for 20 min. Protein denaturation was quantified at 660 nm using UV–Vis spectrophotometry, where reduced absorbance values relative to the control indicated enhanced inhibition and

greater anti-inflammatory potential. Protein denaturation inhibition (%) was calculated according to the equation below:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Where:

Absorbance of Control = Negative control (no samples).

Absorbance of Sample = Value from plant extract or oil-treated samples.

where the absorbance of the control corresponds to the negative control (without sample), and the absorbance of the sample corresponds to the value obtained for the nanoparticle-treated samples. All measurements were independently replicated three times to ensure data reliability. [19].

1.1.8. Cytotoxicity Assessment

The cytotoxic activity of the synthesized nanoparticle formulations was investigated through a yeast growth inhibition and viability assay, employing *Saccharomyces cerevisiae* as the experimental model. The study comprised four pre-prepared nanoparticle samples. For each material, stock suspensions were initially prepared at 20 mg/mL and then serially diluted in a twofold manner to achieve final concentrations ranging from 20 to 0.625 mg/mL.

Yeast cells were harvested from an overnight YPD broth culture, and the cell concentration was adjusted to 2×10^7 cells/mL using 100 mM potassium phosphate buffer (pH 7.0). Equal aliquots of the standardized cell suspension and the nanoparticle preparations were dispensed into sterile 96-well plates and gently combined. The plates were maintained at 37 °C for 30 min to permit nanoparticle–cell interaction. A parallel set of wells containing untreated yeast cells was included as the negative control.

After the exposure period, a semi-quantitative spot test was carried out by dispensing 2.5 µL from each treatment onto YPD agar plates containing 2% glucose. To assess post-treatment recovery and verify that any observed effects were attributable to the nanoparticles, duplicate sets of plates were prepared with and without nanoparticles. Plates were incubated at 37 °C for 48 h, after which colony outgrowth was inspected visually and recorded using a flatbed scanner (Epson®). Growth suppression was evaluated by comparing the intensity and distribution of spots from treated samples against the untreated control, enabling a relative estimation of cytotoxic and/or growth-inhibitory impacts of the nanoparticle preparations on *S. cerevisiae*.

Cytotoxicity was further assessed by comparing growth density and spot patterns relative to the negative control, where reduced growth was considered indicative of cytotoxic or growth-inhibitory effects. Cell viability (%) and mortality (%) data were used to estimate the LC₅₀ value (lethal concentration causing 50% cell death) when applicable, calculated according to the following equation [20]:

$$LC_{50} = C_1 + \frac{(50 - M_1)(C_2 - C_1)}{(M_2 - M_1)}$$

- C1: Concentration yielding mortality above 50%
- C2: Concentration yielding mortality below 50%
- M1: Mortality (%) at C₁
- M2: Mortality (%) at C₂

2. Results and discussion

2.1. Characterization of Zinc Oxide Nanoparticles

2.1.1. UV-Visible Spectroscopy (UV-Vis)

The UV-Visible absorption spectra (200–900 nm range) of zinc oxide nanoparticles dispersed in distilled water revealed an absorption edge at 374 nm for the sample synthesized using pumpkin pulp juice, and at 370 nm for the sample derived from the peel extract. Spectral analysis reveals pronounced absorption in the ultraviolet region, as shown in **Fig.4**.

The optical band gap energies were evaluated from Tauc plots constructed using the UV–Vis absorption spectra. [21], yielding values of 3.32 eV for the pulp-based sample and 3.45 eV for the peel-based sample. These results suggest that the materials possess a nanoscale structure, as ZnO nanoparticles typically do not exhibit significant absorption features within the visible spectral region.[22]

Moreover, the present findings are consistent with previously published reports. For instance, [23] reported an absorption peak at 374 nm, while [15] observed an absorption peak at 373 nm for ZnO nanoparticles. Similarly, [24] identified an absorption peak at 370 nm, and [25] reported a comparable value at 369 nm. This consistency with the literature confirms the validity and reliability of the results obtained in the present study.

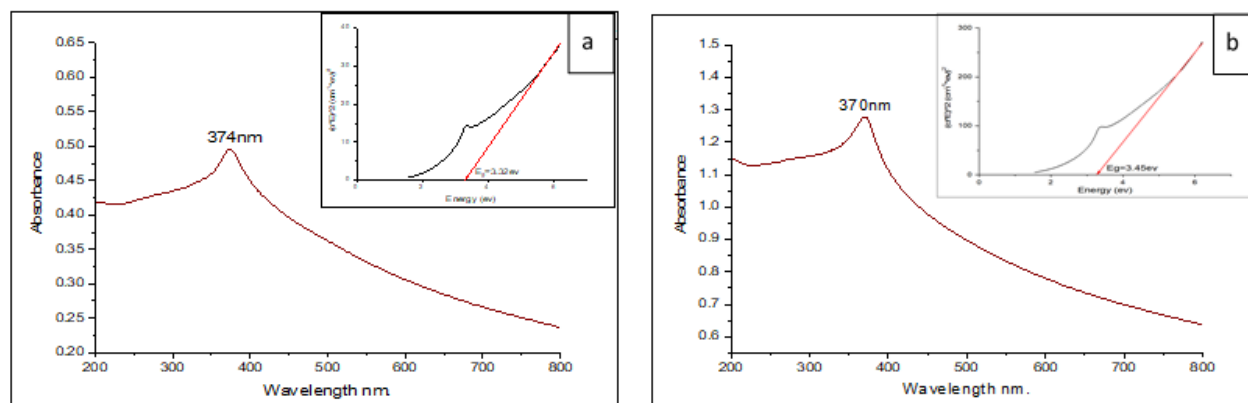


Fig. 4 UV-Vis spectra and corresponding Tauc plots showing the optical band gap of ZnO nanoparticles synthesized from (a) pumpkin pulp juice and (b) pumpkin peel extract.

2.1.2. FTIR Spectral Analysis

FTIR spectra were recorded over the 400–4000 cm^{-1} wavenumber range for zinc oxide nanoparticles synthesized using pumpkin pulp juice and pumpkin peel extract, revealing distinct vibrational features that reflect the chemical structure of the obtained samples. As shown in **Fig. 5**, both spectra exhibit a prominent absorption band in the 450–500 cm^{-1} region, which is typically assigned to Zn–O stretching vibrations, further supporting the formation of ZnO nanoparticles.

Additionally, weak to moderate bands were observed in the 1000–1600 cm^{-1} region, likely corresponding to residual organic compounds from the plant-based precursors (such as C=O or C–O functional groups). A wide, weak absorption band in the 3200–3500 cm^{-1} range is typically attributed to O–H stretching vibrations, indicating the presence of surface hydroxyl functionalities as well as physically adsorbed water.

The strong spectral similarity between the two samples suggests a comparable overall chemical structure of the ZnO nanoparticles synthesized via both methods, with minor variations in peak intensities potentially resulting from differences in organic content or surface interactions. These results highlight the effectiveness of green synthesis approaches and underscore their suitability for potential environmental and biomedical applications due to the purity and surface activity of the resulting nanoparticles.

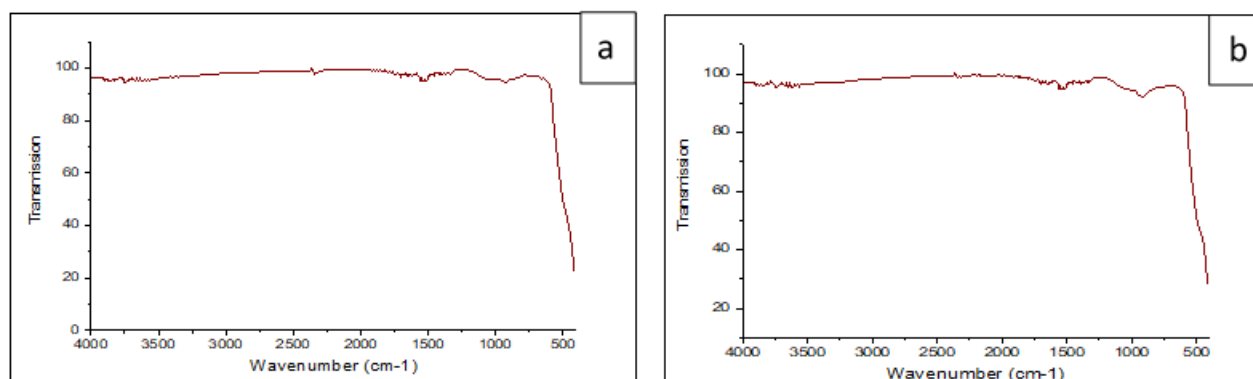


Fig. 5 FTIR spectra illustrating the vibrational characteristics of ZnO nanoparticles synthesized using (a) pumpkin pulp juice and (b) pumpkin peel extract.

The diffraction patterns (**Fig. 6**) match the standard JCPDS file No. 36-1451, confirming that the synthesized nanoparticles crystallize in the hexagonal wurtzite phase of ZnO [26].

Distinct diffraction peaks were detected at 2θ positions of 31.77°, 34.47°, 36.27°, 47.57°, 56.66°, 62.88°, 66.44°, 67.98°, 69.14°, 72.56°, and 76.95°, corresponding to the (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) crystal planes, respectively. The observed diffraction peaks are consistent with the crystallographic planes of hexagonal ZnO. The intensity of the main diffraction peaks, particularly the (101) reflection, showed slight variations between the two samples. The maximum intensity reached approximately 33,782 counts for the peel-based sample and 40,165 counts for the pulp-based sample, suggesting a higher degree of crystallinity in the latter. The observed behavior likely results from the phytochemical composition of the pumpkin pulp extract, which may influence nucleation and subsequent crystal growth during synthesis.

No additional diffraction peaks attributable to secondary crystalline phases were detected in the XRD patterns. The presence of minor background features or weak, non-indexable signals can be ascribed to diffuse scattering and peak broadening effects associated with the nanoscale crystallite size, as well as possible contributions from amorphous or semi-amorphous organic residues originating from the plant-based extract. According to Cullity, amorphous and semi-amorphous materials typically produce broad, diffuse scattering features rather than sharp, well-defined diffraction peaks, and small, highly strained crystallites are known to generate broadened diffraction lines rather than distinct reflections [27].

The average crystallite size was derived from the FWHM of the dominant (101) diffraction peak using the Scherrer equation.[28]. The calculated crystallite sizes were 29.7 nm for the peel-based sample and 29.8 nm for the pulp-based sample, confirming the formation of high-purity nanocrystalline ZnO with comparable structural properties.

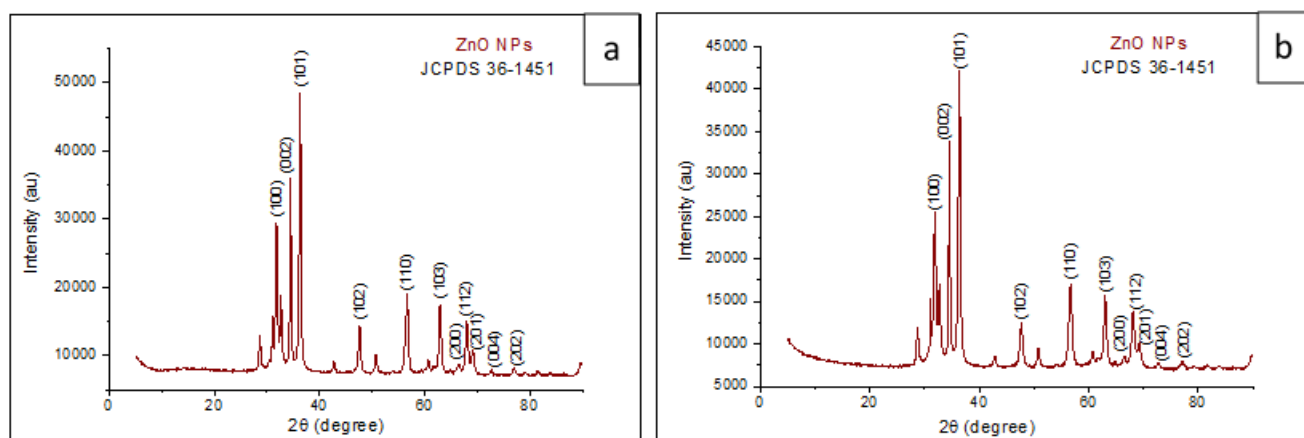


Fig. 6 XRD patterns of ZnO nanoparticles synthesized using (a) pumpkin pulp juice and (b) pumpkin peel extract.

2.1.3. Scanning Electron Microscopy (SEM)

SEM micrographs (Fig. 7 and 8) show that ZnO nanoparticles prepared from pumpkin pulp juice and peel extract consist mainly of nanosized particles with spherical morphology. However, the sample synthesized using pulp juice exhibits a more homogeneous particle distribution and a markedly lower degree of agglomeration compared to the sample prepared with peel extract. This difference is most likely attributed to the presence of bioactive compounds in pumpkin pulp, such as polysaccharides and proteins, which are believed to act as stabilizing and directing agents during the nanoparticle formation process [29].

EDX characterization revealed prominent zinc and oxygen signals, indicating the formation of ZnO nanoparticles, showing comparable atomic ratios of zinc and oxygen in both samples, which indicates the formation of high-purity ZnO structures. The mean particle diameter was approximately 65.34 nm for the sample prepared with pulp juice and 64.28 nm for the sample prepared with peel extract. Despite the similarity in elemental composition, the observed morphological differences suggest that the reaction environment provided by the pumpkin pulp is more favorable for the formation of well-dispersed ZnO nanoparticles, whereas the peel extract may contain compounds that contribute to a higher degree of agglomeration.

The XRD and SEM results collectively confirm the consistency of the structural and morphological attributes of the synthesized particles. While XRD confirms the formation of highly pure nanocrystalline ZnO, SEM highlights differences related to particle agglomeration and distribution. The observed average particle sizes are also consistent with the expected aggregation of individual nanocrystals.

These findings emphasize the crucial role of natural plant-derived compounds present in pumpkin components in controlling the properties of the formed nanoparticles and further support the feasibility of using plant-based materials as environmentally friendly resources for the sustainable synthesis of nanomaterials [30].

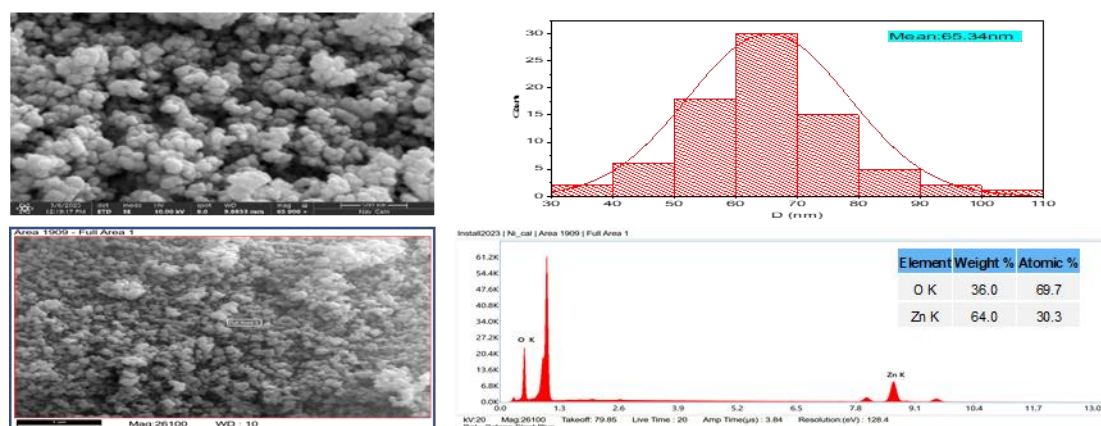


Fig. 7 SEM–EDX analysis and particle size distribution of ZnO nanoparticles synthesized using pumpkin pulp juice.

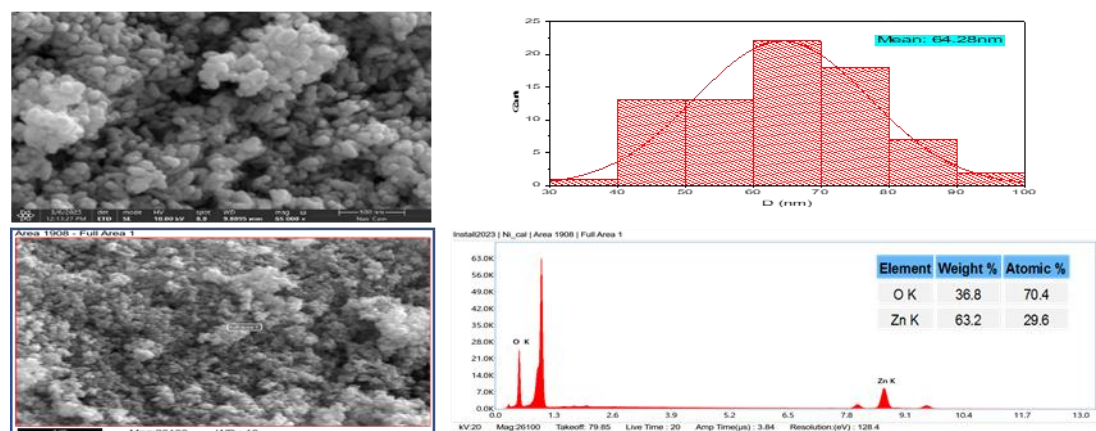


Fig. 8 SEM–EDX analysis and particle size distribution of ZnO nanoparticles synthesized using pumpkin peel extract.

2.1.4. Surface Analysis (BET)

The nitrogen adsorption analysis (BET/BJH) results shown in **Fig. 9** for ZnO nanoparticles derived from pumpkin pulp juice and pumpkin peel extract revealed closely comparable surface properties. The BET-derived specific surface area was approximately 22.61 m²/g for the sample prepared with pulp juice and 22.50 m²/g for the sample prepared with peel extract, indicating similar surface adsorption efficiency for both samples. This similarity is consistent with the XRD results, which demonstrated the predominance of the nanocrystalline ZnO phase, suggesting that the measured surface properties mainly reflect the contribution of the dominant crystalline phase.

Furthermore, the higher surface areas calculated using the Langmuir model showed that the pulp-juice-prepared sample exhibited a surface area of 120.22 m²/g, whereas the peel-extract-prepared sample reached 114.09 m²/g, indicating the availability of active surface sites, particularly in the pulp-based sample. This behavior can be attributed to the nanostructure formed during the green synthesis process, as supported by SEM images, which revealed a more homogeneous particle distribution and a lower degree of agglomeration for the pulp-based sample compared to the peel-based one, thereby enhancing the accessibility of the effective surface area.

BJH analysis indicated that both samples possess a mesoporous structure, with average pore diameters of 39.04 and 36.79 nm for the pulp-based sample during the adsorption and desorption stages, respectively, while the peel-based sample exhibited values of 45.51 and 38.89 nm under the same conditions. Such mesoporosity plays an important role in enhancing adsorption and catalytic properties. The slight differences in pore diameters can be attributed to variations in the nature of plant-derived compounds influencing the growth and aggregation mechanisms, in agreement with the morphological observations from SEM.

The average particle sizes estimated from BET/BJH analysis—265.34 nm for the pulp-based sample and 266.63 nm for the peel-based sample—are larger than the crystallite sizes calculated from XRD, indicating that the formed particles result from the aggregation of primary nanocrystallites. This observation confirms the polycrystalline nature of the particles and is consistent with the agglomerated morphology observed in SEM images.

Overall, these findings demonstrate that green synthesis using pumpkin-derived components yields ZnO nanoparticles with active surface properties and a well-defined mesoporous structure, improving their applicability in adsorption processes, catalysis, and environmental and biological fields.

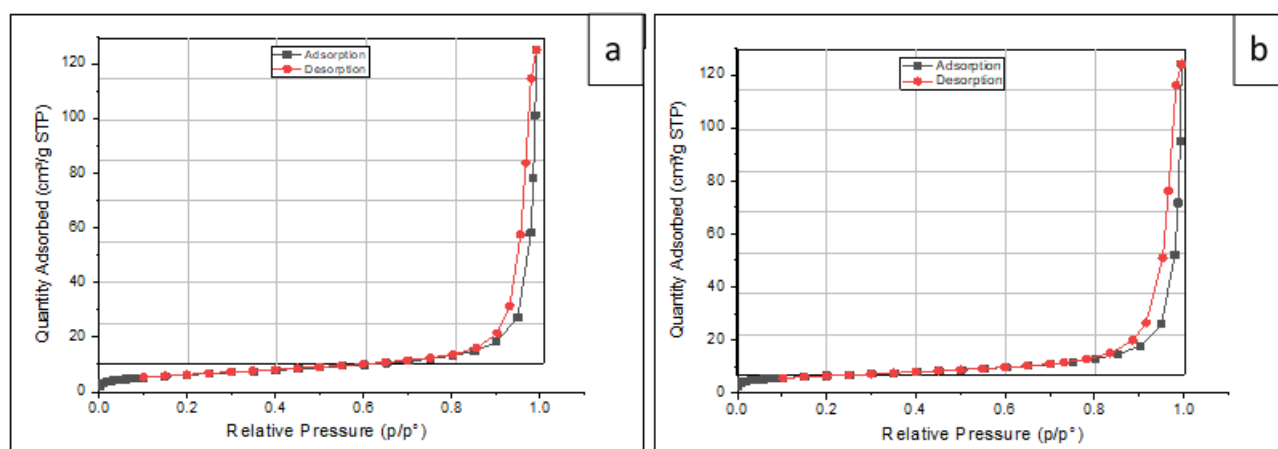


Fig. 9 Adsorption–desorption isotherm curves of ZnO NPs(a) Synthesized using pumpkin pulp juice.(b) Synthesized using pumpkin peel extract.

3. Biological Application of Zinc Oxide Nanoparticles

3.1. Antimicrobial Activity of ZnO NPs

The antimicrobial assay results shown in **Figs. 10** and **11** of zinc oxide nanoparticles synthesized via green methods revealed a clear variation in activity depending on the type of plant extract used (**Table 1**). ZnO nanoparticles prepared using pumpkin pulp juice exhibited antibacterial activity against *Escherichia coli*, with inhibition zones of 10 mm and 11 mm at concentrations of 40 and 20 mg/mL, respectively, while no activity was observed at lower concentrations or against the other tested strains (*Staphylococcus aureus* and *Candida albicans*). These results indicate a selective antimicrobial activity, with higher effectiveness against Gram-negative bacteria, particularly *E. coli*. In contrast, ZnO nanoparticles synthesized using pumpkin peel extract showed limited effectiveness, with growth inhibition detected only against *E. coli* at the highest concentration tested (40 mg/mL), yielding an inhibition zone of 8 mm, and no detectable effect at lower concentrations or against the other microbial strains.

This behavior can be explained by differences in the surface and morphological properties of the two samples. SEM and BET analyses indicate that the sample synthesized using pulp juice is characterized by a more homogeneous particle distribution and a greater availability of active surface sites, which may enhance interactions with Gram-negative bacteria such as *E. coli* [31]. In addition, the generation of reactive oxygen species (ROS) and/or direct interactions between the nanoparticle surface and the bacterial cell membrane are likely to be among the main mechanisms responsible for the observed antibacterial activity [32,33]. Conversely, The reduced antimicrobial activity of the peel-extract-derived ZnO nanoparticles may be due to differences in the surface-associated phytochemical capping and/or a higher tendency toward particle agglomeration, which collectively limits access to reactive surface sites [30].

Overall, these findings confirm that ZnO nanoparticles synthesized using pumpkin pulp juice exhibit promising biological performance, particularly against Gram-negative bacteria. The observed selective antimicrobial activity may be advantageous for targeted applications where non-specific broad-spectrum effects are not required. In contrast, enhancing the antimicrobial activity of ZnO nanoparticles derived from pumpkin peel extract may require optimization of the synthesis conditions to improve their effectiveness.

Table1: Antimicrobial activity results of ZnO NPs

Sample	Microbial strain	40 mg/mL	20 mg/mL	10 mg/mL	5 mg/mL
ZnO NPs (pumpkin pulp juice)	<i>E. coli</i>	10 mm	11 mm	NI	NI
	<i>S. aureus</i>	NI	NI	NI	NI
	<i>Candida albicans</i>	NI	NI	NI	NI
ZnO NPs (pumpkin peel extract)	<i>E. coli</i>	8 mm	NI	NI	NI
	<i>S. aureus</i>	NI	NI	NI	NI
	<i>Candida albicans</i>	NI	NI	NI	NI

NI: No Inhibition detected

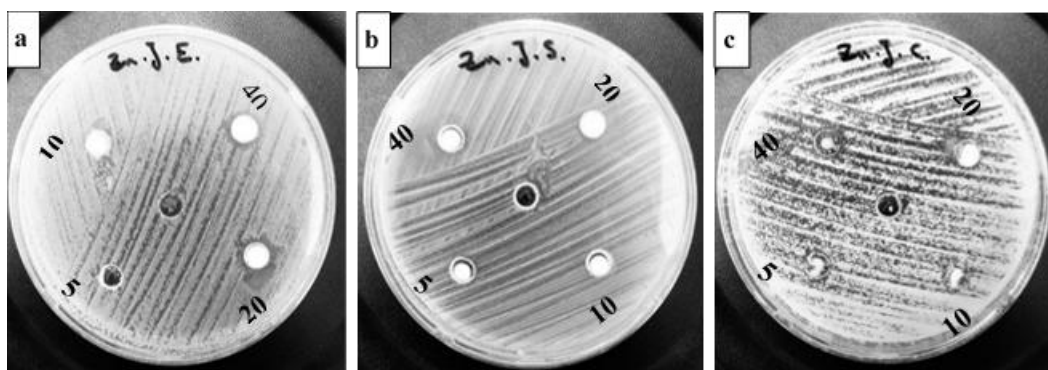


Fig. 10 Antibacterial activity test of ZnO NPs synthesized using pumpkin pulp juicea: Against *E. coli*: Against *S. aureus*: Against *Candida albicans*

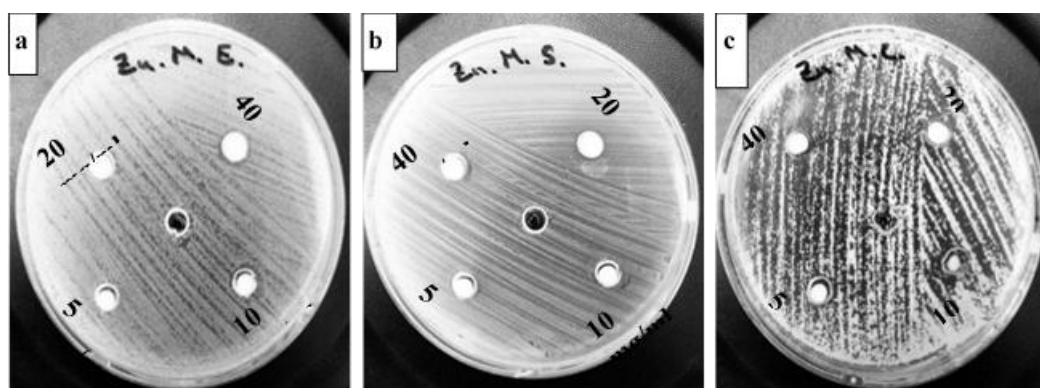


Fig. 11 Antibacterial activity test of ZnO NPs synthesized using pumpkin peel extract a: Against *E. coli* b: Against *S. aureus* c: Against *Candida albicans*

3.2. Anti-inflammatory Activity of ZnO NPs

As illustrated in Fig.12, the ZnO NPs synthesized using pumpkin pulp juice showed relatively stable anti-inflammatory activity across all concentrations, with inhibition rates ranging from 42.6% to 46.2%. In contrast, the peel-based sample exhibited a concentration-dependent trend, peaking at 50.7% inhibition at 20 mg/mL, followed by a gradual decline. These findings highlight the potential of plant-derived ZnO NPs as bioactive ingredients in natural anti-inflammatory formulations. The peel-based ZnO NPs demonstrated superior efficacy at intermediate concentrations, while the pulp-based formulation offered consistent activity across a broader concentration range.

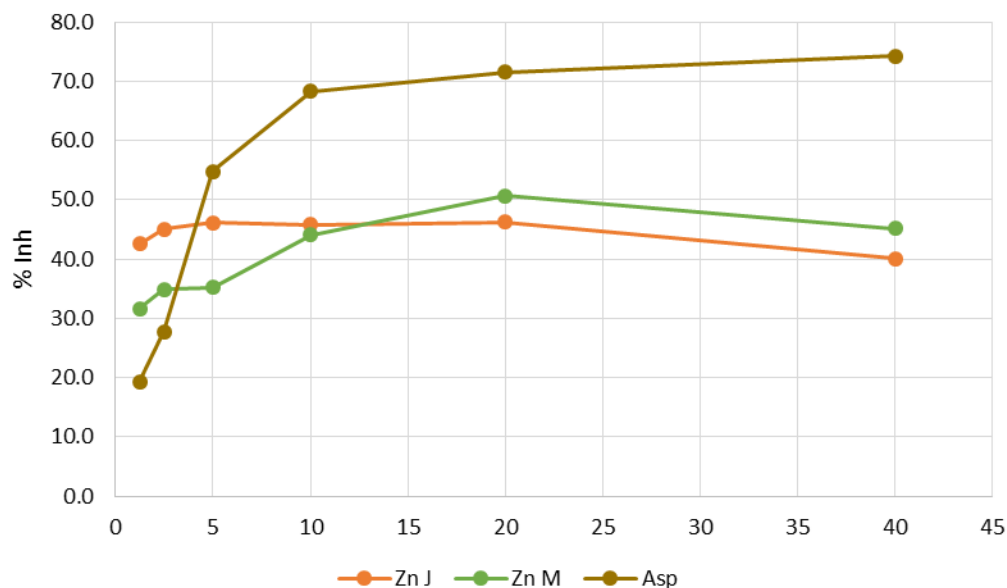


Fig. 12 BSA denaturation inhibition by ZnO NPs synthesized (ZnJ) from pumpkin pulp juice,(ZnM) from pumpkin peel extract in comparison with aspirin (Asp).

3.3. Cytotoxicity of ZnO NPs

The results shown in Fig. 13 revealed noticeable differences in the cytotoxic response between the two investigated samples. ZnO nanoparticles synthesized using pumpkin pulp juice exhibited indications of moderate cytotoxicity, reflected in a dose-dependent reduction in cell viability. The LC_{50} value was estimated to be approximately 7.5 mg/mL, as shown in Table 2, suggesting the presence of a measurable biological interaction between the nanoparticles and the yeast cells under the applied experimental conditions. In contrast, no clear cytotoxic effects were observed for the sample synthesized using pumpkin peel extract within the tested concentration range, indicating a relatively higher degree of biocompatibility in this experimental system.

These findings suggest that the type of plant extract used during synthesis may be an influential factor in determining the biological activity of ZnO nanoparticles. Accordingly, nanoparticles synthesized from pumpkin pulp juice may be more suitable for applications requiring limited or controlled cytotoxicity, whereas those derived from pumpkin peel extract may be preferable for applications demanding a higher level of biological safety. Nevertheless, further investigations involving different cellular models and longer exposure periods are required to substantiate these observations and to assess their broader applicability.

Table 2: Estimated LC_{50} values for tested samples using yeast cell cytotoxicity assay

Sample Code	Estimated LC_{50} (mg/mL)	Toxicological Interpretation
ZnO NPs (pumpkin pulp juice)	≈ 7.5 mg/mL	LC_{50} was estimated to be approximately 7.5 mg/mL, indicating moderate cytotoxicity.
ZnO NPs (pumpkin peel extract)	$\gg 20$ mg/mL	Very low toxicity; no significant reduction in yeast cell viability observed.

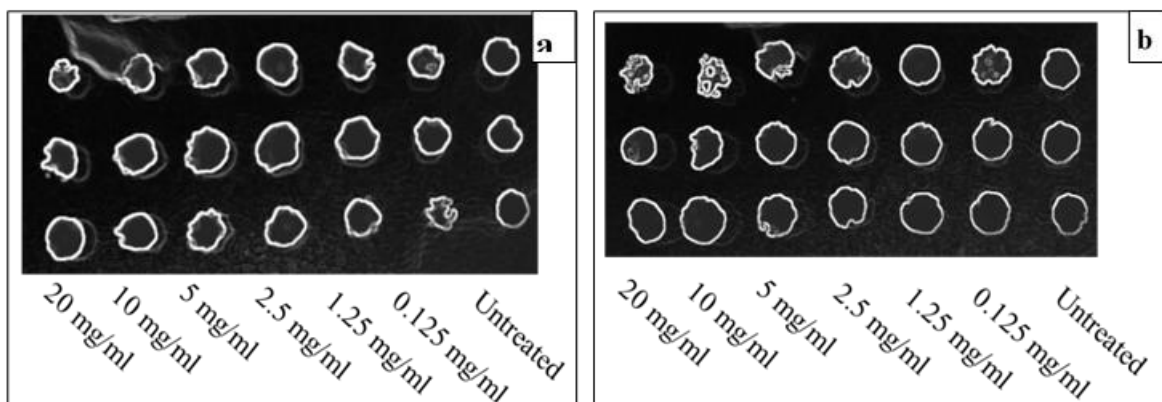


Fig. 13 Cytotoxicity assay of ZnO NPs (a): Synthesized using pumpkin pulp juice. (b): Synthesized using pumpkin peel extract.

4. Conclusion

The present work establishes pumpkin-derived extracts as effective mediators for the bio-assisted synthesis of ZnO nanoparticles, namely pulp juice and peel extract. This strategy allows efficient nanoparticle synthesis under mild conditions, avoiding the use of hazardous chemicals and harsh processing parameters, highlighting its environmentally friendly and sustainable nature.

Comprehensive physicochemical characterization using UV–Vis, FTIR, XRD, SEM, EDX, and BET analyses confirmed the successful formation of hexagonal wurtzite-structured ZnO nanoparticles with mesoporous features and surface properties suitable for biological applications. Notably, ZnO nanoparticles synthesized using pumpkin pulp juice exhibited more uniform morphology and a narrower particle size distribution compared to those obtained from peel extract, emphasizing the influence of biomass composition on nanoparticle formation.

Biological evaluations revealed selective antibacterial activity against *Escherichia coli*, while both samples showed promising anti-inflammatory potential in the BSA denaturation assay. Cytotoxicity assessments indicated moderate, concentration-dependent effects for pulp-derived ZnO NPs, whereas peel-derived nanoparticles exhibited enhanced

biocompatibility, suggesting their suitability for environmentally safe or biomedical applications.

Overall, the findings confirm that plant-mediated green synthesis offers a viable and scalable alternative to conventional routes for ZnO nanoparticle production. Further studies are recommended to investigate long-term biological interactions, elucidate the molecular mechanisms underlying bioactivity, and optimize particle size and morphology for targeted biomedical and environmental applications.

Acknowledgments

The authors gratefully acknowledge the VPRS Laboratory (University of KasdiMerbah, Ouargla, Algeria) and the ACE Laboratory (University of EchahidHammaLakhdar, El Oued, Algeria) for their support and access to research facilities. Special thanks are also extended to Engineer Ali TouatiTliba (ACE Laboratory) for his valuable technical assistance and facilitation during the experimental work.

Author contributions

Conceptualization: Bouzidi Ali, Zerrouki Hayat, and Belfar Mohamed Lakhder; methodology: Bouzidi Ali and RebiaiAbdelkarim; formal analysis and investigation: Bouzidi Ali, RebiaiAbdelkarim, and Bellila Abdelkader; writing-original draft preparation: Bouzidi Ali and Bellila Abdelkader; writing-review and editing: Bouzidi Ali, Zerrouki Hayat, RebiaiAbdelkarim, Belfar Mohamed Lakhder, and Bellila Abdelkader; supervision: Zerrouki Hayat, RebiaiAbdelkarim, and Belfar Mohamed Lakhder. The final manuscript was reviewed and approved by all authors.

Statements and Declarations

Funding

No external funding was received for this study.

Competing Interests

The authors report no competing interests.

Data Availability

The data generated and/or analyzed in this study are available from the corresponding author upon reasonable request.

Ethical Approval

No human participants or vertebrate animals were involved in this research.

Use of AI tools

The authors declare that no generative artificial intelligence tools were used to create or manipulate any figures or images in this manuscript.

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