Research Article

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Green Synthesis and Characterization of *Solanum xanthocarpum*-Derived ZrO_2 Nanoparticles for Antioxidant, Antibacterial, Antifungal, and Anticancer Activities

A. Akalya[1], S. Sundaranayagi[1*] and G. Elatharasan[2]

[1]Department of Chemistry, Periyar Maniammai Institute of Science and Technology (Deemed to be University), Vallam, Thanjavur, TN, India

[2]Department of Mechanical Engineering, University College of Engineering, Pattukkottai, TN, India

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*lavanya14apr@gmail.com

ABSTRACT

This research presents an environmentally sustainable approach for producing zirconium dioxide nanoparticles utilising *Solanum xanthocarpum* leaf extract as both a bioreducing and stabilising agent. The formation of a tetragonal crystalline phase was confirmed by XRD analysis and the functional groups in the plant extract involved in nanoparticle stabilisation were identified by FTIR. UV-Vis spectroscopy indicated strong absorption in the UV region, while photoluminescence analysis showed distinct emission behaviour. Field-emission scanning electron microscopy displayed aggregated nanoparticle morphology, and EDAX confirmed a homogeneous distribution of zirconium and oxygen. The synthesized ZrO_2 nanoparticles demonstrated noteworthy antimicrobial activity against *S. aureus, E. coli, C. albicans*, and *A. niger*. Antioxidant assays using DPPH showed effective free radical scavenging, and cytotoxicity tests against A498 kidney cancer cells indicated promising anticancer activity.

Keywords: Zirconium dioxide nanoparticles; Green synthesis; *Solanum xanthocarpum;* Antimicrobial activity; Anticancer potential; Antioxidant potential.

1. INTRODUCTION

Nanotechnology is a multidisciplinary field rapidly gaining attention for its diverse applications in pharmaceuticals, chemical processing, food technology, and engineering. It is grounded in nanoscience, which focuses on designing, manipulating, and developing materials measuring 1 to 100 nm in size. Nanoparticles, due to their nanoscale dimensions, possess distinctive physical and chemical properties, making them suitable for a variety of innovative technologies, including biomedicine, electronics, and environmental solutions (Nair *et al.* 2022). Specifically, metal and metal oxide nanoparticles are known for their superior antimicrobial potential, attributed to their high surface area-to-volume ratio, which enhances chemical reactivity (Pandiarajan *et al.* 2025). Nonetheless, conventional synthesis approaches for such nanoparticles often involve the use of toxic reducing and stabilising agents, which raise both environmental and safety-related concerns (Szczyglewska *et al.* 2023).

Zirconium dioxide nanoparticles have attracted substantial scientific interest due to their high thermal stability, biocompatibility, and low toxicity. These properties have made them promising candidates for applications in biosensing, drug delivery, and antimicrobial therapy. The unique physicochemical properties of ZrO_2 nanoparticles have led to their widespread application in medical and biotechnological research (Tabassum *et al.* 2021; Goyal *et al.* 2021; Ahmed *et al.* 2021; Tran *et al.* 2022; Bannunah, 2023).

Conventional methods such as sol–gel synthesis, hydrothermal processing, co-precipitation, and vapour-phase methods are commonly employed for producing ZrO_2 Nanoparticles with well-defined morphologies and particle sizes. However, these approaches often require hazardous chemical reagents, substantial energy input, and laborious purification processes, which limit their environmental sustainability and scalability (Sai Saraswathi and Santhakumar, 2017).

To overcome these challenges, green synthesis methods have gained popularity as an eco-friendlier alternative. These strategies utilise natural sources such as enzymes, microbes, and biopolymers as both reducing and stabilizing agents, thereby eliminating the need for hazardous substances (Rafique *et al.* 2017). Besides reducing the environmental footprint, green methods also improve the biocompatibility of the synthesised nanoparticles. Among green methods, plant-based synthesis has emerged as particularly effective due to its low cost, simplicity, and the wide range of

phytochemicals present in plant materials. These compounds including flavonoids, terpenoids, alkaloids, phenolics, and saponins are essential for both reducing metal ions and stabilizing the nanoparticles as they prevent aggregation (Amin *et al.* 2012; Yousaf and Saleh, 2018).

Solanum xanthocarpum, a well-known medicinal herb in Ayurveda, has demonstrated several therapeutic effects, such as antimicrobial, antioxidant, hepatoprotective, anti-inflammatory, and anticancer activities. The phytoconstituents in its leaf extract are instrumental in promoting controlled nucleation and nanoparticle growth. These bioactive molecules not only facilitate metal ion reduction but also provide capping that may enhance functional properties of the nanoparticles. Silver nanoparticles were synthesized using *S. xanthocarpum,* which showed considerable antimicrobial potential, including activity against multidrug-resistant bacterial strains (Pungle *et al.* 2023).

Utilising medicinal plants like *S. xanthocarpum* for nanoparticle synthesis, particularly for fabricating metal oxides such as Ag and Cu, bridges the gap between traditional herbal practices and contemporary nanotechnological advancements (Jessy Mercy *et al.* 2024).

This research focuses on the green synthesis of ZrO_2 nanoparticles using *Solanum xanthocarpum* leaf extract as a natural reducing and stabilizing agent. The study further investigates the structural characteristics, surface morphology, and biological performance of nanoparticles, particularly their antibacterial, antifungal, antioxidant, and anticancer effects. The objective is to establish a sustainable, affordable, and biocompatible method for synthesizing multifunctional ZrO_2 nanoparticles with potential biomedical activities.

2. MATERIALS AND METHODS

Zirconium oxychloride octahydrate ($ZrOCl_2 \cdot 8H_2O$), acquired from Merck, India, served as the zirconium precursor. All chemicals and solvents utilised in this study were of analytical grade and employed directly without further purification. During the experimental procedures, distilled water was utilised as the exclusive solvent.

2.1 Preparation of Leaf Extract

Fresh leaves of *Solanum xanthocarpum* were collected and washed with tap water, then thoroughly rinsed with distilled water to remove residual contaminants. The cleaned leaves were dried in the shade for 5 - 7 days and then ground to a fine powder using a mechanical grinder. About 10 g of the powdered leaves were boiled for 30 minutes at 60-70 ℃ in 200 mL of distilled water to prepare the extract. A clear filtrate was obtained by filtering the solution through Whatman No. 1 filter paper after cooling to room temperature. During the green synthesis of nanoparticles, this plant extract served as a capping and reducing agent, maintained at about 28 ℃.

2.2 Green Synthesis of ZrO_2 Nanoparticles

Zirconium oxychloride was dissolved in 100 mL of distilled water to form a 0.1 M solution, with continuous stirring to ensure complete dissolution. Following this, 100 mL of the *S. xanthocarpum* extract was added dropwise to the zirconium oxychloride solution under continuous stirring at 50-60 ℃. The mixture gradually turned turbid and thickened as phytochemicals interacted with zirconium ions. Continued stirring led to the formation of a uniform gel like mass. A dry solid was formed by drying the resultant gel at 80 ℃ in a hot-air oven. This dried material was calcined at 500 ℃ for three hours to obtain crystalline ZrO_2 nanoparticles. The complete synthesis procedure is illustrated in Fig. 1.

2.3 Antibacterial Activity Assessment

The activity of the green-synthesized ZrO_2 nanoparticles was evaluated through the agar well diffusion method. Nutrient agar plates were sterilised and seeded with *Staphylococcus aureus* and *Escherichia coli* using sterile cotton swabs. Uniform wells were created in the agar, and different concentrations of ZrO_2 nanoparticle suspensions were added to each well. The inoculated plates were incubated at 37 ℃ for 24 hours (Boyanova *et al.* 2005).

2.4 Antifungal Activity Evaluation

The antifungal activity of the synthesised ZrO_2 nanoparticles was assessed against *Candida albicans* and *Aspergillus niger* utilising the agar well diffusion method. The fungal cultures were preserved on PDA plates. Uniform wells were created in the agar, into which varying concentrations of the nanoparticle suspension were introduced (Intra *et al.* 2019).

First, the test strain was transferred into potato dextrose broth (PDB) and incubated at 30 ℃. Subsequently, a well-loaded with 40 µL of test samples was placed onto the inoculated plates using sterile forceps and incubated at 30 ℃ for 24 hours under visible light. Zone of inhibition levels (mm) were measured subsequently for 24 hours at 37 ℃ (Derbalah *et al.* 2019).

2.5 Antioxidant Activity-DPPH Assay

The DPPH radical scavenging assay was employed to assess the antioxidant capacity of ZrO_2 nanoparticles synthesized through eco-friendly methods. About 1 mL of a 0.1 mM methanolic solution was mixed

with nanoparticle dispersions prepared at concentrations between 1 and 10 µg/mL. To accelerate the reaction, these mixtures were maintained at ambient temperature. A UV-Vis spectrophotometer was used to measure absorbance at 517 nm following incubation. The positive control in this experiment was ascorbic acid. The following formula was used to calculate the radical scavenging activity:

$$\% \textbf{ scavenging } = \textbf{100} - \left(\frac{\textbf{Abs }_{\textbf{sample}} - \textbf{Abs }_{\textbf{blank}}}{\textbf{Abs }_{\textbf{control}}}\right) \times \textbf{100} \quad \ldots (1)$$

where Abs_{sample} represents absorbance of the sample, Abs_{blank} corresponds to the absorbance of the blank and $Abs_{control}$ indicates absorbance of the control (Baschieri and Amorati 2021).

2.6 Anticancer Activity – MTT Assay

The MTT assay was employed to assess the cytotoxicity of green-synthesized ZrO_2 nanoparticles on A498 human renal carcinoma cells. Cells were inoculated in 96 well culture plates and incubated for 24 hours to promote optimal adherence. After this interval, varying concentrations of ZrO_2 nanoparticles suspensions were introduced, followed by an additional 24 hour incubation. Thereafter, 20 µL of MTT solution (5 mg/mL) was introduced to each well, and the plates were incubated for an additional 4 hours (Almutary and Sanderson, 2016).

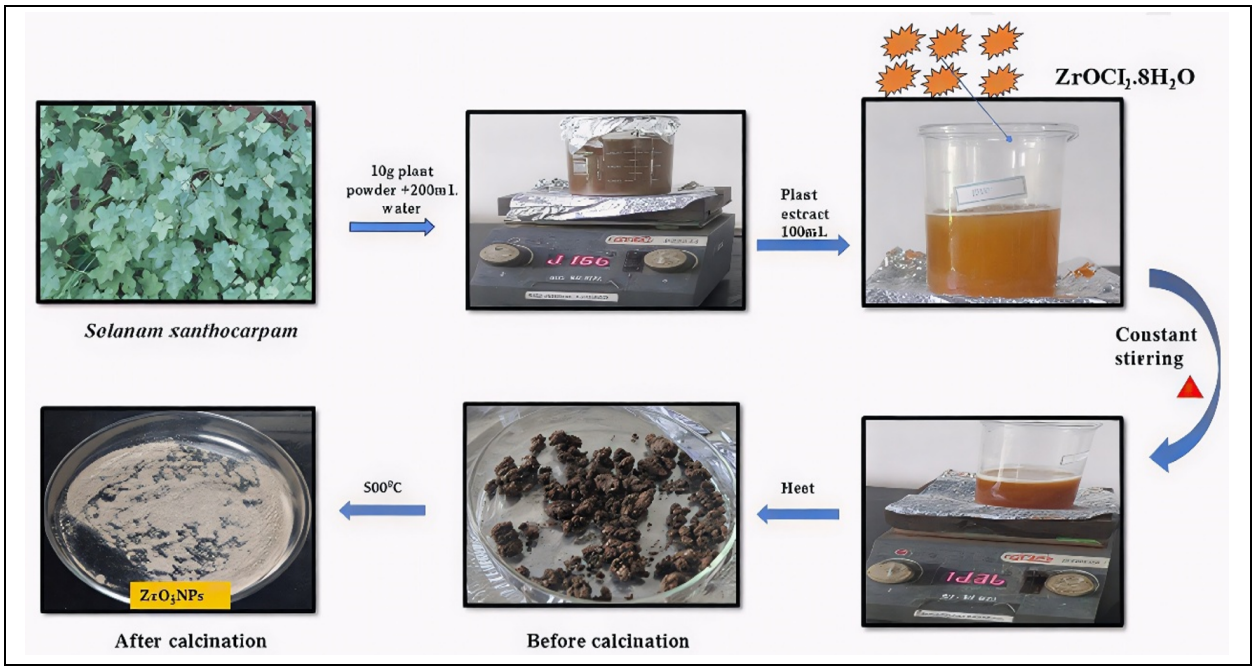


Fig. 1: Synthesis scheme of ZrO_2 Nanoparticles from *Solanum xanthocarpum* leaf extract

3. RESULTS AND DISCUSSION

3.1 XRD Analysis

The crystalline structure and phase composition of the synthesized ZrO_2 nanoparticles were analysed *via* XRD, as illustrated in Fig. 2. Significant diffraction peaks were observed at 2θ angles of approximately 30.2°, 34.2°, 50.3°, 60.2°, and 63.4°, corresponding to the (101), (002), (112), (211), and (202) crystallographic planes, respectively. The diffraction patterns are indicative of the tetragonal phase of zirconium dioxide and correspond with JCPDS card no. 37-1484. The sharpness and intensity of the peaks indicated that the nanoparticles possessed a high degree of crystallinity. The mean crystallite dimension was estimated using the Scherrer formula (Chelliah *et al.* 2023).

$$D = K\lambda / \beta \, Cos \, \theta \quad \ldots (2)$$

3.2 FTIR Spectroscopy

FTIR spectroscopy was employed to determine the functional groups involved in the formation and stabilisation of the nanoparticles (Fig. 3). A broad peak was observed around 3398 cm^{-1} due to O–H stretching vibrations, indicating the likely presence of hydroxyl groups derived from water or polyphenolic compounds. Peaks appearing at 2027 cm^{-1} and 1632 cm^{-1} were attributed to C=C and C=O stretching, respectively, suggesting the involvement of plant-based organic constituents. Additionally, a peak at 1271 cm^{-1} was linked to C–O bending vibrations. Prominent peaks found at 617 cm^{-1} and 512 cm^{-1} were characteristic of Zr–O stretching vibrations, validating the formation of zirconium dioxide nanoparticles. These spectral features highlight the contribution of *S. xanthocarpum*-derived

biomolecules in facilitating both the reduction of zirconium ions and stabilization of the nanoparticles during synthesis (Alagarsamy *et al.* 2022; Chowdhury *et al.* 2023).

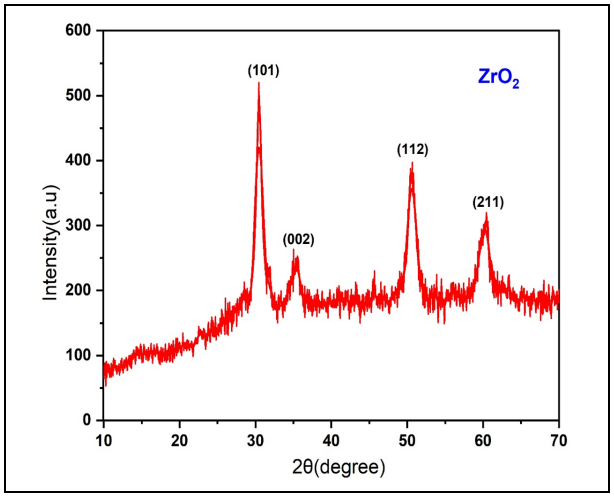


Fig. 2: XRD spectra of the synthesized ZrO_2 nanoparticles

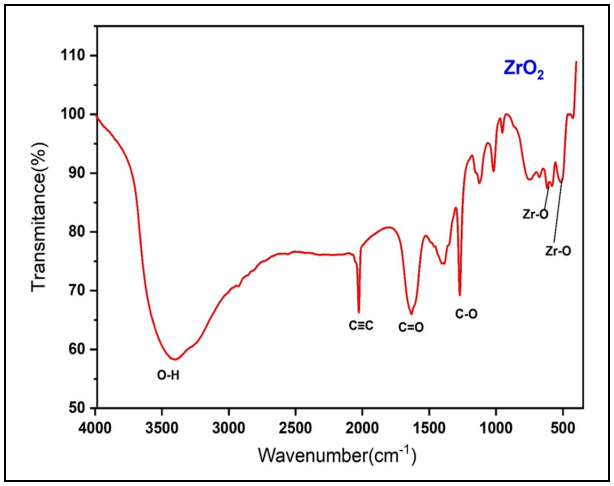


Fig. 3: FT-IR spectrum of ZrO_2 nanoparticles

3.3 UV-Visible Spectroscopy

The optical characteristics of the green-synthesized ZrO_2 nanoparticles were examined utilising UV-Visible absorption spectroscopy (Fig. 4). The spectrum showed distinct absorption bands at 223 nm, 239 nm and 291 nm. These are attributed to charge-transfer transitions between the oxygen 2p and zirconium 4d orbitals, a characteristic feature of Zr–O bonding. The presence of these peaks in the ultraviolet region confirms the nanoscale formation of zirconium dioxide. It indicates that the material exhibits suitable optical behaviour. However, claiming that the UV peaks alone definitively prove nanoscale formation and confirm suitability for biomedical applications is not sufficiently substantiated (Chelliah *et al.* 2023).

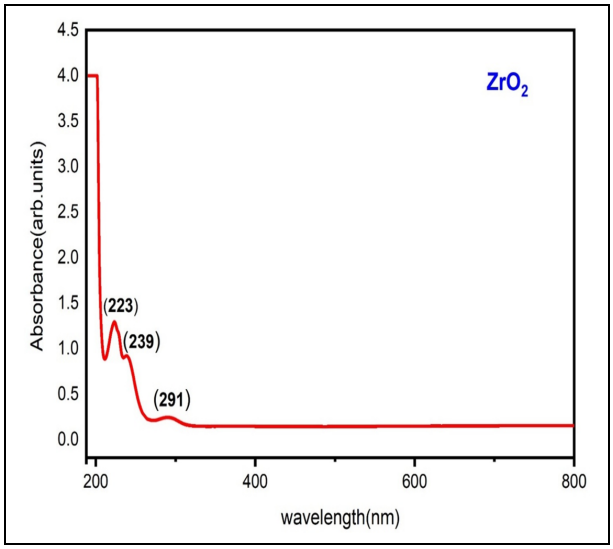


Fig. 4: UV-Visible spectrum of ZrO_2 nanoparticles

3.4 Photoluminescence (PL) Analysis

Photoluminescence spectroscopy was used to investigate the emission characteristics of the synthesized ZrO_2 nanoparticles (Fig. 5). A sharp emission peak was observed at approximately 706 nm, falling within the red region of the visible spectrum. This emission is likely due to oxygen vacancies or inherent structural defects within the nanoparticle lattice, which act as recombination centres for photo-induced charge carriers. The presence of a single, sharp emission peak and the absence of additional bands suggest a relatively pure material with a regulated defect concentration. However, further confirmation could be obtained through PL excitation spectra, lifetime decay measurement or annealing studies. These optical characteristics support the potential of ZrO_2 nanoparticles for optoelectronic and biomedical applications (Sikdar *et al.* 2022).

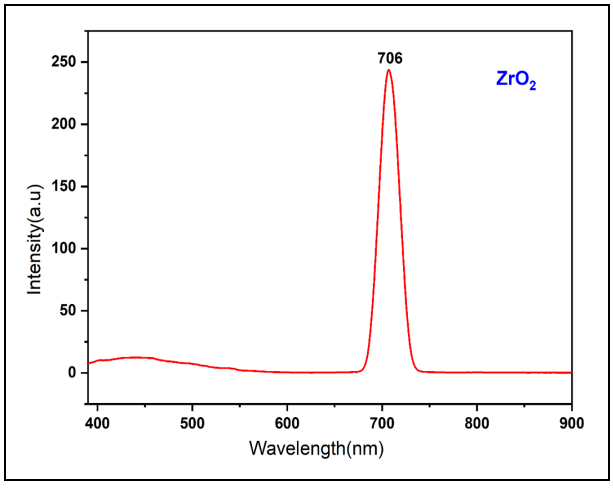


Fig. 5: PL Analysis of ZrO_2 nanoparticles

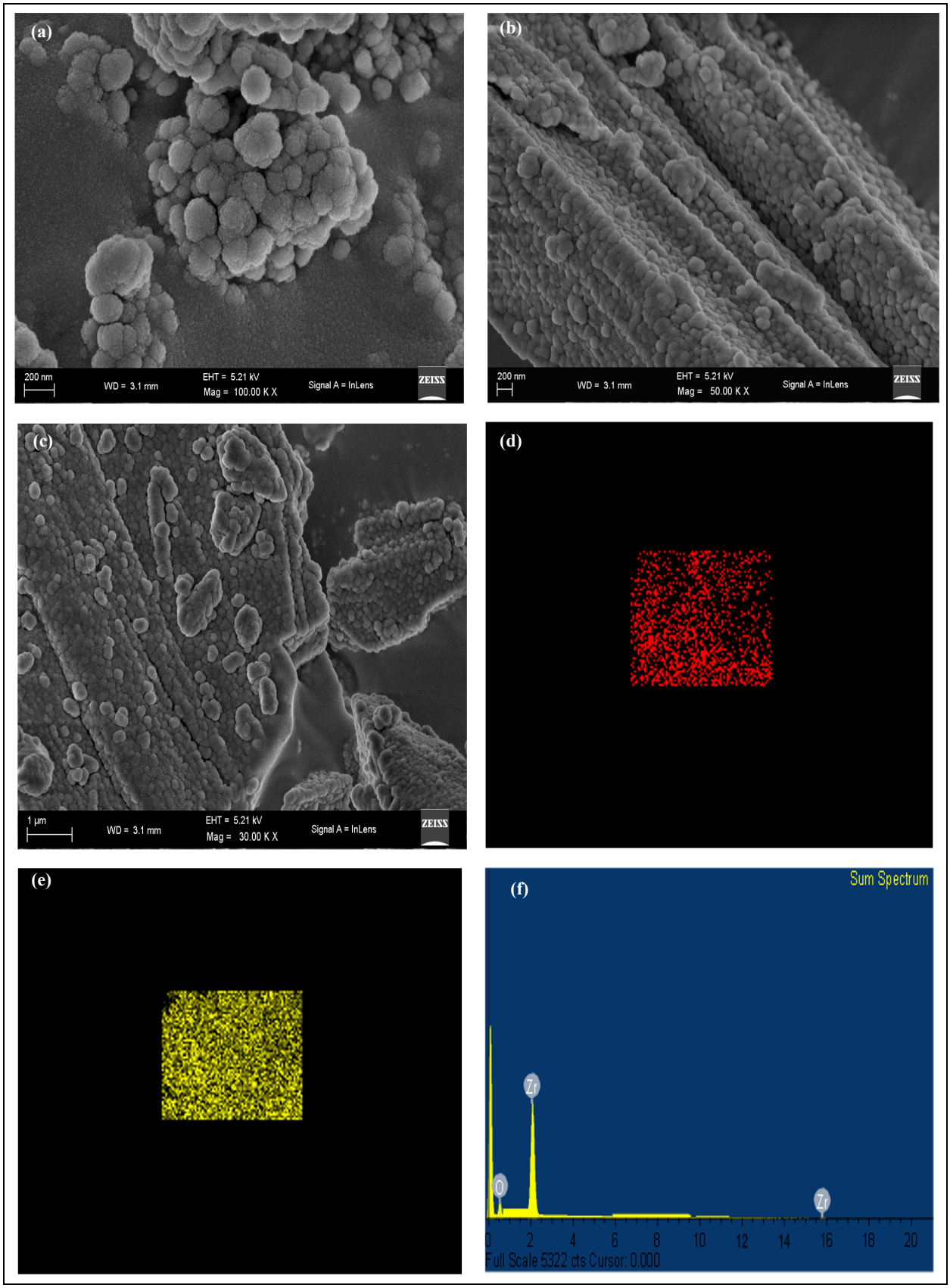


Fig. 6: (a-c) FESEM images, (d and e) Mapping and (f) EDAX spectrum of ZrO_2 Nanoparticles

3.5 FESEM, EDX and Mapping

The morphology and elemental composition of the green-synthesized ZrO_2 nanoparticles were analysed using FESEM, EDX and elemental mapping. FESEM (Fig. a-c) captured at different magnifications revealed predominantly spherical nanoparticles with noticeable agglomeration. This clustering is common in green synthesized nanoparticles because the natural compounds present in the plant extract that act as capping agents. At higher magnification, the particles appear to have a smooth surface, The nucleation and growth of the nanoparticles occurred evenly throughout the sample. Elemental mapping (Fig. d-e) confirmed a uniform distribution of zirconium and oxygen across the sample surface, with zirconium and oxygen represented in yellow and red, respectively. The even dispersion suggested no phase separation or clustering of specific elements. EDX analysis (Fig. f) further validated the elemental composition, showing zirconium and oxygen as the principal constituents, comprising approximately 64.48% and 35.52% by weight, respectively. No foreign elements were detected, confirming the purity and successful green synthesis of ZrO_2 nanoparticles. Variations in properties may be influenced by factors such as synthesis conditions, drying temperature, precursor concentration or natural tendency of nanoparticles to reduce surface energy (Zinatloo-Ajabshir and Salavati-Niasari, 2016; Sagadevan *et al.* 2016).

4. BIOLOGICAL APPLICATIONS

4.1 Antibacterial Activity

The antibacterial activity of ZrO_2 nanoparticles synthesized *via* a green method was evaluated against *Staphylococcus aureus* and *Escherichia coli*. In Fig. 7a, agar well diffusion plates display wells loaded with varying concentrations (25, 50, 75, and 100 µL) of ZrO_2 nanoparticle suspensions.

In the case of *E. coli*, inhibition zones increased from 13 mm at 25 µL to 21 mm at 100 µL. Similarly, for *S. aureus*, the zones expanded from 14 mm to 22 mm across the same concentration range. The results showed a dose-dependent antibacterial effect, with slightly higher efficacy observed against *S. aureus*. Fig. 7b presents a bar graph of inhibition zones (mm). These results affirm the bactericidal potential of green-synthesised ZrO_2 nanoparticles (Tabassum *et al.* 2021).

4.2 Antifungal Activity

To evaluate antifungal efficacy, the synthesised nanoparticles were tested against *Candida albicans* and *Aspergillus niger* using a well diffusion method. Fig. 8a shows four concentrations (25, 50, 75, and 100 µL) of the nanoparticle solution in agar wells. Fig. 8b presents bar graphs comparing the inhibition zones for both fungal strains. The inhibition zones for *C. albicans* ranged from 15 mm to 21 mm and from 13 mm to 22 mm for *A. niger*. *A. niger* exhibited slightly greater sensitivity at higher nanoparticle concentration. The findings confirm the antifungal properties of the ZrO_2 nanoparticles synthesised through green methods.

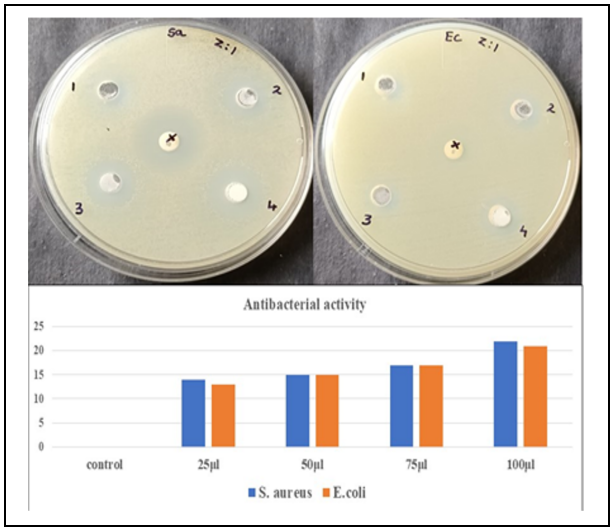


Fig. 7: (a) Antibacterial activity visualized through agar plate and (b) Zone of inhibition for synthesized ZrO_2 nanoparticles against *S. aureus* and *E. coli.*

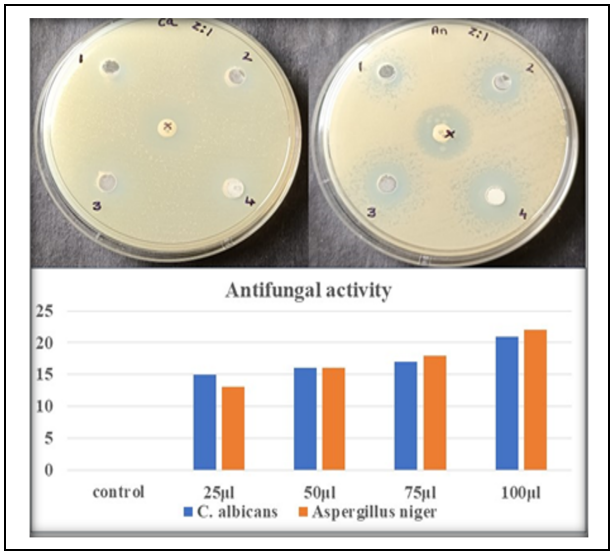


Fig. 8: (a) Antifungal activity visualized through agar plate (b) Zone of inhibition for synthesized ZrO_2 nanoparticles against *C. albicans*, *Aspergillus niger*

4.3 Antioxidant Activity

Fig. 9 shows antioxidant activity of the green-synthesized ZrO_2 nanoparticles evaluated using the DPPH free radical scavenging assay. Vitamin C was employed as the reference standard. An increase in scavenging efficiency was observed with increasing

nanoparticle concentration, indicating a concentration-dependent trend. At 1 µg/mL,

the ZrO_2 nanoparticles showed 24.13% inhibition, while vitamin C showed 27.63%. At 2.5 µg/mL, the nanoparticles displayed 30.69% inhibition, compared with 34.02% for the standard. At 5 µg/mL, inhibition was 36.29% for ZrO_2 and 40.07% for the standard. With 7.5 µg/mL, ZrO_2 nanoparticles reached 41.89%, compared to 45.94% for vitamin C. Finally, at 10 µg/mL, the scavenging activity peaked to 47.88% for ZrO_2 nanoparticles and 51.81% for the control. Although slightly lower than vitamin C, the nanoparticles demonstrated notable antioxidant capacity, supporting their potential biomedical use (Lee *et al.* 2003).

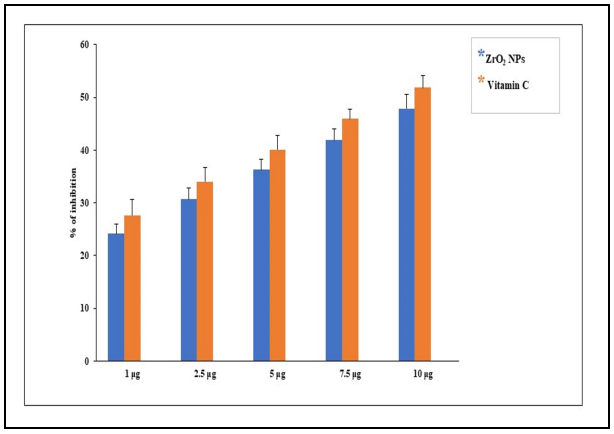


Fig. 9: Antioxidant activity of green-synthesized ZrO_2 nanoparticles

4.4 Anticancer Activity

Fig. 10 shows anticancer activity of ZrO_2 nanoparticles. A progressive decrease in cell viability was observed with increasing nanoparticle concentration (2.5 to 15 µg/mL). The untreated control group exhibited 100 % viability. At 2.5 µg/mL, viability decreased to 92.05 %, followed by 80.03 % at 5 µg/mL and 68.70 % at 7.5 µg/mL. A sharp decline occurred at 10 µg/mL (50.08 %), then 37.56 % at 12.5 µg/mL, and 19.80 % at 15 µg/mL. The results indicate vigorous cytotoxic activity in a concentration-dependent manner.

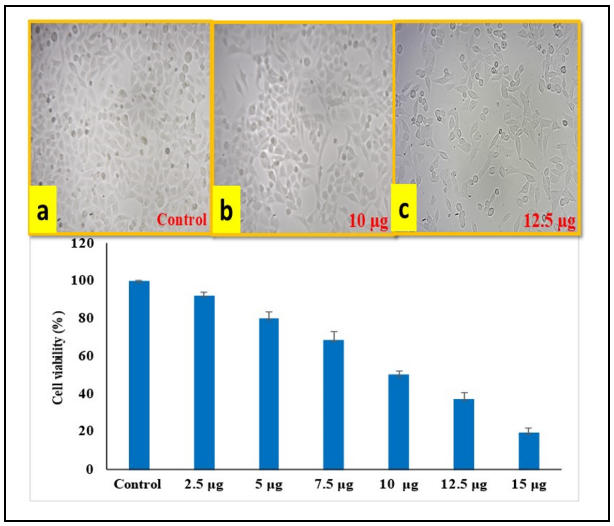


Fig. 10: Anticancer activity of green-synthesized ZrO_2 nanoparticles

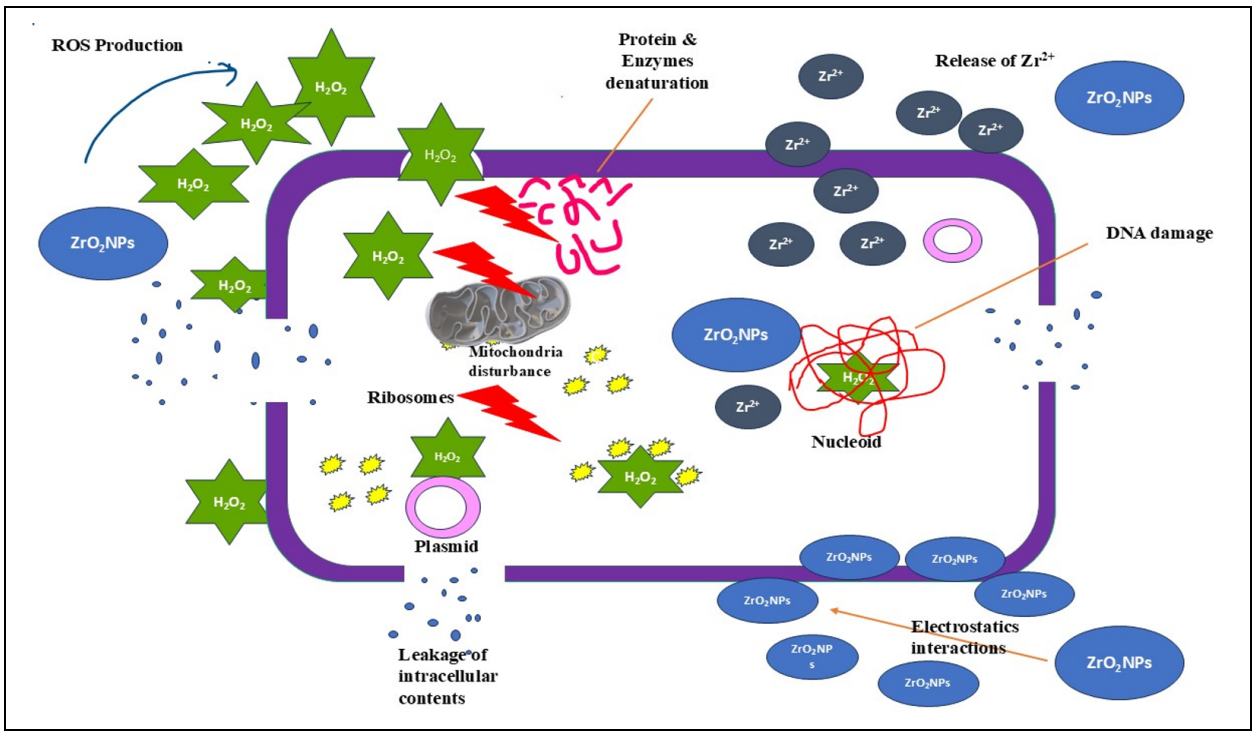


Fig. 11: Proposed cell death mechanism of A498 cell lines induced by ZrO_2 nanoparticles

Fig. 11 illustrates cell- death mechanism induced by ZrO_2 nanoparticles in A498 cells. A combination of oxidative stress, mitochondrial dysfunction and intracellular damage collectively disrupt essential cancer-cell functions. After internalization, ZrO_2 nanoparticles generate excessive reactive oxygen species such as hydrogen peroxide, hydroxyl radicals and superoxide ions, which disturb the cellular redox balance. These ROS attack membrane lipids, structural proteins and DNA, initiating widespread oxidative injury. Mitochondrial membranes are particularly affected, leading to loss of membrane potential and reduced ATP production. This mitochondrial collapse triggers the release of cytochrome-c and activates caspase-mediated apoptotic pathways. Simultaneously, ionic species released from ZrO_2 nanoparticles further intensify toxicity by interfering with enzymes and damaging nuclear components, DNA strand breaks chromatin condensation impair replication and transcription, accelerating apoptotic signaling. Protein denaturation and ribosomal inhibition suppress essential metabolic activities, preventing the cell from repairing accumulated damage. The combined effects of ROS overload, mitochondrial destabilization, DNA injury and metabolic suppression ultimately result in irreversible apoptosis-driven death of A498 renal carcinoma cells (Derbalah *et al.* 2019; Muthulakshmi *et al.* 2023; Tran *et al.* 2022)

5. CONCLUSION

This work successfully demonstrates a sustainable and environmentally friendly technique for synthesizing ZrO_2 nanoparticles using *Solanum xanthocarpum* leaf extract. The synthesized nanoparticles exhibited a well-defined tetragonal crystalline structure, as confirmed through multiple characterization methods. The presence of phytochemicals from the plant extract was evident in facilitating the reduction and stabilization of the nanoparticles during synthesis. Comprehensive analyses including structural, optical, and morphological evaluations confirmed the high purity and desirable properties of the ZrO_2 nanoparticles. Biological investigations revealed strong antibacterial and antifungal activity, along with significant antioxidant and anticancer effects. These findings suggest that green-synthesized ZrO_2 nanoparticles hold considerable promise for use in biomedical applications. Overall, the study presents an efficient, cost-effective, and non-toxic method for fabricating multifunctional nanoparticles suitable for therapeutic purposes.

AUTHOR CONTRIBUTION

AA and SS: Performed experiments, writing, reviewing, and editing; AA: Data analysis

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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