

# Eco-Friendly synthesis, characterization, and biocompatibility of zirconium dioxide nanoparticles using *Calendula officinalis* extract: a possible agent against uropathogenic bacteria

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## Author contributions

Malaichamy HR, Kumaran NKR, Guttapalli T, Subramani K, Girigoswami A, Girigoswami K: Data curation, investigation, methodology, resources; HRM: Writing the original draft; AG, KG: Conceptualization, supervision, visualization, validation, Finalising the manuscript. All the authors contributed to the review and editing of the final version and approved the submission.

## Competing interests

The authors declare no conflicts of interest.

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## Abbreviations

UTI, Urinary tract infections; CO-ZrO<sub>2</sub>-NPs, Calendula officinalis flower mediated zirconium dioxide nanoparticles; UV-vis spectroscopy, UV-visible spectroscopy; FTIR, Fourier transform infrared spectroscopy; XRD, X-ray diffraction; SEM, scanning electron microscopy; EDX, Energy-dispersive X-ray spectroscopy; TEM, transmission electron microscopy; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; ZOI, zone of inhibition; MDR, Multidrug-resistant; ROS, reactive oxygen species; CO, Calendula officinalis; AgNPs, silver nanoparticles; DLS, dynamic light scattering; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DMEM, Dulbecco's minimal essential medium; DMSO, Dimethyl sulfoxide; PDI, polydispersity index.

## Citation

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## Abstract

**Background:** Urinary tract infections (UTIs) are a challenge in the healthcare sector globally, exacerbated by increasing antibiotic resistance. **Methods:** Photophysical characterization of CO-ZrO<sub>2</sub>-NPs was done using UV-vis spectroscopy, XRD, FTIR, SEM, EDX, TEM, and zeta potential measurements. Biocompatibility assessment was performed *in vitro* and *in vivo* using MTT assay, haemolysis assay, and zebrafish embryo toxicity assay, followed by antimicrobial activity estimation using the disc diffusion method. **Results:** The results demonstrated the absorption peak at 300 nm, a hydrodynamic diameter of 156 nm, and a zeta potential of -21 mV, showing moderate stability. The particle size ranged from 163 nm to 224 nm with spherical morphology in SEM, and 50–80 nm in TEM image, with an average particle size of 66 nm. The nanoparticles showed that above 95 % cells were alive, lower than 5 % haemolysis, and no developmental toxicity in zebrafish embryos, indicating a good biocompatibility up to a dose of 50 µg/mL. The antimicrobial activity showed zones of inhibition (ZOIs) of 16 ± 0.14 and 16 ± 0.15, respectively, for *E. coli* and *S. aureus* post-treatment with 100 µg/mL of CO-ZrO<sub>2</sub>-NPs. Anti-biofilm activity was quantified using a crystal violet staining method, which showed a decline in biofilm mass when compared with the untreated control. Antioxidant capacity was determined using the DPPH assay, showing that the CO-ZrO<sub>2</sub>-NPs possessed antioxidant activity, however, lower than that of ascorbic acid at the respective dose. **Conclusion:** Results demonstrate the successful green synthesis of stable CO-ZrO<sub>2</sub>-NPs with significant antimicrobial, moderate antioxidant properties, and moderate anti-biofilm activity against key uropathogens. This study highlights the potential of green-synthesized CO-ZrO<sub>2</sub>-NPs as one of the therapeutic approaches to kill UTI-causing pathogens.

**Keywords:** Healthcare, green synthesis, zirconium dioxide, antibacterial activity, antibiofilm activity

**Highlights**

1. Stable CO-ZrO<sub>2</sub> nanoparticles were successfully green synthesized using *Calendula officinalis* extract.
2. The obtained CO-ZrO<sub>2</sub> nanoparticles possess good biocompatibility, prominent antibacterial, anti-biofilm and moderate antioxidant properties.
3. CO-ZrO<sub>2</sub> nanoparticles have promising application potential for urinary tract infection therapy.

**Medical history of objective**

*Calendula officinalis* is a traditional medicine known for its ability to act as an analgesic, anti-inflammatory, wound-healing, antiviral, and antiemetic. It has been used in traditional medicine since the 12th century. Antimicrobial activity of metal and metal oxide nanoparticles has been identified so far, such as silver nanoparticles, zinc oxide nanoparticles, and copper oxide nanoparticles. Green-synthesized ZrO<sub>2</sub> nanoparticles using *Calendula officinalis* flower extract have not been explored for their antimicrobial activity. Since the flower extract is already known for its antibacterial activity, it will additionally exert the effect when used for the ZrO<sub>2</sub> synthesis. In this study, we have explored the green-synthesized ZrO<sub>2</sub> nanoparticles using *Calendula officinalis* flower extract and investigated their biocompatibility and antimicrobial activity. This study will impart knowledge to future researchers to utilize the nanoparticle as a potent antimicrobial agent.

**Introduction**

Urinary tract infections (UTIs) are commonly known to affect the population worldwide, affecting millions of individuals annually [1]. UTIs encompass a range of conditions, from asymptomatic bacteriuria to severe kidney infections (pyelonephritis), with significant morbidity and healthcare costs [2, 3]. *Escherichia coli* (*E. coli*) is the predominant causative agent, accounting for 70-95% of uncomplicated UTIs [4]. Other common uropathogens include *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus saprophyticus*, and *Proteus mirabilis* [5–7]. The conventional treatment for UTIs relies heavily on antibiotics, and it has also been speculated that a lack of vitamin D can aggravate the UTI conditions [8]. However, the widespread and often inappropriate use of antibiotics has led to a dramatic increase in antibiotic resistance among uropathogens [9, 10]. Multidrug-resistant (MDR) strains are becoming increasingly prevalent, rendering many commonly used antibiotics ineffective [11]. This poses a serious threat to public health, as treatment options for UTIs caused by MDR bacteria are limited and often associated with higher morbidity and mortality rates [1, 12]. Furthermore, recurrent UTIs are common, with a significant proportion of women experiencing multiple infections within a year. These recurrent infections are often caused by the same or closely related strains, highlighting the need for alternative strategies to prevent and treat UTIs [13].

Nanotechnology offers promising solutions for many biomedical applications [14–17] and environmental applications [18], including combating antibiotic resistance [19, 20]. Metal and metal oxide nanoparticles (NPs) have emerged as potential antimicrobial agents due to their unique physicochemical properties and broad-spectrum activity [19, 21–23]. Zirconium dioxide (ZrO<sub>2</sub>) nanoparticles, in particular, have garnered considerable attention due to their biocompatibility, chemical inertness, and stability. ZrO<sub>2</sub>-NPs exhibit a range of favorable properties, including high refractive index, excellent mechanical strength, and catalytic activity [24]. These properties make them suitable for a range of biomedical applications,

which include drug delivery, bioimaging [17], and antimicrobial therapy [25]. Several studies have confirmed the antimicrobial activity of ZrO<sub>2</sub>-NPs against a variety of bacteria, which include Gram-positive and Gram-negative pathogens [26].

Nanoparticles, with their unique properties arising from their size and shape, have become a focal point of scientific research [27–30]. The chemical synthesis of these nanoparticles, however, mostly comprises hazardous chemicals, and the processes are energy-intensive, which raises environmental concerns [31, 32]. Green synthesis, utilizing biological resources like plant extracts, offers a sustainable alternative. Traditional methods for the synthesis of nanoparticles include the method of chemical reduction, the sol-gel method, and the hydrothermal method. These approaches often require toxic precursors and high temperatures, and generate harmful byproducts. Green synthesis, on the other hand, employs environmentally benign reducing agents and stabilizers derived from natural sources, minimizing the environmental impact [32].

Biofilm formation is a major virulence factor for many uropathogens, contributing to chronic and recurrent UTIs [33]. Biofilms are defined as complex bacterial communities entrapped within an extracellular matrix that they produce. Bacteria that reside inside the biofilms are highly antibiotic resistant and more resistant to host immune defenses than planktonic (free-floating) bacteria. The extracellular matrix offers a physical barrier that prevents antibiotics from reaching the bacteria, while also shielding them from phagocytosis and other immune responses [34, 35]. Biofilm-associated UTIs are often difficult to eradicate, leading to persistent infections and increased morbidity. Oxidative stress plays a significant role in the pathogenesis of UTIs. During infection, ROS produced by the host enhances bacterial killing. However, excessive ROS production can also damage host tissues, contributing to inflammation and tissue injury [36]. Uropathogens can also induce oxidative stress within the urinary tract [37]. Antioxidants can help mitigate the damaging effects of oxidative stress by scavenging ROS and protecting cells from oxidative damage. Therefore, agents with antioxidant properties may have therapeutic potential in the management of UTIs.

*Calendula officinalis* (CO), commonly known as marigold, is a medicinal plant belonging to the Compositae family [38]. In traditional medicine, CO extract has been known for its anti-inflammatory, wound-healing, and antimicrobial properties. The flower extract contains various bioactive compounds, including terpenoids, flavonoids, and phenolic acids, which can act as reducing and capping agents in nanoparticle synthesis [39, 40]. *Calendula officinalis* extract was utilized for green synthesis of a range of metal nanoparticles, including silver nanoparticles (AgNPs) [41]. The extract's reducing agents, such as flavonoids and terpenoids, facilitate the reduction of metal ions to their nano-scale form. The use of *Calendula officinalis* offers a biocompatible and sustainable route for nanoparticle production [42].

In order to overcome the use of harsh chemicals, we have used *Calendula officinalis* flower extract for nanoparticle synthesis, which facilitates solid waste utilization along with green synthesis. The flower extract was used to synthesize zirconium dioxide nanoparticles (CO-ZrO<sub>2</sub>-NPs) and was assessed using some characterization techniques, like UV-visible spectroscopy revealing its characteristic absorption spectrum; hydrodynamic diameter using dynamic light scattering (DLS) that provides approximate size; stability in aqueous solution using zeta potential analysis; surface morphology and size, using scanning electron microscopy (SEM), EDAX; nanoparticle size using transmission electron microscopy (TEM); crystalline structure using X-ray diffractometry; and characteristic bonds using Fourier transform infrared spectroscopy (FTIR). The antimicrobial assay was done for *E. coli* and *S. aureus* using the disc diffusion method, and antibiofilm assessment was done for *E. coli* and *S. aureus*. The antioxidant capacity of CO-ZrO<sub>2</sub>-NPs was also estimated by the DPPH assay. The *in vitro* and *in vivo* biocompatibility estimation was performed using MTT assay, haemolysis assay, and zebrafish embryo study.

## Materials and methods

### Materials used

*Calendula officinalis* dried flowers were procured from Amazon India Pvt. Ltd., zirconium (IV) propoxide was obtained from Sigma-Aldrich, USA, ethanol was from E. Merck, India, and other necessary chemicals, including antibiotic antimycotic solution, trypsin-EDTA solution, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Dulbecco's minimal essential medium (DMEM), and Mueller Hinton agar, were procured from HiMedia Pvt. Ltd, India. Cell lines were ordered from NCCS, Pune, India, and zebrafish embryos were procured from a local fish farm, Tarun Fish Farm, Chennai, India. Fetal bovine serum was obtained from Gibco, USA. All the chemicals and reagents were of analytical grade and used directly without further purification.

### Preparation of *Calendula officinalis* flower aqueous extract

The dried *Calendula officinalis* flowers (0.25 g) were added to deionized water (100 mL) and heated at 80 °C for 2 h. Then, the mixture was kept for ultrasonication in a bath sonicator for a further 1h. The mixture was filtered using Whatman filter paper to remove any solid particles and kept at 4 °C, sealing the tube with parafilm for further use. Phytochemicals were identified according to Deepika et al. (2024) [43], and the GC-MS was used to analyze the components, which was done according to Pandiarajan et al. (2025) [29]. The aqueous extract was directly used for the phytochemical analysis, whereas for the GC-MS analysis, the aqueous extract was completely dried in a rotatory evaporator and dissolved in methanol.

### Zirconium dioxide nanoparticle synthesis using the *Calendula officinalis* extract

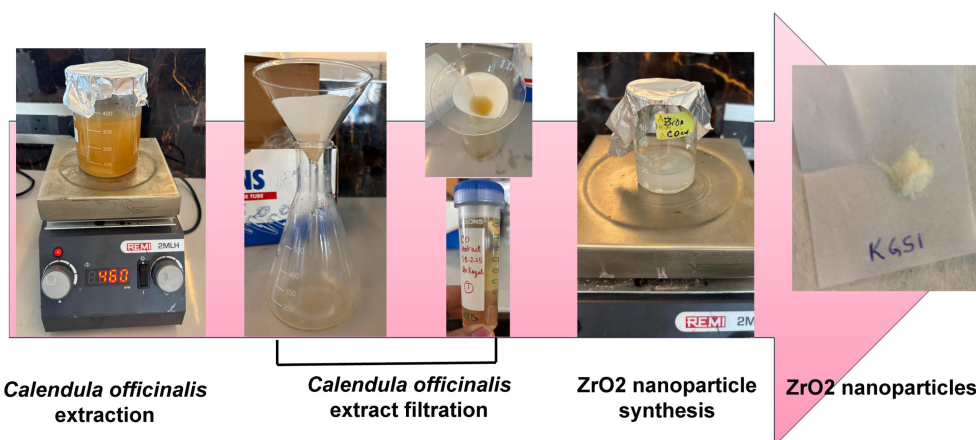
5 mL zirconium (IV) propoxide solution was added to 20 mL of ethanol (95 %) and allowed to stir in a magnetic stirrer for 30 min at 500 rpm. The solution's pH was reduced to 4.0 by gradually adding nitric acid, for the hydrolysis and condensation reactions to take place. The reaction mixture was stirred for an additional hour using a magnetic stirrer. Then, 2 mL of CO extract and 8 mL of ethanol were added and mixed via magnetic stirring for 2h, and a precipitate was observed to appear. For additional aging to take place, stirring was continued for 1 h, and the precipitate was then washed using centrifugation at 8,000 rpm for 10 min, thrice using ethanol. The unreacted chemicals got washed during the washing step, and the precipitate was dried and calcined at 300 °C. Then the powder was ground with the help of a mortar and pestle to get a fine powder, which was our product. The steps of synthesis are shown in Scheme 1.

### Photophysical characterization of the CO-ZrO<sub>2</sub> nanoparticles

Photophysical characterizations were done according to our previous study [44]. The as-synthesized CO-ZrO<sub>2</sub>-NPs were used for the XRD, FTIR, TEM, SEM, and EDAX analysis, whereas a dilute sample in distilled water was prepared for UV-vis spectrophotometry, DLS, and zeta potential analysis. UV-vis spectroscopy was performed to confirm the formation of ZrO<sub>2</sub> nanoparticles and to determine their optical properties. The UV-vis spectrum was recorded using a UV-vis spectrophotometer (Shimadzu UV-1900i UV-visible spectrophotometer, Nakagyo-ku, Kyoto 604-8511, Japan) in the wavelength range of 200-800 nm. DLS was used to measure the hydrodynamic diameter and particle size distribution, and the zeta potential analysis was performed to estimate the colloidal stability of the CO-ZrO<sub>2</sub>-NPs. The measurements were performed using a DLS analyzer (Malvern Zetasizer Nano ZS, Malvern Panalytical Limited, Spectris, Grovewood Road, United Kingdom) at a scattering angle of 90 °. XRD analysis provided information about the crystal structure and phase purity of the CO-ZrO<sub>2</sub>-NPs. The XRD patterns were recorded using an X-ray diffractometer (Bruker X-ray diffractometer (Bruker Corporation, Billerica, MA, USA) with Cu K $\alpha$  radiation (1.54 Å). SEM (SEM-FEI QUANTA 250 FEG, Thermo Fisher (Thermo Fisher Scientific, Waltham, MA, USA) was used to observe the morphology and size of the CO-ZrO<sub>2</sub>-NPs. The SEM images were obtained using a scanning electron microscope set at an accelerating voltage of 15 kV. TEM images were obtained by taking the powdered CO-ZrO<sub>2</sub>-NPs over a copper grid and subjecting them to the TEM analysis using HRSTEM (ThermoFisher - Scientific; MODEL NUMBER: TALOS F200S G2). FTIR spectroscopy (Bruker Alpha FTIR spectrometer, Bruker Corporation, Billerica, MA, USA) was used for functional group detection that were present on the CO-ZrO<sub>2</sub>-NP's surface. The FTIR spectra were recorded using an FTIR spectrometer in the wavenumber range of 400-4000 cm<sup>-1</sup> in transmittance mode.

### Antioxidant, antibacterial, and antibiofilm activity assessment

DPPH radical scavenging assay provided us with the antioxidant activity measurement of CO-ZrO<sub>2</sub>-NPs [45]. A 0.1 mM stock solution was prepared for 2,2-diphenyl-1-picrylhydrazyl (DPPH) using methanol as a solvent. A fresh working solution of CO-ZrO<sub>2</sub>-NPs was prepared (1 mg/mL in methanol). The CO-ZrO<sub>2</sub>-NPs at 10, 20, 30, 40, 50 µg/mL concentrations were added to 200 µL of the DPPH working solution, present inside the wells of a 96-well plate, followed by a 30 min dark incubation. Ascorbic acid was taken as a positive control in the same concentrations as the nanoparticles. The absorbance of the 96-well plate was measured at 517 nm using a plate reader (Tecan), where the blank used was methanol for ascorbic acid and only CO-ZrO<sub>2</sub> + methanol for the nanoparticles to rectify the scattering effect of CO-ZrO<sub>2</sub> nanoparticles. The DPPH scavenging activity (in percentage) was assessed using the formula given below:



**Scheme 1** Synthesis stages of CO-ZrO<sub>2</sub>-NPs.

At the beginning, the extraction of *Calendula officinalis* flower was prepared, followed by filtration. Using this extract, the ZrO<sub>2</sub> nanoparticles were synthesized using the precipitation method, washed, dried, and calcined. Then the obtained powder was ground to get the fine CO-ZrO<sub>2</sub>-NPs.

$$\% \text{ DPPH Scavenging Activity} = \frac{(A_{\text{control}} - A_{\text{sample}})}{(A_{\text{control}})} \times 100$$

where A control is the absorbance of the control (DPPH solution without the sample), and A sample is the absorbance of the sample (DPPH with CO-ZrO<sub>2</sub>-NPs or ascorbic acid).

The antimicrobial activity of the CO-ZrO<sub>2</sub>-NPs was assessed by the disc diffusion technique [46]. Mueller Hinton agar plates were prepared and sterilized by standard autoclaving procedure (121 °C, 15 min). Post sterilization, the medium was gently poured into the sterile Petri plates, avoiding bubble formation, and cooled to room temperature. The microbial suspension (*Escherichia coli* and *Staphylococcus aureus*) was spread uniformly using sterile cotton swabs over the agar plates. Sterile discs of 9 mm diameter were placed on the agar plates using a sterile tweezer. CO-ZrO<sub>2</sub>-NPs were added to the discs at different concentrations (25, 50, 100 µg/mL). The antibiotic disc (Amoxicillin- 10 µL) was used as a standard. Incubation was performed for 24 h, and antimicrobial activity was assessed by measuring the diameter of the inhibition zone around the discs. Sterile PBS (pH = 7.4) was used as a negative control. The diameter of the zone of inhibition was measured using a ruler and recorded in millimeters (mm). The experiment was done in triplicate.

The anti-biofilm assay was done according to the procedure by Biswas et al. 2025 [47]. Biofilms of *S. aureus* and *E. coli* were developed by incubating bacterial suspensions in 96-well microtiter plates for 72 hours. During biofilm formation, 300 µL of CO-ZrO<sub>2</sub>-NPs suspensions at different concentrations were introduced into the wells. The prolonged incubation enabled bacterial adhesion to the well surfaces and the development of mature, three-dimensional biofilm structures. Following exposure to the nanoparticles, non-adherent cells were removed by washing the wells three times with distilled water, as described in our previous studies [21, 47]. Biofilm biomass was quantified using crystal violet staining. The absorbance of the stain solubilized by ethanol was measured at 590 nm using a microplate reader (Tecan). The absorbance readings were used to determine the percentage reduction in biofilm biomass relative to untreated control wells, thereby assessing the nanoparticles' antibiofilm efficacy. All the experiments were performed in triplicate.

#### ***In vitro* and *in vivo* biocompatibility evaluation**

The synthesized CO-ZrO<sub>2</sub>-NPs were aimed at inhibiting the growth of uropathogens. Hence, the biological effect both *in vitro* as well as *in vivo* ought to be assessed to determine the use of this nanoparticle in a safe manner for humans. Normal fibroblasts (3T3-L1) were used to assess *in vitro* biocompatibility using the MTT assay. Briefly, 10<sup>4</sup> exponentially growing cells were added to each well in a 96-well plate, and the cells were allowed to attach for 24 h. Treatment with different concentrations of CO-ZrO<sub>2</sub>-NPs was conducted by suspending the nanoparticles in sterile PBS and filtering the same using a 0.22 µm sterile filter. For 24 h, the treatment was done, keeping the plates in a CO<sub>2</sub> incubator under humid conditions. Then, MTT reagent was added into each well of the 96-well plate (10 µL from a 5 mg/mL stock dissolved in sterile DMEM), and the plate was kept inside a CO<sub>2</sub> incubator in the dark for a further 4 h. Formazan crystals, formed by the metabolically active cells, were solubilized in DMSO, and the absorbance was read using a plate reader (Tecan) at 570 nm. The cell viability was assessed using the formula reported previously [44].

Haemolysis assay was performed using the methodology reported [48]. The assay was done according to the permissions obtained by the Institutional Ethics Committee, Saveetha Medical College and Hospital, SIMATS, Thandalam, Chennai, India. (005/07/2025/IEC/SMCH). The Declaration of Helsinki was followed to conduct the study. Informed consent was obtained at the onset, and 3 mL of venous blood was drawn from a healthy donor (male, 21 years old, Indian) and transferred to an EDTA-containing vacutainer. The blood was diluted 10-fold with normal saline, and 100 µL was added in triplicate to 1.5 mL tubes. The remaining 900 µL was filled with the nanoparticles that yielded final concentrations of 10, 25, and 50 µg/mL. Two-hour incubation was done at a 37 °C incubator, and the

tubes were centrifuged at 12,000 rpm for 1 min. Images were captured of the tubes post-centrifugation, and the absorbance was measured at 540 nm (absorption peak of hemoglobin) to calculate the haemolysis percentage. Distilled water was taken as a positive control, and normal saline was taken as a negative control for the haemolysis assay.

*In vivo* biocompatibility was assessed using zebrafish embryos after obtaining appropriate permission from the Institutional Animal Ethics Committee (IAEC approval No. SU/CLAR/RD/19/2025) of Saveetha Medical College and Hospital. The study was performed in accordance with the OECD guidelines. ARRIVE guidelines were strictly followed. Eight hours post-fertilization (hpf), zebrafish embryos were collected, and healthy embryos were gently transferred to a 24-well plate containing E3 medium. The embryos were treated with different doses of CO-ZrO<sub>2</sub>-NPs, and observed under a microscope for any kind of abnormalities, developmental delay, delayed hatching, mortality, pericardial edema, yolk sac edema, tail bent, spine bent, defect in eye development, and abnormal heart beat. The cumulative hatchability was calculated according to Janani et al. [48].

#### **Statistical analysis**

An unpaired Student's t-test calculated the level of significance in the treated group compared to the untreated control or positive control. All the experiments were performed in triplicate, and the mean ± S.E.M was documented. *P* < 0.05 was recorded as significant compared to the control.

## **Results and discussion**

#### **Phytochemical constituents and GC-MS analysis of *Calendula officinalis* flower extract**

The phytochemicals found in the *Calendula officinalis* flower extract were analyzed using different biochemical tests. The results are depicted in Figure 1a, showing the presence of saponins, carbohydrates, tannins, reducing sugars, flavonoids, glycosides, and coumarins. The GC-MS analysis was performed to further investigate the chemical components present in the extract that can contribute towards the reduction as well as capping of the nanoparticles. The GC-MS spectrum of the CO extract is shown in Figure 1b, and its components are indicated in Figure 1 c–1g, representing the Imidazole, 2-[[[(beta.-carboxy)propionyl]amino]-; Ethyl homovanillate, TMS derivative1-(3,5-Di-tert-butyl-2,6-dihydroxyphenyl)ethenone; Dichloroacetic acid, 2-methyloct-5-yn-4-yl ester; and Silane, chlorodiethylheptyloxy-, respectively. The abundance percentages, chemical formula, and molecular weights are shown in Table 1.

#### **Characterization of the as-synthesized CO-ZrO<sub>2</sub> nanoparticles**

The successful synthesis of any nanoparticle can be assured by the different photophysical analyses. The absorption spectrum of CO-ZrO<sub>2</sub>-NPs was observed at 300 nm (Figure 2a), confirming the UV light absorption capacity. However, the absorption range was wide, from 300 nm to 700 nm, including both the UV and visible regions. This indicated that the synthesized nanoparticles can absorb both UV light and visible light. The hydrodynamic diameter was found to be 156 nm (Figure 2b), ensuring the nano size and the zeta potential to be -21 mV (Figure 2c). The polydispersity index (PDI) value was 0.3808, indicating a relatively broad size distribution. It has been reported earlier that a nanoparticle having a PDI value up to 0.5 can be considered as monodispersed [49, 50], however, according to the strict definition of polydispersity, a value of 0.0-0.1 means monodispersed, > 0.1 and < 0.4 is moderately dispersed, and > 0.4 indicates polydisperse [51]. When a nanoparticle is suspended in water or any aqueous medium, the water molecules adhere to the surface of the nanoparticle. Whereas the suspension liquid flows around the nanoparticles, acting as a mobile fluid. The nanoparticles move in Brownian motion inside the liquid due to high dilution, and a potential difference develops between the slipping plane and the adhered plane of water. Also known as the electrokinetic potential, zeta potential measurement records the potential difference developed

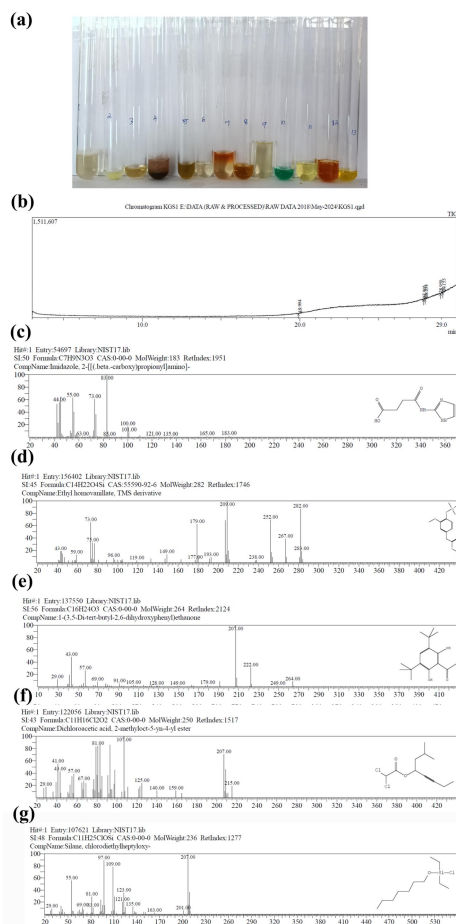


**Figure 1** Phytochemical characterization and GC-MS analysis of the CO extract.

(a) Phytochemical analysis of the CO extract. (b) GC-MS spectrum of the methanol-reconstituted CO extract. (c–g) GC-MS spectra of the identified components: Imidazole, 2-[[(.beta.-carboxy)propionyl]amino]-; Ethyl homovanillate, TMS derivative; 1-(3,5-Di-tert-butyl-2,6-dihydroxyphenyl)ethanone; Dichloroacetic acid, 2-methyloct-5-yn-4-yl ester; and Silane, chlorodiethylheptyloxy-, respectively.

**Table 1** Chemical composition of the methanolic reconstituted dried aqueous extract of *Calendula officinalis*: names, formulas, structures, molecular weights, and relative abundances

| Peak No. | Name                                              | Chemical Formula      | Structural Formula | Molecular weight | Retention time (min) | Abundance (%) |
|----------|---------------------------------------------------|-----------------------|--------------------|------------------|----------------------|---------------|
| 1        | Imidazole, 2-[[(.beta.-carboxy)propionyl]amino]-  | $C_7H_9N_3O_3$        |                    | 183              | 19.994               | 20.08         |
| 2        | Ethyl homovanillate, TMS derivative               | $C_{14}H_{22}O_4Si$   |                    | 282              | 27.905               | 16.97         |
| 3        | 1-(3,5-Di-tert-butyl-2,6-dihydroxyphenyl)ethanone | $C_{16}H_{24}O_3$     |                    | 264              | 28.034               | 21.27         |
| 4        | Dichloroacetic acid, 2-methyloct-5-yn-4-yl ester  | $C_{11}H_{16}Cl_2O_2$ |                    | 250              | 28.950               | 24.77         |
| 5        | Silane, chlorodiethylheptyloxy-                   | $C_{11}H_{25}ClOSi$   |                    | 236              | 29.155               | 16.91         |

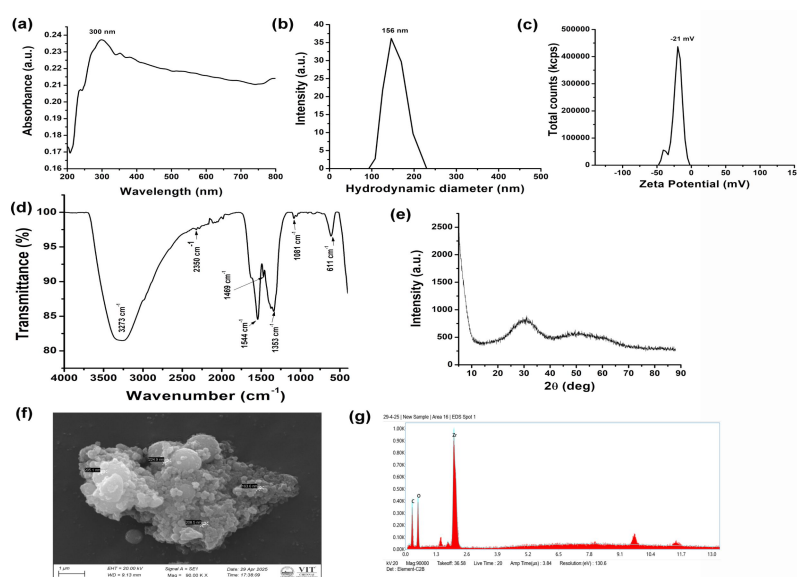


**Figure 2 Characterization of CO-ZrO<sub>2</sub> nanoparticles.**

(a) UV-visible absorption spectrum, (b) hydrodynamic diameter, (c) zeta potential analysis, (d) FTIR spectrum, (e) XRD spectrum, (f) SEM image, and (g) EDAX analysis for as-synthesized CO-ZrO<sub>2</sub> nanoparticles.

between the dispersion medium and the liquid, which is stationary and attached to the surface of the nanoparticle. The zeta potential value above 25 mV (either positive or negative) ensures high stability [52, 53]. Our synthesized CO-ZrO<sub>2</sub>-NPs showed moderate stability. The FTIR peaks (Figure 2d) were observed at 3273 cm<sup>-1</sup> (hydroxyl bond stretching) [21], 2354 cm<sup>-1</sup> (hydroxyl zirconium stretching vibration) [54, 55], 1469 cm<sup>-1</sup> (amide II stretching representing carbonyl stretching present in CO extract) [56], 1544 cm<sup>-1</sup> (C=O stretching) [21], 1353 cm<sup>-1</sup> (C-O bending), 1081 cm<sup>-1</sup> (C-H bending) [57, 58], and 611 cm<sup>-1</sup> (Zr-O vibrations) [55]. This represents a perfect bond formation between the Zr and O elements, incorporating the phytochemicals available in the CO extract. The XRD data (Figure 2e) shows peaks at 32° and 51°, which represent the reflection planes (220) and (311) belonging to tetragonal and monoclinic zirconia. However, the background noise and wide peaks indicated predominantly amorphous structure with a small amount of crystallinity present. The tetragonal and monoclinic phases may be present in very small amounts since the crystallinity was low. The XRD peaks corroborate the peaks for the JCPDS card no. 80-0965. Similar XRD peaks have been observed in previous studies where zirconyl nitrate and ethylenediamine were used to synthesize ZrO<sub>2</sub> nanoparticles [58]. Horti et al. observed a similar XRD pattern while exploring the effect of different calcination temperatures on ZrO<sub>2</sub> crystal formation [54].

The SEM images (Figure 2f) showed spherical morphology of the as-synthesized CO-ZrO<sub>2</sub>-NPs with a size distribution of 163–224 nm, showing a suitable particle size of the nanoparticle that can be used for biomedical applications. The component analysis done by EDAX (Figure 2g) showed the predominant peaks for Zr and O. A similar spherical morphology was observed in previous studies [59–61]. Thus, we could successfully synthesize ZrO<sub>2</sub> nanoparticles using the CO extract.

Figure 3a shows the TEM image of the CO-ZrO<sub>2</sub>-NPs. The shape was observed to be spherical, and the size was in the range of 50–80 nm. The size distribution is shown in Figure 3b, showing an average diameter of 66 nm. There was a slight difference in particle size revealed by DLS (156 nm), SEM (163–224 nm), and TEM (66 nm), which can arise due to various reasons. In TEM during sample preparation, it is uncertain which face of the particle will be attached to the copper grid or in which orientation the particle will be cast. Moreover, in SEM, we coat the sample with gold and palladium alloy for improved conductivity, which contributes to some thickness. In DLS, the particles are surrounded by layers of water molecules, and hence, we get a higher diameter compared to the original particle size.

Therefore, we can conclude that our synthesized CO-ZrO<sub>2</sub>-NPs had a diameter of 66 nm.

#### Antioxidant, antibacterial, and antibiofilm assessment

The antioxidant capacity of the CO-ZrO<sub>2</sub>-NPs was assessed in comparison to that of ascorbic acid. The data, as depicted in Figure 4a, showed that the synthesized nanoparticles exerted a free radical scavenging effect in a dose-dependent fashion. The antioxidant activity of CO-ZrO<sub>2</sub>-NPs was lower than that of ascorbic acid at every respective dose. However, in comparison to ascorbic acid's free radical scavenging capacity, the antioxidant capacity of CO-ZrO<sub>2</sub>-NPs was not significant at the treated doses (10, 20, 30, 40, and 50 µg/mL). The antibacterial effect (Figure 4b, 4c) against *E. coli* and *S. aureus* at a 100 µg/mL concentration of CO-ZrO<sub>2</sub>-NPs was found to be 16 ± 0.14 and 16 ± 0.15, respectively. The antibiotic zones were 10 ± 0.12 and 10 ± 0.13, respectively, for *E. coli* and *S. aureus*. The ZOI for *E. coli* and *S. aureus* was higher than that of the ampicillin disc we used as the standard antibiotic (Figure 4d). These pathogens are the basic pathogens responsible for UTI, and we further investigated the anti-biofilm activity of the CO-ZrO<sub>2</sub>-NPs. The biofilm inhibition was observed by a decrease in biofilm mass as the dose increased for both the Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria (Figure 4e). Thus, the synthesized CO-ZrO<sub>2</sub>-NPs could show significant antibacterial activity and moderate biofilm-disrupting properties.

Zirconium dioxide (ZrO<sub>2</sub>) nanoparticles are present mainly in crystal form, and the crystalline structures commonly found are monoclinic, tetragonal, or cubic phases [62]. Their nanoscale size provides a high surface-to-volume ratio, positively impacting their surface reactivity and interaction with microbial cells. Structurally, ZrO<sub>2</sub> nanoparticles are chemically stable, biocompatible, and possess strong mechanical strength [63]. Their antimicrobial activity is mainly attributed to ROS generation, microbial cell membrane disruption, and interference with cellular metabolism. These nanoparticles can damage bacterial cell walls, leading to leakage of intracellular components and cell death [64]. Due to these properties, ZrO<sub>2</sub> nanoparticles show promising antibacterial and antifungal potential in biomedical and environmental applications.

#### In vitro cytotoxicity assessment and in vivo biocompatibility

The effect of CO-ZrO<sub>2</sub>-NPs treatment on the normal fibroblast cell line, 3T3-L1, is shown in Figure 5a (inverted microscopic images) and Figure 5b (cell viability percentage). The cell viability data indicated that the nanoparticles did not cause any significant toxicity up to a

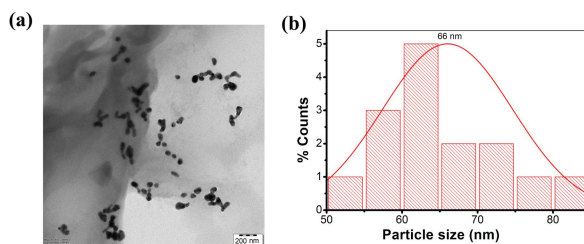


**Figure 3 Morphological characterization of the as-synthesized CO-ZrO<sub>2</sub>-NPs.**

(a) TEM image of CO-ZrO<sub>2</sub>-NPs. (b) Particle size distribution of the CO-ZrO<sub>2</sub>-NPs.

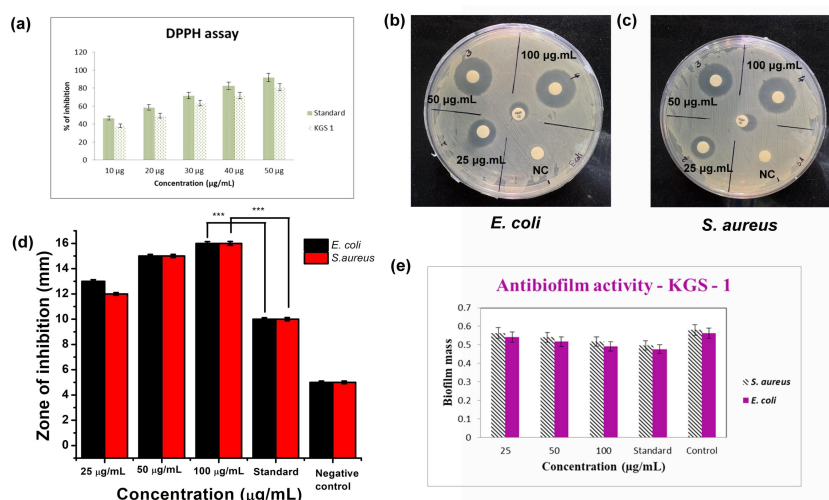


**Figure 4 Antioxidant, antibacterial, and anti-biofilm activities of CO-ZrO<sub>2</sub>-NPs.**

(a) Antioxidant activity. (b) Zone of inhibition for *E. coli*. (c) Zone of inhibition for *S. aureus*. (d) Comparison of inhibition zones at different CO-ZrO<sub>2</sub>-NPs concentrations versus antibiotic (ampicillin) and negative control (nc) (normal saline). (e) Anti-biofilm activity. Data are presented as mean  $\pm$  SD ( $^{***}P < 0.001$ ). NC-negative control.

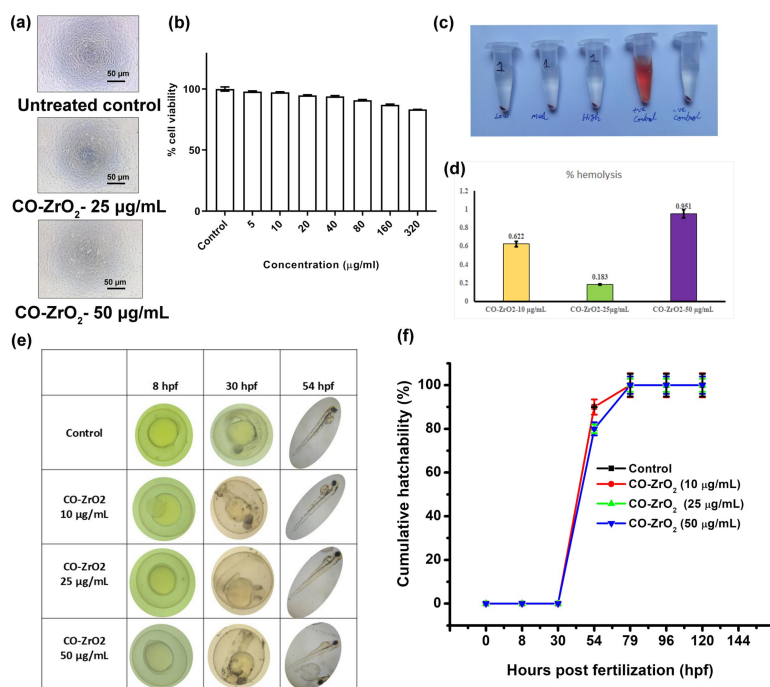


**Figure 5 Cytotoxicity, hemocompatibility, and embryotoxicity assessment of CO-ZrO<sub>2</sub> nanoparticles.**

(a) Inverted microscopic images of 3T3-L1 cells after CO-ZrO<sub>2</sub> nanoparticles treatment. (b) Cell viability assessed by MTT assay 24 h post-treatment with various CO-ZrO<sub>2</sub> nanoparticles concentrations. (c) Tubes containing hemolyzed blood and (d) corresponding hemolysis percentages at different nanoparticle concentrations. (e) Microscopic images of zebrafish embryos at different hours post-fertilization (hpf) and (f) cumulative hatchability percentages following treatment with varying doses of CO-ZrO<sub>2</sub> nanoparticles. Data are presented as mean  $\pm$  S.E.M.

concentration of 320 µg/mL, as evidenced by higher than 95% cell survival. On the other hand, the percentage of haemolysis was below 2% (Figure 5c, 5d) for all the doses exposed. These data suggested that the nanoparticles are tolerant up to 50 µg/mL, and did not cause any significant haemolysis that can be harmful. It is well known that a haemolysis percentage below 5 %, caused by any agent, is acceptable [65, 66].

*In vivo* influence of the CO-ZrO<sub>2</sub>-NPs was assessed using zebrafish embryos. The data revealed that there was no developmental defect elicited by the CO-ZrO<sub>2</sub>-NPs up to a dose of 50 µg/mL (Figure 5e). The cumulative hatchability also showed that there was no delayed hatching or drastic death of the embryos after exposure to CO-ZrO<sub>2</sub>-NPs up to a dose of 50 µg/mL (Figure 5f). The embryos in the untreated control group, as well as the treated group, hatched 100 % without showing any abnormal body bent, pericardial edema, tail bent, yolk sac edema, eye development defect, and abnormal heart rate. Hence, from the biocompatibility assessment, it can be inferred that a dose of 50 µg/mL can be safe for use in humans.

## Conclusion

This study demonstrated that the synthesis of ZrO<sub>2</sub> nanoparticles via the green route using *Calendula officinalis* flower extract was successful. The synthesized nanoparticles were characterized using various techniques, revealing their semi-crystalline and predominantly amorphous nature, nanometer-range morphology, and stability. The *Calendula officinalis* extract plays a crucial role in controlling the nanoparticle size and preventing agglomeration. The use of *Calendula officinalis* extract offers a sustainable and environmentally friendly approach for synthesizing ZrO<sub>2</sub> nanoparticles. The synthesized nanoparticles exhibit significant antimicrobial and moderate anti-biofilm activity against common uropathogens, *E. coli* and *S. aureus*, as well as antioxidant properties. The as-synthesized nanoparticles were also biocompatible till a dose of 50 µg/mL, as assessed by both *in vitro* and *in vivo* studies. These findings suggest that green-synthesized ZrO<sub>2</sub>-NPs have the potential to be a supportive therapeutic approach to combat UTIs. Further studies are needed to evaluate the *in vivo* efficacy for higher mammalian models like mice or rabbits and evaluate the safety of ZrO<sub>2</sub>-NPs in such models for the effective treatment of UTIs.

## References

- Mancuso G, Midiri A, Gerace E, Marra M, Zummo S, Biondo C. Urinary Tract Infections: The Current Scenario and Future Prospects. *Pathogens*. 2023;12(4):623. Available at: <https://doi.org/10.3390/pathogens12040623>
- Kunwar D, Zahavi I, Olchowski J, Dallasheh H, Paul M. Antibiotic class comparisons for the treatment of pyelonephritis and complicated urinary tract infections: A systematic review and meta-analysis. *Int J Antimicrob Agents*. 2026;67(2):107696. Available at: <https://doi.org/10.1016/j.ijantimicag.2025.107696>
- Schwartz L, de Dios Ruiz-Rosado J, Stonebrook E, Becknell B, Spencer JD. Uropathogen and host responses in pyelonephritis. *Nat Rev Nephrol*. 2023;19(10):658–671. Available at: <https://doi.org/10.1038/s41581-023-00737-6>
- Zhou Y, Zhou Z, Zheng L, et al. Urinary Tract Infections Caused by Uropathogenic *Escherichia coli*: Mechanisms of Infection and Treatment Options. *Int J Mol Sci*. 2023;24(13):10537. Available at: <https://doi.org/10.3390/ijms241310537>
- Hackel MA, Karlowsky JA, Sahm DF, West JM, Scangarella-Oman NE. In vitro activity of gepotidacin against urinary tract infection isolates of Enterobacterales, Enterococcus faecalis, and Staphylococcus saprophyticus. *Antimicrob Agents Chemother*. 2025;69(6):e00296-25. Available at: <https://doi.org/10.1128/aac.00296-25>
- Rostkowska OM, Kuthan R, Burban A, et al. Analysis of susceptibility to selected antibiotics in *Klebsiella pneumoniae*, *Escherichia coli*, *Enterococcus faecalis* and *Enterococcus faecium* causing urinary tract infections in kidney transplant recipients over 8 years: single-center study. *Antibiotics*. 2020;9(6):284. Available at: <https://doi.org/10.3390/antibiotics9060284>
- Sokhn ES, Salami A, El Roz A, Salloum L, Bahmad HF, Ghsein G. Antimicrobial susceptibilities and laboratory profiles of *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* isolates as agents of urinary tract infection in Lebanon: paving the way for better diagnostics. *Medical Sciences*. 2020;8(3):32. Available at: <https://doi.org/10.3390/medsci8030032>
- Mercy DJ, Girigoswami A, Girigoswami K. Relationship between urinary tract infections and serum vitamin D level in adults and children- a literature review. *Mol Biol Rep*. 2024;51(1):955. Available at: <https://doi.org/10.1007/s11033-024-09888-6>
- Dutta B, Dutta T, Rima US, et al. Burden of antibiotic-resistant urinary tract infections in rural females: insights from a cross-sectional study in Bangladesh. *APJSA*. 2024;1(2):72–79. Available at: <https://doi.org/10.70818/apjsa.2024.v01i02.012>
- Alhazmi AH, Alameer KM, Abuageelah BM, et al. Epidemiology and antimicrobial resistance patterns of urinary tract infections: a cross-sectional study from southwestern Saudi Arabia. *Medicina (Mex)*. 2023;59(8):1411. Available at: <https://doi.org/10.3390/medicina59081411>
- Filev R, Bogov B, Lyubomirova M, Rostaing L. From pandemic to resistance: addressing multidrug-resistant urinary tract infections in the Balkans. *Antibiotics*. 2025;14(9):849. Available at: <https://doi.org/10.3390/antibiotics14090849>
- El Zein Z, Boutros CF, El Masri M, et al. The challenge of multidrug resistance in hospitalized pediatric patients with urinary tract infections. *Front Cell Infect Microbiol*. 2025;15:1570405. Available at: <https://doi.org/10.3389/fcimb.2025.1570405>
- AbuSara A, Tayyeb N, Matalka L, Almomani B, Abaza H, Nazer L. Prevalence and predictors of multi-drug resistant organisms among ambulatory cancer patients with urinary tract infections. *Infect Drug Resist*. 2023;747-53. Available at: <https://doi.org/10.2147/IDR.S388680>
- Nurjis F, Sarwar U, Ali JS, Fayyaz M, Mazhar S, Shahzad S. Ultrasound triggered in vitro release of co-loaded nanomicelles-based doxorubicin and curcumin delivery in Hep2c cancer cells. *Tradit Med Res*. 2025;10(6):32. Available at: <https://doi.org/10.53388/TMR20241002001>
- Liu FL, Zhang N, Wei M, Li YM, Liu XX, Han QY. A systematic review of the nano-technology applied in traditional Chinese medicines. *Tradit Med Res*. 2025;10(11):68. Available at: <https://doi.org/10.53388/TMR20250124002>
- Alamelu S, Venkatesan KB, Shagirtha K, Srinivasan MK, Pachaiappan P. Chemopreventive potential of brucine-gold nanoparticles (BRU-AuNPs) in DMBA-induced mammary cancer via modulation of PI3K/AKT/mTOR pathway. *Appl Biochem Biotechnol*. 2026;198(6):4161-4187. Available at: <https://doi.org/10.1007/s12010-026-05623-y>
- Srinivasan MK, Packirisamy V. Solvothermal preparation of N,S-doped carbon quantum dots from *Leucas aspera* for sensing and bioimaging applications. *Int J Environ Anal Chem* 2026:1–15. Available at: <https://doi.org/10.1080/03067319.2026.2656702>
- Rekha A, Vijayalakshmi K, Alswieleh A, Sudha PN, Rani JD, Vidhya A. Enhanced removal of Cr(VI) from water using alginate-modified algal biochar: a promising adsorbent. *Biomass*

- Conv Bioref.* 2024;15(17):24135–24148. Available at: <https://doi.org/10.1007/s13399-024-05792-y>
19. Begum MY, Sharma M, Arularasu MV, Vinitha P, Vetrivelan V, Kandasamy G, Manikandan A, Ayanie AG, Gnanasekaran L, Mehta A, Gupta R. Photocatalytic dye degradation and antibacterial activity of gold nanoparticles: a DFT and machine learning study. *RSC Adv.* 2025;15(59):50582-96. Available at: <https://doi.org/10.1039/D5RA08168H>
  20. Suresh U, Panneerselvam C, Aziz AT, Srinivasan MK, Hassan MA, Alasmari A, Mohammedsalem ZM. Synthesis, characterisation and evaluation of polyethylene glycol- $\beta$ -cyclodextrin-curcumin-zinc oxide nanoparticles for mosquitocidal, antibacterial and anticancer applications. *Sci Rep.* 2026;16(1):2658. Available at: <https://doi.org/10.1038/s41598-025-32059-x>
  21. Nishakavya S, Girigoswami A, Gopikrishna A, et al. Size attenuated copper doped zirconia nanoparticles enhances in vitro antimicrobial properties. *Appl Biochem Biotechnol.* 2022;194(8):3435–3452. Available at: <https://doi.org/10.1007/s12010-022-03875-y>
  22. Viswanathan D, Govindasamy R, Kuchipudia JD, Anantharaman R. Green synthesis and characterization of Europium oxide nanoparticles (Eu<sub>2</sub>O<sub>3</sub> NPs) derived from *Caesalpinia bonduca* and its biomedical applications. *Next Research.* 2025;4:101233. Available at: <https://doi.org/10.1016/j.nexres.2025.101233>
  23. Gautam S, Das DK, Kaur J, et al. Transition metal-based nanoparticles as potential antimicrobial agents: recent advancements, mechanistic, challenges, and future prospects. *Discov Nano.* 2023;18(1):84. Available at: <https://doi.org/10.1186/s11671-023-03861-1>
  24. Al-darwesh MY, Babakr KA, Qader IN. Characterization and anticancer evaluation of zirconia nanoparticles synthesized via green route using *sophora flavescens* roots extract. *Nano-Struct. Nano-Objects.* 2024;39:101245. Available at: <https://doi.org/10.1016/j.nanoso.2024.101245>
  25. Modi SK, Nehra M, Kumar S, Mange PL, Modi KB, Dubey S. Synthesis, characterization, and performance of zirconia nanoparticulates as an antibacterial, antifungal, and anticancer activity agent. *BioNanoSci.* 2024;14(3):2529–2540. Available at: <https://doi.org/10.1007/s12668-024-01483-0>
  26. Ziabka M, Wojteczko A, Klesiewicz K, et al. Antimicrobial properties and bioactivity of zirconia-based biocomposites. *Artif. Cells Nanomed. Biotech.* 2025;53(1):361–378. Available at: <https://doi.org/10.1080/21691401.2025.2540647>
  27. Hemanth PR, Srinivasan MK, Packirisamy V. Integrated in silico network pharmacology and  $\beta$ -cyclodextrin nanosponge based delivery of naphthomycin: Mechanistic insights, controlled release, and enhanced antimicrobial activity. *Next Nanotechnol.* 2026;9:100447. Available at: <https://doi.org/10.1016/j.nxnano.2026.100447>
  28. Gomathi M, Kirubakaran D, Malarkodi R. Synthesis, characterization and biomedical environmental applications of Magnesium-Doped zinc oxide nanoparticles using *Lagdera Alata* extract. *Biomed Mater Devices.* 2026;4(2):2606-24. Available at: <https://doi.org/10.1007/s44174-025-00412-3>
  29. Pandiarajan S, Udayakumar S, Janani G, et al. Anticancer effects of biomimetic green-synthesized silver nanoparticles coated lactobacilli species against various cancer cell lines. *3 Biotech.* 2025;15(8):233. Available at: <https://doi.org/10.1007/s13205-025-04393-4>
  30. Kaliyaperumal R, Nagaraj K, Wadaan MA, Poovan VK, Ghodake GS, Badgujar NP, Kumaravel T. Development and Antimicrobial Evaluation of Tin Oxide (SnO<sub>2</sub>) Nanoparticles Stabilized with Corn Cob Ash Extract for Biomedical Applications. *Biomed Mater Devices.* 2026;4:2379–2388. Available at: <https://doi.org/10.1007/s44174-025-00369-3>
  31. Hachem K, Ansari MJ, Saleh RO, et al. Methods of Chemical synthesis in the synthesis of nanomaterial and nanoparticles by the chemical deposition method: a review. *BioNanoSci.* 2022;12(3):1032–1057. Available at: <https://doi.org/10.1007/s12668-022-00996-w>
  32. Kulkarni AG, De Britto S, Jogaiah S. Economic considerations and limitations of green synthesis vs chemical synthesis of nanomaterials. *Advances in Nano-Fertilizers and Nano-Pesticides in Agriculture.* 2021:459–468. Available at: <https://doi.org/10.1016/B978-0-12-820092-6.00018-5>
  33. Lila ASA, Rajab AAH, Abdallah MH, et al. Biofilm lifestyle in recurrent urinary tract infections. *Life.* 2023;13(1):148. Available at: <https://doi.org/10.3390/life13010148>
  34. Perchikov R, Cheliukanov M, Plekhanova Y, et al. Microbial biofilms: features of formation and potential for use in bioelectrochemical devices. *Biosensors.* 2024;14(6):302. Available at: <https://doi.org/10.3390/bios14060302>
  35. Barman S, Kurnaz LB, Leighton R, Hossain MW, Decho AW, Tang C. Intrinsic antimicrobial resistance: Molecular biomaterials to combat microbial biofilms and bacterial persisters. *Biomaterials.* 2024;311:122690. Available at: <https://doi.org/10.1016/j.biomaterials.2024.122690>
  36. Alum EU, Uti DE, Offor CE. Redox signaling disruption and antioxidants in toxicology: from precision therapy to potential hazards. *Cell Biochem Biophys.* 2025;83(4):4253–4274. Available at: <https://doi.org/10.1007/s12013-025-01846-8>
  37. Pettersson C, Wu R, Demirel I. Estrogen-stimulated uropathogenic *E. coli* mediate enhanced neutrophil responses. *Sci Rep.* 2024;14(1):23030. Available at: <https://doi.org/10.1038/s41598-024-74863-x>
  38. Zournatzis I, Liakos V, Papadopoulos S, Wogiatzi E. *Calendula officinalis* - A comprehensive review. *Pharmacol. Res. - Nat. Prod.* 2025;6:100140. Available at: <https://doi.org/10.1016/j.prenap.2024.100140>
  39. Sapkota B, Kunwar P. A review on traditional uses, phytochemistry and pharmacological activities of *Calendula officinalis* Linn. *Nat Prod Commun.* 2024;19(6):1934578X241259021. Available at: <https://doi.org/10.1177/1934578X241259021>
  40. Dey H, Tarannum M, Kumar P, et al. *Calendula officinalis* - A crucial and promising antifungal agent with cytotoxic biological attributes. *Sch Acad J Pharm.* 2024;13(01):30–36. Available at: <https://doi.org/10.36347/sajp.2024.v13i01.004>
  41. Zhangabay Z, Berillo D. Antimicrobial and antioxidant activity of AgNPs stabilized with *Calendula officinalis* flower extract. *Results Surf Interfaces.* 2023;11:100109. Available at: <https://doi.org/10.1016/j.rsufi.2023.100109>
  42. Olfati A, Kahrizi D, Balaky STJ, Sharifi R, Tahir MB, Darvishi E. Green synthesis of nanoparticles using *Calendula officinalis* extract from silver sulfate and their antibacterial effects on *Pectobacterium caratovorum*. *Inorg Chem Commun.* 2021;125:108439. Available at: <https://doi.org/10.1016/j.inoche.2020.108439>
  43. Deepika B, Gowtham P, Raghavan V, et al. Harmony in nature's elixir: a comprehensive exploration of ethanol and nano-formulated extracts from *Passiflora incarnata* leaves: unveiling in vitro cytotoxicity, acute and sub-acute toxicity profiles in Swiss albino mice. *J Mol Histol.* 2024;55(5):977–994. Available at: <https://doi.org/10.1007/s10735-024-10245-x>
  44. Girigoswami A, Deepika B, Udayakumar S, et al. Peony-shaped zinc oxide nanoflower synthesized via hydrothermal route exhibits promising anticancer and anti-amyloid activity. *BMC Pharmacol Toxicol.* 2024;25(1):101. Available at: <https://doi.org/10.1186/s40360-024-00830-x>
  45. Manigandan P, Shanmugam R. Green preparation of fluorescence carbon nanoparticles using honey and its

- biomedical applications- an in vitro study. *J Drug Del Sci Tech*. 2025;108:106919. Available at: <https://doi.org/10.1016/j.jddst.2025.106919>
46. Kumar Singh R, Nallaswamy D, Rajeshkumar S, Varghese SS. Green synthesis of silver nanoparticles using neem and turmeric extract and its antimicrobial activity of plant mediated silver nanoparticles. *J Oral Biol. Craniofacial Res.*. 2025;15(2):395–401. Available at: <https://doi.org/10.1016/j.jobcr.2025.02.005>
  47. Biswas K, Mercy DJ, Udayakumar S, et al. Frustrating the Serenity of Bacterial Biofilms by Bristly Reduced Graphene Oxide Sheets. *BioNanoSci*. 2025;15(2):1–9. Available at: <https://doi.org/10.1007/s12668-025-01866-x>
  48. Janani G, Girigoswami A, Deepika B, Udayakumar S, Mercy DJ, Girigoswami K. Harnessing the anticancer potential of Amphiroa anceps: Folic acid-based liposomal nanocarriers for cancer cell killing in vitro. *Nanomed.: Nanotechnol. Biol*. 2026;72:102901. Available at: <https://doi.org/10.1016/j.nano.2026.102901>
  49. Danaei M, Dehghankhold M, Ataei S, et al. Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems. *Pharmaceutics*. 2018;10(2):57. Available at: <https://doi.org/10.3390/pharmaceutics10020057>
  50. Jaiganesh T, Daisy Vimala Rani J, Girigoswami A. Spectroscopically characterized cadmium sulfide quantum dots lengthening the lag phase of Escherichia coli growth. *Spectrochim. Acta - A: Mol. Biomol. Spectrosc.* 2012;92:29–32. Available at: <https://doi.org/10.1016/j.saa.2012.02.044>
  51. Salgi P, Rajagopalan R. Polydispersity in colloids: implications to static structure and scattering. *Adv Colloid Interface Sci*. 1993;43(2–3):169–288. Available at: [https://doi.org/10.1016/0001-8686\(93\)80017-6](https://doi.org/10.1016/0001-8686(93)80017-6)
  52. Babick F. Dynamic light scattering (DLS). *Characterization of Nanoparticles*. 2020:137–172. Available at: <https://doi.org/10.1016/B978-0-12-814182-3.00010-9>
  53. Bhattacharjee S. DLS and zeta potential – What they are and what they are not? *J Control Release*. 2016;235:337–351. Available at: <https://doi.org/10.1016/j.jconrel.2016.06.017>
  54. Horti NC, Kamatagi MD, Nataraj SK, Wari MN, Inamdar SR. Structural and optical properties of zirconium oxide (ZrO<sub>2</sub>) nanoparticles: effect of calcination temperature. *Nano Ex*. 2020;1(1):010022. Available at: <https://doi.org/10.1088/2632-959X/ab8684>
  55. Dwivedi R, Maurya A, Verma A, Prasad R, Bartwal KS. Microwave assisted sol–gel synthesis of tetragonal zirconia nanoparticles. *J Alloys Compd*. 2011;509(24):6848–6851. Available at: <https://doi.org/10.1016/j.jallcom.2011.03.138>
  56. Hameed H, Waheed A, Sharif MS, et al. Green synthesis of zinc oxide (ZnO) nanoparticles from green algae and their assessment in various biological applications. *Micromachines*. 2023;14(5):928. Available at: <https://doi.org/10.3390/mi14050928>
  57. Salavati-Niasari M, Saleh L, Mohandes F, Ghaemi A. Sonochemical preparation of pure t-LaVO<sub>4</sub> nanoparticles with the aid of tris(acetylacetonato)lanthanum hydrate as a novel precursor. *Ultrason Sonochem*. 2014;21(2):653–662. Available at: <https://doi.org/10.1016/j.ultsonch.2013.09.007>
  58. Ahmad T, Pandey V, Saddam Husain M, Adiba, Munjal S. Structural and spectroscopic analysis of pure phase hexagonal wurtzite ZnO nanoparticles synthesized by sol-gel. *Mater Today: Proc*. 2022;49:1694–1697. Available at: <https://doi.org/10.1016/j.matpr.2021.07.456>
  59. S P, P A, A NJ. Comparative investigation of structural properties and biological applications of chemical and biogenic synthesis of zirconium dioxide (ZrO<sub>2</sub>) nanoparticles using Passiflora edulis. *J Photochem Photobiol B: Biol*. 2025;263:113089. Available at: <https://doi.org/10.1016/j.jphotobiol.2024.113089>
  60. Basappa B, Poonacha LK, Xi Z, et al. Nano-Zirconium Dioxide Catalyzed Multicomponent Synthesis of Bioactive Pyranopyrazoles That Target Cyclin Dependent Kinase 1 in Human Breast Cancer Cells. *Biomedicines*. 2023;11(1):172. Available at: <https://doi.org/10.3390/biomedicines11010172>
  61. Zinatloo-Ajabshir S, Salavati-Niasari M. Facile route to synthesize zirconium dioxide (ZrO<sub>2</sub>) nanostructures: Structural, optical and photocatalytic studies. *J Mol Liq*. 2016;216:545–551. Available at: <https://doi.org/10.1016/j.molliq.2016.01.062>
  62. Tsunekawa S, Ito S, Kawazoe Y, Wang J-T. Critical Size of the Phase Transition from Cubic to Tetragonal in Pure Zirconia Nanoparticles. *Nano Lett*. 2003;3(7):871–875. Available at: <https://doi.org/10.1021/nl034129t>
  63. Behboud AB, Ahmed MK, Kurucu A, Çömlekçi GK, Ordu M. Zirconia nanoparticle coating for high-strength and alkali-resistant glass fibers. *J Non-Cryst Solids*. 2023;619:122571. Available at: <https://doi.org/10.1016/j.jnoncrysol.2023.122571>
  64. Kim HS, Jang W, Im YG, Lim HP. Antibacterial effect of zirconia nanoparticles on polyethyl methacrylate resin for provisional crowns. *Int J Nanomedicine*. 2022;17:6551–6560. Available at: <https://doi.org/10.2147/IJN.S382053>
  65. Sæbø I, Bjørås M, Franzky H, Helgesen E, Booth J. Optimization of the hemolysis assay for the assessment of cytotoxicity. *Int J Mol Sci*. 2023;24(3):2914. Available at: <https://doi.org/10.3390/ijms24032914>
  66. Amin K, Dannenfelser RM. In vitro hemolysis: Guidance for the pharmaceutical scientist. *J Pharm Sci*. 2006;95(6):1173–1176. Available at: <https://doi.org/10.1002/jps.20627>