Recent advances in the Biosynthesis of Zirconium Oxide Nanoparticles and their Biological Applications

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Abstract:
A critical milestone in nano-biotechnology is establishing reliable and ecological friendly methods for fabricating metal oxide NPs. Because of their great biodegradable, electrical, mechanical, and optical qualities, zirconia NPs (ZrO2;NPs) attract much interest among all zirconia NPs (ZrO2;NPs). Zirconium oxide (ZrO2) has piqued the interest of researchers throughout the world, particularly since the development of methods for the manufacture of nano-sized particles. An extensive study into the creation of nanoparticles utilizing various synthetic techniques and their potential uses has been stimulated by their high luminous efficiency, wide bandgap, and high exciton binding energy. Zirconium dioxide nanoparticles may be used as antimicrobial and anticancer agents in food packaging. In response to the growing interest in nano ZrO2, researchers invented and developed methods for synthesizing nanoparticles. ZrO2 nanocomposites with various morphologies have recently been created using biological (green chemistry) methods. Microbes and plants both contribute to the production of zirconia in the laboratory. Capping and stabilizing agents are provided by the biomolecules found in plant extracts, whereas microorganisms provide enzymes as capping and stabilizing agents (intracellular or extracellular). It is possible to analyze the nanoparticles produced using a variety of analytical approaches, including ultraviolet-visible spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), and Fourier transform infrared spectroscopy (FT-IR). When applied to bacteria (both Gram-positive and Gram-negative) and fungi, ZrO2NPs show promising antibacterial capabilities. Normal and malignant cells are sensitive to ZrO2 nanoparticles, which can be explained by the generation of reactive oxygen (ROS). This work discusses and describes many ways of producing ZrO2 nanoparticles, their properties, and various application possibilities.

Keywords: Applications, Characterization, Green synthesis, Nanotechnology, ZrO2NPs.

Introduction:
Nanotechnology deals with structures that range as small as 1-100nm 1. Nanomaterials have different physical, chemical, and structural properties compared to their bigger counterparts. The nanomaterials' remarkable electrical, magnetic, and optical properties and surface activity are due to their nanoscale size and shape. When reduced to nanomaterials, ordinary materials exhibited extraordinary properties with regards to electrical conductivity, chemical reactivity, remarkable strength, super-paramagnetic behaviour, and other characteristics that they do not possess at the macro or micro scale 2. Compared to bulk materials, nanoparticles exhibit distinct or better size, dispersion, and shape characteristics. The quantum effect influences nanomaterial's chemical reactivity, surface heterogeneity (e.g., capping, coating), mechanical, optical, electrical and even magnetic properties. Other associated features, such as antibacterial activity, are influenced by the material's specific surface heterogeneity and area. Due to the wide variety of materials that can be
used to make nanoparticles, they are divided into four categories: 1) metallic nanoparticles (such as gold and silver), 2) metal oxides (such as VO, aluminium oxide, zinc oxide, and zinc chloride), 3) semiconductor nanoparticles (such as zinc sulphide, cadmium sulphide, and cadmium sulphide), 4) carbon nanoparticles.

**Zirconium oxide (ZrO₂) nanoparticles:**
Zirconium oxide is one of the most intriguing and promising metallic nanomaterials currently available (ZrO₂). In addition to being referred to as “ceramic steel”, zirconium (Zr) is a transitional metal element with the electrical configuration [Kr] 4d²5s². It was given this name in honour of the mineral zircon, the primary zirconium source. Naturally, it does not exist in pure form and can only be discovered in large quantities when combined. It may also be found as free oxide zirconia (ZrO₂) in the mineral baddeleyite, zirconium oxide. Minerals in their natural state include a significant quantity of impurities, either elemental or radioactive. So, they cannot serve as the primary source for biomedical research purposes. According to the researchers, these pure powdered forms may one day be utilized in biological applications. It has been widely established that ZrO₂ crystals may exhibit three different crystallographic symmetry types: monoclinic (m), tetragonal (t), and cubic (c) symmetry as seen in Fig. 1. The temperature and pressure used to modify the crystallographic patterns of pure zirconia are the only variables that can be controlled. The monoclinic polymorph (m), the most stable form under ordinary circumstances, is stable up to 1170 °C. This type of deposit is most commonly seen in all-natural deposits. When the temperature is raised to 1170 °C, the monoclinic structure transforms into the tetragonal (t) polymorph, which has a small contraction of ~4–5% in total volume. At extremely high temperatures, the tetragonal form shrinks even more in volume until it eventually transforms into the least helpful cubic (c) symmetry at 2370°C.

In general, volume shrinkage is less prevalent in ceramics as they are heated to higher temperatures; as a result, the unusual features of zirconia enabled scientists to discover its wide range of biological uses. Furthermore, it is discovered that certain lattice modifications are reversible—cooling results in the reversion of tetragonal or cubic symmetry to the monoclinic state. The tetragonal to monoclinic transition begins at about 950 °C with a significant increase in volume (~4–5%), resulting in a much stiffer and harder lattice. Zirconia is a wide bandgap p-type semiconductor with a bandgap 3.25 to 5.1 eV depending on the preparation method. Zirconia nanoparticles are available in nanofluids, nanocrystals, and nanodots. Zirconium oxide is also known as zirconia, zirconic anhydride, and zircosol.

In recent years, metal and metal oxide nanoparticles have been of much interest due to their varied applications, especially their antimicrobial properties. ZrO₂NPs have sparked a lot of study attention amongst transition metal oxide nanoparticles (NPs) because of their inimitable electrical, heat, catalyst, sensing, optical, mechanical, and compatible biological capabilities. Nevertheless, due to the acidic and basic composition, ZrO₂NPs is a well-familiar p-type semiconductor with piezoelectric properties. As a result, ZrO₂NPs are commonly employed in various purposes such as implant materials, dental implants, photocatalyst, refractory, fuel-based cells, gas sensors, solar cells, tissue engineering, biomarkers, drug delivery, theragnostic, water treatment, bio-conjugation and agriculture etc. Furthermore, owing to their inimitable physiochemical characteristics, ZrO₂NPs have antifungal, antioxidant and carcinogenic effects. Zirconia (ZrO₂) is a material of importance with high chemical stability, strength, and corrosion resistance. The applications of ZrO₂ nanoparticles are presented in Fig. 2.
Synthesis of ZrO$_2$ Nanoparticles:

Zirconia was synthesized through various methods like ball-mill assisted, ultrasonic abetted, sol-gel, electrical arc-based discharge, precipitation, hydrothermal, heat plasma path, solvothermal, explosive emulsion, microwave-assisted, and electrochemical deposition. However, these artificial approaches necessitate high temperature and pressures, an extended reaction period, expensive and hazardous chemical forerunners, and the use of specialized tools for investigational work, all of which have a detrimental environmental impact. It is preferable to chemically manufacture nanomaterials to employ biological techniques, such as enzymatic processes, to synthesize small particles like nanoparticles. Fig. 3 illustrates the various ways used to synthesize ZrO$_2$ nanoparticles.
Possible mechanism of formation of ZrO$_2$NPs by using plants:

The underlying molecular pathways that contribute to the creation of NPs, on the other hand, are still poorly understood. Multiple studies have demonstrated that various metabolites can reduce and stabilize metallic NPs and avoid agglomeration and aggregation of new metallic NPs in nonhazardous ways. In general, phenolic chemicals inactivate ions through a process known as chelation. The chelating characteristics of phenolic aromatic rings are likely due to their high nucleophilic natures. The most significant functional groups in metal ion reduction are carbonyl, hydroxyl, amino, and methoxide. These groups connect to the metal ions by electrostatic contact, causing them to be reduced, and the reduction of metal ions in the result. Natural sources respond to heavy metal stress by synthesizing phytochelations or metal-chelating peptides. Metal ions are chemically immobilized and subsequently reduced, sintered, and smelted to produce nanoparticles (NPs). Metal ion concentration and ion penetration site affect the size and shape of nanoparticles. It is possible to manipulate the shape, dispersion, and yield of these biosynthetic NPs by altering the reaction conditions. In the absence of a protective barrier, high polyphenol levels inhibited coalescence and aggregate formation. Metal nanoparticle bioreduction using plant extracts involves three steps. In the first step, metal ions are reduced and nucleated. Second, small adjacent NPs combine to produce larger particles, increasing their thermodynamic stability and finally, the termination phase shapes the NPs. These are then centrifuged with the metal ion precipitates and rinsed with a suitable solvent to remove any leftover impurities before reuse. Fig. 4 presents the possible mechanism of the formation of ZrO$_2$NPs using plant extract.

![Possible mechanism of formation of ZrO$_2$NPs using plant extract.](image)

Green synthesis of ZrO$_2$NPs:

Much research shows that biological production of metallic and metal oxide nanoparticles is more eco-friendly than chemical or physical approaches. Let's consider the biological synthesis process, it employs renewable resources, better solvents and auxiliaries, and produces compounds that are safer to handle than traditional chemical synthesis methods. Plant extracts are prepared by crushing or boiling plant components in appropriate solvents at specific temperatures to generate a concentrated extract. Because of the phytochemicals in plant extracts, zirconia synthesis is made easier by acting as both a reducing and capping agent. The zirconia solution is centrifuged at higher rpm to separate the nanoparticles from the rest of the solution. After that, the pieces are completely rinsed, and the resulting solution is dried. In this process, the solution is subjected to a thermal treatment, and the ZrO$_2$ powder is produced. Much research shows that biological production of metallic and metal oxide nanoparticles is more eco-friendly than chemical or physical approaches. The phytochemicals present in the plants is presented in Fig. 5.
Another characteristic of agglomeration is that the time interval between the heat treatment and the creation of clusters might have an impact on their formation \(^{28}\). Extended agglomerates and particle development were seen by Dhadapani et al. when the period of the heat treatment at 50°C was increased from 30 to 90 minutes, according to their findings\(^{29}\). All of these observations are consistent with the results obtained from other chemical synthesis processes. The lengthening of the time required for nucleation resulted in bigger particles of ZrO\(_2\). It is also known that the pH conditions used during the synthesis process may drastically alter the particle size and shape of metals and metal oxides, which will, in turn, change the characteristics of the nanomaterials produced by the process. The pH of the solutions of the biological extracts used in the green production of ZrO\(_2\) NPs is not considered in much of the previous research.

**Synthesis of ZrO\(_2\) NPs using plants:**

The use of Acalypha Indica leaves for Zirconia nanoparticle formation was noticed, where ZrOCl\(_2\)\(\cdot\)8H\(_2\)O was used as the precursor \(^{30}\). In this work, FTIR results showed a fundamental part in showing the significant functional groups in the ZrO\(_2\) NPs. The SEM and XRD studies showed that the average size of the NPs was recognized as 20-100 nm with cube-shaped ZrO\(_2\) NPs. Gowri et al. \(^{31}\) synthesized flake like nanostructures of ZrO\(_2\) using zirconium oxochloride (0.4M) and aqueous extract of Nyctanthes arbor-tristis. In this study, to evaluate the optimum calcination temperature to generate ZrO\(_2\) crystalline NPs with a characteristic phase, the as-synthesized specimen was then imprecated to calcinations in a muffle furnace at 300°C and 500°C for 3 hrs. From this work, the authors stated that the ZrO\(_2\) NPs (43 nm) at 300 °C exhibit lesser size and adequate crystallinity with tetragonal phase structure. Two bacterial species, Gram +ve (S. aureus) and Gram -ve (E. coli), were used to study the antibacterial activity. However, E. coli bacteria hold more inhibition (30mm) when compared to S. aureus when treated with ZrO\(_2\) NPs synthesis at 300°C on cotton fabric.

Kanda et al. \(^{32}\) synthesized ZrO\(_2\) NPs utilizing Thepesia populnea plant extract to perform on cotton gauze fabric for the antibacterial action of nano-zirconia. In this study, to prepare NPs, zirconyl chloride of 1 mM that is 80 mL, is introduced to the extract of T. populnea by 20 mL. A reaction medium was agitated for a period of 2 hrs at an at of 80 °C temperature as well a reaction mixture leaves for a full night for NPs creation without shaking. After that, sediment is vacuum dried in an oven at a temperature of 200 °C for 1 to 2 hrs to attain ZrO\(_2\) NPs. From UV–Vis spectroscope, the stronger peak formed by ZrO\(_2\) NPs after 200nm designated that formation of ZrO\(_2\) NPs. XRD and TEM analysis, the synthesized NPs were found 10 nm. In the functional group analysis, the stronger bands among 500 and 400 cm\(^{-1}\) were accredited to a stretch of -OH group representing stretch and a bend of H\(_2\)O absorbing through ZrO\(_2\) NPs. The absorbing peaks at 3220,28, 2921.01 and 1608.02 to 698.94 cm\(^{-1}\) are due to its asymmetrical vibrational stretch formed through the -OH group of absorbing H\(_2\)O. The maximum zone of inhibition attained towards E. coli, S. aureus, B. subilis, and P. aeruginosa tested by well diffusion method as 26, 25, 11 and 8 mm, respectively.

Veronika et al. \(^{33}\) developed green methods for producing zirconium oxide-gold (ZrO\(_2\)-Au) core-shell nanocomposites using Equisetum arvenses extract via bio-reduction method. From UV-Vis results, the SPR peaks for the Au/ZrO\(_2\) bi-phasic system centre at 539 nm, but no peak was observed for ZrO\(_2\) NPs. From STEM analysis, the formed AuNPs appeared as spherical and triangular-shaped; the dominant sample shape was spherical rather than triangular. While spherical Au NPs ranged
from 6–44 nm with an average 24 nm diameter. The size distribution of triangular-shaped NPs ranged from 20 to 200 nm. A negative charge (-17.5mV) was observed from zeta potential results due to active phytochemicals with long-term change effects that stabilize NPs and serve as capping agents.

*Aloe vera* extract was employed as a capping and reducing agent in the biological processes used by Gowri et al. 34 to produce nanoparticles. In the UV-Vis spectrum, the seemed at 213 nm was blue lifted from solid ZrO$_2$ substance and distinctive for tetragonal ZrO$_2$NPs. From SEM and AFM analysis, spherical-shaped structures by smoother and attached surfaces and weaker accretion of atoms were evidently determined homogeneously with less than 50 nm. From thermal analysis, an endothermic peak that appeared at below 150°C and 350°C might be linked to the release of surface adsorbing H$_2$O and organic components adsorbed in the as-prepared Zr. The formed ZrO$_2$NPs preserved fabrics exhibited larger antimicrobial action towards *E. coli* (32 mm) microbes than with *S. aureus* (23 mm) bacteria with a zone of inhibition (ZOI) 32 and 23 mm, respectively.

Pragya et al. 35 developed a green, non-toxic and lower-cost creation of monoclinic ZrO$_2$NPs by utilizing a green production study from a methanol-based *Helianthus annuus* seed extract as plummeting substance. The UV-Vis spectrum is sharper and rises at 275 nm owing to its valence to conducting band shift. The zeta potential as -9.32 mV and particle size distribution of ~331 nm is used to illustrate the sustainability of NPs. Because of the transition of enol compounds into ketones, the –H atom is released, which lessens the ionization of the molecules in zirconium salt, which is beneficial. As a result, following the annealing process, it contains zirconium oxide nanoparticles since the other organic compounds are no longer present below the temperature used for the annealing process. The SEM and TEM study of ZrO$_2$NPs displayed spherical shape-based and mean-particle size 35.45 nm. From thermal analysis, an endothermic peak that appeared at below 150°C and 350°C might be linked to the release of surface adsorbing H$_2$O and organic components adsorbed in the as-prepared Zr. The formed ZrO$_2$NPs preserved fabrics exhibited larger antimicrobial action towards *E. coli* (32 mm) microbes than with *S. aureus* (23 mm) bacteria with a zone of inhibition (ZOI) 32 and 23 mm, respectively.

Annu et al. 36 prepared ZrO$_2$NPs through bio-based procedure utilizing *Moringa oleifera* leaf extract. UV-Vis spectrum showed an absorption band at 293 nm that authorizes the blend of tetragonal ZrO$_2$NPs. A spherical-shaped smooth surface with particles size below 10 nm was observed from SEM and XRD analysis. The synthesis ZrO$_2$NPs unveiled 69.4% suppressing action against the free radicals. ZrO$_2$NPs formed by *Moringa oleifera* showed antimicrobial action towards Gram -ve and +ve microbes like *E. coli*, *P. aeruginosa*, and *B. subtilis*. This is due to the fact that the negatively charged cell wall of Gram -ve bacteria is attracted to the positively charged...
zirconium ions contained within the nanoparticles, resulting in the cell death of the organism in the process. In another work, Isacfranklin et al. developed a procedure for the creation of ZrO\textsubscript{2} nanorods by nanorods, which included the use of 10 mL of Nepheleum lappaceum L. fruit peel and hydrothermal treatment to produce the nanorods. From XPS analysis, a protuberant band and a shoulder band are positioned at 183 and 185 eV, resembling Zr3d\textsubscript{5/2} and Zr3d\textsubscript{3/2}, and the O1s spectra attained in the 530–531 eV range that was accountable for the Zr–O/O–H elements. The typical monoclinic structural peaks were caused by Raman peaks detected at 180, 192, and 475 cm\textsuperscript{-1}. The maximum suggests cubic zirconia production at 475 cm\textsuperscript{-1}. The prepared ZrO\textsubscript{2}nanorods were shown antitumor efficiency towards human breast tumor cells (MCF-7) and inhibiting the tumour growth in a dosage-based way at a half-maximal inhibition level of 55.32 μg mL\textsuperscript{-1}.

Kumar et al. prepared a chitosan-based ZrO\textsubscript{2}NPs blend of Zr NPs utilizing an aqueous Aloe vera extract and characterized by UV-Vis, TEM, EDAX, XRD, and FT-IR study. The UV–Vis absorption peak of the produced Zr NPs was 420 nm. The generation of polydispersed NPs varying in size from 18 nm to 42 nm was revealed by TEM. SAED and XRD examination revealed that the Zr crystallites were fcc (facial centred cubic). Zr was found to be an essential component of synthesized NPs, according to EDAX analysis. At pH 7.0, fluoride adsorption on the CNZr composite performed well, with 99 % of fluoride retained. Anderson et al. used Euclea natalensis extract to synthesize zirconia NPs. During the synthesis of NPs in this experiment, the extract concentration was changed from 50 to 75 to 100g/L for precursor doses of 0.01, 0.02, and 0.03 mol/L, respectively. The tautomeristic transition of enol compounds into keto compounds, for example, releases the reactive hydrogen atom, lowering the zirconium ions in the molecule. The calcination produced zirconia nanoparticles since the organic matter created during the process is destroyed at the temperatures used. XRD shows monoclinic and tetragonal phases in zirconia with crystallite diameters of about 5.25 nm. The particles were spherical and had a relatively small average diameter of 5.90 to 8.54 nm. Furthermore, the NPs have executed the tetracycline 30.45 (mg/g) adsorption.

Siripreddy et al. prepared ZrO\textsubscript{2}NPs using Eucalyptus globulus (E. globulus) extract with spherical by the size varying from 9-11nm and with higher zeta potential -45.5 mV. The identified cytotoxic action of ZrO\textsubscript{2}NPs was caused through ROS. Furthermore, green produced ZrO\textsubscript{2}NPs had stronger antioxidant capacity, neutralizing as 85.6 % of free radicals released through the DPPH. The computed IC\textsubscript{50} for non-cancerous Vero cells are 228 g/mL, indicating that ZrO\textsubscript{2}NPs are less harmful to normal cells. Gurushantha et al. produced cubic ZrO\textsubscript{2}: Fe\textsuperscript{3+} (0.5–4 mol%) NPs using Phyllanthus acidus as a reducing agent. Under UV and sunlight irradiation, Fe\textsuperscript{3+} on ZrO\textsubscript{2} matrices influenced photocatalytic depletion of AO7. Shinde et al. carried out an experiment on the Biosynthesis of ZrO\textsubscript{2}NPs by means of Ficus benghalensis extract as capping material for the initial time. The produced ZrO\textsubscript{2}NPs have a spherical shape with a size of 15 nm, which is in good accord with the XRD data. The quantum variation causes a drop of bulk ZrO\textsubscript{2} in a bandgap from 5.3 to 4.9 eV. BET findings indicate as-synthesized ZrO\textsubscript{2}NPs are a larger (88 m\textsuperscript{2}/g) specific surface area. In addition, ZrO\textsubscript{2}catalyst decolorizes the methylene blue, and methyl orange photodegraded nearly 91 and 69 % in 240 minutes. Sai Saraswathi et al. synthesized of ZrO\textsubscript{2}NPs from Lagerstroemia speciosa leaf. The highest absorption spectrum of processed ZrO\textsubscript{2}NPs from a leaf of L. speciosa displayed a peak at 354 nm. The EDX pattern indicates maximum emanation at 1 keV that was the binding energy of Zr (70.4 %), and 0.5 keV have binding energy for O\textsubscript{2}(24.11 %) and enduring creates carbon-based constituent. A photocatalyst action of ZrO\textsubscript{2}NPs was considered for azo dye through revealing to sunlight with 94.58 %. The number of deaths cells rose as the quantity of ZrO\textsubscript{2}NPs doubled. Cells shrank at 500 g/mL, and almost 30–40 % of cells exhibited blebbing (tiny protuberances of the membrane). In the ZrO\textsubscript{2}NPs treated cells, apoptosis bodies were found. Nabil et al. synthesized ZrO\textsubscript{2}NPs using leaf extract of Wrightia tinctoria. An emission spectrum causes an emission of the ZrO\textsubscript{2}NPs at 360 nm, which can be seen in the PL spectrum. An average ZP value of -21.17 eV indicated a capping particle on the surfaces of produced ZrO\textsubscript{2}NPs was primarily made up of negative charges. For 120 minutes, the biologically synthesized ZrO\textsubscript{2}NPs degraded RY 160 dye by 94 %. At a dose of 10 μg/ ml, the aqueous W. tinctoria extract showed the maximum inhibitory zone towards E. coli (12 ± 0.2), S. aureus (10 ± 0.1), P. aeruginosa (9 ± 0.4), and B. subtilis (7 ± 0.3). Biosynthesized ZrO\textsubscript{2}NPs produced by W. tinctoria extract demonstrated excellent antibacterial effectiveness against all tested microbes when compared to leaf extract at 10 g/mL. Inhibition zones were observed for E. coli, S. aureus, P. aeruginosa, and B. subtilis, which had 22.5 mm, 21.5mm, 21.5mm and 20mm, respectively. The nanoparticle's tiny spherical shape and crystallite size may be to blame for their
enhanced antibacterial properties. Vanadium oxide (V₂O₅) or ZrO₂ NC were made by Parsa et al. using Daphne alpine (D. alpine) leaves extract in a green method. The pore space and surface area were investigated utilizing Brunaure-Emmett-Teller (BET) techniques for the N₂ adsorption-desorption process, and SBET was determined to be 214 m²/g. Diffuse reflectance spectroscopy (DRS) was used to investigate the optical property, and the absorption edge was discovered to be 3.93 eV. Around 3499 cm⁻¹, the stretching vibration of the -OH group was noticed. The C-H bending characteristic peaks about 3000 and 2942 cm⁻¹, while the bands at 1725 cm⁻¹ could be attributable to the carbonyl group (C=O) of the ester and carboxylic acid. The -NO bend mode and carbonyl stretch are responsible for the peaks at 1433 and 1220 cm⁻¹, respectively. When methyl orange and picloram were used as photocatalysts, the photocatalytic efficacy of V₂O₅/ZrO₂ NC was tested, and 76.94 % and 86 % were destroyed in 75 minutes, respectively.

Annu et al. prepared ZrO₂ NPs utilizing the pericarp extract of Sapindus mukorossi as a prevailing capping and reducing agent. The particle size was 5–10 nm that, was in accord with the tetragonal stage. Distinct peaks were observed only in EDX spectrum to Zr, and the broad -OH stretching contributes to the large and prominent band at 3180 cm⁻¹. The acute, tight spike at 1655 cm⁻¹ was attributable to the processed specimen's bend vibration adsorbed H₂O structures. The distinctive tetragonal Zr-O-Zr vibration that was predominantly amplified through the calcination method considerably contributes to the peak occurrence in the region of 500-700 cm⁻¹. In batch trials, the adsorptive capabilities of produced NPs for methylene blue (MB) dye were investigated as a function of pH, dose, initial adsorbate level, and time. With an adsorptive capacity of 23.26 mg/g, 94 % removal performance was found, which aligned well with the nonlinear Langmuir isotherm.

Vennila et al. produced ZrO₂ NPs that used a methanol-based extract of Glorisa superba tuber powder. The produced NPs were analyzed using XRD, SEM, and EDX techniques and a solar cell simulator approach to investigate the natural dye-sensitized solar cell activity of ZrO₂ NPs. The photocurrent in the DSSC's photoanode, which accumulates of analyzing stage ZnO/TiO₂ NPs with opuntia dye on the FTO substrate, was studied. Renuka et al. generated ZrO₂ doped with Mg hollow-based microspheres with a 0.1-5 mol% using a simple, environmentally benign, low-cost phytomediated burning technique. The peak related to (-111), (002), and (111) planes marginally migrated to lesser 2θ angle side when Mg²⁺ level increased from 0.1 to 2 mol% in this investigation; however, these planes moved slightly greater angle side as Mg²⁺ concentration increased from 3 to 5 mol %. The bands confirmed the monoclinic phases of ZrO₂ at 100.179, 192, 222, 306, 340, 380, 470, 510, and 540 cm⁻¹. The existence of tetragonal and monoclinic phases in ZrO₂: Mg 4 mol % NPs was confirmed by Raman bandings at 147 and 260 cm⁻¹. Under UV light, the photocatalytic capabilities of photocatalysts are assessed for the destruction of rhodamine B. The photocatalytic performance of 2 mol % Mg enriched ZrO₂ was good, with a dissolution rate of 93 %. Compared to pure ZrO₂, 2 mol % Mg-doped ZrO₂ showed the highest photocatalyst reaction and the largest particular surface area and pore volume. This could aid with dye loading as well as photocatalytic reactivity. Nevertheless, 2 mol % Mg-doped ZrO₂ and 5 mol % Mg-doped ZrO₂ have the highest surface area and pore volume but low photocatalyst activity. In another study, Sathish Kumar et al. developed liner chains of ZrO₂ NPs using Curcuma longa with an average size of range 41-45nm. The TEM image of ZrO₂ particles was presented in Fig. 7.
The elastic modulus of PVA measured amount of Zr NPs and decreased at higher Zr NP content, as per observations. When contrasted to polymeric matrix, the specimen by 1 wt%. ZrO2-PVA had good elastic modulus. The next stage is the H-bonding amid the -OH groups on ZrO2NPs and the -OH functional group of PVA particles. The -OH group in the PVA framework may combine with the surface of ZrO2, which is used as a filler, to produce hydrogen. The nanocomposite was stabilized by hydrogen bonding, which prevented the dissociation of phase 50.

Pandiyan et al. 51 developed CeO2 @ZrO2 core metal oxide (MO) NPs utilizing Justiciaadhatoda extract. The broad peaks at 267, 305 and 615 cm⁻¹ of ZrO2 were noticed in Raman spectra CeO2@ZrO2 core metal oxide NPs at a temperature of 700 °C, which was the characteristic tetragonal stage of Zr. According to the XRD results, CeO2 @ ZrO2 core metal oxide NPs, a proportion of ceria-0.75, Zr-0.25, and two O2 contents demonstrate that CeO2 @ZrO2 core metal oxide NPs has the formula (Ce0.75+ Zr0.25) O2. The nano stick shape of CeO2@ZrO2 core metal oxide was visible in the micrographs. The CeO2@ZrO2 core metal oxide MO inhibited violacein synthesis in C. violaceum in a violacein inhibition assay (ATCC 12472). CeO2@ZrO2 gradually depletes the nutrients bacteria require development, resulting in cell death. The antimicrobial property of CeO2, ZrO2, and CeO2@ZrO2 core metal oxide NPs was tested towards S. aureus and E. coli microbial infections. For both infections in the order S. aureus > E. coli, CeO2 @ZrO2 alone have revealed a diameter of inhibition zone S. aureusas 34 mm, following by E. coli, displayed the best and highest antimicrobial property in the CeO2@ZrO2 core metal oxide NPs as 29 mm. The antioxidant behaviour of core metal oxide has a special characteristic that requires less energy of DPPH radical by up to 89%. S. marcescens was used to assess the antibiofilm action of CeO2 @ZrO2 core metal oxide NPs. Antibiofilm features of CeO2 @ ZrO2 core metal oxide NPs are shown in the study, and they were able to harm the multilayer, 3D biofilm structure. The CeO2 @ZrO2 core metal oxide NPs limit quorum sensing and govern the growth of S. marcescens biofilms.

Raghad et al. 52 green synthesis of ZrO2NPs utilizing different plant extracts: Capsicum annuum, Allium cepa and Lycopersicon esculentum. NPs was produced by C. annuum, their properties according to method one and method two were: average size was 100.25 nm, 86.66 nm, roughness average (Ra) 1.17 nm and 1.08 nm, Root mean square (Sq) 1.98 nm, 1.25 nm. Scherer's equation also calculated crystal’s size; it was 22.09 nm and 13.069 nm, optical band gaps were 5.1 eV and 5.25 eV, and according to SEM, particles size were (<105, 100) nm, respectively. NPs produced by A. cepa, their properties according to method one and method two were: average size was 105.14 nm, 83.00 nm, (Ra) was 0.238 nm, and 1.09 nm, (Sq) was 0.272 nm and 1.27 nm. Crystals sizes were 11.039 mm and 21.97 nm, optical band gaps were 5.3 eV and 3.9 eV, and according to SEM, particles size were (>80, >90) nm, respectively. Zirconium NPs were confirmed for their antimicrobial efficacy towards microbial cultures of E. coli and S. aureus by well diffusion method. Plant mediated synthesis of nano zirconium particles is presented in Table 1.

### Table 1. Plant extract mediated synthesis of nano zirconium particles

| Plant extract               | Characterization | Size (nm) & shape | Functional groups                                      | Applications                  | Ref.     |
|-----------------------------|------------------|-------------------|--------------------------------------------------------|-----------------------------|---------|
| Acalypha Indica (20g, 200ml)| FT-IR, XRD, SEM, EXD | 20-100 & cubic | Zr-O-Zr asymmetrical stretching, -OH stretching and bending vibration | N/A                         | Shanthi et al. 30 |
| Nyctanthes arbor-tristis (5g, 50ml) | TG/DTA, XRD, SEM, EDX, AFM and UV-Vis | 10, Spherical | O-H group of the absorbed water, C-H and C-O groups beta-sitosterol, campasterol, isofucosterol, ascorbic acid; phenolic acids and pollicene acids carboxyl groups, polyphenolic compounds, and -OH stretching vibrations of adsorbed water molecules | Antibacterial | Gowri et al. 31 |
| Thespesia populnea (2.435 g, 20 ml) | UV-Vis, XRD, TEM and FTIR | 24-40, Spherical & triangular | N/A | Antimicrobial & antifungal | Kanda et al. 32 |
| Equisetum arvense (3gm,150ml) | UV-Vis, STEM, DLS and zeta potential | 50-100, Spherical | C-H stretching vibrations, carboxyl group of C-O stretching, and an aliphatic amine group | Antimicrobial activity | Pragya et al. 33 |
| Aloe vera (50g,100ml) | TG/DTA, XRD, SEM with EDX, AFM, UV-Vis and FTIR | 35.45 & spherical | N/A | |
| Helianthus annuus           | XRD, TEM, SEM with EDX, UV-Vis, FTIR and DLS and zeta potential | 35.45 & spherical | N/A | |
| Plant Name                  | Analytical Techniques                                      | Physical Characteristics | Functional Properties                        |
|----------------------------|-------------------------------------------------------------|--------------------------|-----------------------------------------------|
| Moringa oleifera (10g, 100ml) | XRD, SEM-EDX, UV-Vis and FTIR                              | 10 & spherical           | Antibacterial & antioxidant activity          |
| Nephelem lappaceum L. (10ml)   | XPS, TEM, PL, Raman and FTIR                              | 50 & rod                 | Anticancer activity                           |
| Aloevera (5g, 20ml)           | UV-Vis, TEM, EDAX, XRD and FT-IR                           | 18-42 & spherical        | Adsorption of fluoride                        |
| Euclavia natalensis (50, 75, and 100g/l) | XRD, FTIR and TEM                                         | 5.90-8.54 & spherical   | Adsorption of tetracyclin                     |
| Eucalyptus globules (10g/100ml) | UV-Visible and DRS, XRD, FTIR, TEM, SAED, SEM-EDX, DLS and fluorescence spectroscopy | Spherical               | Ellagic acid, gallic acid, caffic acid, chlorogenic acid, methyl gallate, glycosides, and phenolic compounds |
| Phyllanthus acidus            | UV-Vis, XRD, FTIR, HR-TEM, EDAX, SEM and PL spectroscopy   | 4.5 to 5.8 spherical     | Catalytic activity                            |
| Ficus benghalensis (5g/100ml)  | FT-IR, HR-TEM, FT-Raman and BET surface area               | 10-18 & spherical        | Catalytic activity & anticancer activity      |
| Lagerstremia speciosa (15g/100ml) | UV-vis FT-IR, X-XRD, TEM, SEM-EDX, and TGA              | 56.8 & tetragonal        | Catalytic activity                           |
| Wrightia tinctoria (10g/100ml) | XRD, UV-Vis, SEM-EDX, DLS, ZE, PL and FT-IR               | 9.15 & spherical         | Photo catalytic & antibacterial activity      |
| Daphne alpine (20g/1000ml)     | DRS, XRD, TEM, SEM, TG/DTG, and FT-IR                     | 34-50 & spherical        | Photo catalytic                              |
| Sapidnusmukarossi (5g/100ml)   | UV-Vis, FTIR, XRD, SEM-EDX and HR-TEM.                     | 5-10 & spherical         | Adsorption of tetracyclin                     |
| Glorisa superb (10g/100ml)     | XRD, SEM and EAX                                           | NA                       | Solar cell                                   |
| Aloe Vera (3g/20ml)            | M, TEM, PL, UV-Vis, XPS, Raman, and FTIR                  | 7-29 & spherical         | Stretching vibration of hydroxyl group        |
| Curcuma longa 6.8 g            | XRD, TEM, EDX and FTIR                                     | 41-45 & chains           | Photocatalytic activity                       |
| Rosmarinus officinalis         | XRD, FTIR, TGA, FESEM and tensile strength                | 2-30 & spherical         | Vibrations of COOH and C-H vibrations         |
| Justicia adhatoda 10gm/100ml   | UV-Vis, DRS, XRD, FTIR, SEM-EDX, HR-TEM, Raman            | 20-45 & stick-like       | Hydroxyl groups (-OH), which is an indication of alcohol and phenols |
| Capsicum annum, Allium cepa and Lycopersicon esculentum | UV-Vis, XRD, FTIR, SEM, AFM, and HRTEM                 | 80-90 & spherical        | Photocatalytic activity                       |

Note: The table lists the plant name, analytical techniques used, physical characteristics, and functional properties. The functional properties include antibacterial, anticancer, adsorption, catalytic, and photo catalytic activities.
Synthesis of ZrO$_2$NPs using bacteria:

Using microbial culture or biomass, metal and metal oxide nanoparticles can be produced in an extracellular or intracellular context. Extracellular synthesis is a process in which microorganisms manufacture enzymes and proteins that are discharged into the environment. These enzymes and proteins have decreased metal ions and stabilized particles. In contrast to the extracellular biosynthesis pathway, the internal biosynthesis route necessitates the inclusion of a cell lysis step to release the nanoparticles from within the microbe. Thus, intracellular synthesis takes longer and costs more than the extracellular production process, in which metals are reduced or chelated by proteins and enzymes outside the cell. The amino, sulfahydryl, and carboxylic groups found in the main enzymes found in biological materials attach to the metallic ions and cause them to be reduced; however, the exact production method is still not fully known. The possible mechanism is shown in Fig. 8.

Using *Pseudomonas aeruginosa* bacteria, Banhishikha et al. investigated the green synthesis of zirconia nanoparticles. The zirconia NPs, which had a monoclinic and tetragonal crystal structure with a crystallite size of 6.41 nm, had an average particle size of 15 nm and included zirconium and oxygen, as well as functional groups such as O–Zr–OH, Zr–O–Zr, and Zr–O–Zr bonds. There was also a monoclinic and the effectiveness of tetracycline adsorption mediated by zirconia nanoparticles was demonstrated at a pH of 6.0 and a contact period of only 15 min. TEM images show that the zirconium dioxide particles formed are spherical grains with diameters ranging from 5 nm to 25 nm and an average particle size of 15 nm. According to the findings, tetracycline adsorption onto ZrO$_2$NPs synthesized by the researchers followed a pseudo-second-order kinetic model. According to Temoor et al., ZrO$_2$ nanoparticles exhibit SPR spectra with peak ranges 240-350 nm, which is caused by the change from oxide species to zirconium cation (O–Zr$^{4+}$). Zirconium (54.40 %), oxygen (43.49 %), silicon (0.90 %), iron (0.34 %), and aluminium (0.86 %) were all found to be present in biologically formed ZrO$_2$ nanoparticles, according to EDS spectroscopy results. The existence of the hydroxyl (O–H) group was verified by the appearance of a strong signal at 3358 cm$^{-1}$. A high antifungal activity against the *P. versicolor* strain XJ27 was observed in biogenic ZrO$_2$ nanoparticles that were grown in vitro. The electron microscope pictures demonstrated that ZrO$_2$NPs were adsorbed on the *P. versicolor* cell membrane and ruptured pathogen cells, with a continuous peak at 1637 cm$^{-1}$ suggesting C=C alkene stretching, which was seen in the experiments.

One pot of ZrO$_2$ nanoparticles was made at room temperature using an extremophilic *Acinetobacter* KCSI1 strain, according to Shanmugasundaram et al. Researchers found that ZrO$_2$ nanoparticles average size was 44 nm. The crystalline structure of ZrO$_2$ was discovered by the use of XRD and Raman spectra. The HRTEM and SAED pictures revealed ordered crystal lattice nanoparticles that were perfectly aligned. The zeta potential of ZrO$_2$ nanoparticles was measured to be 36.55.46 mV. In this study, the AFM was used to measure the mechanical properties of Bio-ZrO$_2$NPs. The hardness and Young's modulus of the NPs were 9.206 2.22 GPa and 0.285 0.13 GPa, respectively. The Bio-ZrO$_2$ nanoparticles were shown to be cytocompatible, with cell viability of greater than 70% being achieved. When ZrO$_2$ nanoparticles were tested on mouse fibroblast cells, it was shown that they had no substantial cytotoxicity (L929). The highest cell viability was achieved at Bio-ZrO$_2$NPs concentrations of 0.25 mg/mL (98.050.75 %) and 0.5 mg/mL (95.12 0.72 %), respectively (95.12 0.72 %). The dose-dependent cellular response profile of L929 cells treated with different doses of ZrO$_2$ is apparent in the Hoechst pictures taken after the cells were treated with different doses of ZrO$_2$ (Fig. 9).
Fungi and algae:

Similar to the reported molecular method for synthesizing metal and metal oxide nanoparticles from fungus biomass or culture, a bacterial-based green synthesis approach is also used. Green nanoparticles might be created with more efficiency using bacteria, although fungi are thought to have the best chance of success. As a result, fungus cells appear to be more resistant to changes in process conditions and variables such as pressure or flow rate as well as stirring, raising the possibility that they may be used for large-scale synthesis. Heterocyclic compounds identified in proteins from fungal extracts, such as C=O, =C=O, C-O-R, and =C=C=, have been demonstrated to behave as NP capping ligands. Capping ligands in NPs have been shown to include functional groups such as =C=O; C-O; C-O-R; and =C=C=. It was established by Ahmad et al. using AFM micrographs and SEM data that spherical NPs with a diameter of less than 100 nm could be created using this method. Using P. notatum PTCC 5074, P. purpurogenome PTCC 5212, and P. aculeatum PTCC 5167 as sources of colloidal zirconium nanoparticles, the zeta potential of colloidal zirconium nanoparticles was -2.2 mV, -3.87 mV, and 1.72 mV, respectively. Colloidal zirconium NPs produced with P. purpurogenome PTCC 5212 were efficient against Gram-negative bacteria, with MICs of 0.75 mM for E. coli ATCC 27853 and 0.375 mM for P. aeruginosa ATCC 27853, but were unsuccessful against Gram-positive S. aureus (ATCC 27853 and ATCC 27853, respectively). Both the supernatant and the zirconium salt solution failed to exhibit a MIC against Gram-negative or Gram-positive bacteria at a maximum concentration of 1.5 mM.

Golnaraghi-Ghomi et al. evaluated the potential of Penicillium notatum, Penicillium purpurogenum, and Penicillium aculeatum development stages to produce zirconium nanoparticles in three different growth stages. A further finding was that the electrochemical dispersive spectroscopy (EDX) of Zr-NPs displayed an absorption peak at 2.2 keV, indicating that Zr-element is present in the bio produced NPs. For PTCC 5074, PTCC 5167, and PTCC 5212, the hydrodynamic diameters of Zr-NPs at pH 7 were 22.55 nm, 66.6 nm, and 70.9 nm for the three strains studied. The correlation coefficients R2 at each phase (training, validation, and test sets) were calculated using an optimal multilayer perceptron neural network to represent the experimental data, and they were found to be 0.9946, 0.9952, and 0.9997, respectively, when using an optimal multilayer perceptron neural network to represent the experimental data. An innovative approach for the production of ZrO2 nanoparticles was developed by Kavitha et al. by utilizing the plant pathogenic fungus Fusarium solani as a reducing and stabilizing agent. In the HRTEM image, the spherical form of ZrO2 can be seen, with a diameter of 40-50 nm. Vipul et al. used aqueous ZrF6 ions to investigate the fungus Fusarium oxysporum. According to the authors, the hydrolysis of anionic complexes by extracellular proteins results in the simple production of nanocrystalline zirconia at room temperature. Zr–O–Zr bending vibrations can be detected by looking for an absorption band at 819 cm⁻¹. At 1655 and 1544 cm⁻¹, respectively, two absorption bands (amide I and II bands) are centred. The particles have a relatively uniform shape and an overall morphology that is quasi-spherical. According to the particle size histogram, particle sizes range from 3 to 11 nm, with an average of 7.3 ±2.0 nm. Under ambient settings, Uddin et al. employed potassium hexafluorozirconate (K₂ZrF₆) as a precursor to induce extracellular synthesis of zirconia nanoparticles in Humicola spp.

Algal extracts are rich in carbohydrates, proteins, minerals, polysaturated fatty acids, and antioxidants. Chemicals present in algae with similar carboxyl, cysteine, hydroxyl, and amine functional groups may be responsible for metal-ion reduction and capping of freshly formed nanoparticles, according to FTIR investigations. Initial metal ions are deposited on the surface of the algal cell, which is the first step in nanoparticle creation. Depending on the kind of metal ion generated, enzymatic machinery in the cytoplasm, thylakoid membrane, and organelle membrane creates the metal ion either extracellularly or intracellularly (following metal ion intake by transmembrane protein or diffusion). Relying on Sargassum wightii (brown seaweed), Kumaresan et
al. established a simple and ecologically acceptable combustion method for the manufacture of ZrO₂ nanoparticles (S. wightii). The structural, optical, and photoluminescence characteristics of the nanoparticles were determined. A strong absorption peak was seen at 277 nm, according to optical absorption tests. The presence of ionized oxygen vacancies in the material may be detected in PL spectra by the presence of large emission peaks at the interface of the UV and visible wavelength ranges. As seen in the TEM picture, the resultant particles have a spherical shape and a mean particle size of 5 nm, indicating that they are relatively monodisperse. We investigated S. wightii extract before and after it was treated with calcined zirconia nanoparticles for antibacterial activity against Gram-positive and Gram-negative bacteria using the agar well diffusion technique in agar wells (Escherichia coli, Salmonella typhi). Bacteria, algae, and fungi mediated the synthesis of nano zirconium particles in Table 2.

**Table 2. Bacteria, algae and fungi mediated synthesis of nano zirconia particles.**

| Algae/fungi | Characterization | Size (nm) & shape | Functional groups | Applications | Ref. |
|-------------|-----------------|------------------|-------------------|--------------|------|
| Penicillium  | FTIR, XRD, AFM, DLS and TEM | 100 & spherical | O-H and C–C stretching, -OH bending mode, C=O stretching vibration of carboxyl and carboxylic group of amide I | Antibacterial | Ahmad et al. 63 |
| Penicillium spp. | SEM-EDX, DLS and FTIR, | 100 & spherical | O-H and C–C bending, N-H bending of primary mides | NA | Golnaraghi-Ghomi et al. 64 |
| Fusarium oxysporum (MTCC-2671) | FTIR, XRD, and HR-TEM | 40-50 & unvisual spherical | -OH stretching vibrations, amide groups, C-N stretching vibrations of aliphatic or aromatic amines | NA | Kavitha et al. 65 |
| Fusarium oxysporum | TEM, FTIR, SAED and XRD | 3-11 & quasi-spherical | Zr–O–Zr bending vibration, amide I and II bands | NA | Vipul et al. 66 |
| Humicola sp | UV-Vis, TEM, DLS, XPS and FTIR | 13 & quasi-spherical | Zr–O–Zr stretching and bending | NA | Uddin et al. 67 |
| Sargassum wightii | XRD, FTIR, HR-TEM, UV–vis and PL spectroscopy | 5 & spherical | H-bonded hydroxyl groups, stretching band of the carboxylic acid group, asymmetrical and symmetrical vibration of carboxylate ions C=O stretching at alcoholic groups | Antibacterial | Kumaresan et al. 70 |

**Future perspectives**

An eco-friendly nanotechnology is a developing approach that has applications in many areas of life and may be used to generate new, dependable, and long-lasting solutions. Thorough knowledge of the biochemical and molecular mechanisms involved in its formation is required to discover and isolate molecules involved in metal salt reduction into nanoparticles. An in-depth understanding of the distribution and mechanism of green nanoparticles' action is necessary to further biomedical uses of these particles. The most significant difficulty is the evaluation of the possible hazardous aspects of green nanoparticles and the risk management associated with their manufacturing, handling, storage, and eventual disposal. Consequently, more in-depth knowledge of metabolic processes, surface chemistry, and the chemical composition of binding agents will help researchers discover breakthrough methods that make large-scale manufacturing of binding agents possible. This green technology can provide the greatest amount of value to future generations in all sectors of life if it can successfully battle its inherent disadvantages.

**Authors' declaration:**
- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for republication attached with the manuscript.
- Ethical Clearance: NA

**Authors' contributions statement:**
S. S. A.: Writing and outline the study, M. A.: editing, M. A.: data analysis, M. S.: Revising. All authors contributed to data analysis, drafting, or revising the manuscript.

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التطورات الحديثة في التخليق الحيوي لجسيمات أكسيد الزركونيوم النانوية وتطبيقاتها البيولوجية

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الخلاصة:

أثار أكسيد الزركونيوم ZrO2 اهتمام الباحثين في جميع أنحاء العالم، لا سيما منذ تطوير طرق لتصنيع جزيئات بحجم النانو. تم تحسين الدراسة المكثفة في تكوين الجسيمات النانوية باستخدام تقنيات تركيبية مختلفة، بالإضافة إلى استخدامها المحتملة، من خلال كفاءتها الضوئية العالية، وفوائد النطاق العريض، وطاقة ربط الأكسيتن العالية. في تغليف المواد الغذائية، يمكن استخدام الجسيمات النانوية لثاني أكسيد الزركونيوم كعوامل مضادة للميكروبات ومضادة للسرطان. استجابةً للاهتمام المتزايد بنمو ZrO2، ابتكر الباحثون وطوروا طرقًا لتجميع الجسيمات النانوية ذات الأشكال المختلفة باستخدام طرق بيولوجية. في إنزال الزركونيا في المختبر، يتم توفير عوامل التثبيت بواسطة الخلايا الحيوية الموجودة في المستخلصات النباتية، بينما يتم توفير الامكانيات بواسطة الكائنات الحية الدقيقة كعوامل التغطية والتنوع (داخل الخلايا أو خارج الخلايا). من الممكن تحويل الجسيمات النانوية المنتجة باستخدام مجموعة من الأساليب التحليلية، بما في ذلك التحليل الطيفي للأشعة فوق البنفسجية (FT-IR)، والمجهر الإلكتروني للإرسال (TEM)، والتحليل الفلسي على النواة تحت الحمراء (XRD). عند تطبيقها على البكتيريا (موجبة الجرام وسالبة الجرام) والبكتيريا، تظهر ZrO2NPs طرقًا العلمية مماثلة للجزيئات النانوية ZrO2، والتي يمكن تفسيرها من خلال توليد الأكسيتن النشط (ROS)، بإنتاج هذا العامل ووصف الطريقة المماثلة لجزيئات zirconium nanoparticles.

الكلمات المفتاحية: التطبيق، الخصائص، التخليق الأخضر، النانو تكنولوجي، جزيئات الزركونيوم النانوية