

ONE POT POMEGRANATE MEDIATED SYNTHESIS OF ZIRCONIUM &
STRONTIUM NANO PARTICLES AND THEIR ANTIMICROBIAL AND
ANTI-INFLAMMATORY EFFICACY STUDY

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RUNNING TITLE: ANTIMICROBIAL AND ANTI-INFLAMMATORY EFFICACY STUDY OF ZIRCONIUM & STRONTIUM NANO PARTICLES

KEYWORDS	ABSTRACT: INTRODUCTION
Nanotechnology, Green Synthesis, Pomegranate Extract, Zirconium Nanoparticles, Strontium Nanoparticles, Antimicrobial Efficacy, Anti-inflammatory Activity.	Nanotechnology has revolutionized various scientific fields, offering novel approaches for synthesizing and applying nanoparticles, providing innovative solutions for drug delivery, diagnostics, and therapeutic applications. This study focuses on the green synthesis of zirconium and strontium nanoparticles using pomegranate extract, which leverages the natural reducing and stabilizing agents present in pomegranate to simplify the production process and minimize environmental impact.
	METHODS
	Pomegranate peel aqueous extract was prepared by drying and grinding the peels, followed by autoclaving, heating, and centrifugation to extract bioactive compounds. Zirconium and strontium nanoparticles were synthesized by mixing the extract with zirconium chloride and strontium chloride solutions, followed by continuous stirring. The formation of nanoparticles was confirmed by a color change. The nanoparticles were characterized using Fourier Transform Infrared Spectroscopy (FTIR) and UV-Visible Spectroscopy (UV-Vis). Their antimicrobial efficacy was assessed using the agar-well diffusion method, while anti-inflammatory activity was evaluated using the albumin denaturation method.
	RESULTS
	FTIR analysis revealed characteristic peaks indicating the presence of functional groups involved in nanoparticle stabilization. UV-Vis spectroscopy confirmed the formation of nanoparticles with characteristic surface plasmon resonance peaks. The antimicrobial study demonstrated significant activity of the nanoparticles against Methicillin-resistant Staphylococcus aureus (MRSA) and Salmonella, with inhibition zones increasing with nanoparticle concentration. However, no inhibitory effect was observed against Klebsiella. Anti-inflammatory tests indicated that the nanoparticles inhibited protein denaturation effectively.
	CONCLUSION
	The green synthesis of zirconium and strontium nanoparticles using pomegranate extract is an effective method that produces biocompatible nanoparticles with significant antimicrobial and anti-inflammatory properties. This study highlights the potential of these nanoparticles for use in biomedical applications, offering a sustainable and eco-friendly alternative to conventional methods.

INTRODUCTION

Nanotechnology has revolutionized various scientific fields, offering novel approaches for synthesizing and applying nanoparticles, offering innovative solutions for drug delivery, diagnostics, and therapeutic applications [1]. This study focuses on the green synthesis of zirconium and strontium nanoparticles using pomegranate extract, a method that aligns with sustainable and eco-friendly practices. The “one pot” synthesis approach leverages the natural reducing and stabilizing agents present in pomegranate, simplifying the production process and minimizing environmental impact [2]. This study explores a novel, green synthesis method using pomegranate extract to produce zirconium and strontium nanoparticles. The use of pomegranate, a natural and eco-friendly reducing agent, aligns with the increasing emphasis on sustainable and environmentally benign synthesis techniques.

Pomegranate (*Punica granatum*) is a fruit known for its rich nutritional profile and numerous health benefits. It is abundant in vitamins, minerals, and bioactive compounds such as polyphenols, flavonoids, tannins, and anthocyanins, which contribute to its strong antioxidant, anti-inflammatory, and antimicrobial properties [3]. The juice, seeds, and peels of pomegranate have been extensively studied for their therapeutic potential. In addition to its health benefits, pomegranate has emerged as a valuable natural reducing agent in green chemistry, particularly in the synthesis of nanoparticles [4]. The phytochemicals present in pomegranate facilitate the reduction of metal ions to nanoparticles, offering a sustainable and eco-friendly alternative to conventional chemical synthesis methods [3]. The pomegranate-mediated synthesis approach not only simplifies the production process but also enhances the biocompatibility of the nanoparticles, making them suitable for biomedical applications. This study aims to evaluate the antimicrobial and anti-inflammatory efficacy of these green-synthesized nanoparticles. Given the rising concern over antibiotic resistance and the need for new anti-inflammatory agents, the development of effective and safe nanoparticles presents a promising avenue for advancing medical treatments [5].

Zirconium (Zr) and Strontium (Sr) nanoparticles are renowned for their unique properties, including high stability and bioactivity, making them suitable for biomedical applications [5]. The synthesis of zirconium and strontium nanoparticles via pomegranate extract offers a dual advantage: leveraging the medicinal properties of pomegranate while producing nanoparticles with enhanced therapeutic potentials. This research will investigate the structural and functional properties of the synthesized nanoparticles, assessing their effectiveness against microbial pathogens and their capacity to mitigate inflammation, thereby contributing to the broader field of nanomedicine [6]. By utilizing a natural, readily available resource like pomegranate, this method not only reduces the reliance on toxic chemicals but also promotes a more sustainable

approach to nanomaterial synthesis [6]. This research thus contributes to the growing body of knowledge in green nanotechnology and its applications in medicine.

MATERIALS AND METHODS

Preparation of Pomegranate Peel Aqueous Extract - Fresh pomegranate fruits are obtained, and the peels are carefully separated from the seeds. The peels are thoroughly washed with distilled water to remove any dirt, dust, or pesticide residues. The peels were dried at room temperature in a shaded area for seven days to prevent any degradation of bioactive compounds. After drying, the peels were ground into a fine powder using a laboratory blender. This powder underwent autoclaving at 121°C and 15 psi for 20 minutes, ensuring sterility and eliminating any microbial contamination. Following autoclaving, 50 grams of the sterilized pomegranate peel powder were mixed with 500 mL of distilled water and heated at 80°C for 30 minutes with continuous stirring. This step facilitated the extraction of bioactive compounds into the aqueous phase. The mixture was then cooled to room temperature before being centrifuged at 5000 rpm for 20 minutes to separate solid debris from the extract. The supernatant was filtered using Whatman No. 1 filter paper to obtain a clear pomegranate peel aqueous extract. Finally, the extract was subjected to lyophilization (freeze-drying), resulting in a concentrated form rich in bioactive compounds [7].

Synthesis of Zirconium and Strontium Nanoparticles- For the synthesis, zirconium chloride ($ZrCl_4$) and strontium chloride ($SrCl_2$) solutions were prepared by dissolving the respective salts in distilled water to achieve concentrations of 0.01 M. Equal volumes of these metal salt solutions were mixed with the lyophilized pomegranate peel aqueous extract, and the mixture was stirred continuously at room temperature for four hours. The natural reducing and stabilizing agents present in the pomegranate extract facilitated the reduction of metal ions and their stabilization as nanoparticles. The successful formation of nanoparticles was indicated by a color change in the reaction mixture from pale yellow to dark brown. The nanoparticles were separated from the reaction mixture by centrifugation at 10,000 rpm for 30 minutes. The resulting nanoparticle pellet was washed three times with distilled water to remove any unreacted residues and dried at 60°C in a hot air oven.

Characterization of Nanoparticles - The synthesized zirconium and strontium nanoparticles were characterized using various analytical techniques. Fourier Transform Infrared Spectroscopy (FTIR) was employed to characterize zirconium and strontium nanoparticles, focusing on their functional groups and chemical bonding. The methodology began with preparing the nanoparticle samples, typically by mixing them with potassium bromide (KBr) powder to create a pellet, or by using a thin film method if the samples were in a suitable form. The samples were then placed in the FTIR spectrometer, where they were exposed to a broad spectrum of infrared light. The nanoparticles absorb specific wavelengths of this light, corresponding to the vibrational frequencies of their molecular bonds. The FTIR spectrometer measured the absorbed wavelengths and generated an infrared absorption spectrum. This spectrum displayed distinct peaks representing various functional groups and bonding characteristics of the nanoparticles. By analyzing these peaks, researchers could identify the presence of specific chemical bonds and functional groups within the zirconium and strontium nanoparticles, providing essential information about their chemical composition and structural properties. UV-Visible Spectroscopy (UV-Vis) was utilized to analyze the optical properties of zirconium and strontium nanoparticles. The methodology involved preparing a colloidal solution of the nanoparticles by dispersing them in a suitable solvent, such as distilled water or ethanol. The solution was sonicated if necessary to ensure a uniform dispersion of the nanoparticles. This colloidal solution was then transferred into a quartz cuvette, which is transparent to UV and visible light, to avoid any interference during measurement. The cuvette was placed in the UV-Vis spectrophotometer, which scanned the sample over a range of wavelengths, typically from 200 to 800 nm. The spectrophotometer measured the absorbance of light at each wavelength, producing an absorbance spectrum. This spectrum revealed characteristic peaks corresponding to the electronic transitions within the nanoparticles, providing insights into their optical properties, such as band gap energy and surface plasmon resonance [9 -11]. By analyzing these peaks, researchers could infer details about the size, shape, and electronic structure of the zirconium and strontium nanoparticles, aiding in their characterization and potential application development (Figure 1).

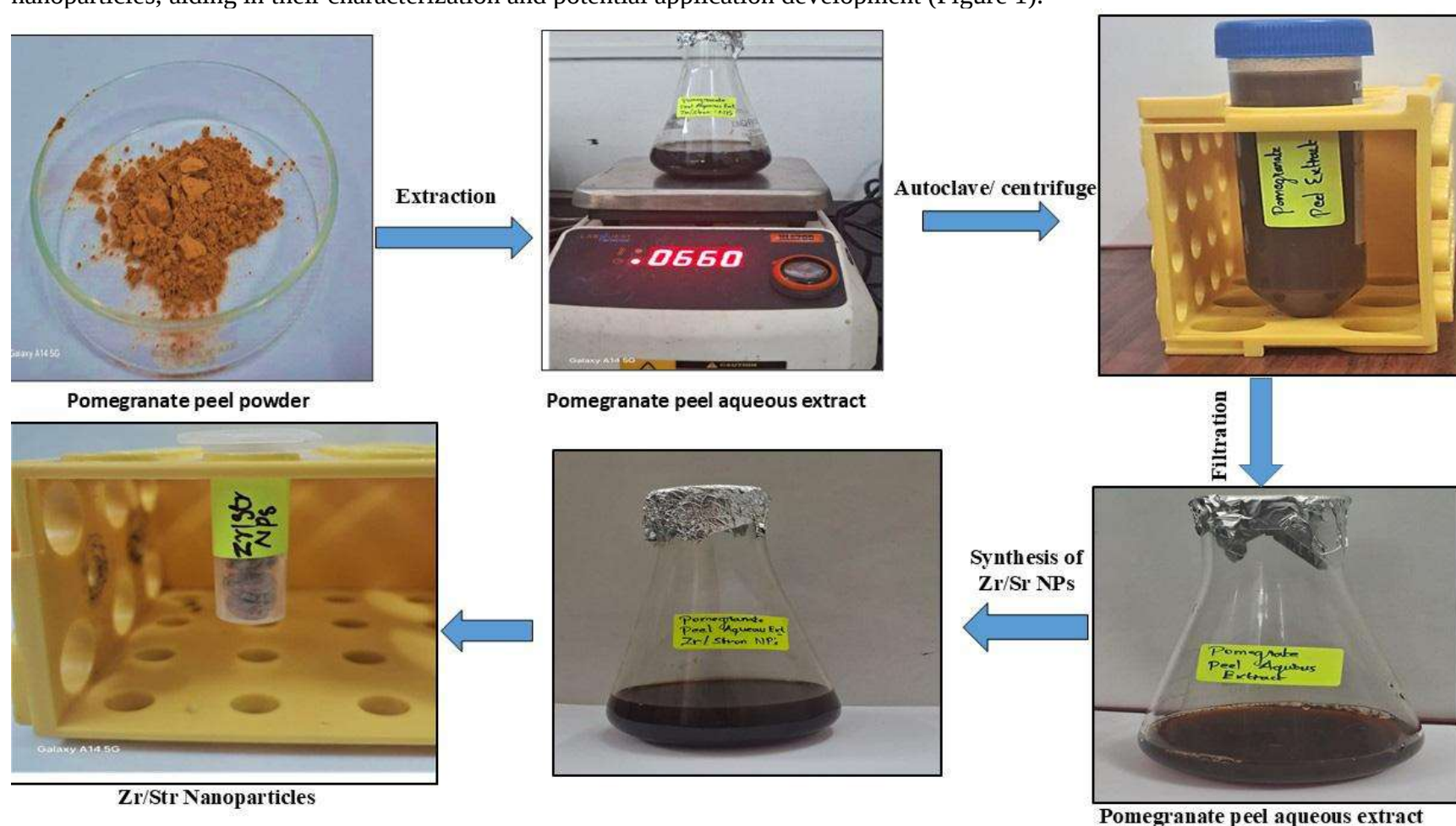


Figure 1: The process of synthesizing zirconium/strontium nanoparticles using pomegranate peel extract begins with the preparation of pomegranate peel powder. This powder undergoes extraction to obtain an aqueous extract rich in active compounds. The extract is then subjected to autoclaving or centrifugation to further purify it. Following this, the purified pomegranate peel extract is filtered to remove any remaining solid particles. The filtered extract is then used in the synthesis process to produce zirconium and strontium nanoparticles. The final outcome of this method is the formation of zirconium/strontium nanoparticles, which are derived from the natural pomegranate peel extract.

Agar-well diffusion method:

The biological efficacy of synthesized zirconium-strontium nanoparticles (Zr-Sr NPs) against various bacterial strains was assessed using the agar well-diffusion method. Initially, Mueller-Hinton agar plates were prepared and inoculated with bacterial cultures. Gram-positive bacteria, specifically Methicillin-resistant *Staphylococcus aureus* (MRSA), and Gram-negative bacteria, including *Escherichia coli* and *Pseudomonas aeruginosa*, were swabbed uniformly across the surface of the agar plates to ensure consistent bacterial growth. Wells measuring approximately 6 mm in diameter were created in the agar using a sterile cork borer. Different concentrations of Zr-Sr NPs (20, 40, 60, 80, and 100 µg/mL) were prepared and carefully added to these wells. Streptomycin served as the positive control to compare the antibacterial efficacy, while Dimethyl sulfoxide (DMSO) was used as the negative control to ensure any observed antibacterial activity was due to the nanoparticles and not the solvent. The inoculated petri plates were incubated at 37°C for 24 hours to allow bacterial growth and interaction with the nanoparticles. After the incubation period, the plates were examined for zones of inhibition, which are clear areas surrounding the wells where bacterial growth has been prevented. These zones were measured in millimeters, and their size indicated the effectiveness of the Zr-Sr NPs in inhibiting the growth of the tested bacterial strains. By comparing the zones of inhibition around the wells containing different concentrations of Zr-Sr NPs with those around the wells with the positive and negative controls, researchers could determine the relative antibacterial potency of the nanoparticles. This method provided a straightforward and effective way to assess the antibacterial properties of the synthesized Zr-Sr nanoparticles against both Gram-positive and Gram-negative bacteria [8].

Anti-inflammatory activity :

The anti-inflammatory activity of the biosynthesized Zr- Sr nanoparticles was assessed using the Albumin denaturation method. The zr- Sr NPs were tested at concentrations ranging from 20 to 100 µg/mL, combined with 1% Bovine Serum Albumin (BSA) at varying concentrations (0, 20, 40, 60, 80 µg/mL) in a microtitre plate. Dimethyl sulfoxide (DMSO) served as the negative control, while diclofenac sodium was used as the standard drug for comparison. The microplates were incubated at room temperature for 15 minutes to allow interaction between the nanoparticles and the BSA, followed by a further incubation at 55°C for 20 minutes to induce protein denaturation. Absorbance readings were taken at 600 nm to measure the extent of protein denaturation. The results were recorded and compared against the controls to determine the anti-inflammatory efficacy of the Zr-Sr NPs, with lower absorbance indicating higher anti-inflammatory activity due to inhibition of albumin denaturation [8].

RESULTS

FTIR analysis of Zr/Str Nanoparticles : The FTIR spectra of zirconium and strontium nanoparticles exhibit several characteristic peaks that provide insights into their functional groups and chemical bonds. The spectrum displays transmittance (%) on the y-axis and wavenumber (cm⁻¹) on the x-axis, ranging from 4000 to 500 cm⁻¹. a broad peak at 3313.46 cm⁻¹ is indicative of O-H stretching vibrations, commonly associated with hydroxyl groups or water molecules adsorbed on the nanoparticle surface. Peak at 2931.61 cm⁻¹ corresponds to C-H stretching vibrations, suggesting the presence of organic moieties or aliphatic chains. Multiple Peaks between 1700-1000 cm⁻¹ are likely due to C=O stretching vibrations from carbonyl groups. The peak at 1654.15 cm⁻¹ and 1454.11 cm⁻¹ is Associated with N-H bending and C=C stretching vibrations, respectively, 1383.65 cm⁻¹ and 1226.02 cm⁻¹ peaks Correspond to C-H bending and C-N stretching vibrations. another peak at 1012.29 cm⁻¹ could be due to C-O stretching vibrations (Figure 2).

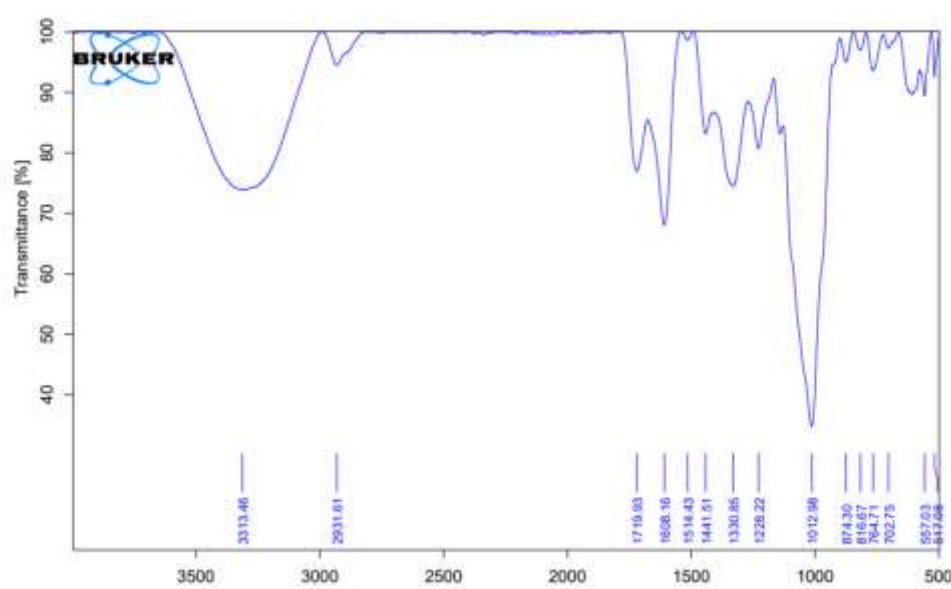


Figure 2: FTIR spectra of zirconium and strontium nanoparticles. The spectrum displays key absorption peaks at 3313.46 cm⁻¹ (O-H stretching), 2931.61 cm⁻¹ (C-H stretching), 1740.93 cm⁻¹ (C=O stretching), 1654.15 cm⁻¹ (N-H bending), 1454.11 cm⁻¹ (C=C stretching), 1383.65 cm⁻¹ (C-H bending), 1226.02 cm⁻¹ (C-N stretching), and 1012.29 cm⁻¹ (C-O stretching). Peaks below 1000 cm⁻¹ suggest metal-oxygen bond vibrations, indicative of the oxide nature of the nanoparticles

The UV-Visible spectra of zirconium and strontium nanoparticles (Zr-Sr NPs) exhibit significant absorption peaks that indicate the presence and characteristics of these nanoparticles. The absorption spectrum is plotted with absorbance (Abs) on the y-axis and wavelength (nm) on the x-axis, covering the range from 200 to 800 nm. The sharp peak at 284.4nm indicates a strong absorption characteristic, which may be attributed to the electronic transitions in the zirconium and strontium nanoparticles. Secondary peak at 271.4nm further confirms the presence of the nanoparticles and suggests additional electronic transitions or interactions within the sample. The spectrum shows a high absorbance in the UV region, gradually decreasing as the wavelength increases towards the visible range, indicating the size and distribution of the nanoparticles (Figure 3).

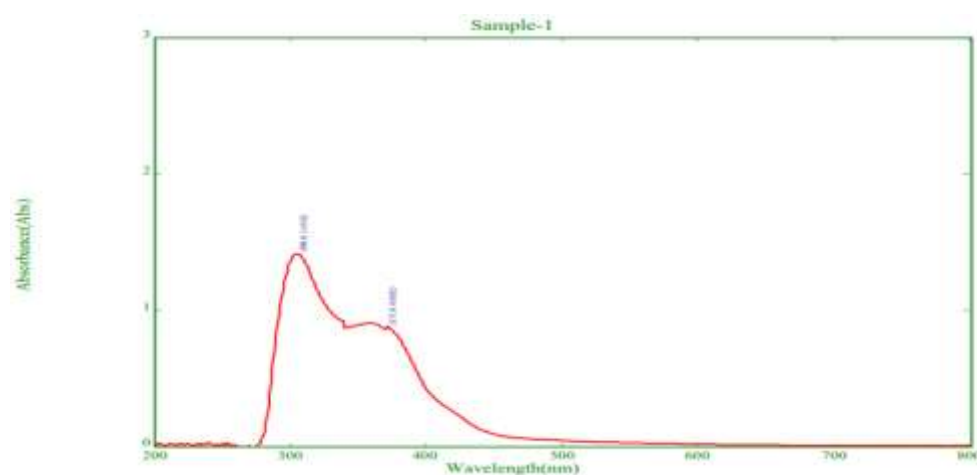


Figure 3: UV-Visible spectra of zirconium and strontium nanoparticles. The spectrum shows significant absorption peaks at 284.4 nm and 271.4 nm, indicating the electronic transitions associated with the nanoparticles. The absorbance decreases gradually from the UV region towards the visible range, providing insights into the optical properties and size distribution of the Zr-Sr nanoparticles.

Antimicrobial Efficacy Results

The antimicrobial efficacy of the synthesized zirconium and strontium nanoparticles was evaluated against four pathogenic microorganisms: *Klebsiella*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Salmonella*, and *Staphylococcus aureus* (Staph). The antimicrobial activity was assessed by measuring the zone of inhibition (mm) around wells containing various concentrations of nanoparticles 20, 40, 60, and 80 µg/mL, along with a positive control (PC) and a negative control (NC). Against *Klebsiella*, the nanoparticles showed no inhibitory effect at all tested concentrations, and neither did the positive or negative controls. For MRSA, the nanoparticles demonstrated significant antibacterial activity, with the zone of inhibition increasing from 25 mm at 20 µg/mL to 27 mm at 80 µg/mL, while the positive control showed a 25 mm zone and the negative control showed none. The nanoparticles were effective against *Salmonella*, exhibiting a 20 mm inhibition zone at 20 µg/mL and a consistent 22 mm zone at higher concentrations, matching the positive control, with no inhibition from the negative control. The highest antibacterial activity was observed against *Staphylococcus aureus*, with inhibition zones ranging from 29 mm at 20 µg/mL to 30 mm at higher concentrations, similar to the positive control, while the negative control showed no inhibition. These results indicate that zirconium and strontium nanoparticles possess strong antimicrobial properties, particularly against MRSA, *Salmonella*, and *Staphylococcus aureus* (Figure 4).

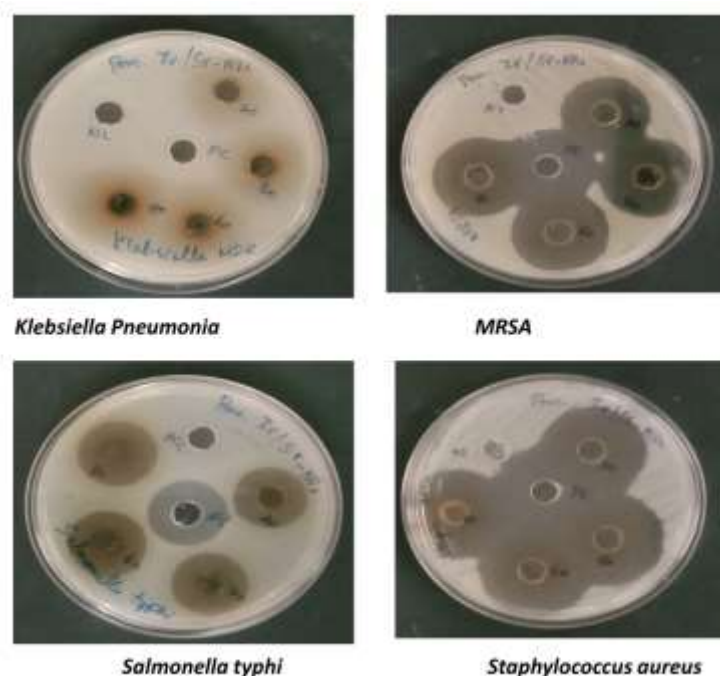


Figure 4: Antimicrobial activity testing: The first dish tests *Klebsiella pneumoniae* with agents labeled 20, 40, 60, and 80, showing zones of inhibition that indicate varying levels of effectiveness. The second dish shows the response of MRSA (Methicillin-resistant *Staphylococcus aureus*) to different agents, with clear zones around some indicating their efficacy. The third dish tests *Salmonella typhi* with agents labeled 20, 40, 60, and 80, displaying inhibition zones that demonstrate the effectiveness of these agents. The fourth dish examines *Staphylococcus aureus*, showing the bacteria's response to multiple agents, with clear zones indicating effectiveness. Each dish includes a negative control (NC) and a positive control (PC) to ensure the validity of the results, where larger inhibition zones signify higher antimicrobial efficacy.

Anti – inflammatory activity:

The anti-inflammatory activity of pomegranate-mediated synthesized Zirconium and Strontium nanoparticles (Zr/Sr-NPs) was evaluated and compared with the standard anti-inflammatory drug, Diclofenac, as shown in Figure 5. The percentage of inhibition was assessed at different concentrations (20, 40, 60, 80, and 100 µg/ml) for both Zr/Sr-NPs and Diclofenac. At 20 µg/ml, Zr/Sr-NPs exhibited a percentage inhibition of approximately 15%, while Diclofenac showed a higher inhibition of around 30%. At 40 µg/ml, Zr/Sr-NPs showed an increased inhibition of approximately 25%, whereas Diclofenac maintained a higher inhibition at around 35%. At 60 µg/ml, the inhibition percentage of Zr/Sr-NPs further increased to about 35%, with Diclofenac showing a slightly higher inhibition at around 40%. At 80 µg/ml, Zr/Sr-NPs demonstrated a significant increase in inhibition to about 45%, while Diclofenac continued to exhibit a higher inhibition of around 55%. At 100 µg/ml, Zr/Sr-NPs reached their maximum inhibition of approximately 55%, whereas Diclofenac exhibited the highest inhibition of around 65% (Figure 5)

Anti-inflammatory activity of Zr/Sr-NPs

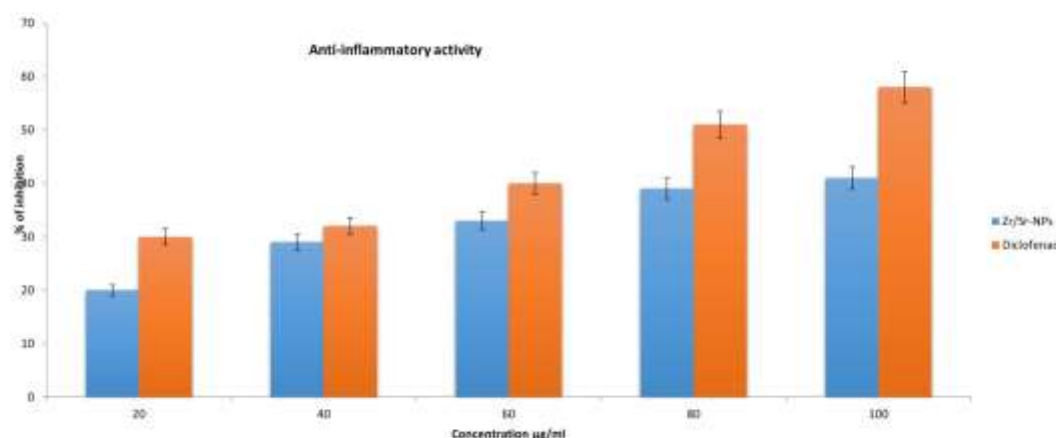


Figure 5: The anti-inflammatory activity of zirconium/strontium nanoparticles (Zr/Sr-NPs) compared to diclofenac, a standard anti-inflammatory drug. The y-axis represents the percentage of inhibition, indicating the extent of anti-inflammatory effect, while the x-axis shows different concentrations (20, 40, 60, 80, 100 µg/ml) of the substances tested. The blue bars represent the Zr/Sr nanoparticles, and the orange bars represent diclofenac. The graph demonstrates that the anti-inflammatory activity of Zr/Sr nanoparticles increases with concentration, showing a comparable, and in some cases, slightly lower inhibitory effect than diclofenac at each concentration level. This suggests that Zr/Sr nanoparticles have significant anti-inflammatory properties.

DISCUSSION

The present study offers a detailed examination of the preparation, synthesis, and characterization of Zirconium and Strontium nanoparticles using pomegranate peel extract along with evaluation of its antimicrobial and anti inflammatory efficacy. The results highlight the efficacy of the green synthesis approach and the potential applications of these nanoparticles in antimicrobial treatments. The preparation of the pomegranate peel aqueous extract involved a meticulous process to ensure the successful extraction of bioactive compounds. Initially, the pomegranate peels were dried and ground to a fine powder. This step is crucial as it increases the surface area for the extraction process, allowing for more efficient leaching of bioactive compounds. The autoclaving process that followed was essential for ensuring sterility and eliminating any microbial contamination that could interfere with the synthesis of nanoparticles. The resulting mixture, after centrifugation and filtration, yielded a clear extract free of solid debris. The clarity of the extract is indicative of effective filtration, which is crucial for preventing any particulate matter from affecting the subsequent synthesis of nanoparticles. Lyophilization was employed to concentrate the bioactive compounds, preparing the extract for use in nanoparticle synthesis. This step ensures that the extract is in a suitable form to interact with metal ions and facilitate their reduction and stabilization [7].

The one-pot synthesis method employed in this study involved using the pomegranate peel extract as both a reducing and stabilizing agent for zirconium and strontium ions. This method is advantageous as it simplifies the synthesis process by combining reduction and stabilization in a single step, thereby reducing the need for additional reagents or complex procedures. Veeraraghavan et al employed a similar method in the synthesis of Ag-Zr nanoparticles [12]. The color change of the reaction mixture from pale yellow to dark brown is a significant indicator of nanoparticle formation. This color transition is attributed to the surface plasmon resonance of the nanoparticles, which occurs due to the collective oscillation of conduction electrons on the nanoparticle surfaces. This observation aligns with the formation of nanoparticles and suggests successful reduction of metal ions. Following synthesis, centrifugation at 10,000 rpm effectively separated the nanoparticles from the reaction mixture. The subsequent washing and drying steps were crucial for removing any residual reactants or by-products, resulting in pure zirconium and strontium nanoparticles with consistent properties. The consistency of the nanoparticle properties is vital for ensuring reproducibility and reliability in their potential applications.

The characterization of the synthesized nanoparticles provided valuable insights into their physical and chemical properties. FTIR analysis identified key functional groups such as hydroxyl, carboxyl, and phenolic groups from the pomegranate extract. These groups are involved in the stabilization and reduction of metal ions during nanoparticle synthesis. The presence of these functional groups indicates that the pomegranate peel extract effectively contributes to the stabilization of the nanoparticles, preventing their agglomeration and ensuring their uniformity. UV-Vis spectroscopy showed characteristic surface plasmon resonance peaks, which are indicative of the formation of zirconium and strontium nanoparticles. The presence of these peaks confirms the successful synthesis of nanoparticles and provides insights into their size and shape based on the position and intensity of the peaks. Studies by Veeraraghavan et al [12], Pandiyan et al [13] shows comparative results on FTIR and UV vis analysis of the nanoparticles

The antimicrobial activity of the synthesized nanoparticles was evaluated against four pathogenic microorganisms: Klebsiella, Methicillin-resistant Staphylococcus aureus (MRSA), Salmonella, and Staphylococcus aureus. The results provide a comprehensive understanding of the effectiveness of the nanoparticles against different bacterial strains. The lack of antimicrobial activity against Klebsiella at all tested concentrations suggests that the nanoparticles are ineffective against this strain. This result could be attributed to intrinsic resistance mechanisms of Klebsiella or specific interactions between the nanoparticles and the bacterial cell walls [14]. Further studies might be necessary to explore potential modifications to enhance the efficacy of the nanoparticles against Klebsiella. The nanoparticles demonstrated significant antibacterial activity against MRSA, with inhibition zones increasing with higher concentrations. This indicates that the nanoparticles are effective against this resistant strain, with activity comparable to the positive control at 20 µg/mL. The effectiveness of the nanoparticles against MRSA is particularly noteworthy given the challenge of treating infections caused by resistant strains. The nanoparticles exhibited consistent antimicrobial activity against Salmonella, with inhibition zones comparable to the positive control [15]. This suggests that the nanoparticles are effective in inhibiting Salmonella growth, which could be beneficial in various applications, including food safety and clinical settings. The nanoparticles showed the highest antibacterial activity against Staphylococcus aureus, with large inhibition zones at all tested concentrations. This suggests a strong potential for these nanoparticles in treating infections caused by Staphylococcus aureus. The consistent high activity indicates that these nanoparticles could be a valuable tool in managing infections caused by this common pathogen. The mechanism by which nanoparticles exert their antimicrobial effects involves a combination of physical and chemical interactions with microbial cells [16]. Nanoparticles interact with microbial cells primarily through physical interactions

with the cell wall or membrane. The high surface area-to-volume ratio of nanoparticles increases their interaction with microbial surfaces. This interaction can lead to cell Wall disruption, membrane damage, and nano-size effects. The small size of nanoparticles allows them to penetrate bacterial cells more easily compared to larger particles. Once inside the cell, nanoparticles can interact with internal structures, potentially leading to further cellular damage [17].

Nanoparticles, especially metal-based ones, can induce oxidative stress in microbial cells by generating reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anions, and hydrogen peroxide. These ROS can damage Oxidative damage to cellular membranes can lead to lipid peroxidation, compromising membrane integrity and function, cause oxidative modifications to proteins, affecting their structure and function, Damage to bacterial DNA can result in mutations, replication errors, and ultimately cell death [18].

Zirconium nanoparticles can exert antimicrobial effects through Direct Interaction - Binding with microbial cell walls or membranes, leading to structural damage and ROS Generation - Zirconium nanoparticles can catalyze reactions that produce ROS, contributing to oxidative stress. Strontium nanoparticles may act through metal Ion Release: Strontium ions can disrupt cellular functions and interfere with enzyme activity. Cell Wall Penetration: Similar to other metal nanoparticles, strontium nanoparticles can physically interact with and penetrate bacterial cell walls [19].

The antimicrobial activity of zirconium and strontium nanoparticles likely involves a combination of the above mechanisms. Synergistic Effects: The combined action of physical disruption, oxidative stress, and ion release can lead to enhanced antimicrobial efficacy. The physical presence of nanoparticles on microbial surfaces, coupled with chemical damage from ROS and metal ions, creates a multifaceted attack on the microorganisms. Particle Size and Shape Dependence: The size, shape, and surface properties of nanoparticles can influence their interaction with microbial cells. Smaller nanoparticles with high surface reactivity are often more effective in penetrating cells and generating ROS [19,20].

CONCLUSION

The findings of this study affirm the effectiveness of zirconium and strontium nanoparticles synthesized using pomegranate peel extract as potent antimicrobial agents. This research highlights the advantages of a green synthesis approach, which employs a natural extract to reduce and stabilize metal ions. This method not only simplifies the synthesis process but also offers an environmentally friendly alternative to traditional chemical synthesis methods, which often involve hazardous substances and complex procedures. The antimicrobial testing demonstrated significant activity against Methicillin-resistant *Staphylococcus aureus* (MRSA), *Salmonella*, and *Staphylococcus aureus*. The nanoparticles exhibited inhibition zones comparable to or exceeding those of positive controls, indicating strong antibacterial effects. This performance underscores the potential applications of these nanoparticles in medical and environmental settings, where they could be utilized to combat a variety of bacterial infections and contribute to the development of effective antimicrobial treatments. However, the lack of observed antimicrobial activity against *Klebsiella* indicates that the current synthesis method may not be effective against all types of pathogens. This limitation suggests the need for further optimization of the synthesis parameters or exploration of alternative methods to enhance the efficacy of the nanoparticles against a broader spectrum of bacteria. In conclusion, the study successfully demonstrates the potential of using natural extracts for nanoparticle synthesis and highlights their promising applications in antimicrobial therapies. Future research should focus on refining the synthesis process to improve efficacy across a wider range of pathogens and fully exploit the benefits of these green-synthesized nanoparticles.

LIMITATIONS

The study demonstrated that the synthesized nanoparticles were ineffective against *Klebsiella*, indicating a limitation in their broad-spectrum antimicrobial activity. Further investigations are needed to understand why certain strains are resistant and how this can be overcome. The use of pomegranate peel extract, a natural product, could introduce variability in the synthesis process due to differences in the composition of bioactive compounds between batches of pomegranates. Standardizing the extract could be challenging. The study relied on common characterization techniques like FTIR, UV-Vis, and TEM. While these provide valuable insights, more advanced techniques such as X-ray photoelectron spectroscopy (XPS) or zeta potential measurements could offer deeper understanding of surface chemistry and stability. Future research should aim to test the efficacy of the nanoparticles against a wider range of microbial strains, including fungi and viruses, to better understand their full antimicrobial potential. Conducting in vivo studies to assess the pharmacokinetics, biodistribution, and long-term biocompatibility of these nanoparticles in animal models would be crucial for advancing towards clinical applications.

REFERENCES

1. Velmurugan P, Anbalagan K, Manosathyadevan, M. *et al.* Green synthesis of silver and gold nanoparticles using *Zingiber officinale* root extract and antibacterial activity of silver nanoparticles against food pathogens. *Bioprocess Biosyst Eng.* 2014; 37, 1935–1943. <https://doi.org/10.1007/s00449-014-1169-6>
2. Albrecht MA, Evan CW, Raston CR. Green chemistry and the health implications of nanoparticles. *Green Chem.* 2006; 8:417–432. <https://doi.org/10.1039/B517131H>
3. Iravani S. Green synthesis of metal nanoparticles using plants. *Green Chemistry.* 2011; 13; 2638-2650. <https://doi.org/10.1039/C1GC15386B>
4. Malviya S, Arvind, Jha A, Hettiarachchy N. Antioxidant and antibacterial potential of pomegranate peel extracts. *J Food Sci Technol.* 2014; 51:4132-7. doi: 10.1007/s13197-013-0956-4.
5. Nagajyothi PC, Muthuraman P, Sreekanth TVM, Kim DH, Shim J, Jun YI. Green synthesis: In-vitro anticancer activity of copper oxide nanoparticles against human lung cancer cell line. *Arabian Journal of Chemistry.* 2015;10: 215-225. <https://doi.org/10.1016/j.jphotobiol.2015.01.001>
6. Rajeshkumar S, Bharath LV. Mechanism of plant-mediated synthesis of silver nanoparticles - A review on biomolecules involved, characterisation and antibacterial activity. *Chem Biol Interact.* 2017 Aug 1;273:219-227. doi: 10.1016/j.cbi.2017.06.019
7. Bolje A, Gobec S. Analytical Techniques for Structural Characterization of Proteins in Solid Pharmaceutical Forms: An Overview. *Pharmaceutics.* 2021; 13:534. <https://doi.org/10.3390/pharmaceutics13040534>
8. Smith, BC. *Fundamentals of Fourier Transform Infrared Spectroscopy* (2nd ed.).2011. CRC Press. <https://doi.org/10.1201/b10777>
9. Haiss W, Thanh NT, Aveyard J, Fernig DG. Determination of size and concentration of gold nanoparticles from UV-vis spectra. *Anal Chem.* 2007 ;79:4215-21. doi: 10.1021/ac0702084.
10. Cappuccino JG, Sherman N. "Microbiology: A Laboratory Manual." 2014; Pearson. ISBN: 9780321840226.

11. Sabu A, Sankaran K, Rengasamy G, Veeraraghavan V.P. One Pot Synthesis of Colloidal Zirconium Nanoparticles using Orthosiphon Stamineus Leaf Extract for Potential Bone Tissue Engineering Applications. *Texila International Journal of Public Health*. 2023; 10.21522/TIJPH.2013.SE.23.01.Art004
12. Pandiyan I, Sri SD, Indiran MA, Rathinavelu PK, Prabakar J, Rajeshkumar S. Antioxidant, anti-inflammatory activity of *Thymus vulgaris*-mediated selenium nanoparticles: An *in vitro* study. *J Conserv Dent*. 2022;25:241-245. doi: 10.4103/JCD.JCD_369_21
13. Zhao G, Stevens SE Jr. Multiple parameters for the comprehensive evaluation of the susceptibility of *Escherichia coli* to the silver ion. *Biometals*. 1998 Jan;11(1):27-32. doi: 10.1023/a:1009253223055
14. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. *Biotechnol Adv*. 2009 Jan-Feb;27(1):76-83. doi: 10.1016/j.biotechadv.2008.09.002
15. Shantkriti S, Pradeep M, Unish K. Biosynthesis of silver nanoparticles using *Dunaliella salina* and its antibacterial applications. *Applied Surf Sci Advance*. 2023;13:100377. <https://doi.org/10.1016/j.apsadv.2023.100377>.
16. Tamanna IS, Gayathri R, Sankaran K. *et al*. Eco-friendly Synthesis of Selenium Nanoparticles Using *Orthosiphon stamineus* Leaf Extract and Its Biocompatibility Studies. *BioNanoSci*.2024; **14**, 37–44. <https://doi.org/10.1007/s12668-023-01277-w>.
17. Naaziya M, Biju TS, Francis AP, Veeraraghavan VP, Gayathri R, Sankaran K. Synthesis, characterization and in-vitro *biological* studies of curcumin decorated biogenic selenium nanoparticles. *Nano LIFE*. 2024;14; 2350013. <https://doi.org/10.1142/S1793984423500137>
18. Kumar A, Arvina R, Rajeshkumar, S. Antifungal Activity of Clove And Cinnamon Mediated Selenium Nanoparticles: An In Vitro Study. *Plant Cell Biotechnology and Molecular Biology*. 2020; 21; 18–23.
19. Shanmugam SB, Gayathri R, Sankaran K. Curcumin Coated Orthosiphon Stamineus Leaf Extract Based Selenium Nanoparticle for Potential Tissue Engineering Applications. *Texila International Journal of Public Health*. 2023; 10.21522/TIJPH.2013.SE.23.01.Art005.
20. Faiz N, Sivaswamy V. Green Synthesis of Gold Nanoparticles Using Eucalyptus and Piper Longum and Its Subsequent Anti- inflammatory Activity Evaluation. *Texila International Journal of Public Health*. 2023; 10.21522/TIJPH.2013.SE.23.01.Art007