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Green Synthesis Of Strontium Oxide Nanoparticles From *Cycas Revoluta* Leaf Extract And Their Antimicrobial Activity

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ABSTRACT

This work investigated the environmentally sustainable production of strontium oxide nanoparticles (SrONPs) using *Cycasrevoluta* leaf extract. We employed UV-Vis spectroscopy, FTIR, XRD, and SEM to examine the SrONPs synthesized by biological entities. The biomolecules in plant leaf extract served effectively as capping and reducing agents. The absorption peak occurred at 300 nm, and the optical band gap measured 4.13 eV. The XRD peaks indicated a cubic structure with average crystallite sizes of 34 nm. The 2θ values ranged from 23.5° to 76.8°. FTIR identified the existence of functional groups (-OH, -C=O, -C-O) and Sr-O stretching vibrations. We evaluated the synthesized SrONPs against *E. coli* and *S. aureus*to determine their efficacy as antibiotics. The inhibitory effect was dose-dependent, with *E. coli* exhibiting the largest inhibition zone of 13 mm and *S. aureus* demonstrating 11 mm at a concentration of 40 mg/mL.

Keywords: Strontium Oxide Nanoparticles, Green Synthesis, *Cycas Revoluta* , Antibacterial Activity, Characterization, Biosynthesis

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Introduction

Nanotechnology research has advanced rapidly worldwide, with the application of nanoparticles (NPs) in various fields, including biomedical applications, cell labeling, drug delivery, plant tissue culture, biomarkers, the automotive industry, and the energy sector, becoming significant areas of inquiry in recent years **[1,2,3].** Different methods can be used to make nanoparticles, which can lead to variances in their size and form **[4].** Chemical and physical approaches are widely used, while biological methods are becoming more popular as an alternative **[5].** Chemical methods often use sodium hydroxide, sodium borohydride, potassium hydroxide, and hydrazine as reducing agents **[6].** These methods for making nanoparticles require the use of several harmful chemicals, organic solvents, and high temperatures, which makes the process dangerous for both people and the environment **[7].** Biological approaches, which include bacteria, fungi, algae, and plants, have been shown to be safe for the environment and non-toxic. They may also be easily improved for large-scale use**[8].**The creation of bio nanoparticles is a long-lasting method in the fields of nanotechnology since it uses ingredients that may be recycled and broken down**[4].**

Strontium oxide nanoparticles (SrO NPs) have attracted considerable attention due to their potential characteristics and applications **[9].**SrO nanoparticles are currently being investigated for fundamental and applied device fabrications due to their well defined morphology, nanoscale size, high thermal and optical properties, low dimensional mechanical strength, electrical nature **[10],** good oxidation resistance, inertness against chemicals and also magnetic behavior. Devices Fabricated from SrONanopowder A range of devices such as custom-made transistors, organic semiconductors, solar cell **[11],** gas sensor **[12**], dye-sensitized solar cells, lithium ion battery and electrodes and catalytic supports goals and capacitors can be easily made by SrO nanoparticles**[13].** Strontium-conjugated nanoparticles demonstrate the antibacterial activity and are efficient in the elimination of hazardous pollutants from industrial effluent. Strontium based nanoparticles is used for applications in sewage treatment and agriculture, as gas sensors to measure high-temperature gas pressure of harmful gasses. The broad impact and potential applications of strontium nanoparticles in various health and environmental sciences. **[14].** Green synthesized SrO nanoparticles possess excellent antibacterial, antifungal and antioxidant activities Low cytotoxicity**. [9].** Limited papers have detailed the synthesis of SrONPs utilizing plants, such as *Ocimum sanctum*, *Vitisvinifera*, and *Elodea canadensis* **[8,15,16].**

The synthesis of NPs using plants is highly feasible, and it can thus be applied as an economic and a useful alternative for the large-scale production of NPs **[17].** The reducing and capping agents are present in the plants **[18].** Plants also posses various types of natural chemicals such as flavonoids, alkaloids, stereo ids, polysaccharides, proteins, phenolic acids and other bio- ligands that are involved in decreasing metal ion to different nano particles **[19].**

The most iconic plants is *Cycasrevoluta***[20]***Cycasrevoluta*, often known as sago palm, king sago, sago cycad, and Japanese sago palm, is not a real palm but a cycad. *C. revoluta* is a common decorative plant that grows in mild temperate and subtropical areas of the United States and indoors in colder areas of the nation. It is native to China and Japan. *C. revoluta* may reach a height of 2 m and a trunk diameter of 20 cm or more. Its crown of dark olive green leaves can be 50 to 150 cm long and

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develop into a feather-like rosette that is up to 1 m in diameter. The leaves are made up of 8 to 18 cm long leaflets that look like spines. *C. revoluta* has a number of poisons in it, including as cycasin, macrozamin, neocycasin, and beta-methylamino-l-alanine **[21].** *C. revoluta* has been used as a traditional medicine to treat vomiting blood, gas, skin disorders, high blood pressure, stomach problems, cough, hair growth, snake bites, dressing wounds, swollen glands, and stomach cleansing. Phytochemical study has shown that it contains flavonoids, glycosides, non-protein amino acids, fatty acids, benzenoids, terpenes, amino acids, diterpenoids, triterpenoids, sterols, esters, and steroids. The chemical components and crude extracts show biological activity including being antibacterial, antioxidant, cytotoxic, antileishmanial, and anticancer **[22].**

Nanoparticles and evaluating their antibacterial characteristics **[23].**Er-AgNPs showed antibacterial effects against a wide range of bacteria**[24].** Nanoparticles obtained from Cu, Zn, and Ag function as antibacterial agents**[25].**

This work highlights the significance of an eco-friendly green synthesis approach employing *Cycasrevoluta* leaf extract for the fabrication of strontium oxide (SrO) nanoparticles and the evaluation of their antibacterial characteristics. The plant extract functions as a reducing, capping, and stabilizing agent, regulating the physicochemical characteristics of the synthesized SrO nanoparticles, which were extensively analyzed using various characterization techniques. The antibacterial efficacy of the produced green SrO nanoparticles was assessed against several harmful microorganisms to determine their inhibitory potential. This work demonstrates the potential antibacterial and therapeutic uses of SrO nanoparticles synthesized using plant-mediated green synthesis, offering significant insights into their unique features.

Materails and method

Strontium nitrate [$Sr(NO_3)_2$] (99% purity, Sigma-Aldrich, USA) as the precursor for the synthesis of strontium oxide nanoparticles. It was solubilized in distilled water. Ethanol was used to clean and sterilize glassware.The pH level of the reaction medium was adjusted using sodium hydroxide (NaOH). Distilled water was consistently utilized throughout the experiment. We acquired fresh *Cycasrevoluta* leaves from Pakistan to generate the plant extract utilized as an agent for stabilization and reduction in the green synthesis process.

Preparation of plant extract

Fresh leaves of *Cycasrevoluta* were procured from the Botanical Garden of Ghazi University, Dera Ghazi Khan, Pakistan. The leaves were meticulously rinsed with tap water and subsequently with distilled water to eliminate dust and surface impurities, then air-dried in the shade for several days at ambient temperature. The desiccated leaves were pulverized into a fine powder with a mechanical grinder.

To prepare the aqueous extract, 20 g of leaf powder was combined with 100 mL of distilled water and heated at 100 °C for 30 minutes with constant stirring. Upon reaching room temperature, the mixture was subjected to filtration using Whatman No. 1 filter paper. The filtrate is used for preparation of SrOnps.

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Synthesis of SrOnps

Acquired from a previous study on SrONP synthesis **[26].** To prepare a 0.1 M strontium nitrate solution, $Sr(NO_3)_2$ was solubilized in distilled water. Approximately 20 mL of *Cycasrevoluta* leaf extract was incrementally introduced to the strontium nitrate solution. A magnetic stirrer was employed to continuously agitate the liquid for several hours at ambient temperature. Following the observation of a color change, the reaction mixture was centrifuged at 8000 × g for 10 minutes at 20 °C. The emitted particle was purified with distilled water and subsequently dried. We employed a UV-Vis spectrophotometer to examine the optical properties of the synthesized strontium oxide nanoparticles within the 200–400 nm range.

Characterization of strontium oxide nanoparticles

The resulting strontium oxide nanoparticles were analyzed by four complimentary analytical methods. UV-Vis spectroscopy (200-800 nm) validated nanoparticle production via surface plasmon resonance peaks and assessed particle size**[27][28]**, concentration, and stability**[29][30][31]**. X-ray diffraction employing Cu-Kα radiation determined crystal structure, composition, and purity, whereas crystallite size was calculated using the Debye-Scherrer equation (K=0.94) **[32][33][34][35]**. FTIR spectroscopy revealed the chemical bonds and functional groups present on nanoparticle surfaces **[36][37][38][39][40][41]**.

Antimicrobial assay

The antibacterial effectiveness of produced strontium oxide nanoparticles was assessed using the agar well diffusion technique**[42][43][44][45][46][47].** The strains of *Staphylococcus aureus*and *Escherichia coli* were incubated overnight in nutrient broth to acquire active cultures. Bacterial suspensions were evenly distributed on prepared agar plates, and wells were formed on the infected surfaces**[48].** Nanoparticle samples at concentrations of 10, 20, and 40 $mg \bullet mL^{-1}$, coupled with streptomycin as a positive control, were added to the specified wells. Plates were incubated under sterile conditions for 24 hours. Measurement was performed on the dimensions of the inhibitory zone associated with each concentration.

Result and discussion

The successful synthesis of strontium oxide nanoparticles (SrONPs) using the *Cycasrevoluta* leaf extract as a reducing agent was evident when there was a visible color change from olive brown to amber yellow after the addition of the strontium nitrate solution (Fig. 1). The leaf extract of *Cycasrevoluta* contains a reducing agent that reduces the amount of strontium nitrate required for the synthesis of SrONPs. This observable color change is attributed to the changes in surface plasmon vibrations of the formed strontium oxide nanoparticles. Recently, researchers have synthesized various types of metallic nanoparticles using different methods**[49][50][51]**, however, the synthesis of strontium oxide nanoparticles using a biological approach for the development of more environmentally friendly synthesis procedures has not yet been discussed at length.

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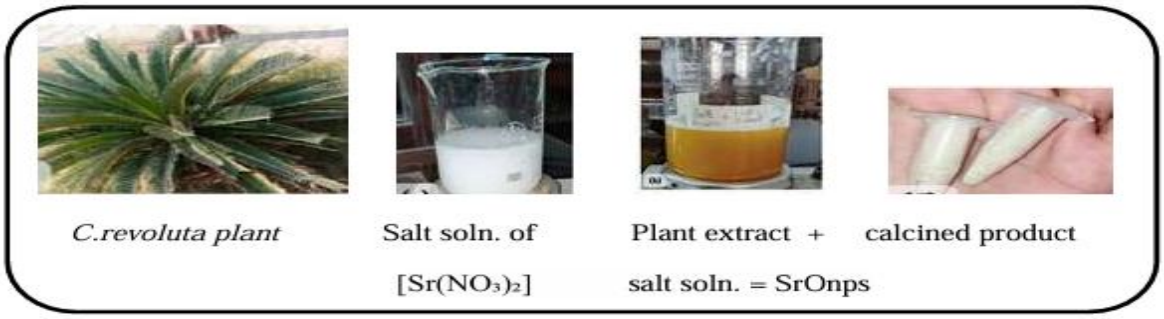

Fig.-1 : Synthesis of SrONPs from C. revoluta Leaves Extracts

Characterization methods

UV-Vis Spectroscopic Analysis

The UV-Visible absorption spectroscopy technique was used to analyze the optical properties of the synthesized strontium oxide nanoparticles (SrONPs) by using the *Cycasrevoluta* leaf extract (Fig. 2). The UV-Vis absorption spectrum shows a strong absorption peak at 200 nm with a maximum absorbance of 3.00, which can be ascribed to the strong electronic transitions due to the excitation of electrons from the valence to the conduction bands. Another absorption peak was noticed at 300 nm with an absorbance of 2.934, which can be ascribed to the inter-band electronic transitions, thus confirming the successful synthesis of crystalline SrO nanoparticles. The previous studies have shown that the strontium oxide nanoparticles synthesized using the Solanumnigrum leaf extract had the absorbance maxima in the range of 250-350 nm, thus indicating the successful synthesis of SrO nanoparticles **[52].** In a similar study, the SrO nanoparticles synthesized using the leaf extract of Albiziajulibrissin had the characteristic UV absorption peak at 300 nm, thus confirming the successful synthesis of SrO nanoparticles **[53].** The above values are in good agreement with the absorption peaks that have been observed in the present study. In the visible region of wavelengths ranging from 400-900 nm, there was a gradual decrease in the absorption, along with the appearance of an amber-yellow coloration in the colloidal solution, thus indicating the absence of defect states or impurity levels in the band structure of the synthesized SrONPs.

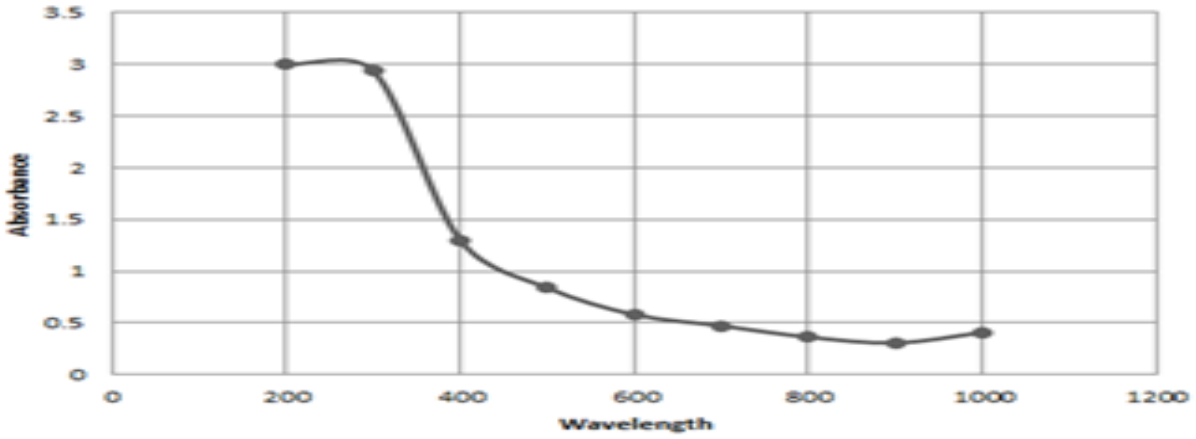

Fig.-2: UV-Vis graph of Strontium Oxide NPs

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The formula Eg = hc/λ to figure out the optical band gap of the produced SrONPs. We assumed that the absorption edge was at 300 nm. The optical band gap of the produced SrONPs was determined to be around 4.13 eV, indicating that the strontium oxide nanoparticles are broad band gap semiconductors. The calculated optical band gap of the prepared SrONPs is comparable to the band gaps of green synthesized SrO nanoparticles reported in earlier literature **[52,53].**The wide band gap also reveals that the synthesized SrONPs have high crystallinity and low electronic defects.

X-Ray Diffraction

In order to study the crystalline structure, phase purity, and average crystallite size of strontium oxide nanoparticles (SrONPs) that were synthesized from *Cycasrevoluta* leaf extract, X-ray diffraction analysis was employed (Fig. 3). The XRD pattern of the material within the 2θ region of 20-80° exhibited many pronounced sharp peaks. This indicated that crystalline SrO nanoparticles had been successfully synthesized. The most prominent diffraction peak occurred at approximately 29.6°, which indicated the optimal direction for crystal growth. The other peaks showed up at 2θ values of about 23.5°, 26.8°, 29.6°, 32.4°, 36.1°, 43.3°, 47.1°, 54.6°, 60.2°, 66.5°, 72.4°, and 76.8°. The values and intensities of the diffraction peaks are very close to the standard JCPDS/ICDD values for the cubic phase of SrO (JCPDS Card No. 96-900-7717). This implies that phase-pure strontium oxide nanoparticles have been successfully synthesized. Prior research on the environmentally benign production of SrO nanoparticles using various plant extracts has revealed similar patterns and peak intensities in X-ray diffraction (XRD) [52], supporting the findings of the current work.

The diffraction pattern did not display supplementary peaks that may suggest contaminants or other phases. Prior research on the environmentally benign production of SrO nanoparticles using various plant extracts has revealed similar patterns and peak intensities in X-ray diffraction (XRD) **[52],** supporting the findings of the current work.

The diffraction pattern did not show any additional peaks that could indicate the presence of contaminants or other phases. This shows that the synthesized SrONPs are free from contamination.This finding further supports the fact that the green synthesis technique using the leaf extract of *Cycasrevoluta* to synthesize chemically pure SrO nanoparticles without any unwanted by-products is advantageous **[8].**Nanocrystalline materials usually have diffraction peaks that are broader. This shows that the particles produced are on the nanometer scale.

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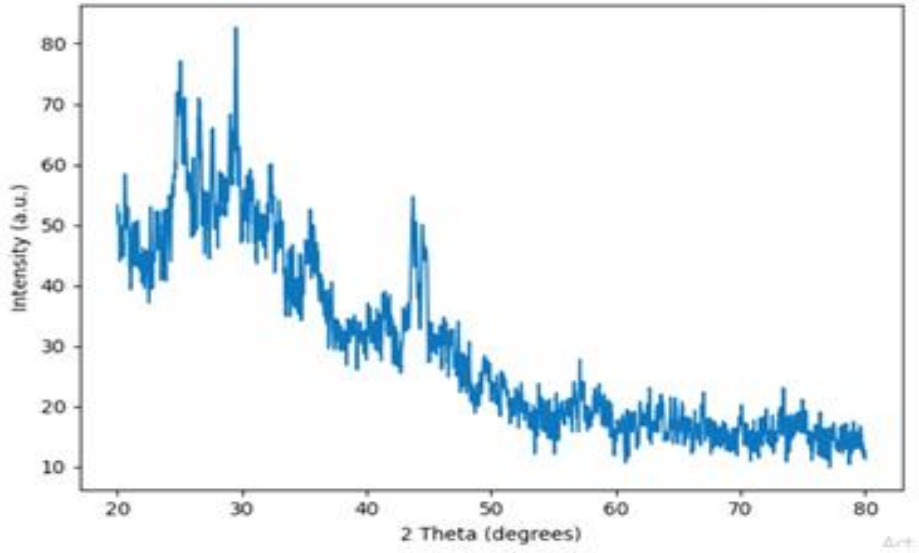

Fig.-3: XRD graph Strontium Oxide NPs

We used the Debye-Scherrer formula to find the average crystallite size (D) of the SrONPs:

$D = K\lambda / \beta \cos \theta$.

where D is the average crystallite size, K is the shape factor (0.9), λ is the wavelength of the Cu Kα X-ray radiation (1.5406 Å), β is the full width at half maximum (FWHM) of the diffraction peak in radians, and θ is the Bragg diffraction angle. Based on the equation above, the average crystallite size of the SrONPs produced was about 34 nm, which shows that the particles were on the nanometer scale. The obtained crystallite size aligns well with existing results for SrO nanoparticles derived from alternative plant extracts, which indicated crystallite sizes between 25-45 nm **[54].** These results clearly indicate that the phytochemical compounds in the *Cycasrevoluta* leaf extract are able to control the nucleation and growth process, resulting in the successful production of well-defined nanocrystallineSrO particles with controlled sizes.

FTIR Spectroscopy Analysis

The FTIR spectrum of *Cycasrevoluta* leaf extract SrONPs was used to identify the functional groups that reduce, stabilize, and cap nanoparticles (Fig. 4). The extensive absorption band at about 3387 cm^{-1} is attributed to O–H stretching vibrations from phenolic compounds and hydroxyl groups, which are crucial for the bioreduction of strontium ions and the surface stability of the particles **[8]**. The absorption peaks at around 1772 cm^{-1} and 1636 cm^{-1} correspond to the stretching frequencies of C=O and C=C groups, respectively, signifying the participation of carbonyl-containing chemicals and aromatic biomolecules in the stability of the nanoparticles. The bands at 1448 cm^{-1}, 1399 cm^{-1}, 1203 cm^{-1}, and 1009 cm^{-1} can be attributed to C-H bending, C-N stretching, and C-O stretching vibrations, thus confirming the adsorption of phytochemical components as effective capping agents **[52]**.

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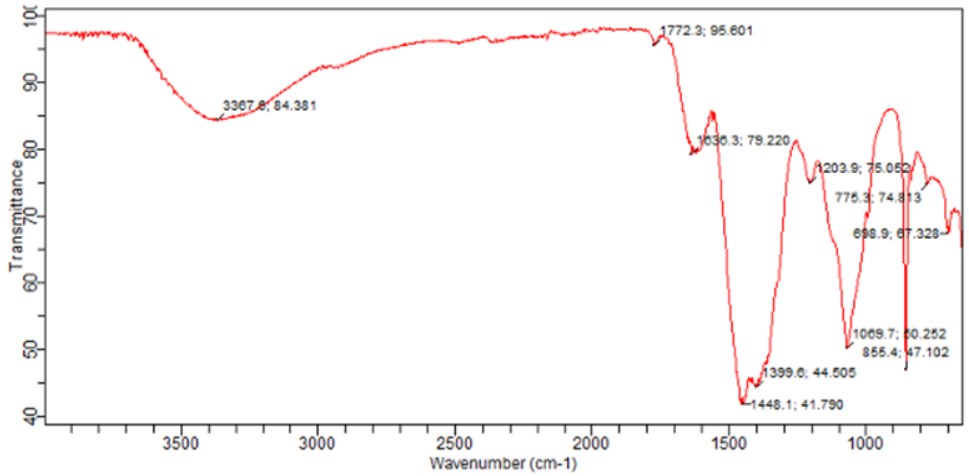

Fig.-4: FTIR Graph of Strontium oxide nanoparticles

The absorption bands at around 855 cm^{-1}, 775 cm^{-1}, and 698 cm^{-1} are attributed to the Sr-O stretching modes, thus verifying the successful creation of strontium oxide nanoparticles **[54].** The unique metal-oxygen stretching modes also verify the fabrication of SrO nanoparticles from the Sr precursor. The FTIR results confirm that *CycasRevoluta* Leaf Extract serves as a versatile bio-resource for the manufacture of stable bio-capped SrONPs by phytochemically induced reduction.

Antimicrobial Assay

The antimicrobial activity of the green synthesized strontium oxide nanoparticles (SrONPs) was evaluated against Gram-positive *Staphylococcus aureus*and Gram-negative *Escherichia coli* using the agar well diffusion method. The zone of inhibition (ZOI) was assessed at three distinct doses of SrONPs (10 mg/mL, 20 mg/mL, and 40 mg/mL), with a standard antibiotic serving as the positive control (Fig. 5). The findings are encapsulated in Table 1, and the comparative analysis is illustrated graphically in (Fig. 6).

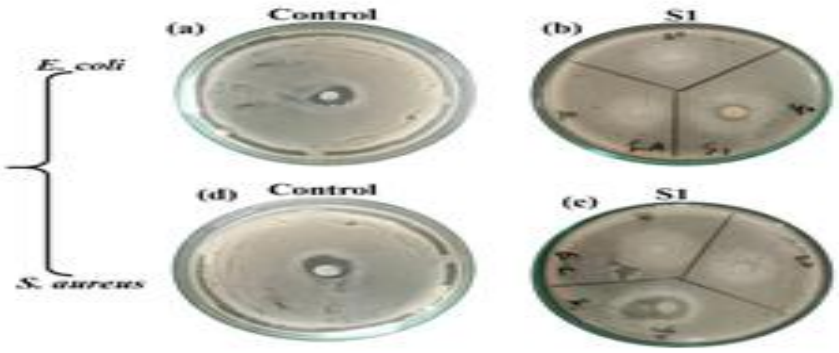

Fig.–5: Antibecterial activity of SrO NPs

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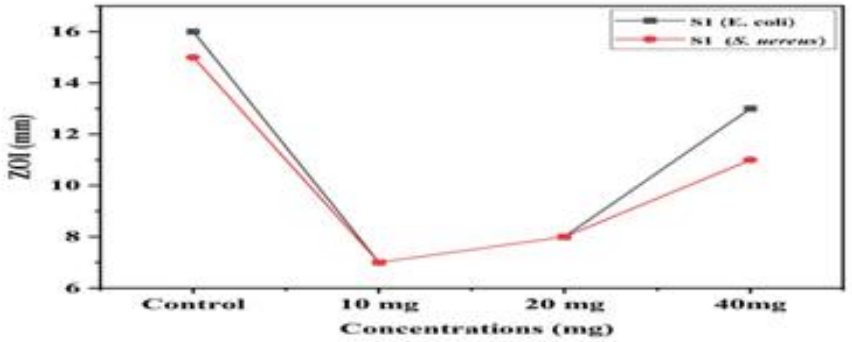

Fig.-6: Antibecterial graph of SrO NPs

The ZOI of SrONPs rose from 10 mg/mL to 40 mg/mL for both bacterial strains, showing that the antibacterial efficacy depends on the dosage. The Zone of Inhibition for *E. coli* is 13 mm and for *S. aureus*it is 11 mm at a dose of 40 mg/mL. This shows that Gram-negative bacteria are more likely to respond to SrONPs treatment. The control group also had a high Zone of Inhibition, with 16 mm for *E. coli* and 15 mm for S. aureus.

Table 1: Antimicrobial Activity of SrONPsAgainst Bacterial Strains

Sr. No	Pathogen	Concentration (mg/mL)	Zone of Inhibition (mm)	Control (mm)
1	*E. coli* (Gram-)	10, 20, 40	13 (at 40 mg/mL)	16
2	*S. aureus*(Gram+)	10, 20, 40	11 (at 40 mg/mL)	15

The phenomenon of concentration-dependent activity is ascribed to the augmented contacts between nanoparticles and membranes at elevated concentrations, hence physically compromising the cell membranes and facilitating cellular leakage. Furthermore, the production of reactive oxygen species (ROS) by SrONPs induces oxidative stress, thus damaging cellular biomolecules like DNA, proteins, and lipids **[46].** An elevated surface area-to-volume ratio enhances the efficacy of nanoparticles as bactericidal agents.The heightened vulnerability of *E. coli* relative to *S. aureus*can be attributed to their cell wall structure. Being a gram-negative bacterium, *E. coli* has a thin peptidoglycan layer, enabling the entry of nanoparticles compared to S. aureus, which has a thick peptidoglycan layer, thereby creating a strong barrier against nanoparticle penetration **[55][56][54].** These results are in conformity with earlier findings based on the increased sensitivity of gram-negative bacteria against metal oxide nanoparticles **[8][52].**

Conclusion

In this work, we synthesized SrONPs using *Cycasrevoluta* leaf extract to reduce the environmental toxicity in the synthesis process and to avoid other disadvantages of chemical methods. The *C. revoluta* extract served as the stabilizing, capping, and reducing agent in the production of SrONPs. Optically, the production of the

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SrONPswas perceived as a color variation from olive brown to amber yellow. Morphological characterization performed on the synthesized SrONPs revealed that the particles were well-defined, nanoscale-sized with controlled morphology. The average crystallite size was estimated as 34 nm. Furthermore, structural characterization revealed the crystallinity of the SrONPs to have a cubic phase structure and their elemental composition to be dominated by strontium and oxygen. In addition, FTIR spectra attested the occurrence of functional groups (-OH, -C=O, -C-O) in the plant extracts and demonstrated that they supported the reduction of strontium ions in the SrONPs. The optical band gap was determined to be 4.13 eV, confirming the semiconductor properties of the nanoparticles. Then, we assessed the antimicrobial activity of such nanoparticles and showed that they were able to exert their inhibition effect in a dose-dependent manner against *E. coli* (inhibition zone diameter - 13 mm) and *S. aureus*(inhibition zone diameter - 11 mm) at concentrations as high as 40 mg•mL^{-1}. The following study justifies that the simple, eco-friendly synthesis of SrONPs using the *Cycasrevoluta* could be further developed into an inexpensive strategy for nanoparticle production. Moreover, SrONPs biosynthesized in the current study can be used in several biomedical applications: antimicrobial coatings, water treatment, and drug delivery. In Future, antifungal, antioxidant, anti-cancer properties of synthesized SrONPs will be checked.

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