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Biogenesis of Zirconium Oxide Nanoparticles by *Momordica charantia* **(Bitter Gourd) Leaf Extract: Characterization and their Antimicrobial Activities**

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Authors' contributions

This work was carried out in collaboration between both authors. Author SAD designed the study, performed the synthesis, characterization, and applications, and wrote the first draught of the manuscript. Author PS managed the literature searches. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To determine the antimicrobial activity of zirconium oxide nanoparticles (ZrO₂ NPs) synthesized by *Justicia Adhatoda* leaf extract.

Study Design: Synthesis, characterization and antibacterial activity determination of ZrO₂ NPs. **Place and Duration of Study:** PG and Research Department of Chemistry, V.O.Chidambaram College, Tuticorin, Tamilnadu, India, between April 2020 and April 2021.

Methodology: *Justicia adhatoda* leaf extract was used to synthesize ZrO₂ NPs. UV-Visible spectroscopy was used to characterize $ZrO₂$ NPs. Using Fourier transform infrared spectroscopy, the function of biomolecules in plant extract in the synthesis of $ZrO₂ NPs$ was identified. XRD was used to determine the particle size of nanparticles. $ZrO₂$ NPs were evaluated for antimicrobial activity.

___ **Results:** The synthesis of ZrO₂ NPs was clearly visible in an absorbance band at 321 nm in the UV-visible spectrum. The absorption peak of $ZrO₂$ NPs in the FTIR was 880 cm⁻¹, confirming the

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 $Zr-O$ vibrational mode in $ZrO₂$ NPs. As evidenced by XRD measurements, the average crystallite size of $ZrO₂$ NPs was found to be 40 nm. The biosynthesized $ZrO₂$ NPs were found to have potent antibacterial action against *Escherichia coli* bacteria and *Staphylococcus aureus* bacteria. **Conclusion:** ZrO₂ NPs mediated by *Justicia adhatoda* leaf extract have demonstrated substantial antibacterial activity

Keywords: Green synthesis; zirconium oxide nanoparticles; Justicia adhatoda; antibacterial activity.

1. INTRODUCTION

Metal oxides have been analysed by intensive investigators due to their low cost, variable oxidation states, excellent physical and chemical properties, and several industrial applications [1,2]. Zirconium oxide $(ZrO₂)$, often known as zirconia, is a versatile material that has been employed in a wide range of applications, including structural reinforcement, antibacterial agents, adsorption, and photodegradation [3,4].

The well-known procedures for the synthesis of nanoparticles necessitate the use of expensive, caustic, hazardous, and combustible chemicals, which are frequently hazardous to the environment and human health. As a result, the development of a green approach for nanoparticle synthesis has recently received a lot of interest. Green synthesis generates nanoparticles that are free of pollution, low cost, biocompatible, and non-toxic, in addition to being environmentally benign as compared to other physiochemical processes.

Plant extracts, bacteria, yeast, fungi, actinomycetes, lichen, or algae have been proposed as green alternatives to physiochemical methods for metal oxide nanoparticle synthesis. Plant extract-based nanoparticle synthesis is less expensive than microbial-based synthesis, which is more expensive due to the high cost of microbe cultivation. Plant extract-mediated nanoparticle synthesis is thought to be more efficient than microbial-mediated nanoparticle synthesis because plant extracts include a variety of bioactive compounds that can be used as reducing and stabilising agents. Thousands of plant extracts have been discovered to have the ability to reduce metal salts to metal oxides.Plant extracts like *Ficus benghalensis, Alium cepa, Eucalyptus globules, Lagerstroemia speciosa, Acalypha indica, Acalypha indica, Euclea racemosa* have been used for the synthesis of $ZrO₂$ nanoparticles [5-8]

Momordica charantia is a subtropical and tropical vine that is widely cultivated for its edible fruit throughout Asia, Africa, South America, and the Caribbean. Bitter gourd, bitter melon, kerela, and balsam pear are all frequent names for this exceedingly bitter fruit plant. It has antidiabetic, antibacterial, antileukemic, anticancerous, antiprotozoal, antitumorous, anti-obesity, antiparasitic, antifungal, anti-ulcer, immune stimulant, hypoglycemia, and antiviral properties and can be used as medicine [9].

Momordica charantia has been used for diabetes, psoriasis, leukaemia, high cholesterol, tumour aetiology, cancer, viral infections, and bacterial infections in African and Asian herbal medicine systems. Triterpenes, proteins, momordicin, charantin, steroids, alkaloids, ascorbic acid, gallic acid, ferulic acid, tannic acid, catechin, caffeic acid, and other phenolic compounds have been found in *Momordica charantia*, which are responsible for the therapeutic effects [10,11].

From a comprehensive literature survey, it is noted that this is the first report on $ZrO₂$ nanoparticle synthesis using the leaf extract of *Momordica charantia.*

2. MATERIALS AND METHODS

2.1 Chemicals Used

Zirconyl chloride $(ZrOCl₂)$ used for the synthesis of $ZrO₂$ NPs was purchased from Sigma Aldrich. Healthy and fresh leaves of *Momordica charantia* were collected from the Thoothukudi, Tamilnadu, India.

2.2 Preparation of *Momordica charantia* **Leaf Extract**

Fresh *Momordica charantia* leaves weighing around 10g were properly cleansed with running tap water and then distilled water to remove dust particles. In a round-bottom flask with a condenser, finely crushed *Momordica charantia* leaves were heated in 100 mL of distilled water for 1 hour at 100°C. The leaf extract was filtered using the Whatman No. 41 filter paper that was used in the $ZrO₂$ NP synthesis.

Zirconyl chloride (ZrOCl2)

Momordica charantia **leaves**

Fig. 1. Chemicals used

2.3 Biosynthesis of ZrO² NPs

For the synthesis of $ZrO₂$ NPs, zirconyl chloride (ZrOCl2) and *Momordica charantia* leaf extract were utilised as the precursor salt and reducing agent, respectively, for the synthesis of $ZrO₂$ NPs.

In a round-bottom flask with a condenser, 25 mL of *Momordica charantia* leaf extract was added to 75 mL of 0.5 M Zirconyl chloride solution and 25 mL of 0.1 M NaOH. This combination was heated for 2 hours at 100 $^{\circ}$ C. The synthesized ZrO₂ NPs were then filtered and dried overnight in an oven at 60°C.

2.4 Characterization of ZrO² NPs

The UV-Visible spectra of the $ZrO₂$ NPs and *Momordica charantia* leaf extract were recorded on JASCO UV-Visible spectrometer. FTIR measurements were performed on a Thermo Scientific Nicolet iS5 instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets. The average particle size of $ZrO₂$ NPs was determined by using XPERT-PRO X-ray diffractometer operating at a voltage of 40 kV and a current of 30 mA with Cu K α radiation.

2.5 Antibacterial Activity

The following approach was used to test $ZrO₂$ NPs biosynthesized from *Momordica charantia* leaf extract for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus.* Muller-Hinton agar medium was prepared and autoclaved for 15 minutes at 121°C. In the

inoculation chamber, the agar medium was transferred to petri plates under aseptic conditions and allowed it to solidify. The test sample-loaded filter paper discs were placed on top of the bacterial liquid culture that has been swabbed uniformly across the agar surface. As a control, ampicillin-loaded discs were used. It was incubated for 24 hours at 37°C. Measurement of the inhibitory zone was taken.

3. RESULTS AND DISCUSSION

Characterizations and applications of $ZrO₂$ NPs were done by various techniques. The results obtained are discussed in detail as follows:

3.1 UV-Visible Spectroscopic Analysis

The optical characteristics of metal oxide nanoparticles can be determined using UV-Visible spectroscopy, which is one of the most potent techniques for characterization of metal oxide nanoparticles.

Two absorption bands at 270 nm and 315 nm observed from *Momordica charantia* leaf extract are attributable to UV absorption of polyphenols, as illustrated in Fig. 2. It demonstrates the presence of polyphenols in *Momordica charantia* leaf extract, which may be responsible for the green production of $ZrO₂$ NPs as a reducing agent.

The UV-visible spectrum of green synthesised $ZrO₂$ NPs is shown in Fig. 3. The spectrum displays one absorption peak at around 321 nm, which corresponds to the

zirconium oxide nanoparticle absorption maxima. The valence-to-conduction band transition of $ZrO₂$ NPs causes the absorption peak at 321 nm [8].

3.2 FTIR Analysis

To identify the probable biomolecules responsible for the synthesis of $ZrO₂$ NPs, FTIR measurements of *Momordica charantia* leaf extract and $ZrO₂$ NPs were taken.

The results of FTIR of *Momordica charantia* leaf extract (Fig. 4) show a number of absorption bands at 619, 1108, 1409, 1572, 2929 and $3409cm^{-1}$. In which, the band at 619cm⁻¹ corresponds to mono substituted aromatic stretching. The band at 1108cm⁻¹ is characteristic of C-C stretching vibrations of aromatic amines. The band at 1409 cm⁻¹ could be due to the C-H stretching vibration. The band at 1572 cm⁻¹ region is characteristic of C-O asymmetric stretching vibration. The band observed at 2929 cm^{-1} may be due to the C-H stretching vibration. The broad band at 3409cm^{-1} is due to the stretching vibration of hydroxyl group. The majority of the FTIR bands are characteristic of alkaloids, flavonoids, terpenoids, steroids, proteins, carbohydrates and other phenolic

compounds present in the *Momordica charantia* leaf extract [9].

The FTIR spectrum of $ZrO₂$ NPs (Fig. 5) shows major peaks at 880, 1456, 1636, 2924 and 3434cm⁻¹. The spectrum clearly shows bands for C-O asymmetric stretching vibration and hydroxyl group stretching vibration at around 1636 and 3434 cm⁻¹ respectively. The band at 1456 cm⁻¹ could be due to the C-H stretching vibration. The band observed at 2924 cm^{-1} may be due to the C-H stretching vibration. The lower absorption bands at about 880 cm $^{-1}$ is attributed to the $Zr-O$ vibrational mode as reported for many $ZrO₂$ NPs [8,9].

3.3 X-ray Diffraction Analysis

The crystallite size can be evaluated using Debye-Scherer equation:

$$
D = \frac{k x \lambda}{\beta \cos \theta}
$$

where D is the thickness (diameter) of the particle, λ is the wavelength of the X-ray beam, β is the full width at half maximum (FWHM) of the peak position in radians, k is the shape factor (0.9) and θ is the Bragg diffraction angle at peak position [8,9].

Fig. 2. UV-Visible spectrum of *Momordica charantia* **leaf extract**

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Fig. 4. FTIR spectrum of *Momordica charantia* **leaf extract**

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Fig. 5. FTIR spectrum of ZrO² NPs

From XRD data, the average crystallite size of the $ZrO₂$ NPs as estimated using the Scherrer formula is 40nm. Fig. 6 show the XRD pattern of the synthesized $ZrO₂$ nano particles. There is a broad peak corresponding to 100% reflection of t -ZrO₂ besides there is a narrow peaks corresponding to $m-ZrO₂$ were also identified. The formation of t -ZrO₂ as the main phase of zirconium nano particles could be related to the presence of sodium hydroxide in the generation stage [12].

3.4 Antimicrobial Activity

Fig. 7 illustrates the antibacterial activity
of biosynthesized ZrO_2 NPs against of biosynthesized $ZrO₂$ NPs against *Escherichia coli* and *Staphylococcus aureus*. Biogenerated $ZrO₂$ NPs have antibacterial efficacy against *Escherichia coli* (zone of inhibition of 11 mm) and *Staphylococcus aureus* (zone of inhibition of 10 mm).

Fig. 7. Antibacterial activity of ZrO² NPs against (I) *Escherichia coli and* **(II)** *Staphylococcus aureus*

4. CONCLUSION

The biosynthesis of ZrO₂ NPs using *Momordica charantia* leaf extract is demonstrated, with phenolic chemicals perhaps acting as a reducing agent. UV-Visible spectra with absorption bands centred around 321nm confirm the production of $ZrO₂$ NPs. The presence of a $Zn-O$ bond is confirmed by bands in the FT-IR spectrum at 880 cm^{-1} . According to the XRD pattern, ZrO₂ NPs have an average particle size of 40 nm. The antimicrobial activity of $ZrO₂$ NPs against *Escherichia coli* and *Bacillus cereus* is substantial.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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