Original Research Article

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

ABSTRACT

Aims: To determine the antimicrobial activity of zirconium oxide nanoparticles (ZrO_2 NPs) synthesized by *Justicia Adhatoda* leaf extract
Study design: Synthesis, characterization and antibacterial activity determination of ZrO_2 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 ZrO_2 NPs. UV-Visible spectroscopy was used to characterize ZrO_2 NPs. Using Fourier transform infrared spectroscopy, the function of biomolecules in plant extract in the synthesis of ZrO_2 NPs was identified. XRD was used to determine the particle size of nanparticles. ZrO_2 NPs were evaluated for antimicrobial activity.
Results: The synthesis of ZrO_2 NPs was clearly visible in an absorbance band at 321 nm in the UV–visible spectrum. The absorption peak of ZrO_2 NPs in the FTIR was 880 cm^{-1}, confirming the Zr–O vibrational mode in ZrO_2 NPs. As evidenced by XRD measurements, the average crystallite size of ZrO_2 NPs was found to be 40 nm. The biosynthesized ZrO_2 NPs were found to have potent antibacterial action against *Escherichia coli* bacteria and *Staphylococcus aureus* bacteria.
Conclusion: ZrO_2 NPs mediated by *Justicia adhatoda* leaf extract have demonstrated substantial antibacterial activity and are regarded as strong antibacterial agents against certain bacteria.

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].

Zirconium oxide (ZrO_2), 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 [2].

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.

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.

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 [3 -5].

From a comprehensive literature survey, it is noted that this is the first report on ZrO_2 nanoparticle synthesis using the leaf extract of *Momordica charantia.*

2. MATERIALS AND METHODS

2.1 Chemicals used

Zirconyl chloride ($ZrOCl_2$)

Momordica charantia leaves

Fig 1. Chemicals used

Zirconyl chloride ($ZrOCl_2$) used for the synthesis of ZrO_2 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 ZrO_2 NP synthesis.

2.3 Biosynthesis of ZrO_2 NPs

For the synthesis of ZrO_2 NPs, zirconyl chloride ($ZrOCl_2$) and *Momordica charantia* leaf extract were utilised as the precursor salt and reducing agent, respectively, for the synthesis of ZrO_2 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°C. The synthesized ZrO_2 NPs were then filtered and dried overnight in an oven at 60°C.

2.4 Characterization of ZrO_2 NPs

The UV-Visible spectra of the ZrO_2 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 ZrO_2 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 $K\alpha$ radiation.

2.5 Antibacterial activity

The following approach was used to test ZrO_2 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 ZrO_2 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.

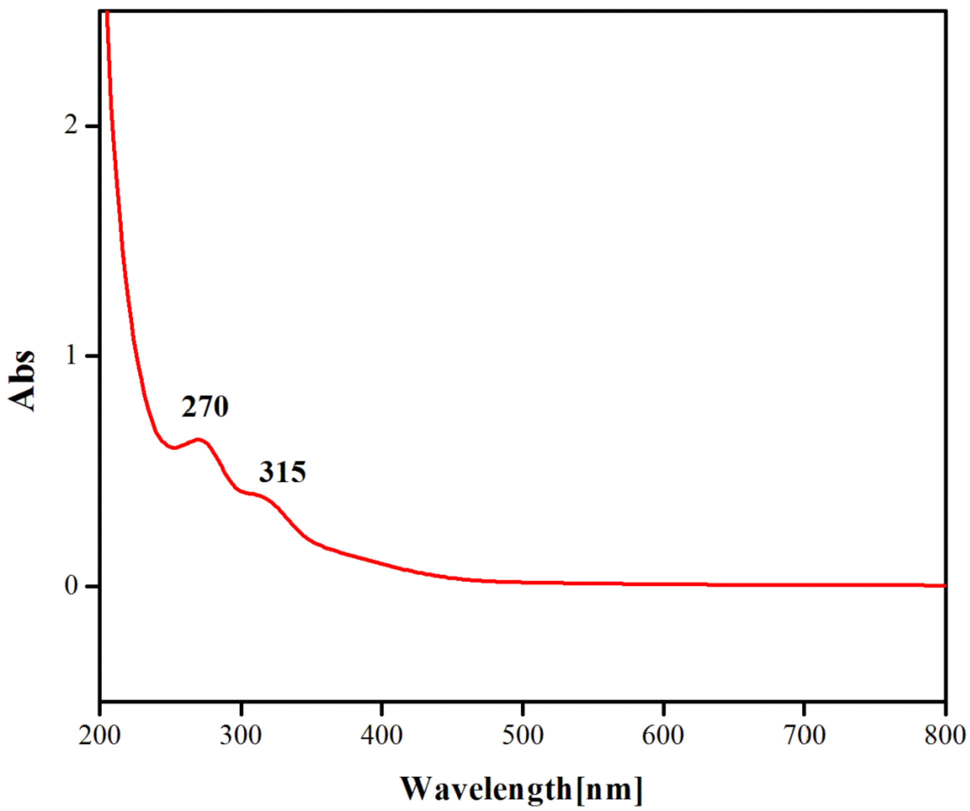


Fig. 2. UV-Visible spectrum of *Momordica charantia* leaf extract

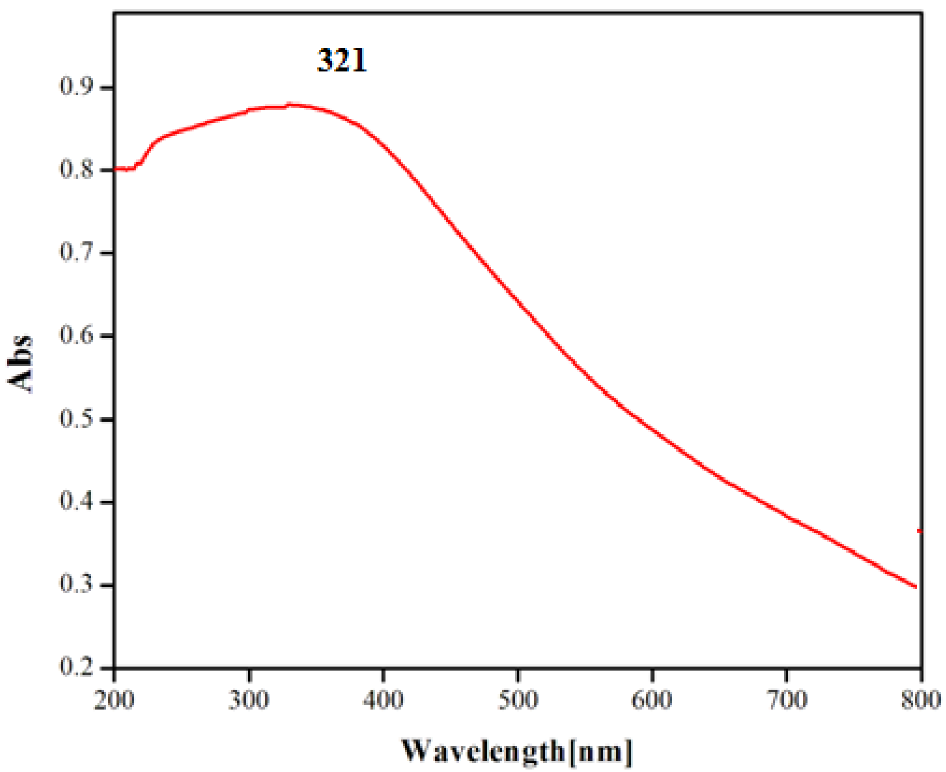


Fig. 3. UV-Visible spectrum of ZrO_2 NPs

Two absorption bands at 270nm and 315nm observed from *Momordica charantia* leaf extract are attributable to UV absorption of polyphenols, as illustrated in figure 2. It demonstrates the presence of polyphenols in *Momordica charantia* leaf extract, which may be responsible for the green production of ZrO_2 NPs as a reducing agent.

The UV-visible spectrum of green synthesised ZrO_2 NPs is shown in figure 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 ZrO_2 NPs causes the absorption peak at 321 nm.

3.2 FTIR analysis

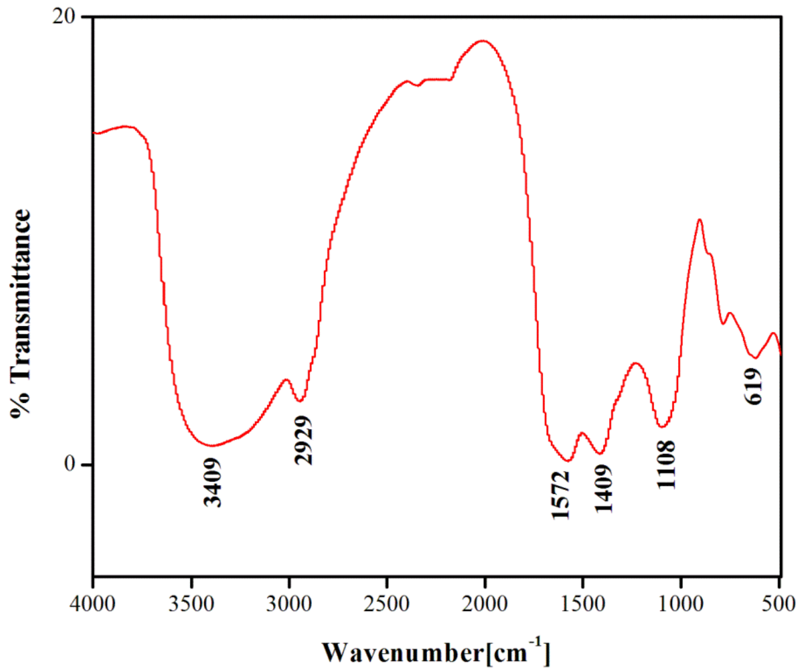


Fig. 4. FTIR spectrum of *Momordica charantia* leaf extract

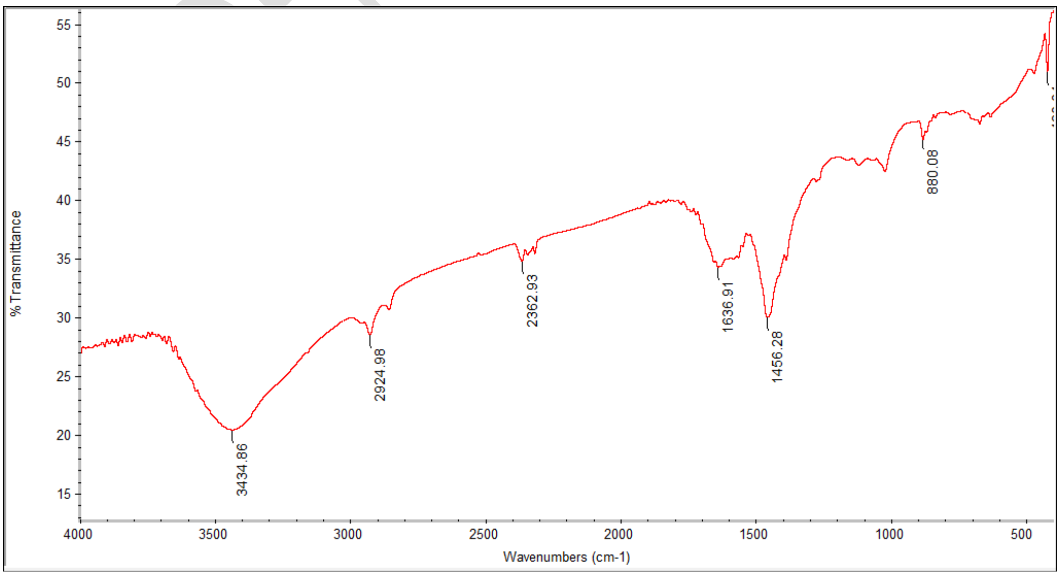


Fig. 5. FTIR spectrum of ZrO_2 NPs

To identify the probable biomolecules responsible for the synthesis of ZrO_2 NPs, FTIR measurements of *Momordica charantia* leaf extract and ZrO_2 NPs were taken.

The results of FTIR of *Momordica charantia* leaf extract (figure 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 C-N stretching of aromatic phenols. 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.

The FTIR spectrum of ZrO_2 NPs (Figure 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 ZrO_2 NPs.

3.3 X-ray diffraction analysis

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

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

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.

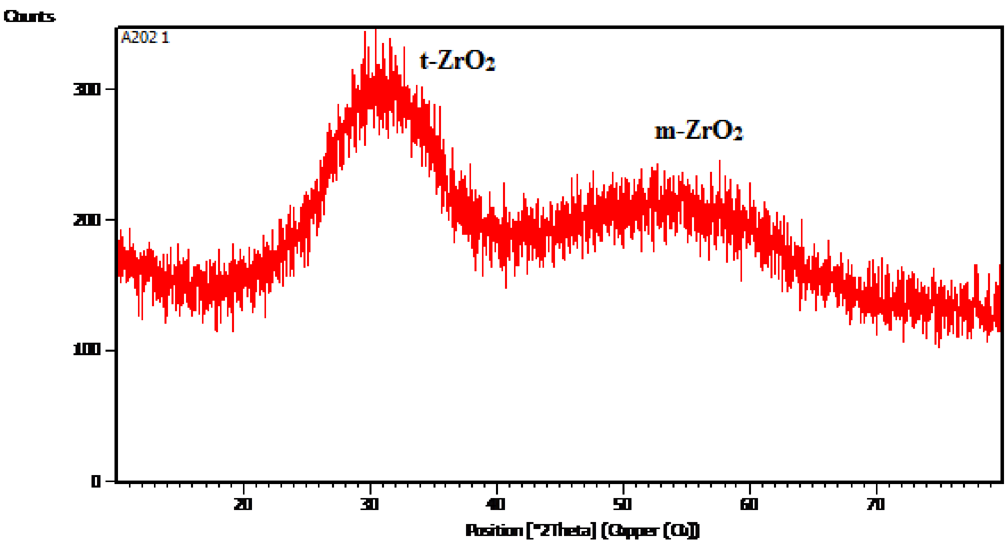


Fig. 6. XRD pattern of ZrO_2 NPs

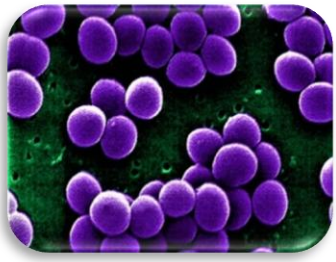
From XRD data, the average crystallite size of the ZrO_2 NPs as estimated using the Scherrer formula is 40nm. Figure 6 show the XRD pattern of the synthesized ZrO_2 nano particles. There is a broad peak corresponding to 100% reflection of t-ZrO_2 besides there is a narrow peaks corresponding to m-ZrO_2 were also identified. The formation of t-ZrO_2 as the main phase of zirconium nano particles could be related to the presence of sodium hydroxide in the generation stage

3.4 Antimicrobial activity

Fig. 7. Antibacterial activity of ZrO_2 NPs against (I) *Escherichia coli and* (II)*Staphylococcus aureus*

Figure 7 illustrates the antibacterial activity of biosynthesized ZrO_2 NPs against *Escherichia coli* and *Staphylococcus aureus*. Bio-generated ZrO_2 NPs have antibacterial efficacy against *Escherichia coli* (zone of inhibition of 11 mm) and *Staphylococcus aureus* (zone of inhibition of 10 mm).

Table. 1. Antibacterial activity data of ZrO_2 NPs

Bacteria	Inhibition zone in mm	
	Concentration 1mg/ml	
	Ab→Ampicillin	S → ZrO_2 NPs
 *Escherichia coli*	14	11
 *Staphylococcus aureus*	19	10

4. CONCLUSION

The biosynthesis of ZrO_2 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 ZrO_2 NPs. The presence of a Zn–O bond is confirmed by bands in the FT-IR spectrum at $880cm^{-1}$. According to the XRD pattern, ZrO_2 NPs have an average particle size of 40 nm. The antimicrobial activity of ZrO_2 NPs against *Escherichia coli* and *Bacillus cereus* is substantial.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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