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BIOGENIC SYNTHESIS OF ZINC OXIDE NANOPARTICLES THROUGH LEAF-BASED EXTRACT OF JAMBOLANA

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Abstract
Zinc oxide nanoparticles (ZnO) were synthesized using aqueous leaf extract of *Syzygium cumini* Linn (Jambolana) through green synthesis. Due to having no toxic effect on an environment green synthesis has become one of the most important methods for the synthesis of particles at nanoscale, therefore plant extract was used for the synthesis of biogenic ZnO Nps. The UV-Vis spectrum was used as a confirmational tool for the prepared particles either in nano scale range or not. Prepared green nanoparticles were further processed for the XRD and SEM analysis so that the exact size and shape of experimental particles were calculated. The presence of the active compounds or functional groups was further calculated using FTIR analysis. Change in color of the mixture (pale yellow to whitish) was the first confirmation of ZnO particles while UV Vis analysis presented a sharp peak at 378 nm which confirmed the synthesized particles in nanoscale. The hexagonal wurtzite structure of ZnO nanoparticles was confirmed through XRD analysis which gives distinct peaks at 2θ position. The average size calculated was around 70 nm with irregular shape of particles. Leaf extract of *S. cumini* was found excellent reducing agent for the synthesis zinc oxide nanoparticles.

Keywords*:* *Syzygium cumini* Linn., Zinc Oxide Nanoparticles, ZnO NPs, XRD, FTIR and SEM.

INTRODUCTION

Nanoscience is the advanced study of structures and molecules at nano scale, ranging between 1 to 100 nm. This advanced technology which is involved in the synthesis and application of these materials at nano range is called nanotechnology (Santhoshkumar *et al.*, 2017). This is a multidisciplinary field which involves other fields such as bio-nanoscience, physical sciences, and materials science. This technology is also fruitful for other sciences such physics, chemistry, and biology (Divya *et al.*, 2018). Nanoparticles are broadly used in a range of applications due to their biological, physiochemical, and optical properties immensely (Karthika *et al.*, 2017) (Benelli *et al.*, 2018). Uses and the applications of these particles are not only limited to medical and material sciences but are recommended to be used in agriculture and is useful for subsistence/organic farmers and will help them to avoid environmental pollution (Badar et al., 2023).

Synthesis of these nanoparticles could be possible through different chemical, physical, and biological methods (Vijayakumar *et al*., 2016; Ishwarya *et al*., 2018). The use of microorganisms like fungi, algae, bacteria, and plants helps in the synthesis of biological nanoparticles, which is termed as green synthesis. This method of synthesis is considered environmentally friendly and considered as unique way of thinking in biology intended to eliminate toxins in environment. Green synthesis of nanoparticles involves the use of many plants or plant parts for the bio reduction of metal ions into their elemental form in the size range 1–100 nm. And thus, the process of green synthesis is simpler, more efficient, and economical, and can easily be scaled up to perform bigger processes (Singh *et al*., 2017; Banumathi *et al*., 2017).

Use of particles at nanoscale range is considered more useful due to numerous advantageous features such as having larger surface to volume ratio, effectiveness, economic and environmentally friendly. This means that the use of material at smaller quantities is required for more effectiveness in contrast to larger particles (Vijayakumar *et al*., 2016). No doubt these particles are becoming a particle/material of choice due to having characteristic features such as catalysis, water treatment, energy storage, medicine, agriculture, etc. (Khot et al. 2012; Gajanan et al., 2018 and Bratovcic, 2019). There are two main properties of nanomaterials due to which these are considered as significant and are different than the same materials at larger dimensions: surface effects and quantum effects (Roduner, 2006). These properties are favorable for these particles exhibiting enhanced or novel mechanical, thermal, magnetic, electronic, optical, and catalytic properties (Gade et al., 2010).

The synthesis of Zinc Oxide nanoparticles from physical and chemical methods has various margins like they are very expensive, highly toxic byproducts, pressure, and temperature critical conditions. To deal with these limitations, eco-friendly and justifiable processes have now become very important for the synthesis of nanoparticles and hence green synthesis method is one of them (Sharma *et al.,* 2020). *S. cumini* naturally has been used as drugs and medicines from last hundreds of years around the world. Jamun has been effectively used against inflammation in the treatment of Diabetes mellitus, diarrhea and ulcers and pre-symptomatic and preclinical studies have also shown anti-neoplastic, radio-protective, and chemo-preventive properties. The *S. cumini* tree is a source of compounds consisting of ellagic acid, glucoside, anthocyanins, kaempferol and myrecetin. The presence of bioactive compounds comprising tannins, alkaloids, lipids, phenols, flavonoids in jamun leaves, fruits, barks, roots, and stems makes contribution to massive potential source of healthy nutrition and drug. The dynamic presence of these compounds withstands pharmacological effects with antidiabetic, antimicrobial, antioxidant, central nervous system activity (CNS), chemo preventive, anti-inflammatory, hepato-protective and anti-allergic properties intake of jamun in human health and metabolism. Jamun is also intensely well-known for its antidiabetic activity as it has been proved to be having the most auspicious nutraceutical value reported by several research papers (Singh *et al*., 2019).

METHODOLOGY

Experimental plant

Fresh leaves of the *Syzygium Cumini* Linn (Jambolana) were used for the green synthesis of Zinc oxide nanoparticles (ZnO NPs).

Synthesis of ZnO Nanoparticles

ZnO nanoparticles were prepared using 10ml of 1 mM $ZnSO_4.7H_2O$ aqueous solution with 3ml leaf extract *S.* c*umini*. Prepared reaction mixture was left on stirrer for 2 hours at 80°C and change in color was noted. Reaction mixture was centrifugated at 6000 rpm for 15 minutes after change in colour; these particles were washed thoroughly with distilled water 4 times. The particles were then dried in a hot air oven at 120˚C for 1 hour. The dried nanoparticles were then stored for further characterization.

Characterization of ZnO Nanoparticles

Characterization of prepared nanoparticles was carried out though the following techniques.

- **UV-Vis Spectrophotometry**
- **Fourier Transform Infrared Spectroscopy (FTIR)**
- **X-ray diffraction (XRD)**
- **Scanning Electron Microscopy (SEM) analysis**

Zinc oxide nanoparticles were sonicated in distilled water for about 15 minutes and wavelength was recorded between 300-800 nm using Thermoscientific, Multiskan Sky plate reader (Mahamuni *et al*., 2018). Fourier Transform Infrared Spectroscopy (FTIR) analysis was performed to assess the presence of active components. Results were recorded in the range of 1000 to 4000 cm^{-1} (Dobrucka *et al*., 2016). X-ray diffraction pattern of the dried biofabricated ZnO NPs were carried out using PANalytical Xpert powder diffractometer (Malvern) for the determination of shape and average size of green nanoparticles.

The average crystallite size of biofabricated ZnO NPs was determined from the highest intense/narrower peak using Debye Sherrer's equation:

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

Where, D is crystallite size of nanoparticles, k is Sherrer's constant, which is 0.94, λ is the wavelength of X-ray sources used in XRD (1.5406Å), β is full width at half maximum (FWHM) of diffraction peak. θ refers to Bragg's angle. The most intense peak was chosen which is <010> and crystalline size of synthesized `biofabricated ZnONPs was determined around 21.63 (Bala *et al*., 2022).

For further confirmations SEM analysis of prepared nanoparticles was performed using Carl Zeiss (model: FE-SEM sigma 500 VP) to assess the surface morphology, shape, and size of biofabricated ZnO NPs.

RESULTS

Synthesis of Zinc Oxide Nanoparticles

A change in colour of reaction mixture from pale yellow to whitish was the first clear indication of zinc oxide nanoparticles (ZnO NPs).

CHARACTERIZATION

UV Analysis

A sharp peak was obtained at 378 nm by UV analysis of experimental particles which confirmed that the synthesized particles were in nano scale range (Figure 1 A). While the band gap energy of biofabricated ZnO NPs was recorded 3.6 eV (Figure 1 B).

FTIR Analysis

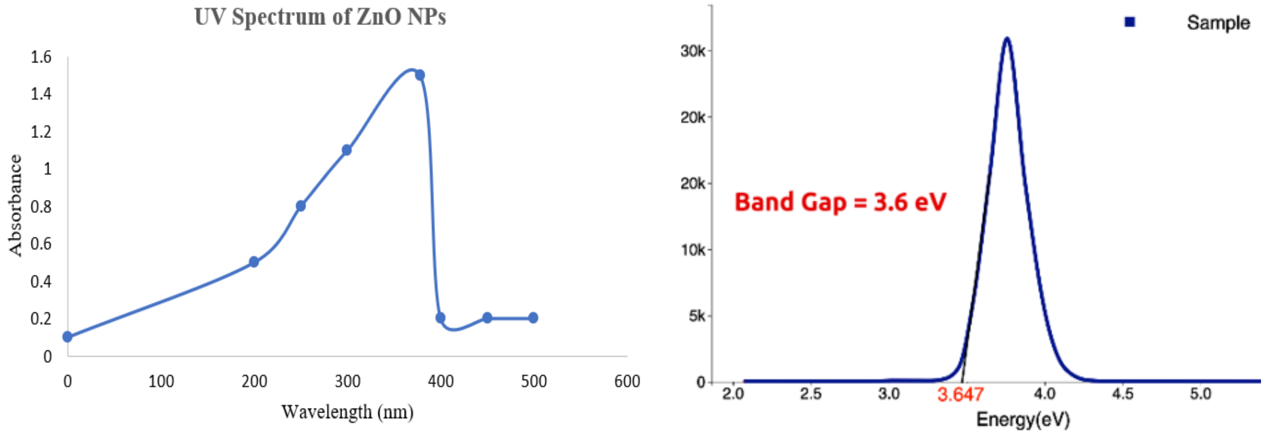
Figure (2) showed the presence of active constituents which were due to the plant extracts. A clear and sharp peak at 2920.85 was due to C-H stretching, while peak positions at 3425.15 and 1433.07 were due to strong O-H stretching. Peaks at 1629.07 and 1112.53 were attributed to the presence of C=C and C-O stretching.

XRD Analysis

The pattern of synthesized biofabricated ZnO NPs clearly indicated the crystalline nature of particles while the diffracted intensities were recorded from 20° to 80° at 2θ. The observed sharp diffraction peaks at 2θ values 31.7, 34.38, 36.18, 47.44, 56.54, 62.98 and 67.8 degrees. These peaks (100), (002), (101), (102), (110), (103) and (112) correspond to JCPDS Card NO. 03-065-2880 confirmed that they were in lattice planes showing hexagonal wurtzite structure (Figure 3).

SEM Analysis

Images recorded from SEM analysis confirmed that the prepared green nanoparticles were in hexagonal shape agglomerated together (Figure 4). These particles were irregular and rough in appearance with average size ranges 25-45 nm.



A **B**

Figure 1 (A) UV Spectrum and (B) presents the Band energy gap calculated by tauc plot of bio fabricated ZnO nanoparticles.

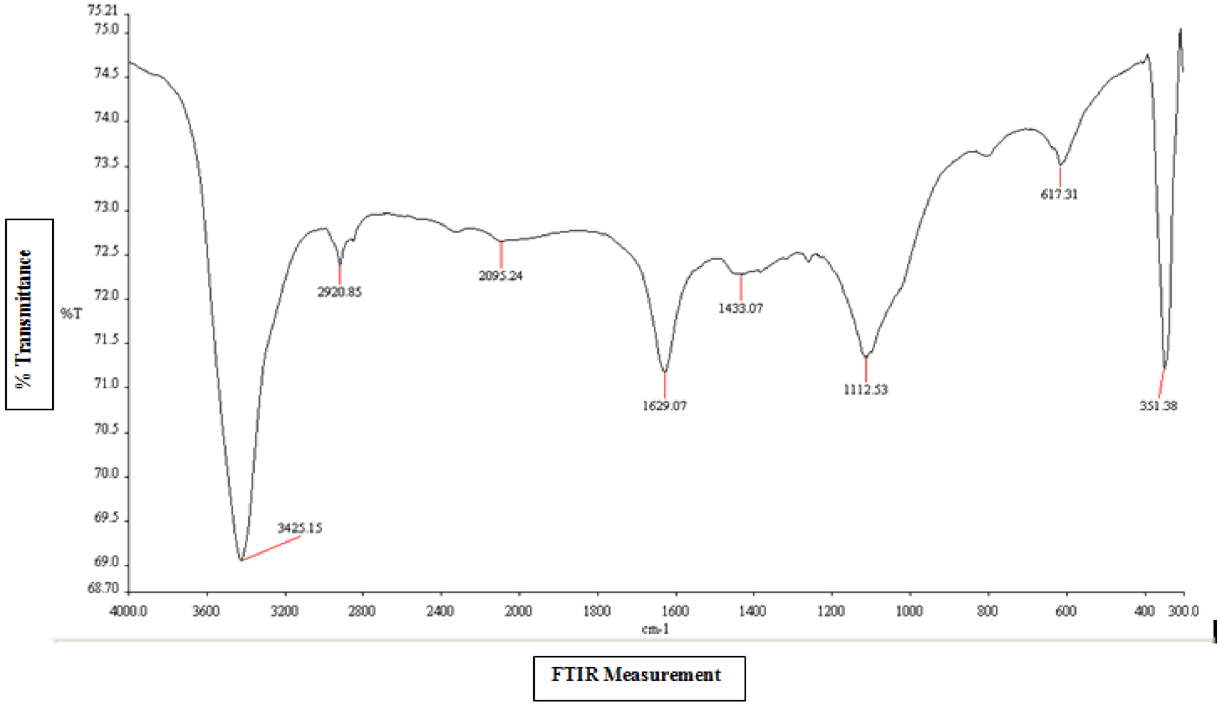
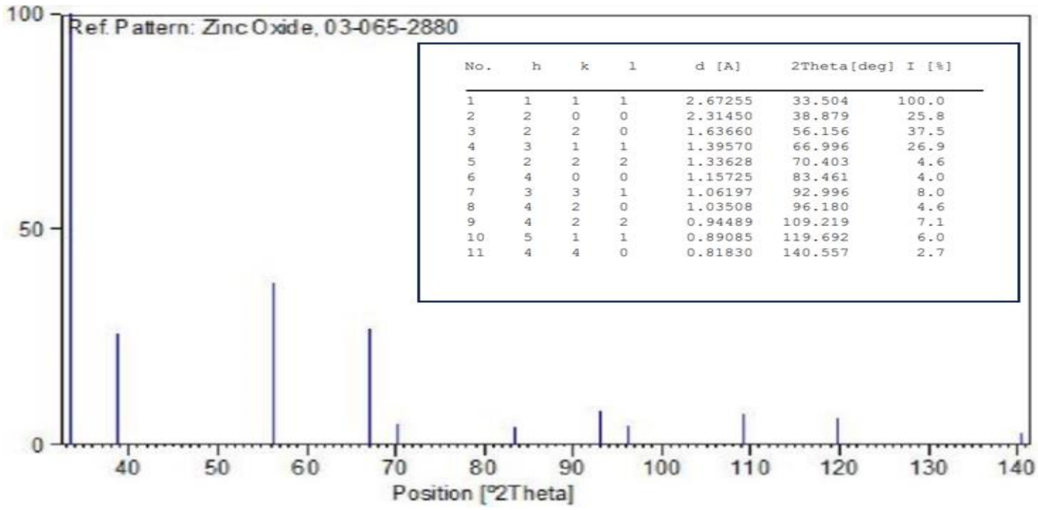


Figure 2 FTIR spectrum of bio-fabricated ZnO nanoparticles



No.	h	k	l	d [A]	2Theta[deg]	I [%]
1	1	1	1	2.67255	33.504	100.0
2	2	0	0	2.31450	38.879	25.8
3	2	2	0	1.63660	56.156	37.5
4	3	1	1	1.39570	66.996	26.9
5	2	2	2	1.33628	70.403	4.6
6	4	0	0	1.15725	83.461	4.0
7	3	3	1	1.06197	92.996	8.0
8	4	2	0	1.03508	96.180	4.6
9	4	2	2	0.94489	109.219	7.1
10	5	1	1	0.89085	119.692	6.0
11	4	4	0	0.81830	140.557	2.7

Figure 3 XRD Analysis presenting JCPDS Card NO. 03-065-2880 of ZnO NPs

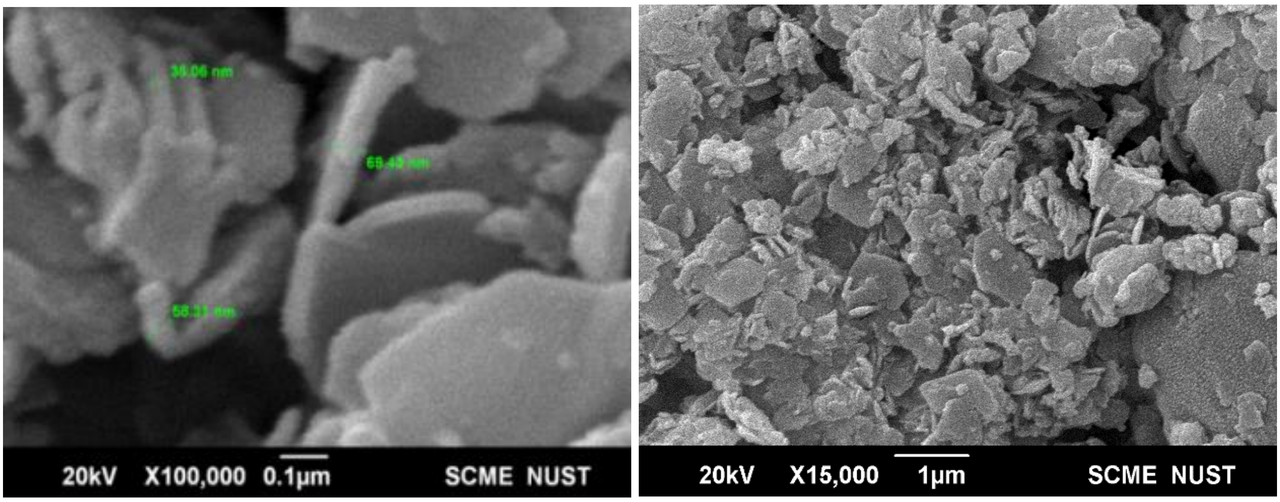


Figure 4 Electron micrograph of bio-fabricated ZnO nanoparticles (SEM)

Discussion

Zinc oxide nano particles had been successfully prepared using green extracts of plant-based origin. Change in color of the prepared reaction is the one of most important feature of zinc oxide nanoparticles. Various studies had been conducted by numerous experts reporting change in color as the first indication of synthesis of these particles. Study conducted by Naseer et al., (2020) reported that ZnO NPs were prepared using leaf extracts of *C. fistula* and *M. azedarach*, and the synthesis of these particles were confirmed by the change of color from yellow to light brown and red to off-white (Dihom et al., 2022). Sharp and strong peaks were recorded by ZnO NPs at peak positions 360-363 nm which was in close coordination with the present study (Salahuddin et al., 2015).

Results of present experimental study were in line with the previous studies (Rajiv et al., 2013).
UV vis spectrometer is the second step for the confirmation of particles whether in nano range or not. Peaks positions by UV analysis had been recorded between range of 200-450 nm commonly. Green synthesis of ZnO NPs recorded peak position at 354 nm which was thought to be due to electron transition of conduction band of bio fabricated ZnO NPs and their large excitation binding energy at room temperature (Fakhari *et al*., 2019). It is well known from absorption spectroscopy that the band gap increases on decreasing particle size. Likewise results of another study suggested that a sharp peak was recorded at 320 nm by ZnO NPs which were prepared using *C. fistula* while the particles synthesized using *M. azadarach* give peak at 324 nm (Naseer et al., 2020). Similar results were also reported by study of Zhang et al (2002) and Swart et al., (2019). Results band

energy gap calculated by Tauc plot for the experimental nanoparticles were also in line with study of Xaba et al., 2019.

Experimental synthesis of these experimental NPs was further confirmed using X-ray diffraction as a tool; peak positions detected by experimental samples were also reported by number of studies. Study conducted by Demissie et al., (2020) reported diffraction peaks at a 2θ value of ≈31.76°, 34.42°, 36.24°, 47.54°, 56.59°, 62.86°, 66.41°, 67.97°, and 69.06° which were corresponding to (100), (002), (101), (102), (110), (103), (200), (112), and (201) crystal planes. Results of present experimental analysis were favored (Getie et al., 2017; Gholamali and Yadollahi, 2021). Results of another study suggested that sharp peaks obtained by XRD analysis were used to calculate average size. Reported results of another study were in line with present study suggested that NPs were hexagonal wurtzite in shape (Dobrucka and Długaszewska 2016).

SEM analysis was used for the morphological study of the experimental nanoparticles, as reported by experts (Rajiv et al., 2013).

CONCLUSION

Leaf extracts of Jambolana were an outstanding material which was used for the synthesis of zinc oxide nanoparticles. These plant extracts were used for the stability of nanoparticles and caping agents. Prepared nanoparticles were further analyzed through UV, FTIR, XRD, and SEM analysis for the confirmation of ZnO NPs. Prepared nanoparticles were in nano range and the FTIR analysis revealed that the stability and the successful synthesis of these nanoparticles were due to the use of plant extracts. While the average size calculated was around 70 nm with irregular shape of particles. These nanoparticles could be further used for various branches of science due to having valuable features and are applicable for all kinds of advanced studies.

Conflict of interests

There are no potential conflicts of interest declared by author (s).

Author contribution

HW performed all experimental work as a part of her PhD project and wrote the 1st draft of manuscript. **AA** designed and supervised the whole project, Edited, revised, and gave final approval to this manuscript. **RB** support for the analysis of data and English editing services and give final shape to the article.

GI provided her technical support for experimental work. **AA** provided her logistic support for a part of experimental work and technical support for the analysis of data.

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Availability of data and materials

All presented data are original.

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