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Green synthesis of zinc oxide nanoparticles using leaf extract of neem (*Azadirachta indica* L.) and their characterization

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Abstract

Plant mediated or green synthesis has emerged as one of the most widely adopted approaches in nanotechnology. Many tropical plant species possess medicinal properties that are closely associated with the synthesis of metallic nanoparticles using plant-derived biomolecules. Nanoparticles are extensively utilized due to their extremely small size, orientation, and unique physiochemical properties which can significantly alter the performance of materials with which they interact. Nanoparticles can be synthesized using different physical, chemical, and biological methods. Green synthesis offers a simple, environment friendly, and sustainable approach. In the present study, green synthesis of zinc oxide nanoparticles was carried out by using neem (*Azadirachta indica*) leaf extract with a zinc acetate solution. The aqueous plant extract served multiple functions by acting as a solvent, reducing agent, stabilizer, and capping agent during nanoparticle synthesis. The synthesized zinc oxide nanoparticles were subsequently characterized for their optical properties, particle size, functional groups, and crystalline structure using UV-Visible spectrophotometry, Transmission Electron Microscopy (TEM), Fourier Transform Infrared spectroscopy (FTIR), and X-ray Diffraction (XRD), respectively.

Keywords: Green synthesis, Neem leaf extract, XRD, TEM, FTIR, Zinc oxide nanoparticles

Introduction

Nanotechnology is science and technology deals with materials at the nanoscale, typically ranging from 1 to 100 nm (Willard *et al.*, 2014) ^[17]. Nanoparticles are described as very small particles that function as a whole unit with respect to their movement and properties (Prathna *et al.*, 2012) ^[8]. A nanoparticle may be defined as a microscopic particle below 100 nm in respect of at least single dimension. Due to their very small dimensions and large surface to volume ratio, it exhibits unique physical and chemical characteristics distinct from their bulk parts. Nanotechnology has emerged as an innovative area of research, offering new opportunities for applications in agriculture as well as other scientific subjects. The effectiveness of nanoparticles largely depends on factors such as their size, chemical composition, surface area, reactivity and the dose of application.

Metal oxide nanoparticles are considered as more remedial than other types material (Sanita Banerjee, 2012) ^[11]. However, their production through conventional physical and chemical ways involves several limitations, like the requirement of temperature and pressure conditions, prolonged reaction times, the use of costly and toxic chemicals and the generation of toxic substance. In view of these drawbacks, increasing attention has been directed toward the development of environmental friendly and sustainable synthesis methods or adoption of biological approaches for nanoparticle synthesis i.e. biomimetics which refers to the application of biological things in the formation of materials.

Various chemical and biological techniques have been used for the synthesis of zinc oxide nanoparticles. Among these, green synthesis has emerged as most environment friendly approach, wherein plant materials are used for the stable and large-scale production of metal oxide nanoparticles (Iravani, 2011) ^[6]. This method has multiple advantages, including procedural simplicity, single-step synthesis, cost efficiency, and high reproducibility (Ahmed, 2016) ^[1]. Plant extracts serve as effective reducing and capping agents, facilitating the formation of stable metal oxide nanoparticles.

Zinc oxide nanoparticles synthesized through green routes are generally regarded as non-toxic and biocompatible, making them more useful for particularly agricultural applications. In addition to zinc oxide, nanoparticles of silver, gold, copper, zinc, carbon, and iron have been successfully synthesized through green method, often exhibiting superior properties than those obtained through conventional chemical processes.

Synthesis of nanoparticles using plant materials offers multiple advantages over synthesis by using microorganism as the maintenance of cell cultures not require and involves minimal use of chemicals. This approach is cost-effective, less time consuming and free from interference by toxic substances, making it environmentally safe. Additionally, the process can be easily scaled up for large-scale nanoparticle production (Bhalerao and Borkar, 2017) [4].

The use of plant extracts for the synthesis of nanoparticles has considerably viable option to conventional physical and chemical synthesis methods, because of its simplicity and environmentally sustainable nature (Balogun *et al.*, 2020) [3]. In recent years plant-mediated synthesis of metal oxide nanoparticles has become a rapidly expanding research focus in nanotechnology (Sathya *et al.*, 2012) [12].

Neem leaf extract contains various phytochemicals that function as biological reducing agents, including flavones, organic acids, ketones, amides and aldehydes. Among these compounds, flavones and organic acids are water soluble in nature and play a key role in the reduction of zinc ions into nanoparticles (Sangeetha *et al.*, 2011) [10].

Plants synthesized some bioactive organic compounds such as polyphenols, flavonoids, alkaloids, terpenes, tannins, steroids and saponins as part of their defence mechanisms to withstand various biotic and abiotic stresses (Tiwari *et al.*, 2014) [15]. These are non-nutritive in nature and function as both reducing and stabilizing agents. Zinc ions present in the precursor solution interact with polyphenols in the plant extract to form intermediate complexes, which upon hydrolysis yield zinc hydroxide $[Zn(OH)_2]$. Subsequent transformation of zinc hydroxide results in the formation of zinc oxide (ZnO) nanoparticles.

Hence the aim of the study is to synthesize Zinc Oxide nanoparticles using neem leaf extract (*Azadirachta indica* L.) and to study its characterization using UV-spectrophotometer, TEM, FTIR and XRD analysis.

Materials and Methods

1. Procedure for green synthesis of zinc oxide nanoparticle (ZnO NPs) using leaf extract of Neem (*Azadirachta indica* L.).

1.1 Preparation of leaf extract of Neem (*Azadirachta indica* L.).

Green synthesis of zinc oxide nanoparticles (ZnO NPs) using neem leaf extract was carried out in two stages, beginning with the preparation of the plant extract. Fresh neem leaves were collected and thoroughly washed several times with tap water to remove adhering dust and impurities, followed by a final rinse with double distilled water and shade dried at room temperature. Five grams of the shade dried leaves were weighed and ground by using a mortar and pestle with 100 ml of distilled water to obtain an aqueous extract. The extract was transferred to a 250 ml beaker and heated at 80 °C for 10 minutes. After heating, the extract was allowed to cool to room temperature and

subsequently filtered through Whatman No. 1 filter paper. The filtrate was then centrifuged at 10000 rpm for 20-25 minutes. The resulting light-yellow supernatant was collected and stored in refrigerated conditions for further use for the synthesis of zinc oxide nanoparticles (Suke, 2022; Dhakane, 2023) [14, 5].

1.2 Synthesis of ZnO nanoparticles

1.2.1 Preparation of 1mM zinc acetate ($Zn(CH_3COO)_2 \cdot (H_2O)_2$ dihydrate) stock solution

Prepared 1mM zinc acetate solution by dissolving 0.219 g zinc acetate ($Zn(CH_3COO)_2 \cdot (H_2O)_2$ dihydrate) in 1000 ml distilled water and stored in ambient condition for further use.

1.2.2 Synthesis of zinc oxide nanoparticles (ZnO NPs)

The synthesis of zinc oxide nanoparticles starts with mixing neem leaf extract with 1mM zinc acetate solution in 3:7 ratio respectively. The mixture was heated at 80 °C for 10 min using hot water bath. Keep mixture for 24 hrs. at room temperature. The colour of the solution turns from light yellow to dark yellow to brown indicating the synthesis of zinc oxide nanoparticles. Then synthesized zinc oxide nanoparticles were separated by centrifuge the reaction mixture at 10000 rpm for 25 min at 4 °C temperature. Pellets or suspended material gathered at the base of centrifuge tube were collected by discarding supernant. Collected material washed repeatedly with distilled water and dried in a hot air oven at 100 to 130 °C for 40-45 min. The light yellow to brown colour powder obtained use as nanoparticles.

2) Characterization of green synthesized ZnO nanoparticles

The green synthesized zinc oxide nanoparticles were characterized for their optical properties, particle size, functional groups, and crystalline structure using UV-Visible spectrophotometry, Transmission Electron Microscopy (TEM), Fourier Transform Infrared spectroscopy (FTIR), and X-ray Diffraction (XRD), respectively.

Optical absorption of the green synthesized zinc oxide nanoparticles was recorded using a UV-visible spectrophotometer measured over a wavelength range of 200-800 nm. This observation was further confirmed by the appearance of sharp absorption peaks in UV-visible spectrum. For this spectral analysis, a small aliquot of the nanoparticle suspension was placed in a quartz cuvette and scanned within the specified wavelength range 200-800 nm. UV-visible spectrum measurements were carried out at the State Level Biotechnology Centre, Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri.

The particle size and morphology of the synthesized ZnO nanoparticles were determined using Transmission Electron Microscopy (TEM). Functional group analysis and identification of biomolecules involved in nanoparticle formation were performed using Fourier Transform Infrared (FTIR) spectroscopy, while the crystalline nature and average crystallite size were evaluated through X-ray Diffraction (XRD) analysis. These characterization studies were conducted at the Common Facility Centre (CFC) and Sophisticated Analytical Instrumentation Facility (SAIF), Shivaji University, Kolhapur.

Results and Discussion

1. UV-visible spectrophotometer (Nanodrop 1000 spectrophotometer)

The formation of green synthesized zinc oxide nanoparticles was confirmed by the presence of distinct and sharp

absorption peaks in the range of 330-400 nm as observed using a Nanodrop 1000 spectrophotometer (Thermo Scientific), thereby validating the successful synthesis of nanoparticles. (Fig. 1)

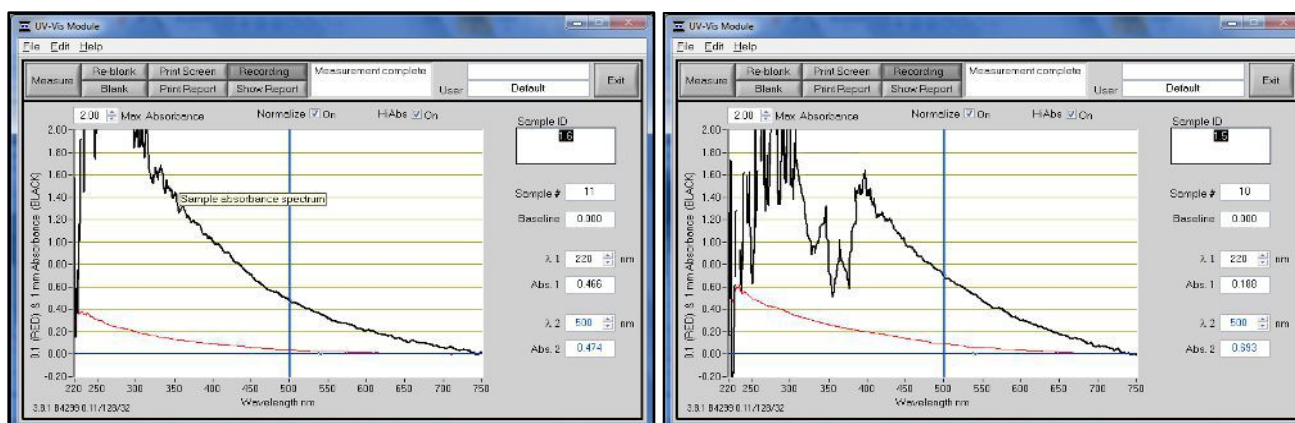


Fig 1: Sharp peaks shown by the absorption spectrum in Nanodrop 1000 spectrophotometer

2. TEM (Transmission Electron Microscopy)

The particle size of the green synthesized zinc oxide nanoparticles was determined in the range of 8.18-11.6 nm using Transmission Electron Microscopy (TEM) with a

JEOL JEM-2100 instrument. The analysis was carried out at the Common Facility Centre (CFC), Sophisticated Analytical Instrumentation Facility (SAIF), Shivaji University, Kolhapur. Fig-2.

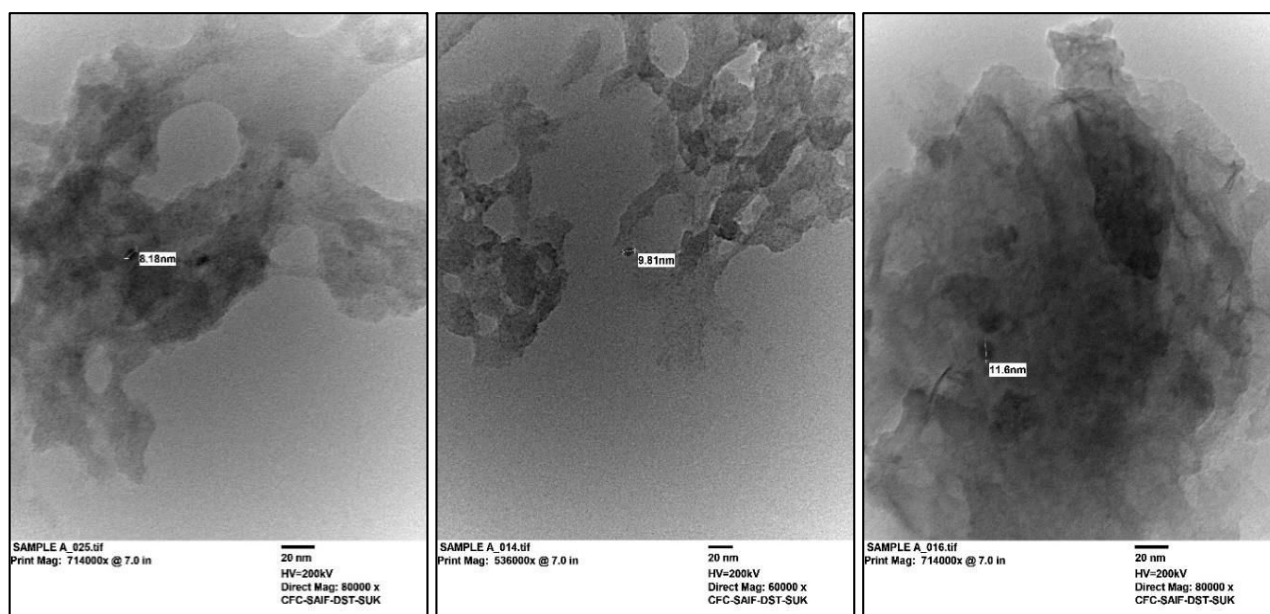


Fig 2: Size of the nanoparticles shown by the TEM-Transmission Electron Microscopy (Jeol-JEM-2100)

3. FTIR-Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared spectroscopy (FTIR) analysis was carried out over a spectral range of 4000-400 cm^{-1} . The presence of zinc oxide nanoparticles was confirmed by the characteristic absorption bands observed at 500-700 cm^{-1} (Sangeetha *et al.*, 2011; Vani *et al.*, 2011; Rajiv *et al.*, 2013) [11, 16, 9]. A broad absorption peak in the range of 3402-3424 cm^{-1} was attributed to O-H band and C = O functional groups, indicating the presence of aliphatic carboxylic acids. Peaks appearing between 1554-1588 cm^{-1} corresponded to presence of aromatic ring, while bands at 1022-1033 cm^{-1} were assigned to saturated primary alcohols. The absorption bands observed in the range of 2854-2931 cm^{-1} were due to presence of C-H stretching vibrations characteristic of aromatic aldehydes.

These functional groups are indication of terpenoid compounds found in the aqueous leaf extract of neem (*Azadirachta indica*) (Jha and Prasad, 2010; Senthilkumar and Sivakumar, 2014) [7, 13, 14]. The FTIR spectra also revealed that polyphenolic functional groups present in neem leaf extract play a significant role in the reduction of zinc ions. Based on the FTIR results, it can be assumed that alcohols, terpenoids, ketones, aldehydes, and carboxylic acids were surrounded with the synthesized nanoparticles. Additionally, phenolic constituents such as flavonoids, lignans, coumarins, tannins, quercetin, alkaloids, and cyanogenic glycosides present in neem leaves likely acted as strong capping agents, contributing to the stabilization of the nanoparticles (Awwad *et al.*, 2013) Fig-3.

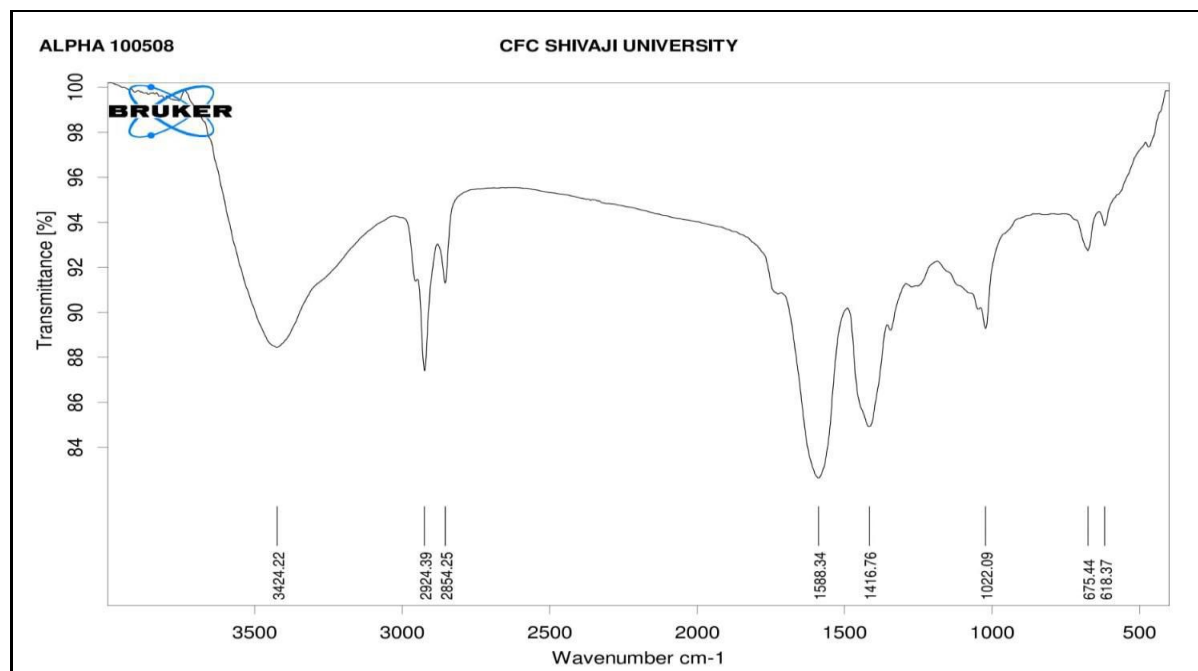


Fig 3: Graph shown by FTIR-Fourier Transform Infrared Spectroscopy

4. XRD-X-ray Diffraction

The average size of green synthesized zinc oxide nanoparticle (ZnO NPs) was found 20.16 nm by XRD-X-ray Diffraction (D8 advance-Bruker Germany). The size of nanoparticles calculated using the Debye-Scherrer equation, Fig.-4.

$$D = (K \times \lambda) / (\beta \times \cos \theta)$$

Where D is the crystallite size, K is the shape factor, λ is the wavelength of X-ray, β is the full width half maxima of XRD peaks in radian, and θ is the peak positions in radian.

Particle size of ZnO-NPs calculated using XRD data.								
SN	2 θ	θ	Cos (θ)	B (Degree)	B (Radian)	Size (Å)	Size (nm)	Avg. Size (nm)
1	12.5	6.25	0.994	0.4	0.00698	199.83	19.983	20.161
2	16.5	8.25	0.9896	0.4	0.00698	200.718	20.0718	
3	20	10	0.9848	0.4	0.00698	201.696	20.1696	
4	22.5	11.25	0.9816	0.4	0.00698	202.354	20.2354	
5	25	12.5	0.9763	0.4	0.00698	203.452	20.3452	

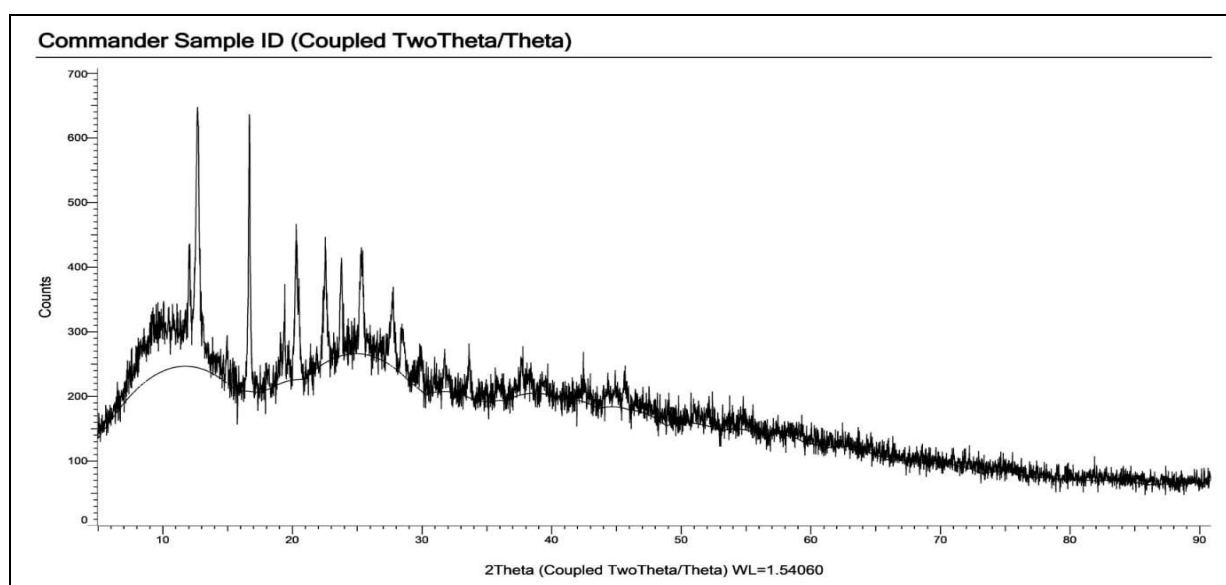


Fig 4: Graph shown by XRD-X-ray Diffraction

Conclusion

In the present investigation, zinc oxide nanoparticles (ZnO NPs) were successfully synthesized using zinc acetate as the precursor and aqueous neem (*Azadirachta indica*) leaf

extract as the reducing, capping, and stabilizing agent. The formation of ZnO nanoparticles was confirmed by the appearance of characteristic absorption peak in the range of 330-400 nm. Transmission Electron Microscopy (TEM)

analysis revealed that the particle size of the green synthesized ZnO nanoparticles ranged from 8.18 to 11.6 nm. Fourier Transform Infrared (FTIR) spectroscopy of the aqueous neem extract indicated the presence of functional groups involved in nanoparticle formation and stabilization. The absorption band observed between 500 and 700 cm^{-1} confirmed the presence of ZnO nanoparticles. A broad peak in the range of 3402-3424 cm^{-1} corresponded to O-H stretching and C = O groups, suggesting aliphatic carboxylic acids. The band at 1554-1588 cm^{-1} were associated with aromatic ring vibrations, while bands at 1022-1033 cm^{-1} indicated saturated primary alcohols. The bands observed at 2854-2931 cm^{-1} were attributed to C-H stretching vibrations of aromatic aldehydes. X-ray Diffraction (XRD) analysis further confirmed the crystalline nature of the synthesized ZnO nanoparticles, with an average particle size of 20.16 nm. The presence of bioactive compounds in neem leaf extract responsible for the bioreduction and stabilization of Zn ions into ZnO nanoparticles highlights the potential of this green synthesis approach. Based on the findings of the present study, neem-mediated ZnO nanoparticles may be effectively utilized in agricultural applications to enhance crop productivity, seed quality, and seed storability.

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