FEAT JOURNAL
FARM ENGINEERING AND AUTOMATION TECHNOLOGY JOURNAL
วารสารวิศวกรรมฟาร์มและเทคโนโลยีการควบคุมอัตโนมัติ

Eco-Friendly Synthesis and Characterization of Zinc Oxide Nanoparticles Using Margosa (*Azadirachta indica*) Leaf Extract for Sustainable Agricultural Nanotechnology

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Received: 10 May 2025
Revised: 10 June 2025
Accepted: 16 June 2025
Available online: 21 June 2025

Abstract

This study presents a sustainable method for synthesizing zinc oxide nanoparticles (ZnO NPs) using aqueous leaf extracts of *Azadirachta indica* (Margosa), serving as natural reducing and capping agents. The green synthesis approach aligns with environmentally conscious practices by eliminating harmful chemicals and minimizing toxic byproducts. The physicochemical characteristics of the ZnO NPs were thoroughly investigated using established analytical techniques. UV-Vis spectroscopy identified a distinct absorption peak at 363 nm, and the direct band-gap energy was calculated as 3.4 eV. FTIR analysis confirmed the involvement of biomolecules, such as proteins, aromatic compounds, and alcohols, in the reduction and stabilization processes. SEM imaging revealed the formation of agglomerated nanoparticles, while EDX confirmed the elemental composition of zinc and oxygen. DLS measurements estimated the average particle size to be approximately 47.2 nm. The results support the viability of using agricultural plant-based materials for the eco-friendly and cost-efficient production of metal oxide nanoparticles, contributing to advancements in green nanotechnology within agricultural and farm engineering applications.

Keywords : Green synthesis: Zinc oxide nanoparticles: Margosa leaves: *Azadirachta indica*

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1. Introduction

Nanotechnology is an emerging field that has garnered considerable attention due to its broad spectrum of applications across various sectors, including textiles, cosmetics, food processing, and agriculture. Among the nanomaterials explored, zinc oxide nanoparticles (ZnO NPs) have been extensively investigated for their multifunctional properties. These include their roles as antimicrobial agents [1] and their applications in catalysis [2-4], electronics [5], optoelectronics [6], and photochemical responses [7]. Additionally, ZnO NPs function as essential cofactors in plant metabolic processes, particularly in protein and tryptophan synthesis [8]

In recent years, the agro-industrial sector has increasingly utilized ZnO nanoparticles (ZnO NPs) as plant growth enhancers [8] and nano-scale fertilizers [9]. Due to their ultra-small size, these nanoparticles are more readily absorbed by plants compared to conventional bulk materials, enabling easier penetration into plant tissues. The application of ZnO NPs can lead to a range of physiological responses in plants, including the stimulation or inhibition of seed germination and seedling development [10,11], activation of metabolic genes [12-14], enhancement of photosynthetic activity [15-18], generation of reactive oxygen species [19], and even chromosomal alterations [20]. Zinc, a vital micronutrient for plant development, is instrumental in regulating key metabolic functions and activating enzymes such as carbonic anhydrase, aldolase, and hydrogenase [21] A deficiency in zinc, particularly during the early stages of growth, can significantly hinder seedling development and water uptake. Pre-soaking seeds in zinc-enriched solutions has been shown to improve moisture retention and promote more effective germination [8] Moreover, zinc contributes to the biosynthesis of tryptophan and auxin, crucial plant hormones that play a significant role in improving crop yield and quality [22]

Zinc oxide nanoparticles (ZnO NPs) can be synthesized through various chemical and biological methods, each offering distinct advantages and limitations. Chemical methods typically involve precipitation, sol-gel, or hydrothermal techniques using zinc salts and strong chemical reducing agents [23-26], which often require high energy input and may produce toxic byproducts. In contrast, biological or green synthesis methods employ microorganisms or plant extracts as eco-friendly reducing and capping agents, minimizing environmental

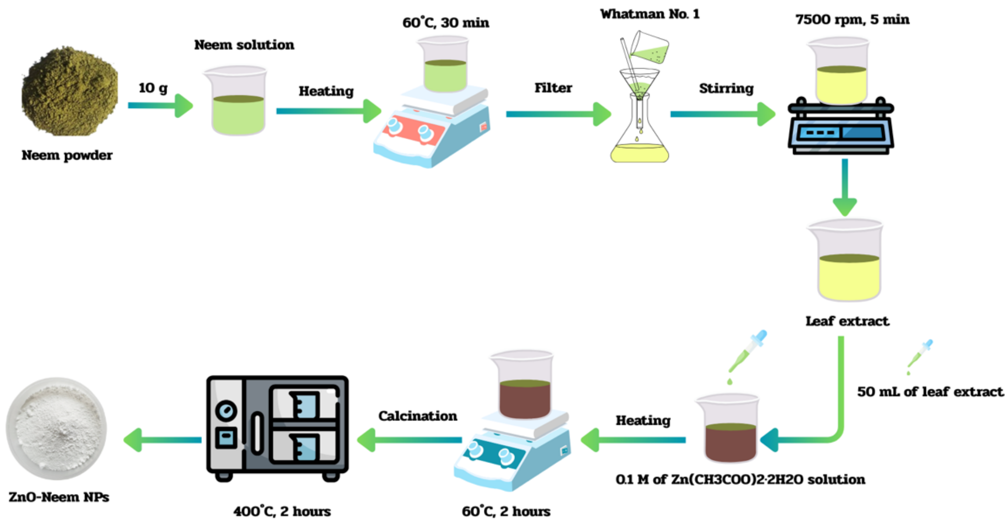


Figure 2 Procedure for synthesizing ZnO from Margosa leaf.

impact [27,28]. Among these, plant-mediated synthesis is gaining popularity due to its simplicity, cost-effectiveness, and the rich presence of bioactive compounds that facilitate nanoparticle formation [29]. The abundance and diversity of plant species provide a wide range of natural reagents for ZnO NP synthesis, making this method both scalable and sustainable. In particular, Thailand is rich in Margosa trees (in Thai: สะเดา), whose leaves (Fig. 1) are known to contain phytochemicals suitable for green synthesis, presenting a valuable local resource for environmentally friendly ZnO nanoparticle production.

Figure 1 Margosa leaves (*Azadirachta indica*)

Margosa, commonly referred to as neem (*Azadirachta indica*), is a tree species within the mahogany family (*Meliaceae*), native to the Indian subcontinent. It has a long history of use in traditional medicine, owing to its wide range of therapeutic properties. The leaves of the Margosa tree are rich in bioactive compounds, including flavonoids, terpenoids, and alkaloids, which have been shown to possess anti-inflammatory, antifungal, antibacterial, antiviral, and anticancer effects [30]

The primary aim of this study is to develop zinc oxide nanoparticles (ZnO NPs) through an environmentally friendly and simple green synthesis approach using leaf extracts from Margosa. This method offers a sustainable and cost-efficient alternative to conventional nanoparticle synthesis techniques. To evaluate the characteristics of the resulting ZnO NPs, several advanced analytical instruments were employed, including Scanning Electron Microscopy (SEM) with Energy Dispersive X-ray Spectroscopy (EDX), Dynamic Light Scattering (DLS), and Fourier Transform Infrared Spectroscopy (FTIR). These tools provide essential insights into the structural and physicochemical properties of the nanoparticles, enhancing the overall understanding of the synthesized material.

2. Materials and Methods

2.1. Preparation of extract

Leaves of Margosa were collected from Nakhon Phanom University, located in Nakhon Phanom, Thailand. The aqueous extract was prepared following the procedure outlined by Sekhar et al. [31], with minor adjustments. Initially, the leaves were thoroughly cleaned multiple times using double-distilled water to remove surface contaminants. Then, Margosa leaves was dried in ambient temperature (25 - 30°C) for 7 – 10 days. Note that, this process must avoid direct sunlight to preserve bioactive phytochemicals. To ensure complete moisture removal, the dried leaves were further dried in a hot-air oven at 50°C for 4 hr. The fully dried leaves were ground using a clean electric grinder into fine powder, sieved through a 60-mesh sieve to ensure uniformity. The resulting Margosa powder was stored in an airtight amber glass bottle at room temperature until used for aqueous extraction.

2.2. Green Synthesis of ZnO NPs

The synthesis of zinc oxide nanoparticles (ZnO NPs) using Neem (Margosa) leaf extract, as illustrated in Fig. 2, begins with 10 grams of Neem powder, which is mixed with distilled water and heated at 60°C for 30 minutes to obtain a Neem solution. This solution is then filtered using Whatman No. 1 filter paper to remove solid residues, followed by centrifugation at 7500 rpm for 5 minutes to separate the supernatant, which serves as the Neem leaf extract. Subsequently, 50 mL of this extract is added to 100 mL of 0.1 M zinc acetate dihydrate $[Zn(CH_3COO)_2 \cdot 2H_2O]$ solution. The mixture is then heated at 60°C for 2 hours under constant stirring to facilitate the reaction between the phytochemicals in the

Neem extract and zinc ions. The resulting mixture is subjected to calcination at 400°C for 2 hours in a hot air oven to convert the reaction product into a white ZnO nanopowder. The final product, ZnO-Neem nanoparticles, is collected and ready for further characterization and application.

2.3. Characterization of ZnO NPs

The green-synthesized ZnO nanoparticles were characterized using a UV-Vis spectrophotometer, with measurements taken in the wavelength range of 300–700 nm (Avaspec-EDU). The surface morphology and elemental composition were analyzed using a scanning electron microscope (SEM) coupled with an energy dispersive X-ray spectrometer (EDX, Quanta 250 FEI Company, Eindhoven, The Netherlands). Particle size distribution was determined using dynamic light scattering (Malvern Zetasizer ZSP, Worcestershire, UK). The functional groups present in both the synthesized nanoparticles and the leaf extract were identified using a Fourier Transform Infrared (FT-IR) spectrophotometer (PerkinElmer Inc., Buckinghamshire, UK), employing the KBr pellet method within the spectral range of 4000–500 cm^{-1} for comparative analysis.

3. Results and Discussion

Plants, due to the presence of secondary metabolites, can reduce zinc ions in a solution to form zinc oxide. The plant extract not only acts as a reducing agent but also stabilizes the nanoparticles. This was confirmed through UV-Vis spectroscopic analysis conducted in the range of 280 nm to 800 nm. The ZnO nanoparticles displayed a characteristic absorption peak at 363 nm, attributed to the surface plasmon resonance effect and the electronic transitions between the conduction and valence bands, corresponding to the intrinsic band gap of the ZnO nanoparticles as shown in Fig. 3. The band gap energy was calculated using the equation $Eg = 1240/\lambda$ eV and found to be 3.4 eV, which is consistent with previously reported values for ZnO nanoparticles. Additionally, the purity of the synthesized ZnO nanoparticles was validated by the absence of any extra peaks in the absorption spectra that could be linked to bioactive compounds from the extract. This finding aligns with earlier studies where the absorption peaks of ZnO nanoparticles synthesized using *Monsonia burkeana* were observed at 325 nm [32], 360 nm for *Aloe barbadensis* (Aloe vera) [33], and 389 nm for *Trianthema portulacastrum* (Desert horsepurslane) [34] extract.

As suggested by Khan et al. [35], the hydroxyl and phenolic compounds in the leaf extracts play a critical role in reducing Zn^{2+} ions to Zn^{+} ions, facilitating the nanoparticle formation. Similarly, Ezealisiji et al. [36] proposed that zinc nitrate undergoes ionization in the aqueous medium and is reduced to Zn^{+} by the phytochemicals in the extract, with the hydroxyl group in polyphenols potentially forming zinc hydroxide via hydrolysis.

The observed surface morphology confirms the successful synthesis of nanoparticles, which appear in an agglomerated form. Several studies have highlighted the influence of nanoparticle morphology on the enhanced functional properties of ZnO.

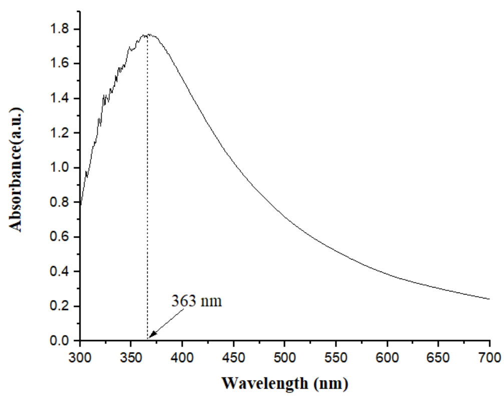
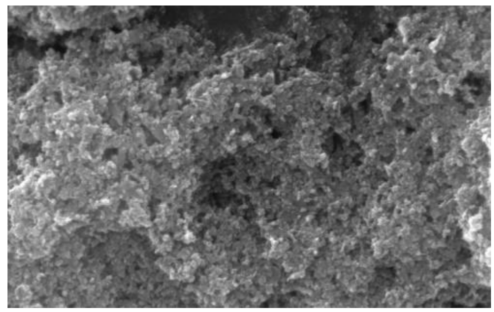
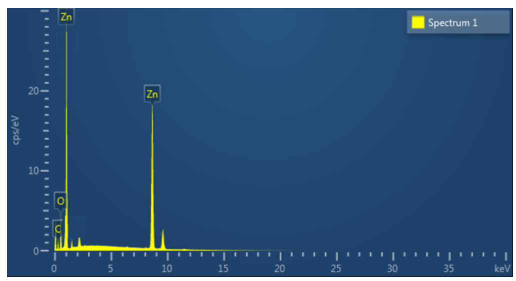


Figure 3 UV-Vis absorption spectra of ZnO NPs.

The SEM analysis in this study is consistent with previous research, which reported that extracts from *Albizia lebbeck* (Indian siris) stem bark [37] also led to the formation of similarly spherical ZnO nanoparticles. A key advantage of spherical nanoparticles is their improved ability to penetrate cellular walls more effectively. ZnO nanoparticles have also been widely recognized for their potent antibacterial, antifungal, and anticancer properties.



(A)



(B)

Figure 4 (A) SEM photographs and (B) EDX pattern of ZnO NPs.

Energy Dispersive X-ray (EDX) analysis was performed to assess the elemental composition and structural characteristics of the synthesized ZnO nanoparticles. The detected signals for zinc and oxygen, as shown in Fig. 4, confirm the chemical purity of the nanoparticles. The elemental composition was determined to be 80.24% zinc and 19.76% oxygen.

These results are in close agreement with the findings of Saputra and Yulizar [38], who reported Zn (80.3%) and O (19.65%) during the green synthesis of ZnO NPs using Imperata cylindrica (Cogon grass) leaf extract. In contrast, Ngoepe et al. [39] observed a higher zinc content relative to oxygen when synthesizing ZnO NPs with Trianthema portulacastrum (Desert horsepurslane) extract. The underlying mechanism of ZnO nanoparticle formation involves the reduction of Zn2+ ions through interactions with bioactive compounds present in the plant extracts. This includes electron donation from carbonyl groups via ligand bonding, which facilitates the conversion of Zn^{2+} into ZnO nanoparticles. The mechanism of Zn^{2+} reduction to ZnO is shown in Fig. 5.

Ionization of Zinc Acetate nAqueous Medium

$$Zn(CH_3COO)_2 \cdot 2H_2O \longrightarrow Zn^{2+} + 2CH_3COO^- + 2H_2O$$

Reduction by Phytochemicals (e.g., carbonyls, phenolics)

$$Zn^{2+} + R-C=O + 2OH^- \longrightarrow Zn(OH)_2 + R-C=O^{*+}$$

Let R-C-O represent a phytochemical with a carbonyl group capable of electron dona

Thermal Decomposition during Calcination(at 400 °C)

$$Zn(OH)_2 \xrightarrow{\Delta} ZnO + H_2O$$

Net Reaction (Simplified Overall Equation)

$$Zn^{2+} + 2OH^- \xrightarrow{\text{Plant extract, } \Delta} ZnO + H_2O$$

Figure 5 Reduction mechanism of Zn^{2+} to ZnO

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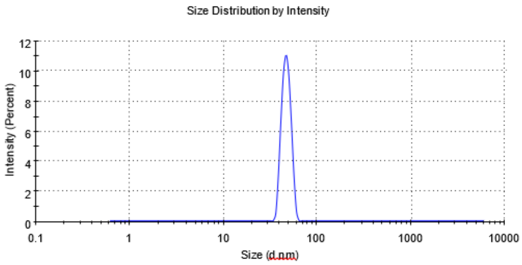

Figure 6 Size distribution of green synthesized Zinc oxide nanoparticles.

Particle size of ZnO NPs was characterized by DLS. Fig. 6 shows particle size distribution which mean size is 47.2 nm. Moreover, the deviation of the size is monomodal distribution. This implies that the synthesizing is archive to obtained monodisperse ZnO NPs.

Fourier Transform Infrared (FT-IR) spectroscopy was employed to identify the functional groups involved in the synthesis of ZnO nanoparticles. The results indicate that the formation of ZnO NPs is facilitated by interactions with bioactive compounds such as phenolics, alkynes, terpenoids, and flavonoids. As shown in Fig. 7, the FT-IR spectrum of the synthesized nanoparticles, recorded in the 400–4000 cm^{-1} range, displays characteristic absorption bands. These bands correspond to various vibrational modes of functional groups, which played a crucial role in the reduction of zinc ions and the stabilization of ZnO nanoparticles.

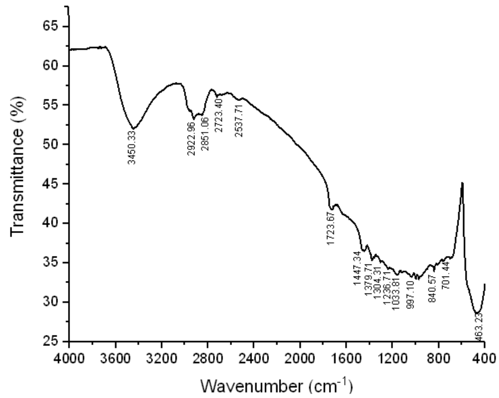

Figure 7 FTIR spectrum of green synthesized Zinc oxide nanoparticles.

The broad absorption band near 3450 cm^{-1} is associated with the O–H stretching vibration of phenolic compounds. A peak around 1723 cm^{-1} indicates the presence of alkene groups, while the band at 1379 cm^{-1} is related to C–N stretching vibrations of amine groups. The C–O stretching vibrations of ester and carboxylic acid functional groups are observed between 1000 and 1300 cm^{-1}. Additionally, the sharp peaks at 701 cm^{-1} and 463 cm^{-1} confirm the presence of Zn–O stretching vibrations, indicating successful formation of zinc oxide nanoparticles.

4. Conclusion

This study effectively demonstrated an eco-friendly synthesis of zinc oxide nanoparticles (ZnO NPs) utilizing Margosa (*Azadirachta indica*) leaf extract as both a reducing and stabilizing agent.

The structural and chemical characteristics of the nanoparticles were analyzed using various techniques such as UV–Vis spectroscopy, FTIR, SEM, EDX, and DLS. The synthesized ZnO NPs showed an average particle size of approximately 47.2 nm and a distinct UV–Vis absorption peak at 363 nm. FTIR analysis suggested the involvement of proteins, aromatic compounds, and alcohols in the nanoparticle formation. SEM and EDX confirmed the agglomerated structure of the nanoparticles and their composition of zinc and oxygen. These findings support the viability of Margosa leaf extract as a sustainable, low-cost method for producing ZnO NPs, with potential applications across sectors like agriculture, medicine, and industry. Future investigations are recommended to refine the synthesis protocol and further explore the biocompatibility and safety of the nanoparticles.

5. Acknowledgement

The authors gratefully acknowledge the financial support provided by Thailand Science Research and Innovation (TSRI) on the Fundamental Fund Project.

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