Uttar Pradesh Journal of Zoology

Volume 45, Issue 16, Page 345-352, 2024; Article no.UPJOZ.3843
ISSN: 0256-971X (P)

Green Synthesis and Characterization of Zinc Oxide Nanoparticles from *Mukia maderaspatana* (L.) Roem. Leaf Extract and their Antibacterial Activity

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i164315

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3843

Original Research Article

Received: 19/05/2024
Accepted: 23/07/2024
Published: 01/08/2024

ABSTRACT

Objective: To green synthesize and characterize zinc oxide nanoparticles from *Mukia maderaspatana* (L.) Roem. Leaf extract and study its antibacterial activity.
Materials and Methods: The leaves of *Mukia maderaspatana* were collected, shade dried, powdered and leaf extract was prepared using standard procedure. Zinc oxide nanoparticles was

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Cite as: *V., Catherine Sheeja, Anami Augustus Arul A., Ani Besant S., Blessy R., and Hanna Jeeja Alexander. 2024. "Green Synthesis and Characterization of Zinc Oxide Nanoparticles from Mukia Maderaspatana (L.) Roem. Leaf Extract and Their Antibacterial Activity". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (16):345-52. https://doi.org/10.56557/upjoz/2024/v45i164315.*

synthesized using the leaf extract, followed by characterization studies such as UV-Vis, FT-IR and SEM were carried out. The synthesized zinc oxide nanoparticles was used for anti-microbial activity.
Results and Discussion: Morphological, structural and antimicrobial properties of synthesized nanoparticles are characterized by using UV-Vis Spectrophotometer, FT-IR and SEM followed by Antimicrobial study using Disc Diffusion Method. The UV Vis spectroscopic analysis of synthesized zinc nanoparticles showed the maximum absorbance at 265.50 nm. SEM analysis shows that synthesized nanoparticles are found to be predominantly hexagonal in shape. Zinc oxide nanoparticles inhibited *Escherichia coli* with an inhibition zone of 16 mm.
Conclusion: The study reveals an efficient, ecofriendly and simple method of green synthesis of ZnO nanoparticles using green synthetic approach.

Keywords: Mukia maderaspatana; zinc oxide nanoparticles; green synthesis; SEM; Anti-bacterial activities; FT-IR analysis; UV-Vis spectrophotometer; Escherichia coli.

1. INTRODUCTION

In recent years, nanoparticles have garnered significant interest and have been widely utilized in biological research [1,2]. As one of the fastest-growing fields within nanotechnology, engineering technology and nanosystems have seen rapid development [3]. This progress has opened new horizons in nanoscience, particularly in areas such as drug delivery, gene delivery, nanomedicine, and biosensing [4,5]. Various synthesis techniques, including chemical, physical, and biogenic methods, have been developed over the years.

Traditionally, *Mukia maderaspatana* (L.) Roem, an annual monoecious plant from the Cucurbitaceae family, has been used as an herbal medicine in the Siddha and Ayurvedic systems. In Tamil Nadu, it is commonly found along roadside trails and railway lines, particularly in South India. Historically, this herb has been employed to repel monsoon diseases [6]. In the Kaniyakumari district, the leaves are widely used as greens [7] and consumed as decoctions [8,9]. According to Ayurveda, the leaves and roots of *M. maderaspatana* are prescribed for various ailments, including asthma, cough, burning sensations, dyspepsia, flatulence, colic, constipation, ulcers, neuralgia, odontalgia, and vertigo [10].

Zinc, an essential micronutrient, plays a crucial role as an enzyme component and cofactor. Zinc oxide nanoparticles (ZnO NPs) are particularly important for plants due to their role in chlorophyll production, fertilization, pollen function, and germination [11]. The unique properties of nanoparticles, such as "redox" activity, sedimentation rate, and agglomeration, can induce morphological changes in neuron cells and cultured rodent microglia [12]. Biological sources used in the green synthesis of nanoparticles contain biologically active compounds such as enzymes, proteins, polyphenols, flavonoids, and terpenoids. These compounds act as catalyzing, reducing, stabilizing, or capping agents, enabling a one-step synthesis process [13,14]. ZnO nanoparticles exhibit a variety of properties due to their distinctive size and shape [15].

Given these unique properties, there is a need to develop new synthesis methods for nanoparticles that require fewer reaction conditions, use cheaper reagents, and minimize environmental harm. This study aims to examine the efficacy of green-synthesized zinc nanoparticles and the aqueous extract of *M. maderaspatana* leaves against two bacterial pathogens, Escherichia coli and Staphylococcus aureus, using two different concentrations: T1 (50 µl) and T2 (100 µl).

2. MATERIALS AND METHODS

2.1 Collection of Plant Leaf

Mukia maderaspatana leaf was collected from Marthandam, Kaniyakumari district. Polyethene bags were tightly packed with collected leaves, then the leaves were transferred to the laboratory for analysis. A herbarium specimen was prepared and deposited at the Botany Department Herbarium, Holy Cross College (Autonomous), Nagercoil.

2.2 Preparation of Leaf Extract

Plant leaf extract of *Mukia maderaspatana* was prepared by first washing the surface of the leaves under running tap water and then with distilled water. The leaves were kept in the dark at room temperature for drying. Then it was

ground into powder using a blender. Two grams of the ground sample were dissolved in 100 ml of water and autoclaved for five minutes in pressure cooker. The extract was left, until it reaches the room temperature and then filtered through Whatman No.1 filter paper. The filtrate obtained was stored in the refrigerator for further experiments.

2.3 Synthesis of Zinc Oxide Nanoparticles Using *Mukia maderaspatana* Leaf Extract

In 100 millilitres of Milli-Q-Water, 0.378 g of Zinc oxide has been dissolved using a magnetic stirrer for 1 hour to ensure complete solubilisation to prepare 0.1M Zinc oxide solution. From that 80 ml solution was added to 20 ml of aqueous leaf extract of *M. maderaspatana* and the solution was stirred continuously. 2 M NaOH was added drop-by-drop while stirring to maintain pH 12. After 2 hours five minutes centrifugation at 4000 rpm was performed. Typically, nanoparticles are detected by centrifugation when the mixture changes the colour [16]. The synthesized Zinc oxide nanoparticles changes from green to fine white powder by visual inspection followed by characterization such as UV-visible spectrum, Fourier transform infrared (FTIR) and scanning electron microscope (SEM).

2.4 Characterization

An UV-Vis spectrophotometer (JASCO V-630) was used to record the bioreduction of synthesized zinc nanoparticle solutions, which was scanned from 200 to 800nm using a Shimadzu UV 1700 series at a scanning speed of 1nm. A UV–vis spectrometer was used to analyze ZnO's optical absorption properties. Plant extracts were analyzed using FT-IR spectroscopy for the formation of zinc nanoparticles and their different functional groups. By analyzing samples of synthesized NPs using Kemp's [17] Fourier Transform Infrared (FTIR) method, functional groups in the NPs were identified and their bonding was studied. The instrument used to FTIR analysis was IR Affinity – 1 (FTIR spectrophotometer) Shimadzu, Japan and the spectra was scanned in the range from 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1}. The spectrum was recorded as a graphical chart. SEM analysis was used to characterize surface morphology and were operated at an accelerated voltage at 20 kV.

2.5 Antibacterial Assay by Agar well Diffusion Method of Green Synthesized Zinc Oxide Nanoparticles

The Agar well diffusion method was used to determine the antibacterial activity of Zinc oxide nanoparticles synthesized from *Mukia maderaspatana* leaf extract. Zinc oxide nanoparticles were tested against clinical strains of *Escherichia coli* and *Staphylococcus aureus* for their antibacterial activity by the following method of Valgas et al., [18]. Muller and Hinton agar (MHA) medium (Hi-Media Pvt. Ltd., Mumbai) was used as a medium for this study. The inoculums were obtained from the Microbial Type Culture Collection (MTCC) in Chandigarh. An MHA medium solution is poured into the petri plate after some time. A sterile swab moistened with the bacterial suspension was used to spread the inoculums onto the solid plates after the media was solidified. The plates were incubated at 37°C for 24 hrs. Gentamycin was used as a control in our study. After incubation, the plates were observed and the zone of bacterial growth inhibition around the wells was measured in mm.

3. RESULTS AND DISCUSSION

3.1 UV-analysis

The UV-Vis spectroscopy is used to determine the amount of light extinction (scatter + absorption) that passes through the sample. Due to the unique optical properties of nanoparticles, UV-Vis is a valuable tool for identifying, characterizing and studying nanomaterials based on their size, shape, concentration, and agglomeration state. In a UV-spectrophotometer, the absorbance of biosynthesized ZnO nanoparticles ranged from 200 to 800 nm. According to spectroscopic analysis, biosynthesized ZnO nanoparticles in the reaction mixture had a maximum absorbance at 265.50 nm. There was strong agreement between the experimental results of Oladiran and Olabisi, [19] and the results of these experimental investigations on synthesized ZnO nanoparticles from $ZnCl_2$ using the wet chemical method at room temperature (Fig. 1).

3.2 FT-IR Analysis

FT-IR reveals the composition and formation of functional groups in synthesised ZnO nanoparticles. Synthesized ZnO nanoparticles

show an FTIR spectrum which reveals the possible biomolecules responsible for the reduction of zinc ions and their interaction with ZnO nanoparticles (Fig. 2). Flavonoids, alkynes, terpenoids and phenolic compounds are also suspected to interact with ZnO to form nanoparticles. The IR spectrum of ZnO NPs shows intense bands at 482.17 cm^{-1}, 714.58 cm^{-1}, 770.51 cm^{-1}, 844.76 cm^{-1}, 1029.92 cm^{-1}, 1088.74 cm^{-1}, 1168.78 cm^{-1}, 1384.79 cm^{-1}, 1526.55 cm^{-1}, 1524.62 cm^{-1}, 2106.12 cm^{-1} and 3240.19 cm^{-1}.

As described by Rad et al., [20], the peaks that appeared at 3200–3600 cm^{-1} in the FTIR spectrum can be corroborated by the O−H stretching alcohols, stretching vibrations of the primary and secondary amines as well as C−H stretching of alkanes. The absorption peak at 482.17cm^{-1} is associated with metal-oxygen (ZnO stretching vibrations) vibration mode, whereas a similar peak has been observed in the findings on the synthesis of ZnO nanoparticles by sonochemistry [21]. The peak at 1565 cm^{-1} indicated the (C-C) aromatic stretching which was confirmed by Ramesh and others in their synthesis of Zinc Oxide nanoparticles in combination with *Cassia auriculata* leaf extract in [22]. In *Cyathea* plant, there is a peak at 1526.55 cm^{-1}, which indicates nitro compound stretching,

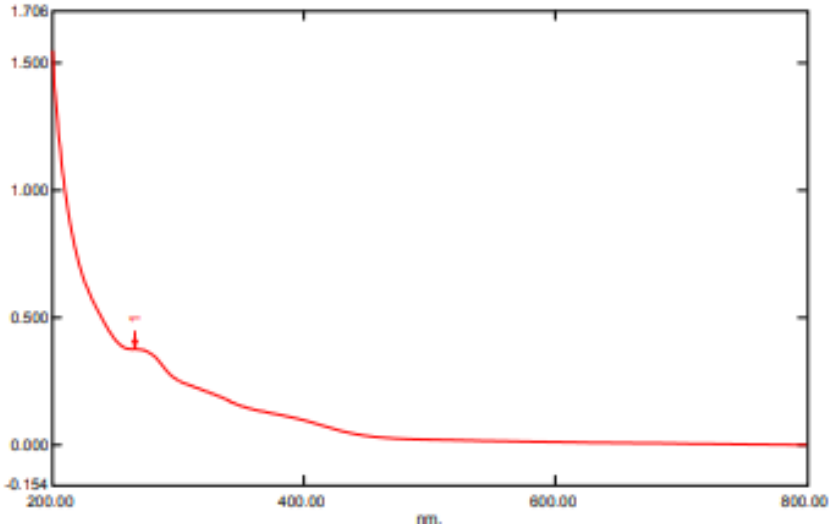


Fig. 1. UV-Vis spectrum of synthesised zinc oxide nanoparticles

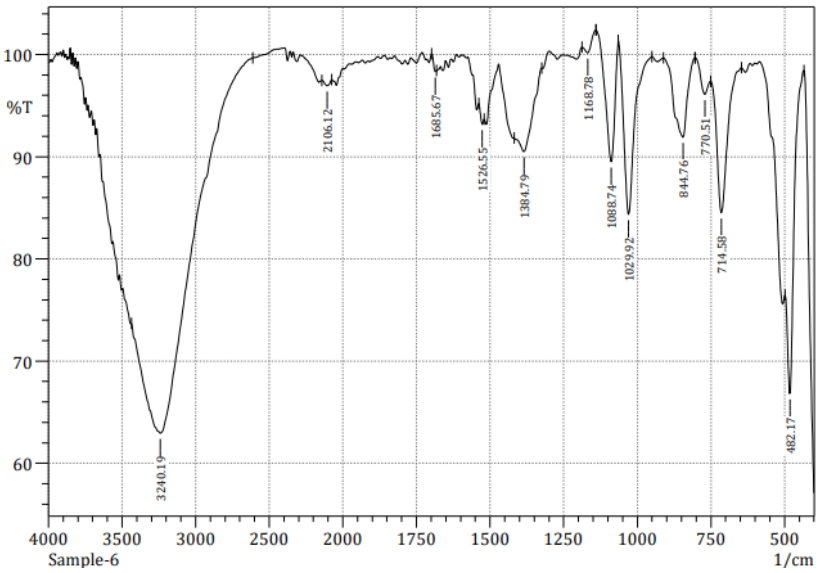


Fig. 2. FTIR spectrum of synthesised Zinc Oxide nanoparticles

in agreement with Janakiraman and Johnson, [23] and in *Embelia ribes* by Kamble and Gaikwad, [24]. The strong peak at 1384cm^{-1} can be attributed to asymmetric stretching vibrations of nitrate ions. There have already been reports of zinc oxide nanoparticles having beneficial effects [25], zinc oxide synthesis from *Sesbania grandiflora* leaf extract [26], as well as zinc oxide nanoparticles synthesized from *Pongamia Pinnata* leaf extract [27].

3.3 SEM

The green method of synthesizing zinc nanoparticles was analysed using a scanning electron microscope. According to the SEM image Plate 1, the ZnO nanoparticles are hexagonal in shape, and their sizes are < 50nm in range. Based on the results of Parthasarathy et al., [1] it appears that hexagonal-shaped nanoparticles and aggregated molecules are formed during the green synthesize of ZnO nanoparticles from *Ocimum basilicum* leaf extract.

3.4 Antibacterial Activity of Green Synthesized Zinc Oxide Nanoparticles

At two different concentrations T_1 (50 µl) and T_2 (100 µl), green synthesized zinc oxide nanoparticles and aqueous extract of *Mukia maderaspatna* leaves were assessed for antibacterial activity against selected bacterial pathogens such as *Escherichia coli* and *Staphylococcus aureus* and gentamycin was the control.

At T_2 (100 µl), green synthesized Zinc Oxide nanoparticles exhibit an inhibition zone (16 mm) against *E. coli*, while no inhibition was detected at T_1 (50 µl). The zinc oxide nanoparticles shown inhibition zones of 12 mm and 15 mm at T_1 (50 µl) and T_2 (100 µl) concentrations against *S. aureus*. In both concentrations of *M. maderaspatna* leaf extract, no zone of inhibition was observed (Plate 2 and Table 1).

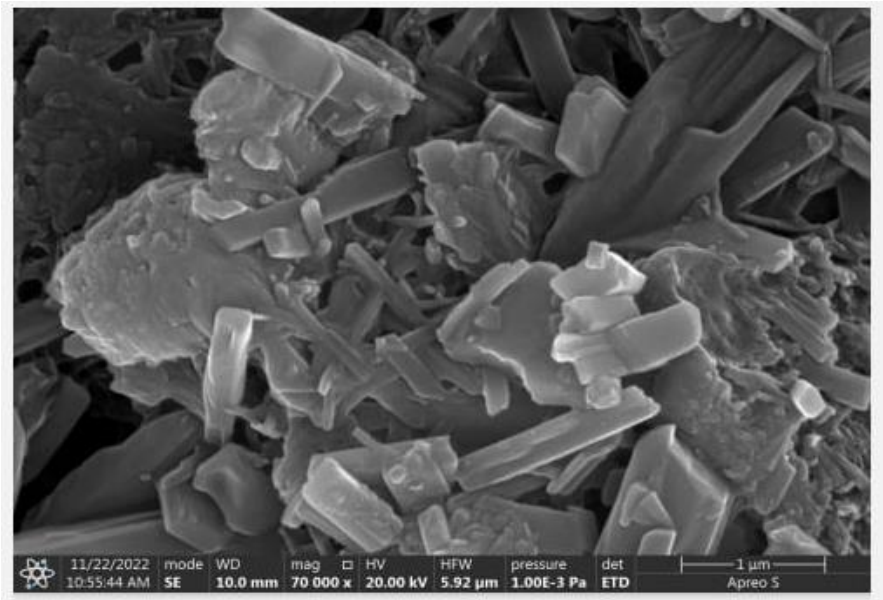


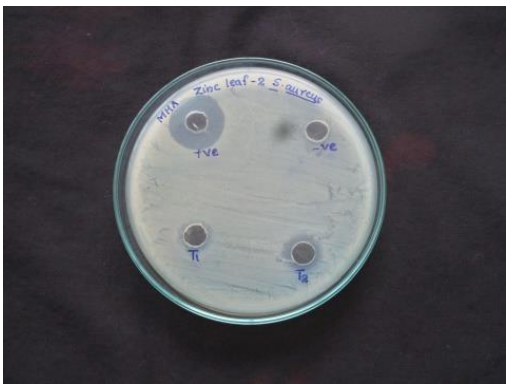
Plate 1. SEM images of Zinc Oxide nanoparticles

Table 1. Antibacterial activity of synthesised Zinc oxide nanoparticles and leaf extract

Sl.No	Sample	Micro organisms	Zone of inhibition (mm)			
			Standard Gentamycin (160 mcg)	Negative control	T1 (50 µl)	T_2 (100 µl)
1.	Zinc Oxide	*E. coli*	21 mm	-	-	(16 mm)
2.	Nanoparticle	*S. aureus*	22 mm	-	(12 mm)	(15 mm)
3.	Aqueous Leaf	*E. coli*	24 mm	-	-	-
4.	Extract	*S. aureus*	18 mm	-	-	-

Zinc Oxide Nanoparicles - *Escherichia coli*

Leaf extract - *Escherichia coli*



Zinc Oxide Nanoparicles - *Staphylococcus aureus*

Leaf extract - Staphylococcus aureus

Plate 2. Antibacterial activity of synthesised Zinc Oxide nanoparticles and leaf extract

ZnO NPs have antibacterial activity caused by penetration and disintegration of membranes by smaller NPs, leading to lysis of cells [28,29]. Inactive plants or inactive constituents do not indicate absence of therapeutic constituents [30]. The crude extract may contain inadequate amounts of active compounds. As a result, only high doses can prove a lack of activity [31]. A large zone of inhibition indicates a susceptible organism, whereas a small or no zone of inhibition indicates a resistant organism.

4. CONCLUSION

Finally, nanoscience and nanotechnology are concerned with developing eco-friendly processes for synthesizing zinc nanoparticles. Our study demonstrates that the successful green synthesis of Zinc Oxide nanoparticles from *Mukia maderaspatna* leaf extract and its anti-microbial activity. A UV-Vis analysis was done on Zinc Oxide nanoparticles to determine their optical properties. According to the absorption spectrum, presence of Zinc Oxide nanoparticles was conformed. By using FT-IR analysis, it was confirmed that the leaf extract contains a functional group and by using the disc diffusion method, *M maderaspatna* leaf extract and zinc nanoparticles were further investigated for their antimicrobial activity. According to the results, Zinc Oxide nanoparticles derived from *M. maderaspatna* leaf extract inhibit the growth of various pathogenic microorganisms such as *Escherichia coli*, *Staphylococcus aureus* and it was not observed in leaf extract.

ACKNOWLEDGEMENT

We sincerely acknowledge the University Grants Commission (UGC), New Delhi, India for financial assistance in the form of JRF to first author. The authors also thank the FIST Lab and the Department of Botany, Holy Cross College (Autonomous), Nagercoil for providing the necessary infrastructure for being able to carry out this research.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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