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Biomimetic Approach to Phyto-Mediated Synthesis of Zinc Oxide Nanoparticles from *Waltheria Indica* Root Extract and Exploring their Potential *In-Vitro* Antibactertial Activity

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KEYWORDS

Biofabrication, *Waltheria indica* roots, FTIR, XRD, SEM-EDAX, *In-vitro* antibacterial activity.

ABSTRACT:

This study investigates biogenic ZnO NPs synthesised from *Waltheria indica* root extract and their antibacterial properties. UV-visible, FTIR, XRD, and SEM-EDAX confirmed ZnO NP formation. UV-Vis spectrophotometers show ZnO NP formation with a 374 nm SPR peak. XRD confirmed the NPs' structural crystallinity. SEM micrographs show hexagonal ZnO NPs. *W. indica* root extract-mediated ZnO NPs from biological synthesis inhibited many microbial strains. The study found that ZnO NPs have antibacterial properties against all tested microorganisms, with a 13-mm zone of inhibition for *S. flexneri* at 100 µg/mL concentration. This suggests that biologically synthesised ZnO nanoparticles may be utilised in therapeutics to improve effectiveness, owing to their significant antibacterial properties. Moreover, further investigation may reveal new opportunities for developing innovative and secure treatments for bacterial infections through the use of biofabricated ZnO nanoparticles.

1. Introduction

Nanotechnology is one of quickly fascinating interdisciplinary research, chemistry, aerospace etc. The objective encompasses the synthesis and application of nanoparticles, characterized by at least one dimension ranging from 1 to 100 nm. This study examines these particles in terms of design, manufacturing, characterization, and the application of small functional systems derived from these materials. These nanoparticles exhibit 0D, 1D, 2D, and 3D structures, contingent upon their size [1]. The optical property is a principal and fundamental characteristic of nanoparticles. The importance of these nanoparticles became evident when researchers discovered that size influences the physicochemical properties of the material. The active surface phenomenon and increased surface area of metal nanoparticles have demonstrated effectiveness in biomedical research. Nonetheless, the fundamental potential characteristics of nanoparticles (metals) are inherently enduring, and their surface properties can be altered based on their application in environmental and biological sciences [2].

Due to the stable extensive properties are unique properties of the (metal) nanoparticle these can be used in various fields. Nanoparticles can be categorized into various types based on their morphology, size, and shape; several examples are provided below. Organic-nanoparticles. Inorganic nanoparticles include metal nanoparticles, ceramic nanoparticles, metal oxide nanoparticles, and biological nanoparticles, also referred to as bio nanoparticles [3]. Metallic nanoparticles synthesized through biological methods find applications in biomedical fields, including protection against harmful microorganisms, bio-mining, therapies for cancer, and healthcare diagnosis, their unique properties, such as anti-metastasis, biocompatibility, stability, and manipulability, enhance their utility. Additionally, metallic nanoparticles exhibit catalytic activity, making them significant in various industrial applications in contemporary settings [4].

The synthesis of nanoparticles can involve either natural or synthetic origins, resulting in materials that exhibit

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unique properties at the nanoscale. Two fundamental approaches exist: the top-down approach and the bottom-up approach. In the top-down approach, nanoparticles are produced through size reduction, which is accomplished using various physical and chemical methods [5]. In bottom up approach nanoparticles are synthesized by gathering and combing liquid atoms or molecules, where the main reaction is reduction or oxidation. But these methods are rarely preferred due to their relative pros and cons, so phyto-nanotechnology the synthesis of nanoparticles using fresh plants or plant extract or microorganisms, also known as green or biological synthesis is the best. Researchers prefer biological nanoparticle synthesis because it is eco-friendly, simple, economical, and reproducible for faster metal nanoparticle production [6, 7].

The green synthesis method represents a bottom-up approach akin to chemical reduction, wherein costly chemical reducing agents are substituted with extracts from natural sources, such as tree leaves or fruit crops, for the synthesis of metals or metal oxides. Additionally, microorganisms may also be employed in this process. Among biological entities, plants or plant extracts appear to be the most effective agents due to their accessibility, suitability for large-scale nanoparticle production, and the eco-friendly nature of their waste products [4, 8]. Plants or their extract are rapidly used as a substitute of chemical reducing agent in the production of nanoparticle because of their heavy metal accumulation, detoxification and reduction properties and the secondary metabolites in the plants also acts as reducing agent. Zinc oxide, gold, silver nanoparticles are already produced and they are used in various fields [9].

The biological approach of reducing metal precursors to create corresponding nanoparticles is environmentally friendly, cost-effective, and devoid of chemical contaminants. This method is particularly suitable for medical and natural applications, where the purity of the NPs is of utmost significance. An alternative synthesis technique should be developed, that avoids the use of hazardous and toxic substances. Therefore, Biosynthesis of Nanoparticles is more advantageous than physico-chemical approaches [5]. While numerous nanoparticles have been utilised for many purposes, Zinc oxide nanoparticles (ZnO NPs) have garnered the highest level of interest due to their potential applications in cancer treatment and diagnosis [2, 8]. These nanomaterials are highly important and intriguing nanoparticles used in bio-medical applications. Due to their distinctive physic-chemical characteristics, they are considered highly attractive nanomaterials in the field. The ZnO NPs produced through biosynthesis possess distinct biological characteristics, including pronounced scattering, minute particle size, and a large surface area. ZnO NPs have been found to possess morphological structures that can effectively combat infections in wounds and burns. This is likely due to the Ag component, which provides ZnO NPs with antibacterial, antifungal, anti-platelet, and antiviral properties [10, 11].

Similar findings were made regarding the biosynthesis and characterization of zinc oxide nanoparticles using a plant extract from *Cinnamomum verum* that facilitated green synthesis. Samples that were prepared were validated for their nanoscale dimensions through the application of sophisticated characterization methods, including powder XRD and various microscopic techniques, specifically SEM and TEM. The SEM images illustrate the distinct agglomeration of particles, a finding that was corroborated by TEM studies. The green synthesized ZnO nanoparticles demonstrated inhibitory effects on the growth of *E. coli* and *S. aureus*, with minimum inhibitory concentrations (MIC) recorded at 125 µg/mL and 62.5 µg/mL, respectively. The findings suggest that the synthesized ZnO nanoparticles may serve as a viable antimicrobial agent against pathogenic microorganisms [12]. Previous studies found that they successfully synthesized the, synthesis of ZnO nanoparticles utilizing the extract from the seeds and barks of *Azadirachta indica* for antibacterial applications is supported by XRD patterns and FTIR spectra, which confirm the hexagonal wurtzite crystalline structure and the photochemical coating of the nanoparticles, respectively. The antimicrobial activity of nanoparticles is examined against four bacterial strains and it was observed that ZnO nanoparticles exhibit a greater inhibitory effect on the growth of gram-positive microbes compared to gram-negative microbes, as indicated by the minimum inhibitory concentration (MIC) values [13].

Waltheria indica is highly valued medicinal plant used to treat the cancer. *W. indica* is a species of flowering plant in the mallow family Malvaceae that has pan tropical distribution. It is believed to have originated in the Neotropics. *W. indica* also known as Velvet leaf, Marsh yellow, Monkey bush, and many other names. It is found throughout the tropics and warmer sub tropics. *W. indica* is a plant growing in many regions of the world. It has been used in traditional medicine for the treatment of several diseases in Hawaii and South Africa [14, 15].

To best of our knowledge, till now there is no research has been done on the bio-friendly production of ZnO NPs using *Waltheria indica* and its parts. As a result, the objective of this research work was to synthesise

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ZnO NPs by utilizing *W. indica* roots extract, and to assess their potential for *in vitro* antibacterial activity.

2. Material and methods

Collection of chemicals, plant sample and pathogens

Zinc nitrate hexahydrate Zn $(NO_3)_2$ $6H_2O$ was purchased from Hi-media laboratory, Mumbai Pvt Ltd, India. *W. indica* (Figure. 1) were collected from the surroundings of Chandravalli forest, Chitradurga and the plant specimen was authenticated by the institution taxonomist (Voucher No: KU/BOT/2021-22/MALV-SNKNS-08). The microbial pathogens such as, *E. coli*, *S. flexneri*, *B. subtilis*, and *S. aureus* were procured from IMTECH, Chandigarh, India.

Figure 1: Morphology of *Waltheria indica* A) Whole Plant, B) Stem, C) Leaves, D) Unopened Flowers, E) Flowers, and F) Fruits.

Preparation of *W. indica* roots extract

The collected roots were removed and properly cleaned three times with tap water and with sterile distilled water, and subsequently permitted to air dry in a shaded environment for a duration of ten days. The fine mixture was prepared using an electric blender and subsequently stored in zip-lock polythene bags. Roots that had been ground into a fine powder were roughly weighed (25 g), mixed with 500 mL of distilled water, and then boiled at 80 °C for about 5 hours. The obtained mixture was filtered using Whatman No. 1 filter paper, and the prepared filtrate was kept at 4 °C for further experimental use [12].

Synthesis of ZnO nanoparticles

2 grams of concentrated *W. indica* root extract were dissolved in 100 mL of distilled water. Precisely 2.9 grams of $Zn(NO_3)_2{\cdot}6H_2O$ were combined with 5 milliliters of diluted plant extract. The mixture was maintained in a pre-heated muffle furnace at 400 °C and underwent combustion. The reaction was completed within a duration of 5 minutes. The synthesis of nanoparticles was conducted using varying concentrations of the plant extract, specifically 10, 15, 20, and 25 mL. The resultant product was maintained in an airtight container until subsequent utilization (Ogunyemi et al., 2019) [16].

Characterization of synthesized ZnO nanoparticles

A spectrophotometer (Double beam: METASH UV-9600A) was used to scan the particles for UV-Visible spectrum in the range of 300 and 700 nm to measure ZnO NPs (Nayaka et al., 2020) [17]. The synthesised NPs were analysed using FTIR to recognize the functional groups that cap and stabilise the nanoparticles. Water was removed from *W. indica* root extract mediated ZnO NPs by thermostatic desiccation at 40 °C for 12 h. They were then finely mixed with 5% KBr and compressed into thin discs. The Thermo Fisher Scientific NICOLET 6700 instrument measured the transmittance spectrum of ZnO NPs. For powder crystal size determination, the power level was 40 Kv with 30 mA, and the diffraction angle was 20º to 80º for two-theta (2θ). Measurements of X-ray intensities and scattering angles determined the crystallographic structure. From the XRD pattern using Rigaku Miniflex 600, Smart-Lab SE XRD instrument. ZnONPs were characterised using SEM and EDAX (VEGA3, TESCAN) for morphological features and to check the topology of synthesised ZnO NPs.

Antibacterial activity ofsynthesized ZnO nanoparticles

The agar well diffusion method described by Nagaraja et al., (2023) [18] was used to test the synthesised ZnO NPs antibacterial activity against selected two Gram negative and two Gram positive bacterial strains, such as, *S. flexneri* (MTCC 1457) and *E. coli* (MTCC 40), *B. subtilis* (MTCC 6633) and *S. aureus* (MTCC 6908) were chosen for activity testing, respectively. A sterile cotton swab spread each culture evenly on nutrient agar plates. A gel-hole puncher created 6 mm wells in 4 mm agar plates. The wells were loaded with ZnO NPs at 25 to 100 µg/µL concentration. For 24 h, the plates were incubated at 37 ℃. Streptomycin was used as positive control. With a transparent ruler, the inhibition zone around each well was measured in millimetres after incubation.

Statistical analysis

The experiments were administered in triplicate, and the data is furnished as the mean value along with the standard deviation. The analyses were performed using Excel (2010) and OriginPro 2022b software.

3. Results and Discussion

Biosynthesis of ZnO nanoparticles and their characterizations

The synthesis of zinc oxide nanoparticles utilising aqueous *W. indica* root extract was validated through visual observation. The combination of colourless zinc

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nitrate with brown root extract resulted in the formation of a yellowish-white suspension, thereby indicating the successful synthesis of zinc oxide nanoparticles. The notable alteration in colour provided a definitive signal that the synthesis of ZnO nanoparticles had taken place (Figure 2A-C). UV-Visible spectroscopy was conducted on the *W. indica* root extract mediated ZnO NPs and the reacted solution to verify the production of NPs. The presence of a distinct SPR band at 374 nm confirmed the synthesis of *W. indica* root extract mediated ZnO NPs in the reaction mixture and it is depicted in Figure 3. The synthesis methods for green nanoparticles were highly reliable, efficient, easily accessible, and environmentally sustainable. The synthesis of NPs using this technique has garnered prominent attention from researchers across various disciplines, including medicine [19, 20].

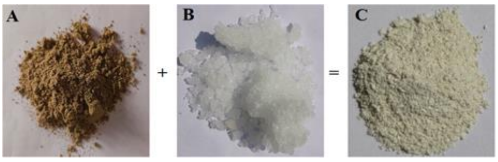

Figure 2: Visual observation of ZnO NPs. A) Root extract B) Zn $(NO_3)_2 \cdot 6H_2O$ C) Formation of ZnO NPs.

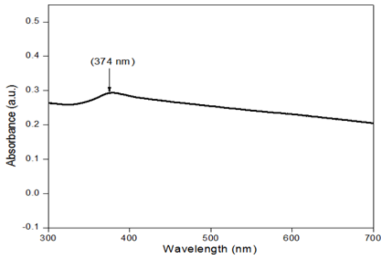

Figure 3: UV-Visible absorption spectrum of synthesised ZnO NPs from *W. indica* root extract.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR analysis identified the likely bio-molecules responsible for capping the ZnO NPs in *W. indica* root, which effectively stabilised the ZnO NPs (Figure 4), where 8 vibrational peaks in the 4000 to 400 cm^{-1}. The analysis of the ZnO NPs using spectral analysis reveals noticeable changes. The peak 3281 cm^{-1} can be corresponded to the strectching of alcohol (O-H) bonds. Likewise, the existence of alkane stretching (C-H) is confirmed by the band detected at 2926 cm^{-1} in the spectrum. The presence of a (C=C) strectching cyclic alkene is indicated by the noticed band ranging from 1723 cm^{-1}.The band at 1629 cm^{-1} to 1378 cm^{-1} suggests the bending of the (O-H) group in phenol and the bending of the (C=H) group in alkane. The small shift in the band position of 1022 cm^{-1} suggests involvement primary alcohol with stretching. The vibrational bands notified at band's position at 427 cm^{-1} noticed to be caused by the strectching of the (C=C) bending in tri-substituted alkenes. The noticed changes in the bands of FTIR spectra correlate that the functional groups of bio-constituents play a crucial role in the synthesis of NPs from the extract of plant parts. Following bio-reduction, the absorption bands indicated the presence of ZnO NPs, which were coated with bio-moieties. In a study conducted by Razanamahandry et al., (2020) and El-Belely et al., (2021), the researchers observed how the functional groups of plants interacted with biological components, resulting in the reduction of metal salts to NPs [21, 22].

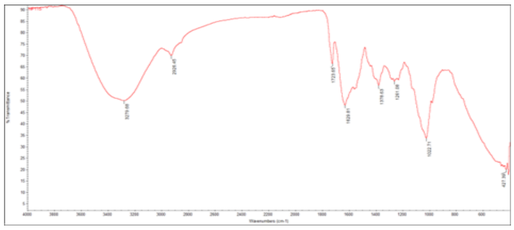

Figure 4: FTIR analysis spectrum of synthesized ZnO NPs from *W. indica* root extract.

X-ray Diffraction Analysis

X-ray diffraction (XRD) measurement was ustilised to ascertain the dimensions and morphology of ZnO NPs crystals. The synthesised ZnO NPs using the roots extract of θ were analysed using XRD patterns, which showed distinct peaks (Figure 5). An XRD investigation affirmed the crystalline property of ZnO NPs, which exhibited prominent bands at 31.74°, 34.38°, 36.30°, 47.51°, 56.61°, 62.81°, 66.36°, 68.98°, 69.10°, can be indexed to the (100), (102), (101), (102), (110), (103), (112) planes of ZnO. In a similar manner, the synthesis of ZnO nanoparticles utilizing orange peels resulted in comparable XRD patterns, exhibiting analogous Bragg reflection indices at specific 2θ angles. These findings correspond to reference code number 01-075-9742, which revealed a hexagonal structure, with no additional phases detected [23].

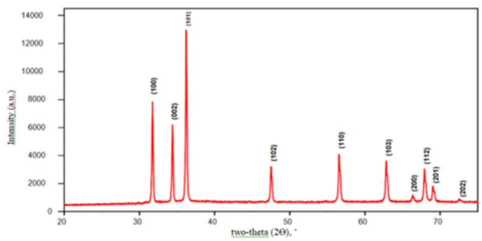

Figure 5: XRD analysis spectrum of biosynthesized ZnO NPs from *W. indica* root extract.

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Scanning Electron Microscopy with Energy Dispersive Spectroscopic Analysis

The SEM analysis was utilised to visualize the morphological features of the synthesised ZnO NPs. The investigation demonstrated the ZnO NPs have a hexagonal in nature and are distributed in a poly-dispersed manner, as it is depicted in Figure 6. The EDAX spectra (Figure 7) shows that no impurities are present in the synthesized ZnO nanoparticles as no peaks were seen for any impurities the elemental analysis showed 66.89% of zinc and 33.11% of oxygen respectively. The intrinsic chemicals present in the plant may have been responsible for the alterations in particle sizes. The EDAX detector was used to determine the elemental topology of powdered materials. In addition, the presence of the SPR caused the Zinc oxide nanoparticles to exhibit a distinct optical absorption bands/peaks at around 1-10.5 keV [24, 25]

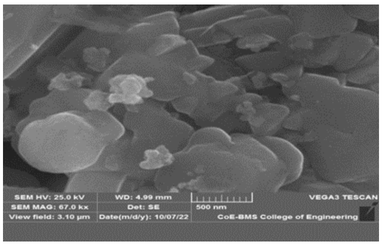

Figure 6: SEM image of biosynthesized ZnO NPs from *W. indica* root extract.

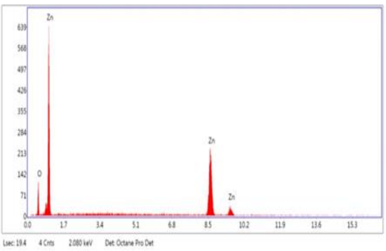

Figure 7: EDS analysis of biosynthesized ZnO NPs from *W. indica* root extract.

Antibacterial activity ofsynthesized ZnO nanoparticles

Synthesised ZnO NP's antibacterial activity was assessed using the agar well diffusion assay. Culture plates showed circular inhibition zones. *W. indica* root extract mediated ZnO NPs treated Gram-positive and Gram-negative. According to the results, synthesised ZnO NPs showed the maximum growth inhibition for when treated with 100 µL of synthesised ZnO NPs, *S. flexneri* and *E. coli* showed the highest growth inhibition with zones measuring 13 mm, and 11 mm, respectively. *S. auerus* and *B. subtilis*, showed the lowest growth inhibition with zones measuring 10 mm and 9 mm. **Figure 8(A-D)** displayed zone of inhibition against tested pathogens in various concentrations. Overall results revealed that, metal complexes are an effective antibacterial agent for bacterial infection control. The simultaneous release of ions to bioactive compounds increased the suppression of bacterial growth at low levels [26, 27].

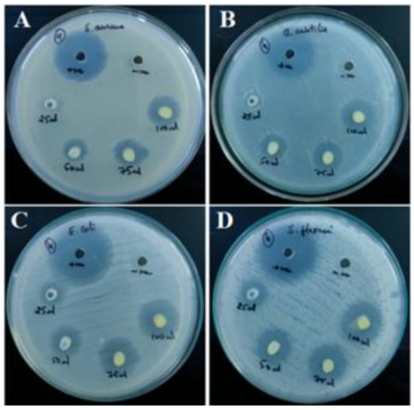

Figure 8: Antibacterial activity of different concentration of synthesised ZnO NPs from *W. indica* root extract: A) *S. aureus*, B) *B. subtilis*, C) *E. coli*, and D) *S. flexneri*.

4. Conclusion

A green synthesis method using *W. indica* roots extract as the reducing agent produced ZnO nanoparticles in this study. The annealing temperature and synthesis pH greatly affected ZnO nanoparticle microstructure, morphology, and bactericidal activity against *S. aureus*, *B. subtilis*, *E. coli*, and *S. flexneri*. ZnO nanoparticles show significant antibacterial activity against all tested microorganisms, with *S. flexneri* showing the highest inhibition zone at 100 µg/mL (13 mm). The study found that zinc oxide nanoparticles (ZnO NPs) kill bacteria by damaging DNA and cell walls through cell membrane interactions with reactive oxygen species (ROS). This study proposes a green synthesis of ZnO nanoparticles using root extract, which may reduce chemical use and nanoparticle production costs.

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that there is no conflict of interests among them.

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