



Biosynthesis, characterization, and antibacterial activity of zinc oxide nanoparticles derived from *Bauhinia tomentosa* leaf extract

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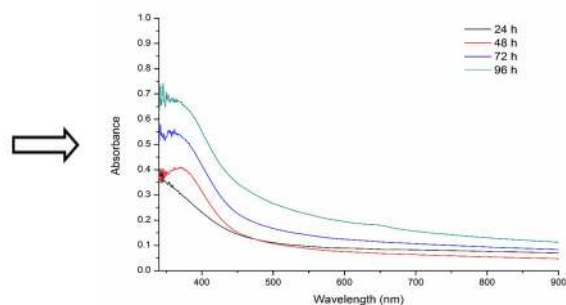
Abstract

A facile, eco-friendly synthesis of zinc oxide nanoparticles (ZnO NPs) employing *Bauhinia tomentosa* leaf extract as bioreducing agent was reported. The green-synthesized ZnO NPs were characterized by UV–Vis, TEM, EDX, XRD, and FTIR analyses. The formation of ZnO NPs was confirmed by the appearance of characteristic SPR peak at 370 nm due to the collective oscillation of electrons in the conduction band in UV–Vis spectra. The hexagonal morphology exhibiting nanosized ZnO was observed from the TEM and XRD analyses. The chemical bonds present in the as-synthesized ZnO NPs were identified by FTIR analysis. ZnO NPs showed a significant antibacterial activity against Gram-negative bacteria *P. aeruginosa* and *E. coli* than Gram-positive bacteria. Results of this study demonstrated that *B. tomentosa* leaf extract containing phytochemicals such as alkaloids, terpenoids, flavonoids, tannins, carbohydrates, and sterols possess bioreducing property for ZnO synthesis and the obtained ZnO NPs could be employed effectively as a better bactericidal agent for biological applications.

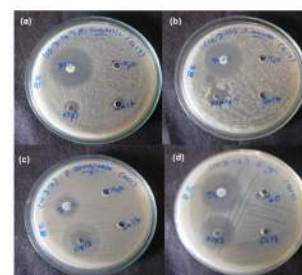
Graphical abstract



B. tomentosa leaves



Synthesis of ZnO NPs



Antibacterial activity of ZnO NPs

Keywords Green synthesis · ZnO · Nanoparticles · Antibacterial activity · *Bauhinia tomentosa*

Introduction

The combination of nanotechnology and biological science has been created a new path for the researchers to exploit in various biological fields. Nowadays, synthesis of metal nanoparticles becomes growing attention in nanotechnology research

due to its tremendous applications in medicine, biomedical, pharmaceutical, drug delivery, and photocatalytic activity [1]. In the past few decades, there is an increased demand for the metallic nanoparticles which can be synthesized by various traditional physical and chemical methods in different shapes and sizes. Irradiation [2], microwave processing [3], solvothermal [4], and ultrasound processing [5] are some of the physical approaches for the metallic nanoparticle synthesis. The disadvantages of physical method involve the processing done at elevated temperature and pressure, requires large space

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to set the machines and also the cost is high [6]. On the other hand, utilization of hazardous substances such as reducing agents, organic solvents, and stabilizers in chemical approach causes hazardous for the labourers as well as environment during synthesis. It has been reported that the toxic substances reside inside the nanoparticles if it is prepared either physical or chemical method induces hazard in the field of their applications [7]. The alternative method to synthesize nanoparticles in an eco-friendly, biocompatible, safe, and cost-effective is green approach, which allows large-scale production of nanoparticles through bacteria, fungi, algae, and plants. Different plant parts such as root, stem, leaf, flowers, and fruits are rich in phytochemical substances and act as a stabilizing and reducing agent in the production of nanoparticles [8–10]. Among all other metal nanoparticles, ZnO nanoparticles possess tremendous applications in medical field such as UV filtering in preparation of sunscreen lotions, anti-inflammatory, wound-healing, anti-cancer, antifungal, antibacterial, and targeted drug delivery [11, 12]. ZnO NPs can be produced in different nanoscale sizes and structures in the form of flakes, flowers, belts, rods, and wires [13], thereby employed flexibly in heavy metal removal and biomedical applications.

Plants are the major source involved in the synthesis of stable nanoparticles for large-scale production. In this study, *Bauhinia tomentosa* leaf extract acts as a bioreducing agent for the synthesis of ZnO NPs. The plant belongs to “Fabaceae” family, with 4 m height, multi-stemmed, slender branches, and contains elliptical shaped leaves with entire margin divided deeply at centre. The barks are grey colour with smooth and hairy texture; the flowers are yellowish bell-shaped structure with dark reddish brown patches at the base. The fruit is a pod-shaped and appears as pale brown colour. This plant is widely distributed in India, Sri Lanka, Zimbabwe, and Mozambique which contains therapeutic active components such as quercetin, isoquercetin, phytohemagglutinins, protocatechuic acid, and rutin [14]. The current study focussed on the green synthesis of ZnO NPs using *Bauhinia tomentosa* leaf extract and its characterization was investigated along with the evaluation of antibacterial property for the synthesized ZnO NPs.

Experimental

Materials

Zinc sulphate (ZnSO_4) was purchased from HiMedia (India) Ltd. *Bauhinia tomentosa* leaves were collected from Vanjipalayam, Tirupur district, Tamilnadu. All the chemicals used in this study were of analytical grade.

Preparation of plant extract

Bauhinia tomentosa leaves were washed with tap water, shade dried, and powdered. 5 g of the dried fine powder of *B. tomentosa* leaves was mixed with 50 ml of distilled water. The mixture was kept in water bath at 60 °C for 15 min. After boiling, the mixture was stirred using magnetic stirrer for 30 min. The extract was then filtered using Whatman No. 1 filter paper and the filtrate extract was used for further studies.

Biosynthesis of ZnO NPs

Twenty millilitres of plant extract prepared in the previous step were mixed well with 80 ml of 2 mM ZnSO_4 solution and incubated at room temperature. The mixture was kept for 4 days and was monitored at regular interval by UV–Vis spectrophotometer. After incubation, the mixture was centrifuged at 5000 rpm for 15 min. The obtained pellet was resuspended in distilled water and centrifuged again. This process was repeated twice or thrice to remove the impurities. The finally obtained pellet was dried and stored for further analysis.

Characterization of ZnO NPs

UV–Vis spectral analysis

A bioreduction of Zn^{2+} ion to ZnO NPs by *B. tomentosa* leaf extract was analyzed using UV spectrophotometer (Analytikjena) for 4 days with the interval of 24 h.

TEM analysis

The shape and size distributions of synthesized ZnO nanoparticles were characterized using transmission electron microscope (TEM, TECNAI). The analysis was performed by sonicating the liquid sample of ZnO NPs for 10 min using citizen digital ultrasonic bath before placing it on the carbon-coated copper grid.

EDX analysis

The elemental composition of ZnO NPs was analyzed by EDX analysis using the instrument coupled with TEM.

XRD analysis

The crystalline structure of the ZnO NPs was determined by X-ray diffraction analysis using PAN analytical diffractometer in the 2θ range of 10°–40°.

FTIR analysis

The chemical bonds present in the green-synthesized ZnO nanoparticles were identified by FTIR spectroscopy analysis. FTIR analysis was performed in Perkin-Elmer Spectrum Two FTIR instrument using KBr pellet method at a resolution of 2 cm^{-1} .

Antibacterial activity

The antibacterial activity of ZnO NPs was tested against two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) by well-diffusion method. Three wells were made in the agar plate and loaded with 20 μl of ZnO nanoparticles (1 $\mu\text{g}/\mu\text{l}$), distilled water (negative control), and ZnSO_4 solution (1 $\mu\text{g}/\mu\text{l}$). Antibiotic disc Levofloxacin was used as positive control. The plates were incubated for 24 h at 35 °C. The antibacterial activity of ZnO NPs on the tested bacterial strains was determined by formation of inhibition zone around the wells.

Results and discussion

Characterization

UV–Vis analysis

The formation of ZnO NPs by the bioreducing activity of *B. tomentosa* leaf extract was assessed by UV–Vis spectroscopy. The time course profile of ZnO NPs synthesis is shown in Fig. 1a. Oscillation of electrons in the conduction band at a particular wavelength was due to the effect of surface plasmon resonance (SPR). The SPR peak obtained at 375 nm was observed after 24 h of incubation and it confirmed the formation of ZnO NPs. A steady increase in the

absorbance intensities in the UV–Vis spectra clearly indicates the increased formation of ZnO NPs. The phytochemicals present in the *B. tomentosa* leaf extract reduces Zn^{2+} ions to zero valent Zn atoms initially. These zero valent Zn atoms initiates the nucleation and involved in the reduction of residual Zn^{2+} to ZnO and its further growth leads to cluster formation [15]. The possible reaction mechanism of ZnO formation is the reduction of zinc ion by the phytochemicals such as alkaloids, flavonoids, terpenoids, and tannins present in the plant extract and acts as bioreductant as well as a stabilizing and capping agent to finally produce ZnO nanoparticles. The obtained SPR peak at 375 nm was highly correlated with the previous studies on green synthesis of ZnO NPs [12, 16]. The optical band-gap energy was calculated using Tauc's equation (Eq. 1):

$$ah\nu = k(h\nu - E_g)^n, \quad (1)$$

where k is a constant, $h\nu$ is the photon energy, E_g is the allowed energy gap, $n = 1/2$ for allowed direct transition, and $n = 2$ for allowed indirect transition. Tauc plot ($ah\nu$ versus $h\nu$) is shown in Fig. 1b and the band-gap energy was calculated as 2.8 eV for ZnO NPs synthesized using *B. tomentosa* leaf extract which was in good agreement with the result reported by Khuili et al. [17].

TEM and EDX analyses

The shape and size of the *B. tomentosa* leaf extract-derived ZnO NPs were characterized by TEM. Individually separated hexagonal-shaped ZnO NPs were observed in TEM image (Fig. 2a). The size of the synthesized ZnO nanoparticles was found to be in the range of 22–94 nm and particle size distribution histogram is shown in Fig. 2b. Similar results were reported by Rajiv et al. [18] and Qian et al. [19] for the green synthesis of ZnO NPs using *Parthenium hysterophorus* and *Aloe vera* extracts, respectively. The chemical composition of prepared ZnO NPs

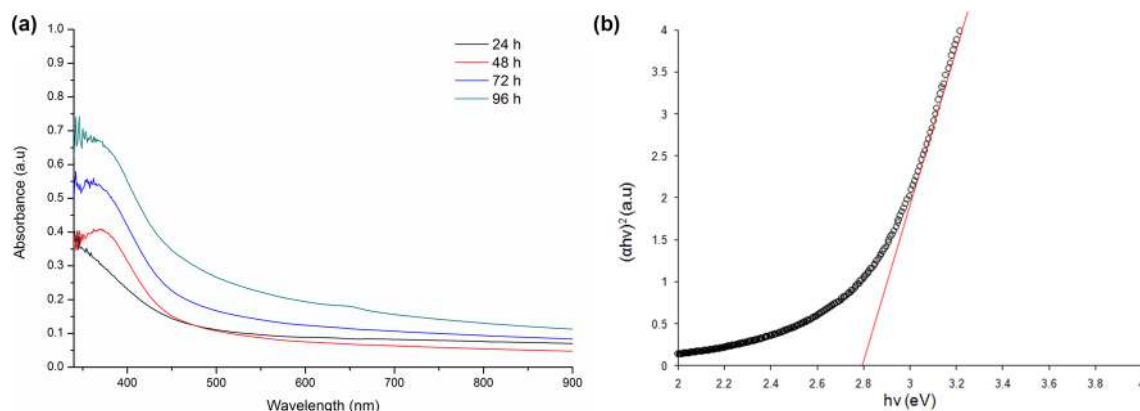


Fig. 1 a UV–Vis spectrum and b Tauc's plot of green-synthesized ZnO NPs using *B. tomentosa* leaf extract



Fig. 2 **a** TEM image and **b** histogram showing the size distribution of *B. tomentosa* leaf extract-derived ZnO NPs

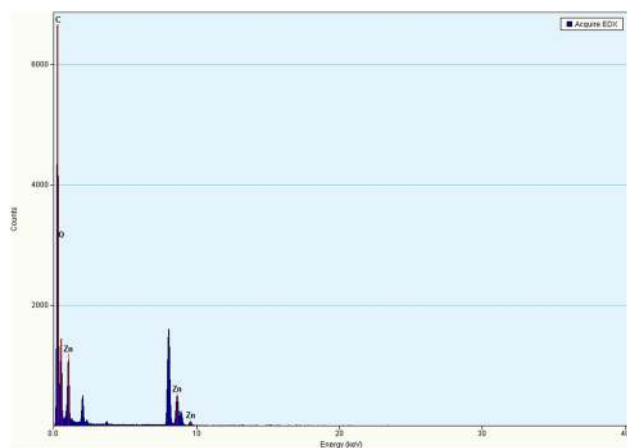
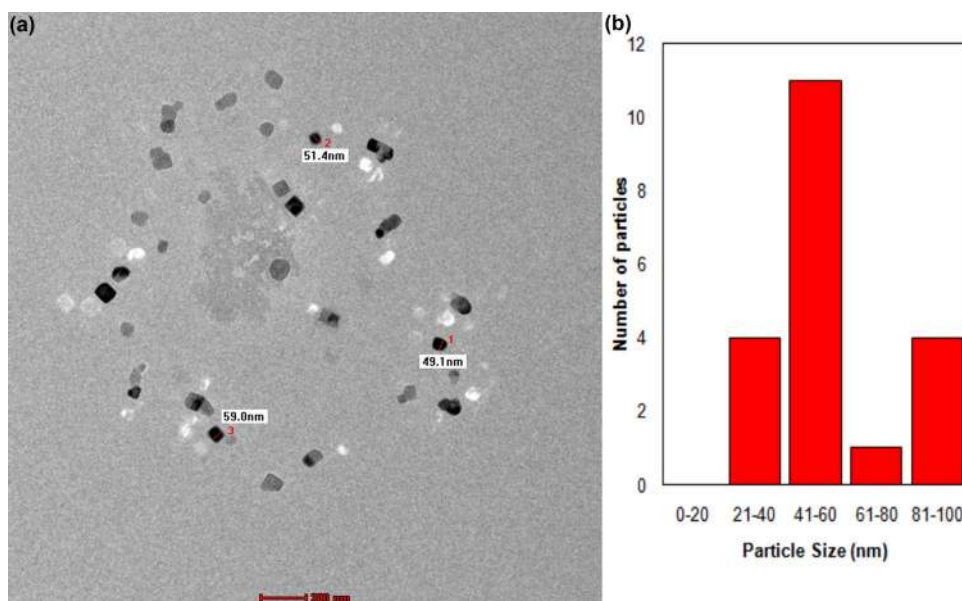


Fig. 3 EDAX profile of *B. tomentosa* leaf extract-derived ZnO NPs

was determined by EDX analysis. EDAX spectrum (Fig. 3) revealed the presence of zinc and oxygen signals in the ZnO NPs prepared using *B. tomentosa* leaf extract. EDX analysis confirmed that the synthesized nanoparticles are ZnO NPs.

XRD analysis

XRD spectrum of green-synthesized ZnO NPs is shown in Fig. 4. The hexagonal wurtzite crystalline structure of ZnO NPs was confirmed by the observation of distinct diffraction peaks at 14.07° , 16.84° , and 38.82° in the spectra which corresponds to the Miller indices of (100), (101), and (210), respectively [20]. The obtained results had good agreement with the standard JCPDS card no 01-1136.

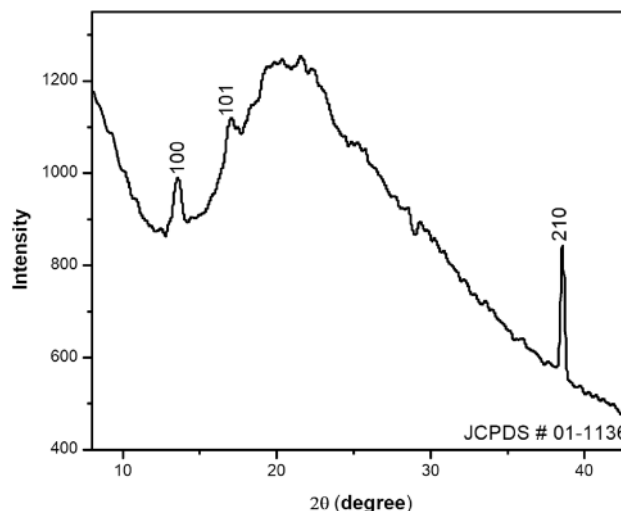


Fig. 4 XRD pattern of *B. tomentosa* leaf extract-derived ZnO NPs

FTIR analysis

The chemical groups present in the biosynthesized ZnO NPs are identified by FTIR analysis and the obtained spectrum is represented in Fig. 5. The absorption band appeared at 3278 and 1339 cm^{-1} was attributed to O–H group of alcohols or plant phenolics. The alkane (C–H) bond in stretching mode was identified by the peaks observed at 2917 and 2850 cm^{-1} . The peaks 1655 and 1543 cm^{-1} were corresponded to the amine (N–H) group of proteins or enzymes present in the *B. tomentosa* leaf extract. The absorption peaks observed in the lower wavenumbers $400\text{--}600\text{ cm}^{-1}$ confirmed the presence of Zn–O bond in the synthesized NPs [21]. The peaks at 448 and 406 cm^{-1} were corresponded to the stretching frequency



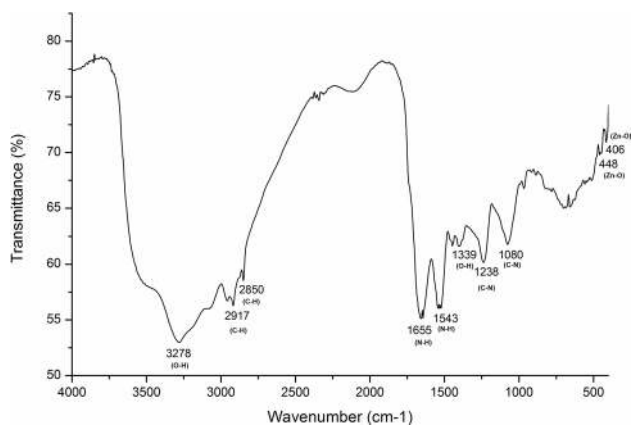


Fig. 5 FTIR spectrum of *B. tomentosa* leaf extract-derived ZnO NPs

of Zn–O bond [22]. The peaks at 1238 and 1080 cm^{-1} represent the C–N stretching mode of aromatic or aliphatic amines. The presence of N–H and O–H bonds in the FTIR spectrum revealed that the proteins or phenolic compounds in the *B. tomentosa* leaf extract involved in the bioreduction of Zn^{2+} ions to ZnO NPs.

Antibacterial activity

The antibacterial potential of the biosynthesized ZnO NPs using *B. tomentosa* leaf extract is tested against *B. subtilis*, *S. aureus*, *P. aeruginosa*, and *E. coli* and shown in Fig. 6. The biosynthesized ZnO NPs showed antibacterial effect on all the tested bacterial strains. The bactericidal effect of ZnO NPs was found higher for Gram-negative bacteria than Gram-positive bacteria and was based on the difference in the structural composition of Gram-positive and Gram-negative bacteria [23]. *B. tomentosa* leaf extract-derived ZnO NPs showed a significant zone of inhibition for *P. aeruginosa* (20.3 mm) and *E. coli* (19.8 mm), whereas the zone of inhibition was observed less for *B. subtilis* (8.1 mm) and *S. aureus* (10.7 mm). Similar results on antibacterial effect for *P. aeruginosa* and *E. coli* by ZnO NPs were reported previously in the literature [24]. The mechanism of antibacterial activity of ZnO NPs may be attributed to the penetration and disintegration of the membrane by smaller sized NPs which lead to cell lysis [25, 26]. The release of H_2O_2 from the surface of ZnO also reported as the possible mechanism for bactericidal activity. The generation of H_2O_2 is highly depended on the surface area of ZnO and the generated H_2O_2 penetrates the cell membrane and cause damage to kill the bacteria [27, 28]. Due to the presence of alkaloids, terpenoids, flavonoids, tannins, carbohydrates, sterols, saponins, proteins, and amino acids in *B. tomentosa* leaf extract showed potential bioreducing activity and also bactericidal activity against the tested bacteria which could be useful for biomedical applications.

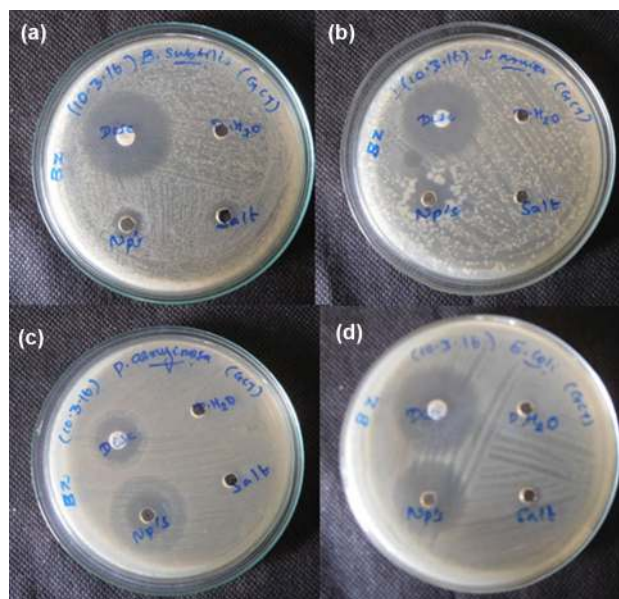


Fig. 6 Antibacterial activity of *B. tomentosa* leaf extract-derived ZnO NPs **a** *B. subtilis*, **b** *S. aureus*, **c** *P. aeruginosa*, and **d** *E. coli*

Conclusion

Zinc oxide nanoparticles were successfully synthesized by biogenic route using *B. tomentosa* leaf extract. UV–Visible spectroscopy analysis confirmed the synthesis of ZnO by the existence of SPR band at 375 nm. TEM analysis revealed that the synthesized ZnO NPs were in nanoscale range. Hexagonal structure of the ZnO NPs was confirmed by XRD analysis. FTIR spectrum explained that the phenolics or proteins may involve in bioreduction process for the nanoparticles synthesis. ZnO NPs showed better antibacterial activity against Gram-negative bacteria than Gram-positive bacteria. ZnO NPs synthesized using *B. tomentosa* leaf extract could be utilized as antibacterial agent in biomedical, textile, and food industries.

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Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.


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