Green route synthesis of ZnO nanoparticles mediated by Melia azedarach for microbiological applications

Adeela Ahmad¹, Waqar A A Syed², Muhammad A Ghufra³, Zafar Iqbal¹ and Wiqar H Shah²

¹ Department of Physics, Riphah International University, Islamabad, 44000, Pakistan
² Department of Physics, International Islamic University, Islamabad, 44000, Pakistan
³ Department of Environmental Sciences, International Islamic University, Islamabad, 44000, Pakistan

E-mail: adil.syed@iiu.edu.pk

Keywords: green synthesis, Melia azedarach, ZnO nanoparticles, antibacterial activity

Abstract

Green synthesis technique of nanoparticles has advantages over physical and chemical methods due to its simplicity in preparation, environment friendly nature and less time consuming. We report a green approach for the synthesis of zinc oxide nanoparticles using leave extract from Melia azedarach. Melia azedarach plant has been traditionally famous for its antibacterial properties. The plant extract acts as reducing and capping agent, while zinc nitrate hexahydrate [Zn(NO₃)₂.6H₂O] was used as a precursor. The formation of ZnO nanoparticles was observed by the color changes during the reaction. Structural, optical and morphological properties of ZnO nanoparticles were determined using x-ray diffraction, scanning electron microscope, Fourier transform spectroscopy and UV–vis spectroscopy. XRD analysis confirms the formation of wurtzite hexagonal structure NPs with significantly small average crystallite size of 7.0 nm. The energy band gap determined by UV–vis spectroscopy was measured between 2.9 eV to 5.4 eV. The antibacterial activity of ZnO nanoparticles was tested against E. coli bacteria and significant results were obtained. The anti-bacterial study proved that green-rout prepared ZnO nanoparticles are the best choice for future research concerning antibacterial activity.

Introduction

Zinc oxide is considered as environmentally safe as its toxicities are either limited or unknown in most of the ecosystems. As an environment friendly material, its uses are in various biological systems particularly in agricultural production as micronutrient. Zinc oxide has good antibacterial properties so that it is used in the packing of food, herbal medicine and for the protection from UV light [1]. The ZnO powder is also utilized as an added substance in different items such as clay, glass, concrete, oils paints, cement, plastic, sealants, batteries, ferrites and imperviousness to fire [2]. Zinc oxide is viewed as a standout with its wide band gap and extensive excitation energy making it essential for modern applications [3]. It is stable under diverse environmental conditions, and fabrication at low temperature.

ZnO NPs have shown antimicrobial activity against bacteria and even antibacterial activity against spores [4–7]. Amongst all the inorganic semiconducting nanoparticles, ZnO NPs have attracted the scientific community on the grounds that these nanoparticles can effectively be synthesized and are believed to be biocompatible, biodegradable, and nontoxic for clinical applications [8]. It is taken as an alternating material for TiO₂ due to its properties, for example strength and its tendency to be easily synthesized in various shapes and sizes. ZnO NPs are utilized in medication field as it explicitly destructs the bacterial cell layer and the elevated bactericidal impact. The mechanisms of antibacterial activity of ZnO particles are not well understood, however the generation of hydrogen peroxide may be a probable factor for antibacterial activity or electrostatic binding of nanoparticles on bacterial surface could be a mechanism [9, 10], therefore, the present study is an attempt to synthesize ZnO through a green route and check its potential antimicrobial activity against E. coli pure bacterial culture.
Different physical, chemical and biological methods have been used for synthesizing ZnO nanoparticles, such as homogenous precipitation, direct precipitation, green synthesis, sol-gel method, thermal decomposition, sono-chemical method and hydrothermal process [11]. These approaches utilize inorganic solvents and harmful reducing agent and a greater part of which are profoundly reactive and dangerous to the environment. In order to avoid harmful reactions synthesis of ZnO nanoparticles with biological methods is preferred. Biosynthesis of nanoparticles is a bottom up approach where the plants extract and microorganisms are utilized for obtaining metal nano particles; in contrast to chemical methods. Several biological frame works including microorganisms, parasites and yeast have been utilized in synthesis of nanoparticles. Right now ‘green’ method in the synthesis of ZnO nanoparticles has progressively become a subject of interests. Since there is no need of energy, high pressure, temperature or harmful chemicals, scientists have depicted that the use of eco-accommodating favorable materials like leaf extract, bacteria and fungus for the synthesis of nanoparticles offers significant interests of Eco friendliness and corresponding pharmaceutical and other biomedical applications. In this work green synthesis route has been adopted which is a bottom up approach and yields large scale production [12, ]. Leaves extracts of *melia azedarach* are used as reducing agent; the plant has medicinal background with good antibacterial properties and it is expected that by using zinc oxide nanoparticles, the antibacterial properties will be significantly improved [13]. *Melia azedarach* is commonly known as persian lilac and has a place within the family of meliaceae and local to India, Indochina, southeast Asia and Australia. This plant is naturalized in the greater part of the tropics and subtropical nations and is portrayed by the nearness of thick and dim green leaves. Its bark is of brown colored and the leaves are interchange, pamphlets are short stalked. Blooms are white with purple lines and described by the nearness of a common scent and the fruits are yellow berries [14].

Many phytochemical constituents are present in the extract of the different parts of the plant. These are alkaloid, carbohydrate, steroidal glycosides, tannin, phenol, chlorogenic acid, flavonoids and terpenoid [15]. Customarily this plant has been utilized for the treatment of infection, cardiovascular ailments, intestinal sickness, diabetes, back, strangury, amenorrhoea, bronchitis, dermatitis, asthma, fever, gonorrhea, heaps, gum disease, tuberculosis and ailment. The different pieces of *melia azedarach* are investigated for its monstrous organic exercises, for example, anthelmintic, antiviral, antifertility action, ovicidal, larvicidal, cancer chemopreventive agent, antibacterial, antimalarial, antiparasitic, anti hyperglycemic, anticancer and cytotoxic activities [16–18].

The upside of utilizing plants for the synthesis of nanoparticles is its effective accessibility, harmless to deal with and changeability of metabolites which helps in reduction. Much work has been done on ‘plant mediated reduction’ of metal nanoparticles and the scrupulous role of phytochemicals. Biogenic synthesis of ZnO nanoparticles utilize entire plant extracts and effectively attained Calotropis procera [19], Calotropis gigantean [20], Passiflorae oedtida [21], Duranta erecta [22], Acalypha indica [23], Hemidesmus indicus [22].

To our understanding, this is the first study reporting synthesis of zinc oxide nanoparticles using *melia azedarach* leaf extract. ZnO is an antibacterial agent and nanoparticles are found effective to bacteria. Sensitivity tests utilizing various bacterial strains specially *E. coli* culture scan were used to test the activity of the nanoparticles against the bacterial growth. The activities of the nanoparticles of various elements specifically of metals against the bacterial strains are usually based on their toxicities. However the ZnO nanoparticles are hypothetically nontoxic, therefore the restriction to the germination of the bacterial colonies in the presence of ZnO nanoparticles is expected to be the factor of its nontoxic nature. Yet inhibition activity to the growth of *E. coli* and other bacterial strains proves it an environmentally safe material.

**Synthesis of zinc oxide nanoparticles**

Fresh, disease free leaves of *melia azedarach* plant were obtained throug pruning. Zinc nitrate hexahydrate [Zn (NO$_3$)$_2$·6H$_2$O] is used as a precursor with distilled water and ethanol to prepare extract. *Melia azedarach* leaf (25 g) were washed with distilled water to remove the impurities and dust particles. The surface water was dried and leaves were chopped into small pieces. The chopped leaves were boiled in the clean and sterilized conical flask with the distilled water. The color of the water changes to light green which was was cooled at room temperature and then filtered.

The 0.59 g of zincnitrate hexahydrate is added in 20 ml of the extract for the preparation of Sample 1. This solution is kept on the magnetic stirrer hot plate at 100 °C for 20 min. The color of the solution changes to thick light yellow after heating. The leaf extract act as a catalyst and reduces the nitrates present in the solution. After the reaction, the solution is kept for one day at room temperature and than placed in the electrical furnace at 300 °C for 4–5 h for drying. Finally the nanoparticles were obtained in the form of light yellow color powder. Other samples with number 2, 3, and 4 were prepared by taking the same amount of extract with varying precursor concentration. The rest of the procedure was similar as that of Sample 1., and summed up in the figure 1.
Structural analysis
The structural properties of fabricated nanoparticles were studied by XRD and the phase structure and the crystallinity parameters were determined. The diffraction pattern of thin films was recorded using D8 advanced Bruker XRD with copper Kα source of 1.54 Å at National Centre for Physics, Islamabad. The diffraction angle varied from 10 to 80 degrees. The diffraction patterns for all four samples are shown in figure 2, relieving the structure of samples and the peak positions confirm the crystallographic nature of nanoparticles as hexagonal. The crystallite size was calculated using Scherrer formula,

\[ D = \frac{(0.89)}{\cos \theta} \]  

where 0.89 is a constant, \( \lambda \) is the wavelength used, \( \beta \) is full width at half maximum and \( \theta \) is the diffraction angle. All other parameters including dislocation density, strain etc can be obtained using the following two equations,

\[ \varepsilon = \frac{\beta \cos \theta}{4} \]  
\[ \delta = \frac{n}{D^2} \]

The major peak for the preferred orientation is of plane (101), which is observed in the spectra of all the samples S1, S2, S3 and S4. Peaks for other planes i.e. (100), (002), (102), (110), (103) and (112) are also observed. These peaks confirm the formation of zinc oxide nanoparticles. These formations were matched with the JCPDS cards 00-025-1402. There is a slight angular shift in the in the peaks. The diffraction peak (101) decreases with the increasing concentration of the precursor. This indicates that the molar ratio of the precursor is the
dominant factor in the synthesis of zinc oxide nanoparticles. Using Sherrer’s formula the average crystallite size is obtained of the order of 0.8 nm, whereas the minimum crystallite size was 0.7 nm. The lattice spacing is of the order of 2.48 Å, and the lattice strain is 1.28%. The structural parameters obtained by XRD analysis are summed up in table 1.

Morphological and compositional studies
The surface morphological study was carried out using scanning electron microscope at National Centre for Physics, Islamabad. SEM image were taken at magnification of 5 μm and shown in figure 3. Small amount of agglomeration were observed. SEM image reveals the formation of spongy cave like structure of sample S1, S2 and S3, while the image of sample S4 reveals the formation of nano flakes.

The elemental analysis of ZnO nanoparticles samples were carried out by energy dispersive X-rays (EDX) attached with the SEM. The elemental results show the atomic contents of elements, present in the ZnO nanoparticles. The elemental results by EDX show the increasing occurrence of Zn which reach to 88 wt% in the sample S4. Other constituents’ oxygen, potassium and calcium are also observed. The K and Ca came from the extract of plant, these elements act as stabilizing, capping and reducing agent. The elemental composition is given in table 2.

Functional groups by Fourier transform infrared spectroscopy
The IR spectra of fabricated samples is observed over the range of 400–4000 cm⁻¹ using Shimadzu IR Tracer-100 FTIR at Department of Physics, IIUI, and presented in figure 4. The selected peaks of ZnO are in the region from 400 to 500 cm⁻¹. The OH peaks are observed for all samples, which were due to the moisture in the sample. Amide group bond with N–H stretching was observed in all four samples; this shows that the prepared ZnO nanoparticles were surrounded by proteins and metabolites. The C=O polyphenol groups stretching vibration is also observed for all samples.

Energy band gap by UV–vis spectroscopy
ZnO nanoparticles shows only direct band gap, conforming a phase change. Tauc’s plot method is used to measure the direct band gap energy for all four samples in figure 5. An increasing trend from 2.93 eV to 5.46 eV was observed. We could not observe indirect band phase.
Culture sensitivity test for *E. coli*

The *Escherichia coli* pure bacterial culture was used to test the activity of the nanoparticles over the bacterial growth. *E. coli* strain was grown in Luria–Bertani (LB) medium in a composition of 1000 ml of H2O, 10 gram Bacto-tryptone, 5 gram yeast extract and 10 gram of NaCl. Strain was grown aerobically at 37°C, in 10 ml of medium glass culture tubes (Fisher Scientific) with shaking at 200 rpm. To examine the bacterial growth rate and determine growth behavior in the presence of the zinc oxide nanoparticles, *E. coli* were grown in liquid medium supplemented with different concentration of nanoparticle colloidal suspensions. Cultures of nanoparticles free medium under the same growth conditions were used as a control. These culture media were then transferred to petri dishes and incubated for 24 h with repeated screening of bacterial growth. This method was designed to have an understanding that how much the growth is restricted by putting a known concentration of the nanoparticles into the pure bacterial culture. The concentration of the ZnO nanoparticles was used in a way that the material mixed directly with the bacterial culture and be used for inoculation and subsequent incubation. The mix was applied by streaking under sterile conditions, on the plates containing media and they were incubated for some initial duration to check the growth.

Initially 5–6 h incubation was done in order to know the initial growth with the mixed inoculum and nanomaterials. Initially no preliminary growth/bacterial colonies were observed; next 24 h incubation was carried out to see the growth with the mixed inoculum and nanomaterials; shown in figure 6. Again, growth/bacterial colonies were not observed confirming that the growth was restricted either by the toxicity of the ZnO nanoparticles or...
nanoparticles, their higher concentration and/or their interference with the biological pathways of the *E. coli* cells. If this is the result of nanoparticles toxicity there is little use of the nanomaterial in restricting the incubation, and to be used as a medicine/remedy. Whereas if this restricted bacterial growth is a result of biological pathways, then this will need additional systematic studies to understand the action of the nanomaterial on bacterial germination.

Figure 4. Fourier transform infrared spectroscopy of all the samples S1, S2, S3, and S4.

Figure 5. Tauc Plots for the measurements of energy band gap of (a) S1, (b) S2, (c) S3, (d) S4.
Conclusion

The current research work was aimed to analyze the effective synthesis of ZnO nanoparticles by utilizing *Melia azedarach*, through green synthesis method. Additionally the study is supported by the application of fabricated ZnO nanoparticles against one of the wide spread pathogen the *E. coli* and to address the potential use of these ZnO NPs for antibacterial activity. The discussion was on the antibacterial activity of ZnO-NPs with various elements affecting the activity, for example ZnO particle size, surface morphology and amazing antibacterial results were obtained. The green method reflected as naturally sheltered as eco-accommodating, less tedious and cost effective technique. In our experiment, a constant amount of extract was maintained while the precursor concentration was increased in order to decrease the amount of capping agent. Due to this, the crystallite size was decreased showing the dependence of the particle size on the nature of the used salt. ZnO-NPs with large surface to volume ratio possess unique properties and excellent stability with long life compared with organic based commonly used disinfectants. The prepared nanoparticles were found stable after a period of three months, with crystallite size ranging between 8.7 nm to 7.0 nm, which is the minimum size of reported zinc oxide nanoparticles. The anti-bacterial study supported that green route prepared ZnO nanoparticles potentially and successfully hinder the bacterial growth, particularly against one of the most widespread environmental hazard i.e. *E. coli*. These nanoparticles can be used in various forms to control the *E. coli* prevalence in various Eco-zones and ecosystems around the world, especially in drinking and food causing diarrhea, dysentery and other gastrointestinal track disorders as environmentally safe material.

Acknowledgments

The authors would like to thank Umair Ibrahim, Muhammad Afran for their support and help during the experiment.

Data availability statement

Any data that support the findings of this study are included within the article.

ORCID iDs

Waqar A A Syed  https://orcid.org/0000-0001-8917-520X
References

[1] Rana S, Rawat J, Sorensson M M and Misra R D K 2006 Antimicrobial function of Nd\textsuperscript{4+} doped anatase titania-coated nickel ferrite composite nanoparticles P. A Biomaterial System. Acta Biomaterialia 2 421–32

[2] Wishart D, Arndt D, Pon A, Sajed T, Guo A C, Djoumbou Y, Knox C, Wilson M, Liang Y, Grant J, Liu Y, Goldansaz S A and Rappaport S M 2015 T3DB: the toxic exposomedatabase Nucleic Acid Res. 43 1929–34

[3] Jones M R, Osberg K D, Macfarlane R J, Langille M R and Mirkin C A 2011 Templated techniques for the synthesis and assembly of plasmic nanostructures Chem. Rev. 111 3736–827

[4] Rahghunath A and Perumal E 2017 Metal oxide nanoparticles as antimicrobialagents: a promise for the future Int. J. Antimicrob. Agents 49 137–52

[5] Guo B L et al 2015 The antibacterial activity of Ta-doped ZnO nanoparticles Nanoscale Res. Lett. 10 1047

[6] Reddy I S, Nisha M M, Joice M and Shilpa P N 2014 Antimicrobial activity of zinc oxide (ZnO) nanoparticle against Klebsiellapneumoniea Pharm. Biol. 52 1388–97

[7] Wagner G, Korenkov V, Judy J D and Bertsch P M 2016 Nanoparticles composed of Zn and ZnO inhibit Peronosporatabacina spore germination in vitro and P. tabacina infectivity on tobacco leaves Nanomaterials (Basel) 6 30

[8] Hameed A S et al 2016 In vitro antibacterial activity of ZnO and Nd doped ZnO nanoparticlesagainst ESBL producing Escherichia coli and Klebsiellapneumoniea Sci. Rep. 6 24312

[9] Sirelkhaitim A et al 2015 Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism Nano–Micro Lett. 7 219–42

[10] Stoimenov P K, Klinger R L, Marchin G L and Klabunde K J 2002 Metal oxide nanoparticles as bactericidal agents Langmuir 18 6679–86

[11] Kolekar T V, Bandgari S S, shirguppikar S S and Ganachari V S 2013 Synthesis and characterization of ZnO nanoparticles for efficient gas sensors Arch. Appl. Sci. Res 5 20–8

[12] Iravani S 2011 Green synthesis of metal nanoparticles using plants Green Chem. 13 2638–50

[13] Russell A B, Hardin J W and Grand L 1997 Melia azedarach Int. Poisonous Plants of North Carolina

[14] M A M 2013 Pharmacological potentials of Melia Azedarach L. – a review American Journal of BioScience 1 44

[15] Azam M M, Mamunor R A, Towfiqee N M, Sen M K L and Nasrin S 2013 Pharmacological potentials of Melia azedarach L A JBIO 1 44

[16] Sumathi A 2013 Evaluation of physicochemical and phytotoxicity of Melia Azedarach. Leaves (family: meliaceae) Int. J. Pharm. Pharm. Sci. Supplement 2 104–107

[17] Khan A V, Farveen G, Alam M M and Singh V K 2002 Ethnomedical uses of Neem in rural areas of Uttar Pradesh, India Ethnomed and Pharmacou. II Rec. Prog. Med. Plants 7 319–30

[18] Wishart D, Arndt D, Pon A, Sajed T, Guo A C, Djoumbou Y, Knox C, Wilson M, Liang Y, Grant J, Liu Y, Goldansaz S A and Rappaport S M 2015 T3DB: the toxic exposomedatabase Nucleic Acid Res. 43 1929–34

[19] Vijendra N and Kumar K P 2010 Traditional knowledge on ethno-medicinal uses prevailing in tribal pockets of Chhindwara and Betul Districts, Madhya Pradesh, India African Journal of Pharmacy and Pharmacology 4 662–70

[20] Vidya C A, Hirematha M N, Antonyraja M A L, Gopala I V, Jaina A and Bansala K 2013 Green synthesis of ZnO nanoparticles by Calotropis Gigantea International of Current Engineering and Technology 118 120 http://scholar.google.com/scholar_javauthor=Hiremath%20A%20C&as_sdt=0,5

[21] Singh R P, Shukla V K, Yadav R S, Sharma K P, Singh P K and Pandey A C 2011 Biological approach of zinc oxide nanoparticles formation and its characterization Advanced Materials Letters 2 513–7

[22] Shukhawat M S, Ravindran C P and Manokari M 2014 Biogenesis of zinc oxide nanoparticles from Passiflora foetida L. extracts and their characterization International Journal of Green and Herbal Chemistry 3 518–23

[23] Ravindran C P, Manokari M and Shukhawat M S 2016 Biogenic production of zinc oxide nanoparticles from aqueous extracts of Duranta erecta L. World Scientific News 28 30–40

[24] Gnanasangeetha D and Thambavani S D 2013a Biogenic production of zinc oxide nanoparticles using Alcypha indica Journal of Chemical, Biological and Physical Sciences 4 438–46