



BIOGENIC SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING LEAF EXTRACT OF *JUSTICIA ADHATODA* AND THEIR ANTIMICROBIAL ACTIVITY

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Abstract: In the present study, an inexpensive green route has been demonstrated for the formation of ZnO nanoparticles by biogenic method using aqueous leaf extract of *Justicia adhatoda* which acts as a reducing and stabilizing agent. The synthesized ZnO nanoparticles were preliminarily characterized by UV-VIS followed by using different analytical techniques such as X-ray diffraction (XRD) and fourier transform infrared spectroscopy (FT-IR). The XRD pattern confirmed that, the synthesized ZnO nanoparticles are of hexagonal wurtzite structure with average calculated grain size less than 9.40 nm. The FT-IR spectra indicated the presence of hydroxyl groups, carboxylic acids which may be responsible for biochemical reaction. The clear zone of inhibition against both gram- positive and gram-negative bacteria confirmed the antimicrobial potential of synthesized ZnO nanoparticles.

Key words: Biogenic, Zinc nitrate, Nanoparticles, XRD, FTIR, Antimicrobial activity.

Introduction

Nanotechnology, one of the modern techniques of material science is based on the manipulation of individual atom or molecule to produce new material at nanoscale level (1-100 nm) for function well below sub microscopic level (Jemal et al., 2017). New synthesized nano molecule exhibits completely new or improved properties based on size, distribution and morphology, with enhanced catalytic activity, thermal conductivity and non-linear optical performance (Sangeetha et al., 2011 and Sharma et al., 2018). Biosynthesized ZnO nanoparticles has drawn worldwide interest in past two-three years due to their facilitating properties i.e., biocompatible, biodegradable, less hazardous, non toxic, eco-friendly and wide range of applicability in different fields i.e., Agriculture, medical, electronic, optic and other material sciences (Sun et al., 2000; Schaffer et al., 2009; Balgobind et al., 2016 and Sharma et al., 2018).

Further, among all inorganic semiconductor nanoparticles, ZnO nanoparticles have attracted increasing attention because they are easy, inexpensive and safe to prepare. Besides, US FDA considered and enlisted ZnO nanoparticles as “Generally recognized as safe” (GRAS) metal oxide (Jayaseelan et al., 2012 and Agarwal et al., 2017).

Justicia adhatoda commonly known as Vasaka is a small evergreen, sub herbaceous bush of Acanthaceae family. The plant is distributed in the open sparse tree canopy habitat in tropical to subtropical areas upto 1450 masl (Khan et al., 2018). The plant is widely used in Ayurvedic, homeopathy and Unani systems of medicine (Bisht and Khajuria, 2014). The plant is equally popular in folk system of medicine and used to treat number of ailments (Sharma et al., 1992; Haider et al., 2011 and Khan et al., 2018).



Pharmacologically plant is known to have antioxidant (Khan et al., 2018), antibacterial (Pa and Mathew, 2012; Bose and Chatterjee, 2015; Sharma and Kumar, 2016), antifungal (Fatima et al., 2016), antitussive (Barth et al., 2015), cardioprotective (Atal, 1980 and Chandhoke, 1982), anthelmintic activity (Sobia et al., 2018), hepatoprotective Activity (Afzal et al., 2013), haemagglutination (Khan et al., 2013) and anticancerous activity (Batool et al., 2017 and Latha et al., 2018).

Besides its medicinal value, plant is used as a hedge plant because the leaves of the plants are unpalatable to livestock thus its cultivation as a hedge in areas where free-roaming, browsing livestock can be a problem for field crops. Further, the value of the plant to be a bee forage plant is reported in different states (Sobia et al., 2018).

Thus in the present work, an attempt has been made to synthesize nanoparticles using leaf extract of *Justicia adhatoda* and evaluate their antimicrobial potential against gram positive and gram negative bacteria.

Material and Methods

Collection and processing of plant material:

Leaves of *Justicia adhatoda* were collected from its natural population at village Kaptiyal, Tehsil Indora area of District Kangra, Himachal Pradesh, India. Collected leaves were washed thoroughly under tap water to remove all adhering soil particles. The washed leaves were then shade dried for more than 15 -20 days. The dried leaf material was crushed into fine powder with the help of motor and pestle. Powdered leaves (8 gm) were soaked in 100 ml distilled water in conical flask, followed by heating on the hotplate at constant temperature of 60°C for 10 minutes. The prepared extract was allowed to cool at room temperature and finally filtered with the help of filter paper. The final volume of the extract was made to 100 ml.

Green synthesis of Zinc Oxide nanoparticles:

Zinc nitrate hexahydrate was used as precursor of

Zinc. The synthesis of ZnO nanoparticles was carried by mixing 100 ml of the plant extract with 100 ml of zinc nitrate hexahydrate extract in 500 ml flask. Prepared leaf extract was warmed for few minutes before the addition of Zinc extract in the flask slowly. After complete pouring, the mixture of both solutions was kept for vigorous stirring at constant temperature (60 °C) for 1hour. The change in colour of the solution was considered as a visual marker for the synthesis of nanoparticles, followed by their precipitate formation. The precipitate formed in the reaction was allowed to settled down for overnight and collected by centrifuging the solution at high speed of 8000 rpm for 10 mins. The collected pellets were washed with the help of double distilled water thrice followed by drying the pellets in oven for 10 hrs. at 60 °C. Finally nanoparticles were homogenized using mortar and pestle and then collected in air tight bottles for further use.

Characterization of synthesized nanoparticle:

Preliminary characterization of synthesized nanoparticles was made by visual markers. Secondary characterization of synthesized ZnO nanoparticles was carried out using different analytical tools i.e., UV-Vis, XRD (X-Ray Diffraction) and FTIR (Fourier Transform Infrared Spectroscopy).

Antibacterial assay: To study bactericidal effect of synthesized nanoparticles, four bacterial strains two gram positive (*Staphylococcus aureus* and *Streptococcus pneumonia*) and two gram negative (*Klebsiella pneumoniae* and *Escherichia coli*) were assayed by agar well diffusion method.

Results and discussion

During the synthesis of ZnO nanoparticles, change in the colour of reaction mixture from its initial stage (dark brown) to final stage (yellow brown) was used as positive indicator for the green synthesis of ZnO nanoparticles (Figure 1 a&b). Further, confirmation of ZnO synthesis was carried out by using UV-VIS technique and



obtained results showed peaks in the region at 340 to 390 nm, which could be attributed to the ZnO nanoparticles.

The synthesized nanoparticles were then subjected to conformational details about the shape, size and functional group attached if any which may be responsible for reduction and capping of synthesized nanoparticles by the following methods.

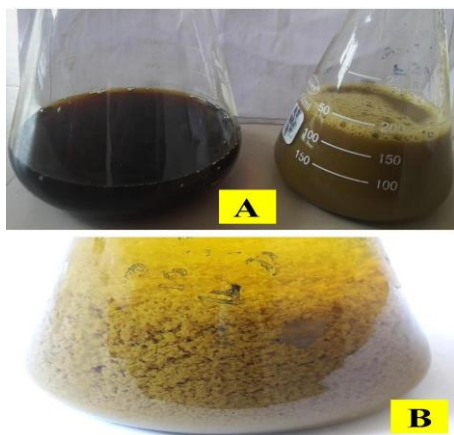


Figure 1: a & b: A= Change in colour indicating synthesis of nanoparticles. B=Image showing precipitate formation.

XRD analysis was carried out to study the crystallinity, size and shape of the unit cell of the

synthesized nanoparticles using the diffraction of X-ray. X-ray penetrates into the nano material and obtained diffraction patterns were compared with standards (ICSD) to get structural information. Each crystalline has its unique identity, thus used to determine, how the atoms packed together at what angle and what is the inter-atomic distance between them.

Size of nanoparticles were estimated by Scherrer's equation

$$D = \frac{0.9\lambda}{\beta \cos\theta}$$

Where, D is crystallite size; λ is wavelength; θ is Bragg angle; β is Full width at half maxima

Where, Bragg law is

$$2d \sin \theta = n \lambda$$

d= interplaner distance and n= Order of diffraction.

The XRD spectra of leaf extract mediated ZnO nanoparticle showed crystalline nature of ZnO nanoparticles and the average calculated size using Debye-Scherrer's equation was less than 9.40 nm. XRD spectra showed distinct peaks at $2\theta = 31.87, 31.95, 34.52, 34.61, 36.36, 36.46, 47.57, 47.70, 56.66, 56.82, 62.95, 63.13$, confirming the hexagonal closed pack nano crystals (Figure 2).

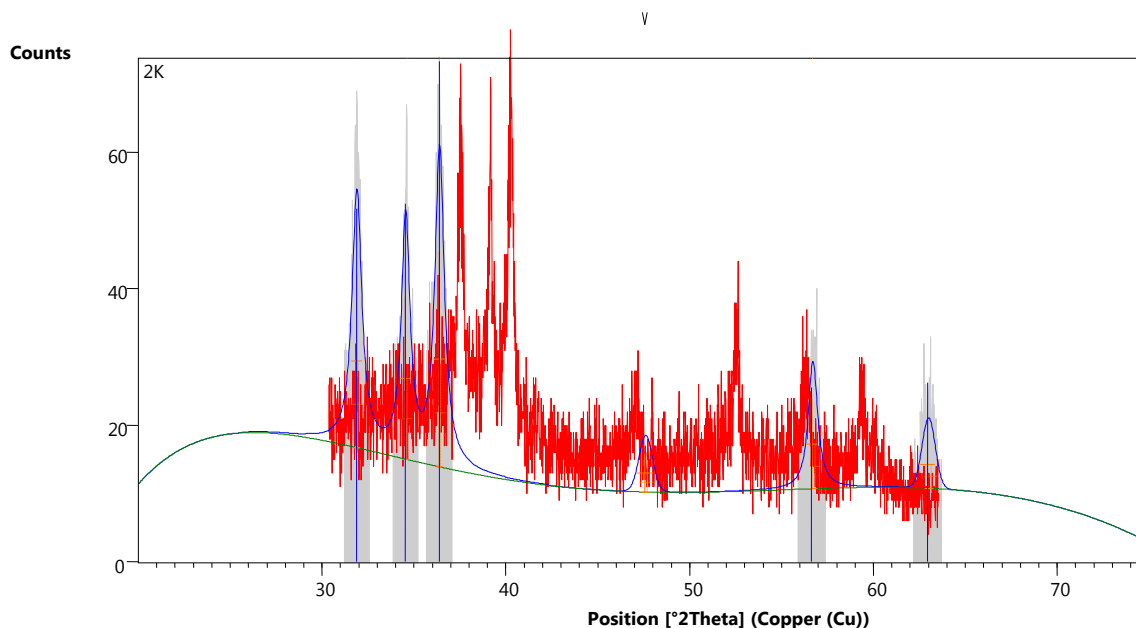


Figure 2: XRD Spectrum of ZnO nanoparticles synthesized using leaves of *Justicia adhatoda*



FTIR spectra is used to analyse the presence of functional groups on the surface of synthesized nanoparticles. FTIR spectrogram of leaf based synthesized ZnO nanoparticles showed various peaks which corresponds to different organic groups. Broad band at 3400.18 cm⁻¹ corresponds to the stretching vibrations -OH group (hydroxyl

group). Further, peaks at 1595.70 cm⁻¹, 1513.40 cm⁻¹, 1386 cm⁻¹, and 1080 cm⁻¹ which again corresponds to C=O frequency of extensive conjugated system, C=C aromatic stretchings, and C-O stretchings respectively (Figure 3). Further, presence of band at 538.40 cm⁻¹ attributed to ZnO hexagonal structure.

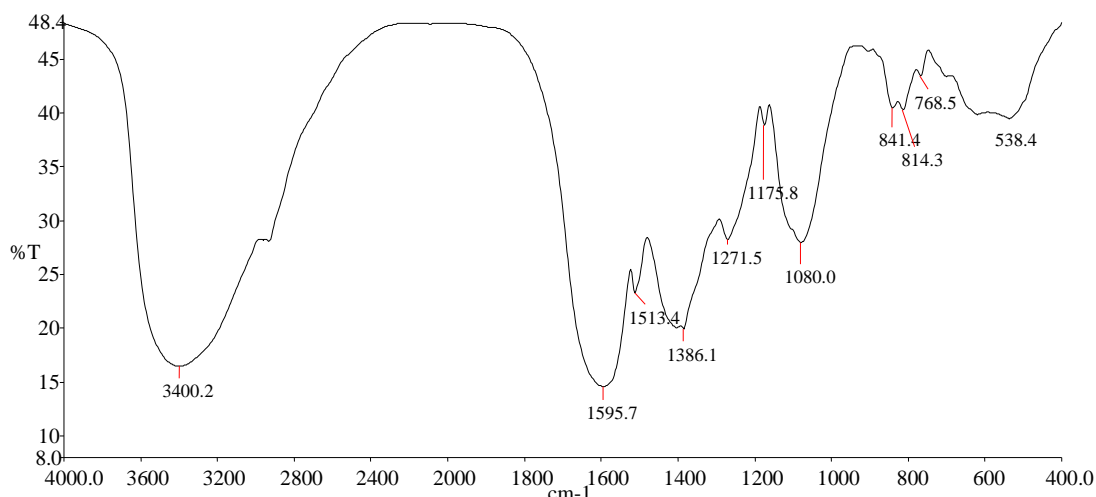


Figure 3: FTIR Spectrum of ZnO nanoparticles synthesized using leaves of *Justicia adhatoda*.

Synthesized ZnO nanoparticles (100 µg) were used by well diffusion method against gram positive (*Staphylococcus aureus* and *Streptococcus pneumoniae*) and gram negative (*Klebsiella pneumoniae* and *Escherichia coli*) bacteria. Saline was used as control in the present study and it was found that saline have no effect

on bacterial growth and gives no zone of inhibition. While ZnO nanoparticles showed antimicrobial potential against all test organisms. The maximum zone of inhibition (12.90±0.36 mm) was reported for *Staphylococcus aureus*, while the minimum zone of inhibition (9.77±0.40) was reported for *E. coli* (Table 1).

Table 1: Antimicrobial activity of leaf based ZnO nanoparticles against test organisms.

Concentration	Test organisms				
	Control	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>E. coli</i>	<i>Streptococcus pneumoniae</i>
100 µg	0.00	12.90±0.36	12.60±0.79	9.77±0.40	12.47±0.83

± Standard deviation

The possible pathway may involve in this bactericidal activity production of reactive oxygen species, which cause dis-functioning of cell membrane of bacteria resulting in death of bacterial cell by oxidizing its membrane lipid layer (Akhtar *et al.*, 2012; Setyawati *et al.*, 2013 and Soenen *et al.*, 2015). Findings of the present work are in correlation with the work of other

workers i.e., Raghupathi *et al.*, (2011), Senthkumar *et al.* (2014) and Naqvi *et al.*, (2019) who reported that ZnO nanoparticles induce antibactericidal activity.



Conclusion

The synthesis of crystalline ZnO nanoparticles with an average size of about 9.40 nm by green and environment friendly pathway using the plant extract of *Justicia adhatoda* as an effective reducing and capping chemical agent has been demonstrated. The role of phytochemical in reduction and capping was further confirmed by presence of phytochemicals on the surface of nanoparticles in FTIR studies. Nanoparticles showed good antimicrobial activity against tested micro-organisms. Thus, this route may contribute in the synthesis of stable ZnO nanoparticles to substitute to the physical and chemical methods and developing some reliable drug in the era of multi-drug resistance.

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