Antibacterial Studies of Synthesized Doped Zinc Oxide Nanoparticles by Using Triumfetta rotundifolia Plant Extract

P. Ananthi[1],S. Mary Jelastin Kala[2],A. Maria Clara[3]

[1] Assistant Professor of Chemistry, PET Engineering College, Vallioor, Tirunelveli, [2] Assistant Professor of Chemistry, St. Xavier's College, Palayamkottai, Tirunelveli, [3] PG Department of Chemistry, St.Xavier'College, Palayamkottai, Tirunelveli.

Abstract- **Eco-friendly green methods for the production of metallic nanoparticles have many advantages in the field of nano-technology. The aim of the present work, describes a cost effective and environment safe technique for green synthesis of doped zinc oxide nanoparticles by using the plant extract as reducing agent. The synthesized nanoparticles have been characterized by UV-VIS spectra, FTIR, XRD, SEM, EDX, and CV. The absorption maximum was scanned by UV-VIS spectroscopy. Further these biologically synthesized nanoparticles exhibit a tremendous anti bacterial activity. Hence, the plant based route could be considered as fast and easy bio process of nanoparticles production.**

Keywords: Doped zinc oxide nanoparticles, UV-VIS, FTIR, XRD, SEM, EDX, Bacterial studies

INTRODUCTION

In recent years, metal and metal oxide nanoparticles have been intensively studied in the past decade. The field of nanotechnology is one of the most active researches in modern material science. Nanotechnology is emerging as a rapid growing field with its applications in science and technology for the purpose of manufacturing new materials at the nano scale level. The nano sized materials have been an important subject in the field of basic and applied sciences. Nanomaterials have different size and shapes so, its appealed considerable attention because of their peculiar physicochemical properties compared to the bulk materials. Nowadays, there are plenty of opportunities to fully employ in modern clinical technology the novel concepts and phenomena that have appeared in nanoparticles research field [1]. The bio synthesized doped zinc oxide nanoparticles are trusted to be friendly with environment, non toxic, bio safe and bio-compatible. It is desirable for bio medical application such as drug carriers, cosmetics and filling the medical materials [2]. Based on the review of literature, the bio synthesis of doped zinc oxide nanoparticles using various plants has been carried out. In this present study, bio synthesized doped zinc oxide nanoparticles using T.rotundifolia plant aqueous extract. (Family: Tiliaceae). The size, structure and morphology of the synthesized nanoparticles have been established on the basis of elemental analysis and spectral data.

MATERIALS

Z Zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$),Ammonium heptamolybdate ($(NH_4)_6Mo_7O_{24}$)) were purchased from Hi-Media chemicals. Ultra pure deionised water was used throughout the reactions. All glass wares were washed with concentrated sulphuric acid (H_2SO_4) and deionised water then dried in hot air oven.

Collection of plants: The plant of T.rotundifolia was collected from Kovilpatti, Tuticorin District, Tamil Nadu, India during the month of March. The plant was identified and authenticated by Botanist Dr. V. Chelladurai, Research Officer-Botany (Scientist-C) Central Council for Research in Ayurveda & Siddha, Government of India (Rtd). The collected plant materials were thoroughly washed several times using normal water and then followed by distilled water to remove impurities. The cleaned plant materials were subsequently dried under sunshade to remove moisture completely, powdered by using mechanical grinder and then stored.

METHODS

Preparation of Plant extracts: 5g of the collected plant T.rotundifolia powder was mixed with 100 mL of double distilled water and refluxed for 30 minutes and cooled down to room temperature. The solution was then filtered through whatman No.1 filter paper and the filtrate obtained was stored then at 4°C for future work.

Synthesis of Mo ion-doped zinc oxide nanoparticles using plant extract of T.rotundifolia:

For the synthesis of Mo ion-doped zinc oxide nanoparticles, 50 mL of T.rotundifolia plant extract was taken and boiled to 60-80ºC using a stirrer-heater. 5g of zinc nitrate and 1% of ammonium molybdate solution were added to this extract and the temperature reached 60ºC. The solution was then boiled until a deep yellow coloured paste was formed. This paste was then collected in a ceramic crucible and heated in an air heated furnace at 400ºC for 2 hours. A light yellow coloured powder of Mo ion-doped zinc oxide nanoparticles was obtained and this was carefully collected and preserved in the air-tight vials for further studies. The powder was mashed in a mortar-pestle so as to get a finer nature of the sample for further characterization studies. Similar procedure was adopted for the synthesis of different percentage of Mo ion content in the doped samples by varying the amount of ammonium molybdate in the range of 2% and 3%.

Characterization Techniques:
The absorption spectra of the synthesized nanoparticles were measured by using UV-1800 SHIMADZU UV-spectrophotometer instrument in 200 to 500. The possible functional groups involved in the synthesis and stabilization of nanoparticles was identified by performing FTIR analysis in the range 400-4000 cm-1 using SHIMADZU-8400S model instrument, and the potassium bromide (KBr) pellet method was applied for spectral analysis. Crystalline size and structure of the synthesized nanoparticles were carried out by XRD using BRUKER, ECO D8 Advance model instrument. Detailed analysis of the morphology, size and distribution of the nanoparticles was documented by Scanning Electron Microscopy (SEM) using CARL ZEISS, EVO 18 model instrument. Elemental analysis of nanoparticles measured by EDX was carried out on BRUKER, X flash 6130 model instrument. In AFM analysis, the sample was analysed using a Veeco di Caliber set to tapping mode with a Veeco TESP silicon tip. Cyclic Voltammetry was carried out by using Chi 650C electrochemical work station, (USA) instrument.

Antibacterial activity: The antibacterial screening of the synthesized nanoparticles was assessed against clinical bacterial strains such as Pseudomonas aeruginosa, Escherichia coli, Staphyloccus aureus, Klebsiella pneumonia and Salmonella typhi. In order to determine the antibacterial activity of the synthesized Mo-ion doped nanoparticles using T. rotundifolia at different concentrations (5µl, 10µl and 15 µl) by nutrient agar well diffusion method [3]. Sterile nutrient agar medium was inoculated with 0.1ml of fresh overnight sterile nutrient broth culture of bacterial strains (approx.107CFU/ml) and poured into sterile petri plates. Synthesized Mo-ion doped nanoparticles' extracts at three different concentrations were poured by using sterile pipette and in the middle well a known antibacterial drug Ofloxin (1mg) was poured, and act as a positive control.

After holding the plate at room temperature for 2 hours to allow diffusion of the extracts and controls in to the nutrient agar medium. The plates were incubated at 370C for 24 – 48h. The sensitivity of the test organisms to each of the extracts were indicated by clear halo around the well. The halos' diameter as an index of the degree of sensitivity, were measured with a transparent plastic ruler. Then they were examined for inhibition of the bacterial growth. The diameters of the zones of inhibition in each case were expressed as mm sensitivity [4].

RESULT AND DISCUSSION

UV-Visible Spectroscopy: The UV-Vis spectrum of synthesized Mo ion-doped zinc oxide nanoparticles using T.rotundifolia plant extract is shown in Figure 1. The absorption band at 302 nm is observed for Mo ion-doped zinc oxide nanoparticles for using T.rotundifolia plant extract. The absorption wavelengths are seen to be slightly shifted towards lower wavelength. This blue shift might be attributed to the smaller size of nanoparticles [5].

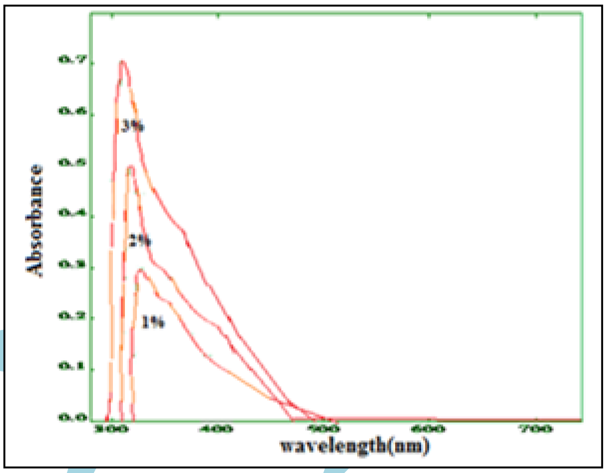


Figure 1 UV-Visible spectrum of synthesized Mo ion-doped zinc oxide nanoparticles T.rotundifolia plant extract

Fourier Transform Infrared Spectroscopy: Figure 2-4 shows the FTIR spectrum of Mo ion-doped zinc oxide nanoparticles synthesized using plant extract of *T.rotundifolia*. The peak at 3510 cm^{-1}corresponds to O-H stretching vibration of phenols. The peak observed at 3390 $cm^{-1}$is assigned to N-H stretching vibrations of amide. The peak at 2833 cm^{-1} corresponds to C-H stretching of aldehydes. The peaks at 2428 cm^{-1}, 2397 cm^{-1}, 2339 cm^{-1} represent O-H stretching of carboxylic acids. The peak at 1762 $cm^{-1}$is assigned to C=O stretching of carbonyls. The peak at 1494 cm^{-1} corresponds to C-C stretching of aromatics. The peaks at 1384 cm^{-1}, 1355 cm^{-1}, 1319 cm^{-1} represent C-N stretching vibrations of aromatic amines. The peak observed at 950 cm^{-1} corresponds to C–N stretching vibrations of the amine or –C–O–C or –C–O groups of carboxylic acids [6]. The peak at 893 cm^{-1}can be ascribed to the aromatic C-H out of plane bending. The peaks at 869 cm^{-1}, 621 cm^{-1} and 748 $cm^{-1}$are due to the stretching vibrations of zinc oxide nanoparticles [7]. The peak observed at 439 cm^{-1} corresponds to the Mo ion bending vibration band [8]. The bonds or functional groups such as –C–O–C–, –C–O, and –C=C– derived from heterocyclic compounds. e.g. alkaloid or flavones and the amide bond derived from the proteins that are present in the plant extract are the capping ligands of the nanoparticles [9].

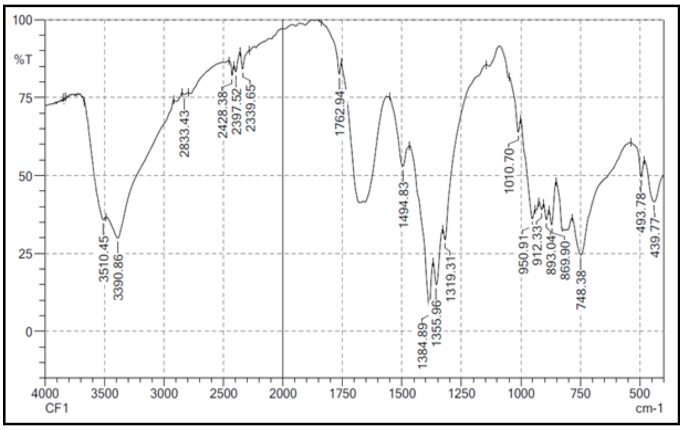


Figure 2 FTIR spectrum of 1% Mo ion-doped zinc oxide nanoparticles synthesized using T.rotundifolia plant extract

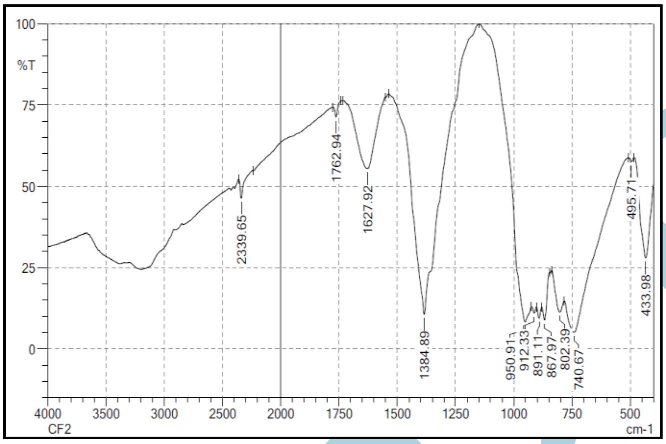


Figure 3 FTIR spectrum of 2% Mo ion-doped zinc oxide nanoparticles synthesized using T.rotundifolia plant extract

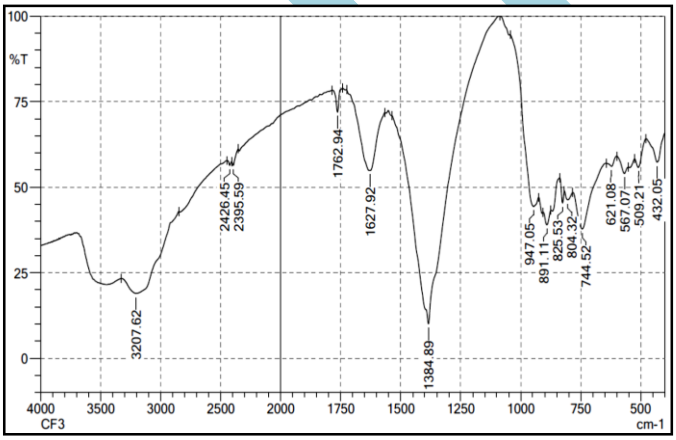


Figure 4 FTIR spectrum of 3% Mo ion-doped zinc oxide nanoparticles synthesized using T.rotundifolia plant extract

X-Ray Diffraction analysis: Structural parameters of doped zinc oxide nanoparticles synthesized using T.rotundifolia extract is calculated from the XRD pattern. The prepared Mo-ion doped zinc oxide nanoparticle is crystalline structure. Calcination at 4000C is essential for complete removal of water and to obtain higher crystallinity. The X-ray diffraction pattern of doped zinc oxide nanoparticles synthesized using T.rotundifolia plant extract is shown in Figure 5. The spectrum of doped zinc oxide nanoparticles exhibits sharp peaks at 2θ equal to 23.94º, 24.47º,26.33º,28.30º,33.24º,41.47 º,and 57.48 º. These peaks are identified to originate from (111), (100), (101), (110), (103), (112), and (201) which are in good agreement with those of powder Mo-ion doped zinc oxide obtained from the International Centre of Diffraction Data card (JCPDS-36-145117). The average crystallite size of the synthesized zinc oxide nanoparticles is calculated to be 28.89 nm using Debye-Scherrer equation as shown in Table 1 [10].

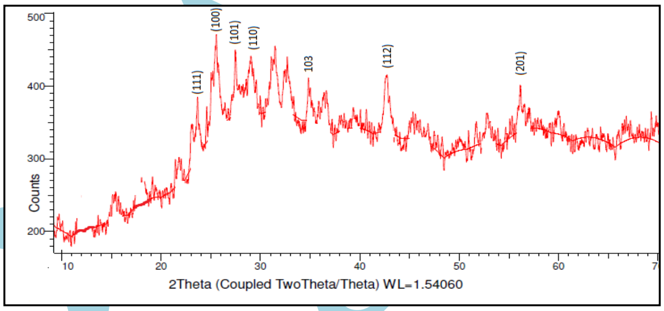


Figure 5 XRD Spectrum of Mo-ion doped zinc oxide nanoparticles synthesized using T.rotundifolia plant extract

Table 1 XRD parameters of Mo-ion doped synthesized zinc oxide nanoparticles using T.rotundifolia plant extract

2θ(°)	**FWHM(β)**	**Crystalline Size(nm)**	**Plane**
23.94	0.31	28.29	111
24.47	0.34	28.31	100
26.33	0.30	28.41	101
28.30	0.33	28.54	110
33.24	0.36	28.88	103
41.47	0.34	29.58	112
57.48	0.30	31.35	201

Scanning Electron Microscopy: SEM was employed to analyze the morphology and the growth features of the prepared nanoparticles. Figure 6 shows the SEM image of Mo ion-doped zinc oxide nanoparticles synthesized using T.rotundifolia. This exhibited spherical as well as rod crystalline like structure. From SEM images, the crystallite size range of Mo ion-doped zinc oxide nanoparticles synthesized using T.rotundifolia is 10-100 nm

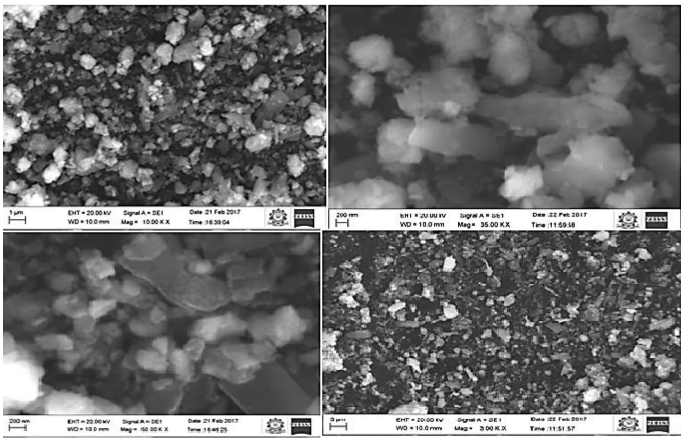


Figure 6 SEM photographs of Mo ion doped zinc oxide nanoparticles synthesized using T.rotundifolia plant extract at different magnification level

Energy Dispersive X-Ray analysis: The elemental composition of the Mo-ion doped zinc oxide nanoparticles are carried out by EDAX analysis. Figure 7 shows the EDAX spectrum of Mo ion-doped zinc oxide nanoparticles synthesized using T.rotundifolia. Mo ion-doped zinc oxide nanoparticles are found to have atomic percentage 43.71 of Zn, 12.20 of O, 22.52 of Mo as shown in Table 2. This confirms the doping of Mo ion in zinc oxide lattice.

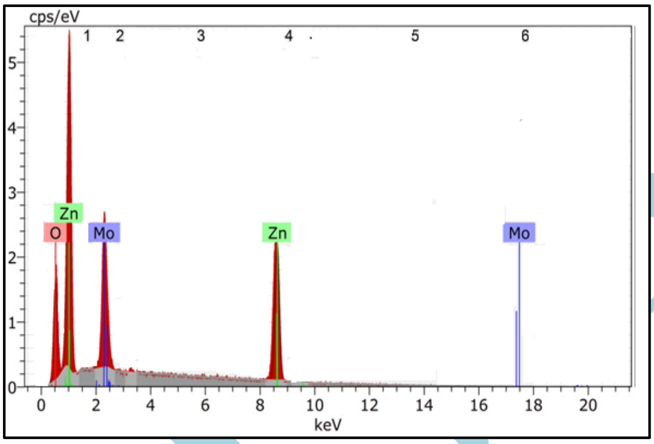


Figure 7 EDAX Spectrum of synthesized Mo ion-doped zinc oxide nanoparticles using T.rotundifolia plant extract

Table 2 Elemental composition of Mo ion-doped zinc oxide nanoparticles synthesized using T.rotundifolia plant extract

Element	**Unn.C [wt.%]**	**Norm.C [wt.%]**
Zinc	43.71	54.57
Oxygen	12.20	13.35
Molybdenum	22.52	32.08
Total	**78.43**	**100.00**

Cyclic Voltammetry: Cyclic Voltammogram of Mo-ion doped zinc oxide nanoparticles using T.rotundifolia is shown in Figure 8. In this cyclic voltammogram, cathodic and anodic peaks are observed in the potential range -0.8 to 1.0 V. After the cathodic peak position, it is readily shifted to anodic peak position at -0.5 V. So, it is a redox reaction.

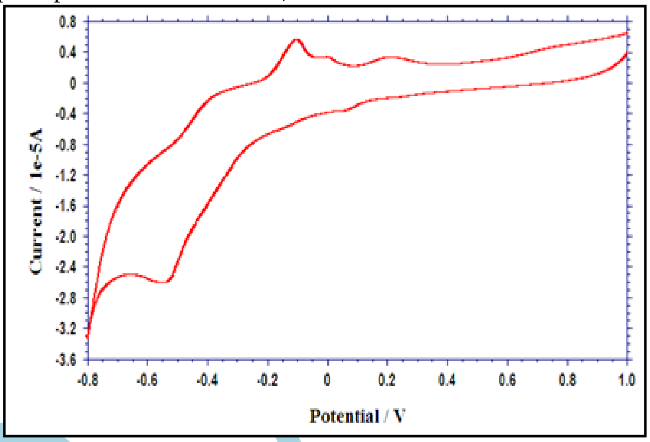


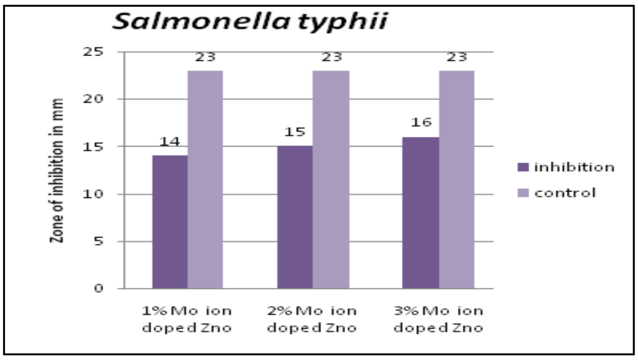
Figure 8 Cyclic Voltammogram of Mo ion-doped zinc oxide nanoparticles synthesized using T.rotundifolia plant extract

Antibacterial activity: In-vitro antibacterial activity of the synthesized Mo ion-doped zinc oxide nanoparticles using T.rotundifolia shows significant antibacterial activity against Bacillus subtilis, Staphylococcus aureus, Staphylococcus albus (Gram positive) and Pseudomonas aeruginosa, Salmonella typhii (Gram negative) bacterial are was shown in Figure 9. The results expressed as zones of inhibition in mm are noted in Table 3. Amikacin is used as a control. All the bacterial pathogens show a perceptible activity against the synthesized Mo ion-doped zinc oxide nanoparticles. Several researches confirming antibacterial activity of zinc oxide nanoparticles against the food related bacteria Bacillus subtilis, Escherichia coli, and Staphylococcus aureus have been reported [11&12]. Zinc oxide nanoparticles are also known to exhibit antibacterial activities against E. coli [11]. The formation of hydrogen peroxide from the surface of zinc oxide is considered to be mainly responsible for its antibacterial property [13]. Thus from this study it can be concluded that T.rotundifolia extracts can be used for the synthesis of Mo-ion doped zinc oxide nanoparticles. This study also suggests that synthesized Mo-ion doped zinc oxide nanoparticles can be used as an alternative to the existing antibacterial agents.

Table 3 The zone of inhibition values (in mm) of synthesized Mo-ion doped zinc oxide nanoparticles using T.rotundifolia plant extract

Bacterial Strains	**1% Mo ion-doped ZnONPs**	**2% Mo ion-doped ZnONPs**	**3% Mo ion-doped ZnONPs**	**Control Amikacin**
Pseudomonasaeruginosa	13	12	14	21
Bacillus subtilis	11	13	11	22

Staphylococcus aureus	15	16	17	20
Staphylococcus albus	18	20	25	26
Salmonella typhi	14	15	16	23



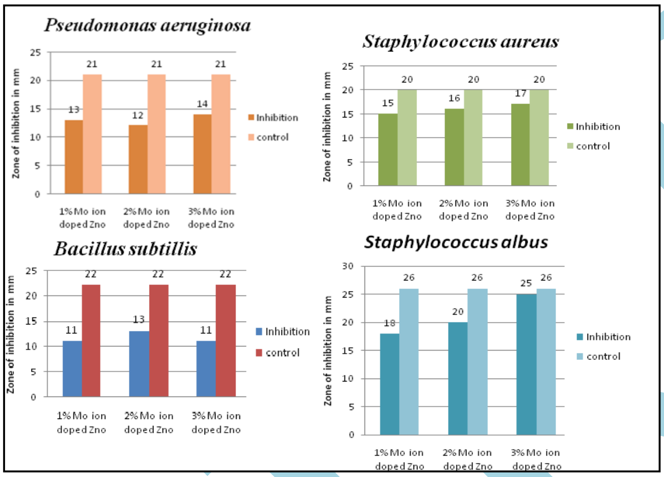


Figure 9 Antibacterial activities of Mo-ion doped zinc oxide nanoparticles using T.rotundifolia against different bacterial strains

CONCLUSION :

Green synthetic strategy should be adopted for a healthy future of nanotechnology. Colloidal based nanotechnology has been developed to control the size, shape, uniformity and functionality. Green chemistry approach towards the synthesis of nanoparticles has many advantages such as ease with which the process can be scaled up and economic viability. A fast, eco-friendly and convenient method has been developed for the synthesis of metal, and metal oxide nanoparticles, are attracting attention as they do not involve the harsh conditions that are required in the chemical and physical synthetic methods. Eco-friendly nanoparticles, bactericidal, antioxidant, anti-inflammatory, wound healing and other medical and electronic applications, make this method potentially exciting for the large-scale synthesis of other nanomaterials.

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