

## Evaluation of Synergistic Potential of Zinc Oxide Nanoparticles from Plant Sources in Combination with Antibiotics on Multiple Drug-Resistant *Pseudomonas aeruginosa*

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### ABSTRACT

*Pseudomonas aeruginosa* is a multiple drug resistant (MDR) bacteria which shows resistant to different classes of antibiotics and it causes severe complications in humans as well as animals. Such an organism – *Pseudomonas aeruginosa* was isolated from the hospital environment. Drug resistance concerning different classes of antibiotics was tested. For the treatment of the same antibiotic-resistant *Pseudomonas* bacteria, an alternative eco-friendly biochemical was being searched. Green nanoparticles are known to have immense applications in the field of medicine and biology which are eco-friendly, safe and stable. The zinc oxide nanoparticles were synthesized from plant leaves of *Pongamia pinnata*, *Parthenium hysterophorus*, *Clematis montana* using Zn (NO<sub>3</sub>)<sub>2</sub> hexahydrate salt. The nanoparticles were analysed by UV-visible spectroscopy (UV-Vis), Scanning electron microscopy (SEM), Transmission Electron Microscopy (TEM), Energy Dispersive Analysis Of X-Ray (EDAX), Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD) methods. It was observed that these nanoparticles acted as strong microbicidal agents against antibiotic-resistant *Pseudomonas aeruginosa*. Synergistic effects of antibiotic with zinc oxide nanoparticles were also observed in *Pseudomonas aeruginosa* and found to be effective. Thus, Zinc oxide nanoparticles (ZnONPs) in combination with antibiotics could be an interesting tool to control multiple antibiotic-resistant *Pseudomonas aeruginosa*.

**Keywords:** MDR, Antibiotics, *Pseudomonas aeruginosa*, Green Nanoparticles, Zinc oxide Nanoparticles

### INTRODUCTION

The discovery and clinical use of many antibiotics have been paralleled by the emergence of bacteria that resist their action. At present naturally occurring compounds

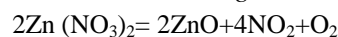
(Tiwari, et al., 2009) which are structurally and functionally unique are the choice of antimicrobial agents to treat drug-resistant pathogens.

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Resources against multidrug-resistant pathogenic infections are now limited. There are over 1340 plants with defined antimicrobial activities, and over 30,000 antimicrobial compounds have been isolated from plants (Tajkarimi et al., 2010). The nanoparticles possess increased structural integrity as well as unique chemical, mechanical, optical, electrical and magnetic properties (Alagumuthu, & Kirubha 2012 & Bhattacharya & Rajinder, 2005) and it is interesting to study such particles. An important branch of nanotechnology has emerged to study biological processes. The central molecule in the area of nanotechnology are nanoparticles, the minute structures usually ranging from 1 – 100 nm. The unique property of nanoparticle is their specific surface area to volume ratios, which gives strong antimicrobial action (Raghupathi et al., 2011). An important area of research in nanotechnology is the biosynthesis of gold, silver, zinc nanoparticles which are found to have wide applications. Among microorganisms, bacteria and fungi and various plant extracts have received the most attention for the biosynthesis of nanoparticles (Koper, 2002). Antibiotic resistance can be decreased using nanoparticles in combination therapy (Nan-Yao Lee et al, 2019). Zinc oxide (ZnO) is considered as a multi-tasking metal oxide which can be used as Nanoscale (Sunada, & Kikuchi, Hashimoto & Fujishima 1998 & He, Liu, Mustapha, & Lin, 2011) due to its unique physical, chemical and biological properties. Moreover, zinc oxide nanoparticles have tremendous potential in biological applications like biological sensing, biological labelling, gene delivery, drug delivery and Nanomedicines (Kolar, Urbanek, & Latal, 2001). It was shown that Zinc Oxide nanoparticles (ZnONPs) causes a decrease of the antibiotic resistance and enhance the antibacterial activity of Ciprofloxacin against microorganism, by interfering with various proteins that are interacting in the antibiotic resistance (Banoee, 2010). ZnO shows significant antimicrobial properties against a wide range of pathogens (Rasmussen et al.,

2010). It shows that ZnO nanoparticles are promising antimicrobial agents (Emily Schifano et al 2020) having a wide application because ZnONPs are reported by several researchers as non-toxic to human cells (Seil and Webster, 2012). The mechanism of the ZnONPs antimicrobial activity is related to the disruption of the bacterial cell membrane integrity, the diminishing cell surface hydrophobicity, and the downregulation of the transcription of bacteria (Wróblewska, 2006).

A major challenge has aroused regarding the treatment of infections caused by opportunistic pathogens, predominantly those with high-level resistance to all antibiotic classes and extreme ability to acquire resistance such as *Pseudomonas aeruginosa* (Neu, 1983 & Bonomo, 2006). This bacterium causes the nosocomial infection and develops resistance to antibiotics rapidly over several generations. The current study has been focused on Zinc oxide nanoparticles synthesis using plant extracts and Zinc Nitrate salt and to observe its effect on multiple antibiotic resistant *Pseudomonas aeruginosa* in vitro.



## MATERIALS AND METHODS

### 2.1 Preparation of Leaf Extract

Three commonly available plants viz. *Pongamia pinnata*, *Parthenium hysterophorus*, *Clematis montana* were selected for the synthesis of zinc oxide nanoparticles. These plants were present in college campus only. Aqueous extracts of dry leaves were prepared by taking 10gms of leaves in 100 ml of distilled water. The obtained extract was filtered through Whatman number-1 filter paper, the filtrate was collected in a 250 ml conical flask and then stored at 4°C for further use (Bala et al 2015).

### 2.2 Green Synthesis of Zinc Oxide Nanoparticles

A ratio of 1:1 of 0.5M concentrations of Zinc nitrate [ $\text{Zn}(\text{NO}_3)_2$ ] solution and aqueous leaf extracts was mixed in a flask. The solutions were heated at 65°C for 10 to 15 minutes. A colour change from pale yellow to honey colour was observed. This was further dried in

a hot air oven at 80°C followed by powdered for characterization (Sundararajan, et al 2015).

### 2.3 Characterization of Zinc Oxide Nanoparticles

#### 2.3.1 UV-Visible (UV-Vis) Spectra Analysis

Optical properties of the prepared ZnO nanostructure sample (10mg/ml of distilled water) were revealed by UV-Visible Spectroscopy at room temperature at the absorption wavelength at a range of 208 to 215 nm. (Dobrucka et al 2016)

#### 2.3.2 Scanning Electron Microscopy (SEM) Analysis

SEM results provided information about morphology and size of the nanoparticles. The nanoparticles analysis was done by using Vega3 Tuscan Scanning Electron Microscope machine (Geetha et al 2016).

#### 2.3.4 Energy Dispersive Analysis of X-Ray Diffraction Spectroscopy (EDAX) Analysis

The EDAX spectrum of ZnO nanoparticles prepared by an aqueous solution method. The strong peaks observed in the spectrum related to Zinc and oxygen (Gajjar et al 2009)

#### 2.3.5 Transmission Electron Microscopy (TEM) Analysis

The size and shape of the synthesized ZnONPs were determined by transmission electron microscopy (TEM) (Geetha et al 2016).

#### 2.3.6 X-Ray Diffraction (XRD) Analysis

This technique is employed to identify and quantitatively examine various crystalline forms of nanoparticles. The structure and lattice parameters of the diffracted powder specimen were analysed by measuring the angle of diffraction when the X-ray beam is made to an incident on them (Mishra et al 2015)

#### 2.3.7 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Various peaks of functional groups of nanoparticles can be identified through this technique. (Mishra et al 2015)

### 2.4 Catalytic action of ZnO NPs against Malachite green Dye Reduction

10mg of malachite green dye was added to 1000ml of distilled water was used as a stock solution. To 10mg/ml of a plant extract containing ZnO nanoparticles, 0.1ml of the

diluted dye was added. The tubes were incubated at 37°C. The absorbance at the end of 24 and 48 hours was recorded at 670nm. The decolourisation of the dye was due to the presence of ZnO nanoparticles which acted as reducing agents (El-Kemary et al., 2011). A control was also maintained without the addition of ZnO nanoparticles. The percentage of decolourisation was calculated by using the following formula.

Dye removal percentage =  $\frac{C_i - C_f}{C_i} \times 100$

Where,  $C_i$ - initial concentration of dye.  $C_f$ - final concentration of dye.

### 2.5 Determination of Antimicrobial Activity by Well Diffusion Method

The zinc nanoparticles synthesized from green plants were tested for their antimicrobial activity against *Pseudomonas aeruginosa* by well diffusion method. The organisms were spread uniformly on the sterile Nutrient Agar plates using sterile cotton swabs. Wells of 10mm in sizes were made on the Nutrient agar. Various concentration of nanoparticles (10µg/ml to 10010µg/ml) were poured into the wells. The plates were incubated at 37°C for 24 hours and the growth pattern of the microorganisms was observed after incubation and the antimicrobial activity of ZnO nanoparticles was determined (Ginocchio, 2002).

### 2.6 Synergistic Effect of Zinc Nanoparticles and Antibiotics

The antimicrobial activities of the synthesized zinc nanoparticles were tested against *Pseudomonas aeruginosa*. The overnight culture was swabbed on Muller-Hinton agar medium along with antibiotic discs of Ampicillin, Chloramphenicol, Erythromycin and Tetracycline and ZnO NPs. The zone of inhibition was measured after overnight incubation at 37°C (Nachiyar et al., 2015)

Statistical Analysis Numerical data were analyzed for significance using the student's t-test (N=3). Experiments were repeated three times. The significance of the experiment was set at  $p < 0.05$ .

### 2.8 Estimation of Protein

Luria-Bertani broth was sterilized and 10ml of the sterile medium was distributed in sterile test tubes. The medium was incubated with

MIC concentration of antibiotics (Ampicillin, Chloramphenicol, Erythromycin and Tetracycline) and 100µg/1ml of ZnONPs. All the tubes were inoculated with 100µl of *Pseudomonas aeruginosa* culture (24 hrs). The tubes were incubated at 37°C for 24 hours. Following incubation, the bacterial culture was centrifuged at 6000 rpm for about 10 minutes. To the pellet 1ml of lysis buffer (10mM Tris-HCl, pH 8; 0.5M EDTA; 0.5% SDS; 1M NaCl) was added and vortexed. These aliquots were estimated for proteins by Folin-Ciocalteu method (Lowry et al., 1951).

## RESULTS AND DISCUSSIONS

### 3.1.1 Green Synthesis of Zinc Oxide Nanoparticles

The leaves of *Pongamia pinnata*, *Parthenium hysterophorus*, *Clematis montana* after processing (Fig1) was mixed with Zinc nitrate salt (0.5M). The powdered (Fig2) Zinc oxide Nanoparticles (ZnONPs) were subjected to analysis

### 3.1.2 UV-Visible Spectrophotometry

The absorption peaks are obtained at the wavelength 282.7, 282.08 and 289.93nm for ZnO particles ZnO nanoparticles prepared using the leaf extracts of *Parthenium*, *Pongamia* and *Clematis* respectively (Fig3, Fig4& Fig5). From the UV-Vis graphs, the energy bandgap was obtained as 4.38, 4.39 and 4.27eV respectively using the formula  $E_{bg} = 1240/\lambda$  (eV)

$E_{bg}$  = band-gap energy (eV)

$\lambda$  = absorption maximum wavelength (nm)

### 3.1.3 Scanning Electron Microscopy (SEM) Analysis of Zinc Oxide Nanoparticles

The synthesized ZnO nanoparticles were agglomerated with a particle size ranging from 94-110nm. SEM images were seen in different magnification ranges (20 µm–200 nm) which demonstrated the presence of rod-shaped nanoparticle. The size of the zinc oxide nanoparticles synthesized from *C. montana*, *P. pinnata* and *P. hysterophorus* was 94nm, 100nm and 110nm in length (Fig 6.1, Fig 6.2 & Fig 6.3)

### 3.1.4 Transmission Electron Microscopy (TEM) Analysis of Zinc Oxide Nanoparticles

Metal oxide nanoparticles prepared using plant extracts revealed fine particle morphology with significantly low agglomeration. The morphology of zinc nanoparticles revealed that particles of ZnONPs were rod-shaped structure. The particle size was average of 12nm by 28nm. The phytochemicals obtained were used for reduction as well as stabilization of the nanoparticles. The selected area electron diffraction (SAED) patterns demonstrated the concentric diffraction rings as bright spots corresponding to the presence of zinc oxide nanoparticles. The pattern showed the highest crystalline structures of ZnONPs in *Parthenium*, *Pongamia* followed by *Clematis* (Fig 7.1, Fig 7.2 & Fig 7.3)

### 3.1.5 Energy Dispersive Analysis of X-Ray Diffraction Spectroscopy (EDAX) Analysis

This EDAX results further confirmed the biosynthesis of the ZnO nanoparticles and the occurrence of the zinc nanoparticles in its oxide form rather than in pure zinc form. The elemental constitution of ZnO nanoparticles obtained from *P. hysterophorus* with two major peaks found to have weight percentage at 75.20 of Zn and 6.66 of oxygen. Presence of 18.15% of Cu indicated the grid components of the experiment (Fig 8.1).

The elemental constitution of ZnO nanoparticles obtained from *P. pinnata* with two major peaks found to have weight percentage at 62.23 of Zn and 22.28 of oxygen. Presence of 15.50% of Cu indicated the grid components of the experiment (Fig 8.2).

The elemental constitution of ZnO nanoparticles obtained from *C. montana* with two major peaks found to have weight percentage at 62.00 of Zn and 27.14 of oxygen. Presence of 11.86% of Cu indicated the grid components of the experiment (Fig8.3).

### 3.1.6 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR identification and characterises a substance through the rotational as well as vibrational modes of motion of a molecule.

The Infrared spectrum of an organic compound can differ from the absorption patterns of all other compounds and therefore it identifies that compound uniquely.

The FTIR spectra of ZnO NPs taken in the range (400–4500  $\text{cm}^{-1}$ ). The FTIR broad peak at 3436  $\text{cm}^{-1}$  represented O–H group stretching of O–H, H-bonded single bridge. 2106  $\text{cm}^{-1}$  peak may be due to the absorption of atmospheric carbon dioxide by metallic cations. The peaks at 528  $\text{cm}^{-1}$  (*P. pinnata*) and 530  $\text{cm}^{-1}$  (*P. hestrophorus* and *C. montana*) corresponds to ZnO (Fig 9.1, Fig 9.2 & Fig 9.3) bonding which confirmed the presence of ZnO particles

### 3.1.7 X-ray Diffraction (XRD) Analysis

X-ray diffraction identifies the crystalline forms quantitatively present in powder and solid samples. Diffraction forms as waves interact with a regular structure whose repeat distance is about the same as the wavelength. For example, light can be diffracted by a grating having scribed lines spaced on the order of a few thousand angstroms, about the wavelength of light. It happens that X-rays have wavelengths on the order of a few angstroms, the same as typical inter-atomic distances in crystalline solids. In 1912, W. L. Bragg recognized a predictable relationship among several factors. 1. The distance between similar atomic planes in a mineral (the interatomic spacing) is the d-spacing and measure in angstroms. 2. The angle of diffraction which is the theta angle and measure in degrees. The diffractometer quantifies an angle twice that of the theta angle, the measured angle is '2-theta'. 3. The wavelength of the incident X-radiation, symbolized by the Greek letter lambda and equal to 1.54 angstroms.  $n\lambda=2d\sin\theta$ , where,  $\lambda$ -wavelength of X-ray d-interplanar spacing,  $\theta$ -diffraction angle n-0,1,2,3....

The XRD of ZnONPs synthesized from *P.pinnata*, *P. hestrophorus* and *C.montana* showed similar results (Fig.10.1, Fig 10.2& Fig 10.3). The major peaks correspond to Bragg reflections with  $2\theta$  values of 31.41°, 34.31°, 36.12°, 47.79°, 56.54°, 62.81°, 67.97°, 72.91° and 77.17°. These locations of the characteristic Bragg reflections were indexed to (1 1 1), (2 0 2), (3 1 1), (2 2 2), (4 0 0), (3 3 1), (4 2 0) and (4 2 2) planes of ZnO wurtzite structure and this confirmed the presence of zinc oxide nanoparticles. The appearance of sharp diffraction patterns confirms the small size as well as high crystallinity of the synthesized nanoparticles. Particularly XRD spectrum showed diffraction peaks around 37°, which are indexed the (102) of the wurtzite crystal structure of ZnO. These sharp Bragg peaks might have resulted due to capping agent stabilizing the nanoparticle. Therefore, XRD results also suggested that the crystallization of the bio-organic phase occurs on the surface of the ZnO nanoparticles or vice versa. Generally, the broadening of peaks in the XRD patterns of solids is attributed to particle size effects. Broader peaks signify smaller particle size due to experimental conditions on the nucleation and growth of the crystal nuclei.

### 3.2 The catalytic action of Dye Reduction of ZnONPs against Malachite green

Percentage of dye reduction was highest in ZnONP synthesized from *P. hystrophorus* followed by *P. pinnata* and *C. montana*. *P. hystrophorus* ZnONPs showed 66.67% dye reductions, followed by a 50% reduction with *P. pinnata* and 33.33% reduction with *C. Montana* (Fig 11).

### 3.3 Antimicrobial Activity by Well Diffusion Method

Table1: Zone of Inhibition Diameter (Well-Diffusion)

Test Organism	Concentration	Diameter on the Zone of Inhibition(mm)
<i>Pseudomonas aeruginosa</i>	0.5M (Parthenium ZnONP)	17.66±.03
	0.5M (Pongemia ZnONP)	16.01±.01
	0.5M (Clematis ZnONP)	14.01±.01

The incubated nutrient agar plates containing bacteria and the leaf extract with the nanoparticles were observed and found that ZnONPs synthesized from all three extracts are inhibitory against *P. aeruginosa* – a multiple drug-resistant bacteria. *P. hysterothorus* synthesized ZnONP were more effective than *P. pinnata* and *C. montana*. The maximum zone of inhibition was seen for *P. hysterothorus* ZnONP which was about 17.66 mm and the minimum zone of inhibition was seen for *C. montana* ZnONP ranging 14mm (Table1& Fig13)

### 3.3.1 Synergistic Effect of Antibiotics and Nanoparticles (Disc Diffusion)

The Zinc oxide nanoparticles synthesized from the plants were tested along with Ampicillin, Chloramphenicol, Erythromycin and Tetracycline to study their Synergistic effect on *P. aeruginosa*. Trtracycline and ZnONPs gave maximum synergistic effect followed by Ampicillin, Chloramphenicol and Erythromycin respectively. This showed that ZnONPs synergistically effective with antibiotics against resistance *P.aeruginosa* (Table2).

**Table 2: Synergistic Effect of Antibiotics and ZnONPs**

Antibiotic	ZnONPs Concentration	Zone of Inhibition(mm)
Ampicillin	10µg	5.96±0.08
Ampicillin+ZnONPs(Parthenium)	0.5M	24.22±0.03
Ampicillin+ZnONPs(Pongemia)	0.5M	21.97±0.02
Ampicillin+ZnONPs(Clematis)	0.5M	19.97±0.01
Chloramphenicol	30µg	3.9±0.05
Chloramphenicol +ZnONPs(Parthenium)	0.5M	21.56±0.01
Chloramphenicol + ZnONPs(Pongemia)	0.5M	19.91±0.02
Chloramphenicol + ZnONPs(Clematis)	0.5M	17.91±0.02
Erythromycin	15µg	0.003±0.00
Erythromycin+ ZnONPs(Parthenium)	0.5M	17.663±0.0
Erythromycin+ ZnONPs(Pongemia)	0.5M	27.9±0.06
Erythromycin+ZnONPs(Clematis)	0.5M	16.013±0.02
Tetracycline	30µg	7.83±0.08
Tetracycline+ZnONPs(Parthenium)	0.5M	25.49±.03
Tetracycline+ZnONPs(Pongemia)	0.5M	23.84±.04
Tetracycline+ZnONPs(Clematis)	0.5M	21.84±.03

### 3.3.2 Estimation of Protein Concentration of Antibiotics and ZnONPs

There was the highest decrease in the protein concentration when Tetracycline and zinc oxide nanoparticles were added followed by Ampicillin but the combination of zinc nanoparticles along with erythromycin had the least effect on the protein content(Fig12). Possibly the effects of nanoparticles and antibiotics were not only on protein machinery but probably on the other aspects of the cell of *Pseudomonas aeruginosa*. Overall the effect of zinc nanoparticles and antibiotics had a

significant effect on the decrease of the protein content of *P. aeruginosa* (Tiwari et al., 2018). Leaching of outer membrane proteins by ZnO nanoparticles was quite inevitable causing decrease total protein content of *Pseudomonas*. During the interaction, after incubation of ZnONP and antibiotics of two different concentrations, it was observed that protein content was decreased. The results demonstrated that ZnO nanoparticle enhanced the permeability of the outer cell membrane in cells. So it could be conferred that turbulence of membranous permeability would be an important factor to inhibit the bacterial growth.



Fig. 1: Collected Leaves of Plants for Nanoparticles Synthesis

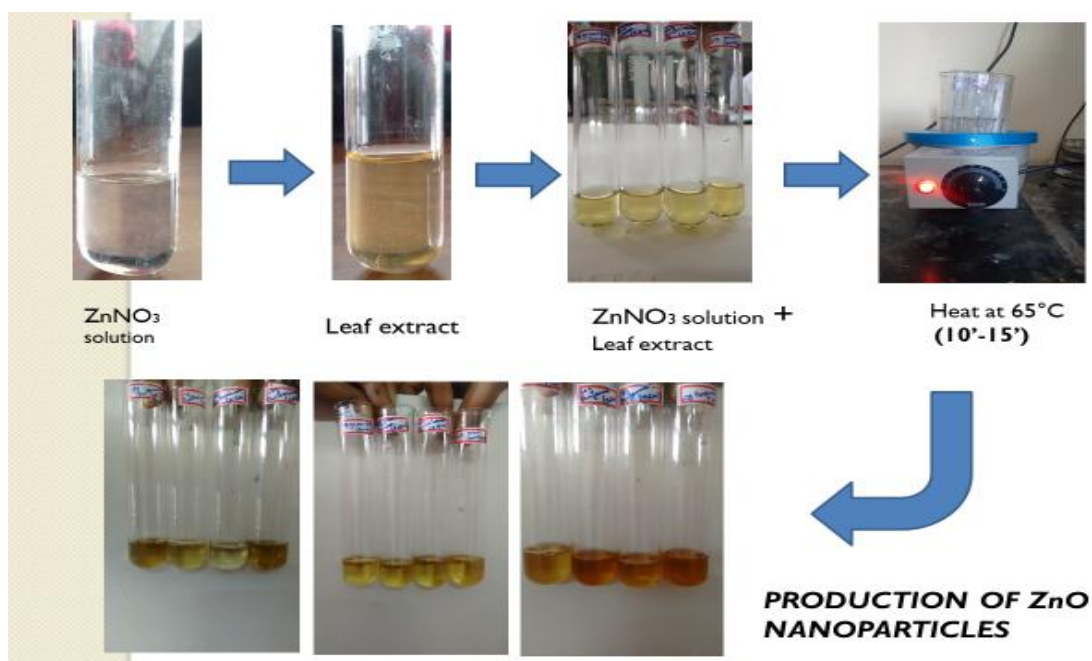


Fig. 2: Process of Production ZnO Nanoparticles

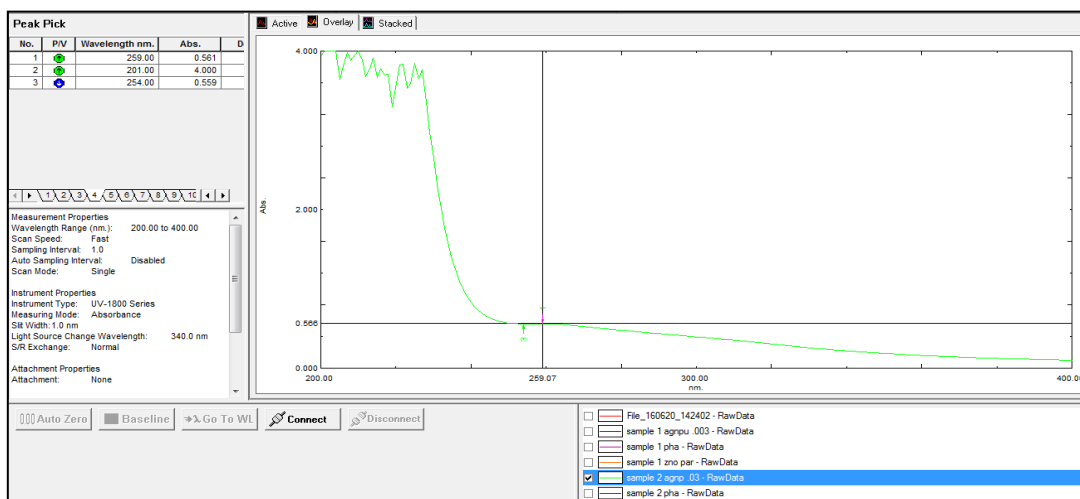


Fig. 3: ZnO-Parthenium hysterophorus

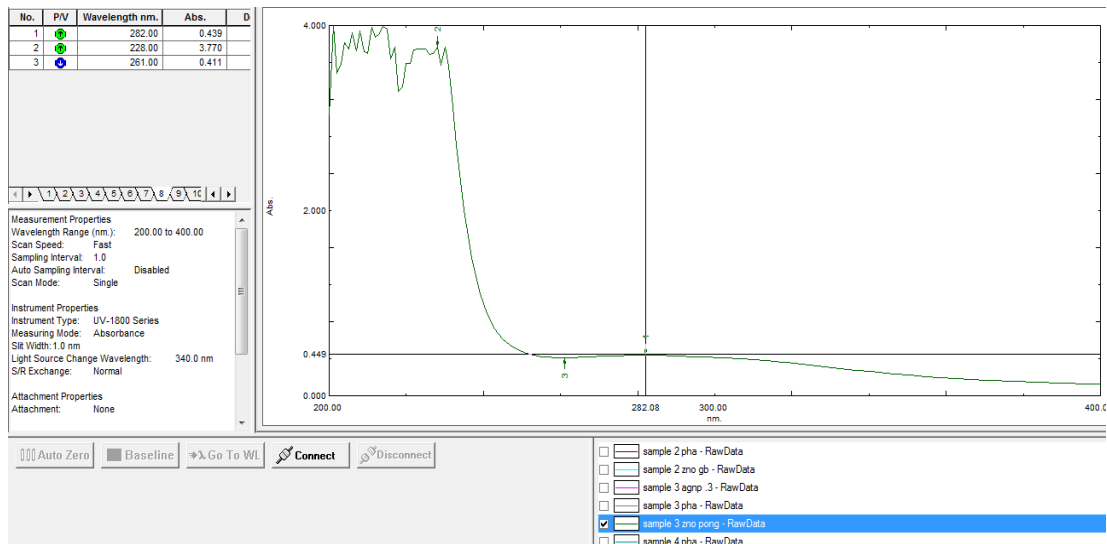


Fig. 4: ZnO-Pongemia pinnata

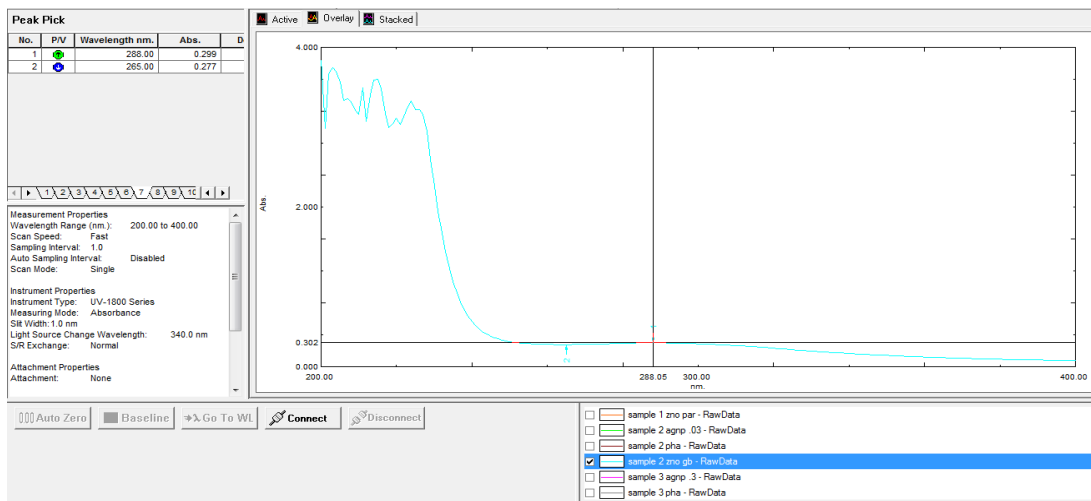


Fig. 5: ZnO-Clematis montana

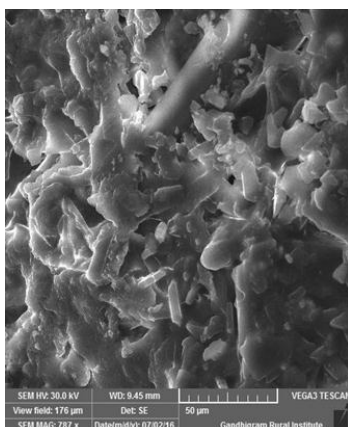


Fig. 6.1: SEM-Parthenium

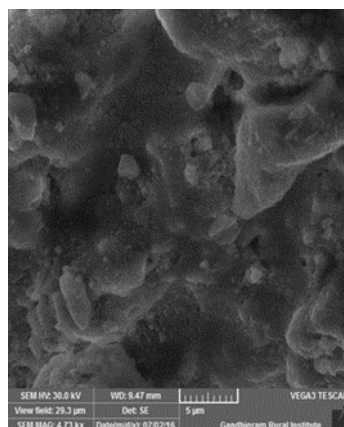


Fig. 6.2: SEM-Pongemia

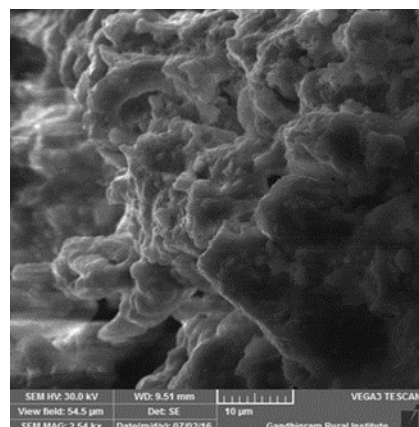


Fig. 6.3: SEM-Clematis



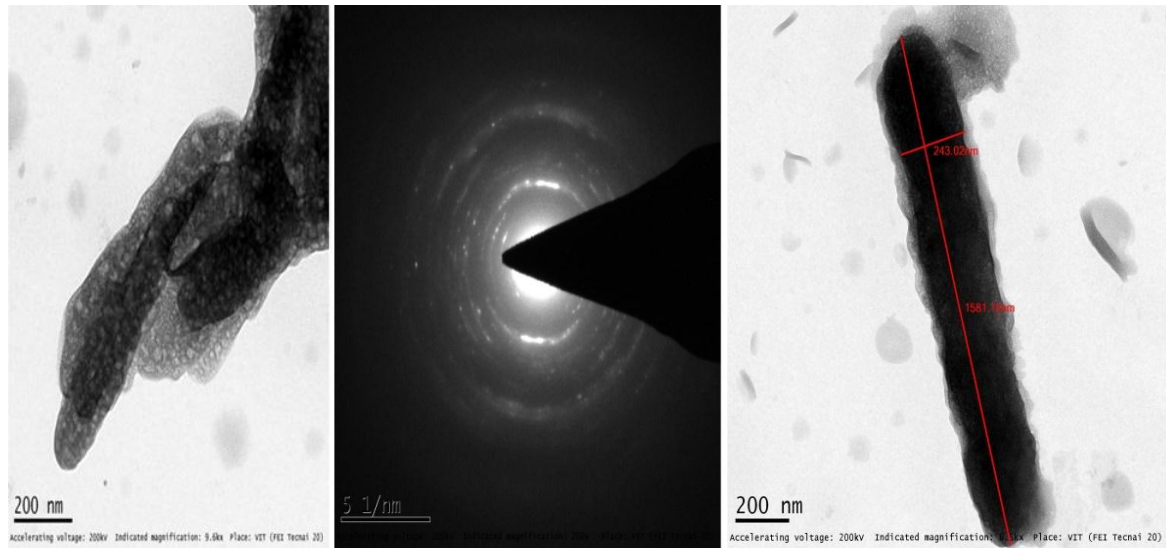


Fig. 7.1: TEM-Parthenium

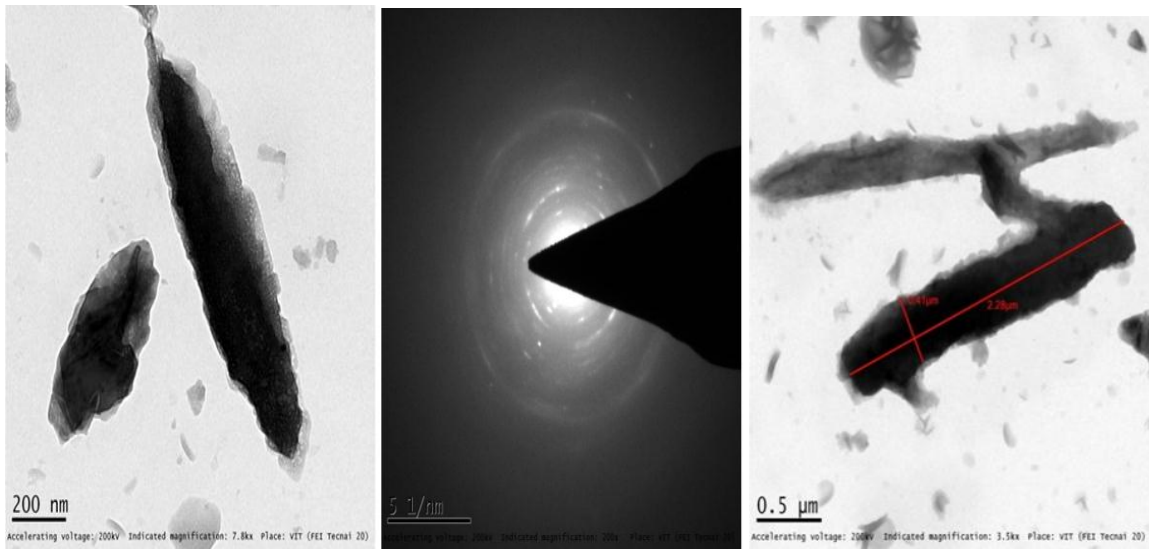


Fig. 7.2: TEM-Pongamia

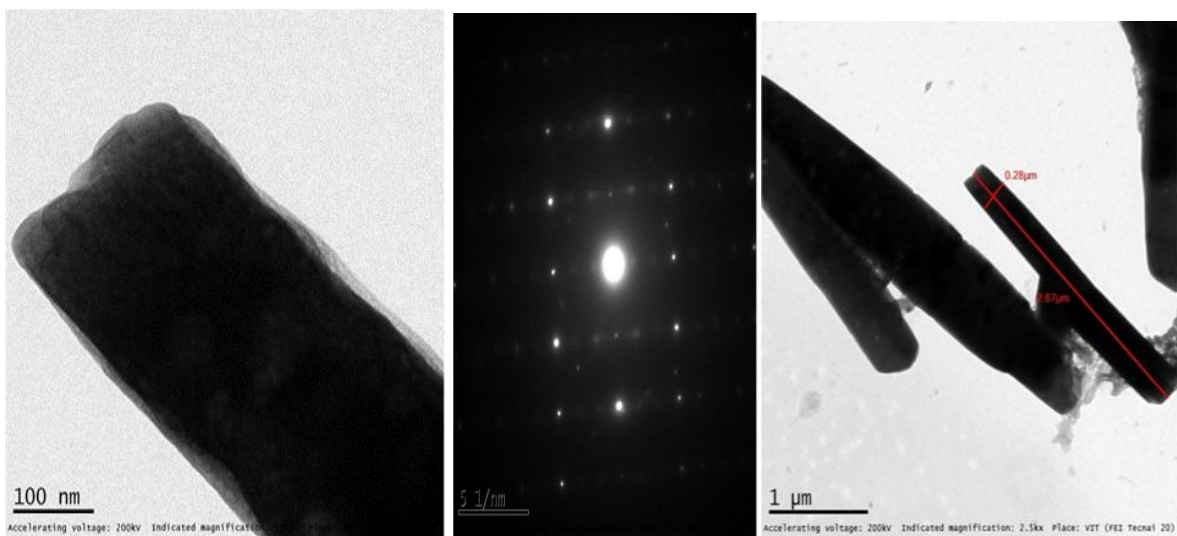


Fig. 7.3: TEM-Clematis

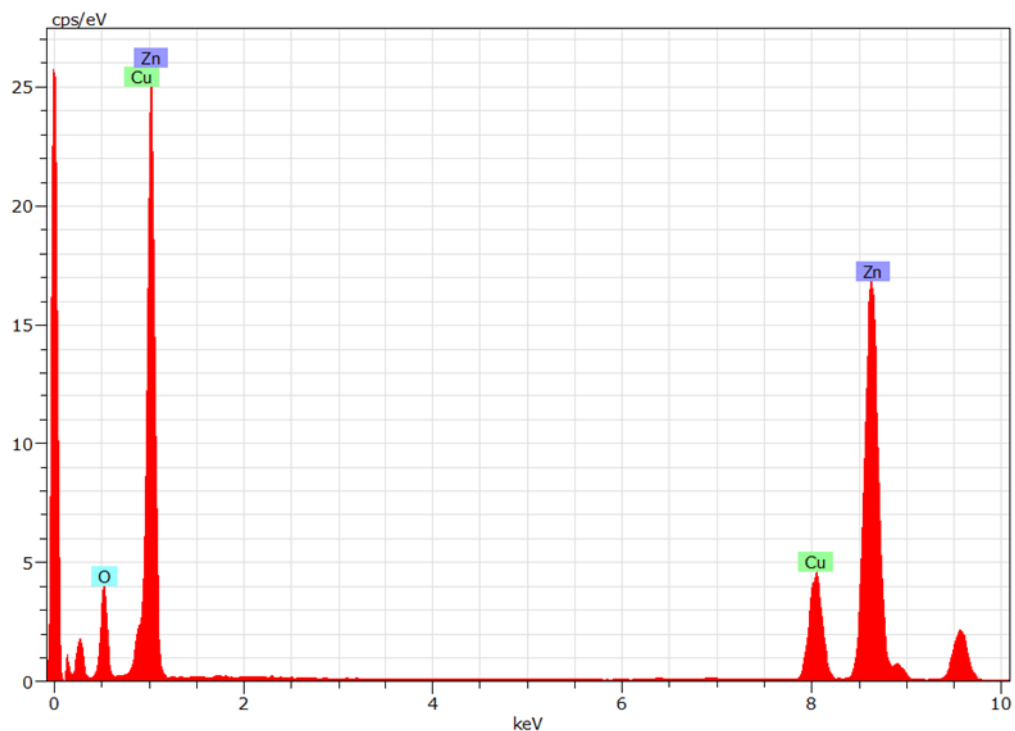


Fig. 8.1: EDAX- Parthenium

Spectrum: Parthinium ZnO

El	AN	Series	Net un.	C norm.	C Atom.	C Error (1 Sigma)
			[wt. %]	[wt. %]	[at. %]	[wt. %]
O	8	K-series	7945	6.66	6.66	0.24
Cu	29	K-series	24260	18.15	15.42	0.58
Zn	30	K-series	94173	75.20	62.10	2.30
Total:			100.00	100.00	100.00	

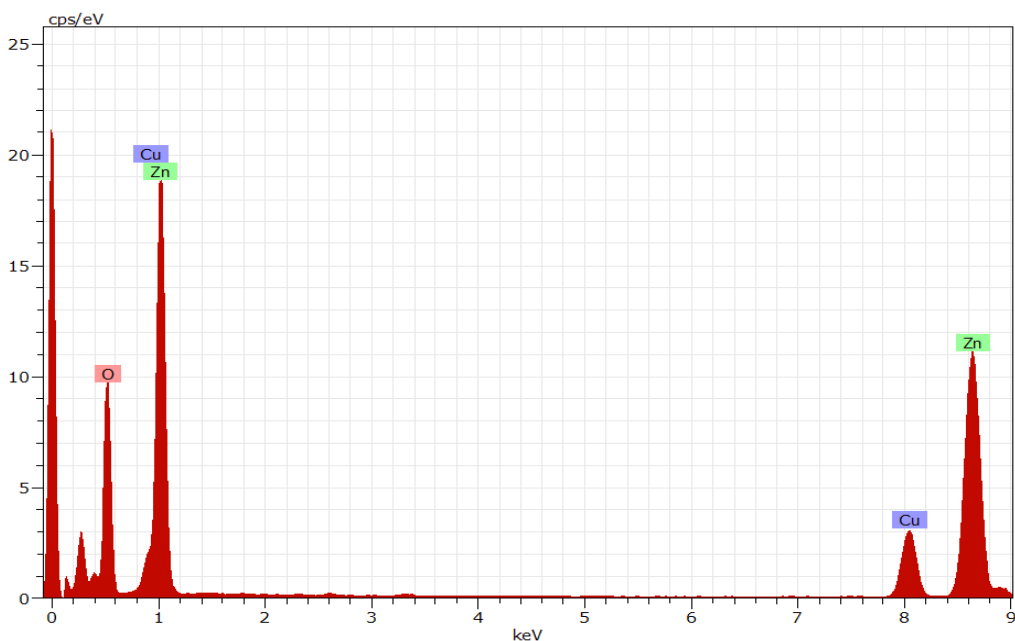


Fig. 8.2: EDAX-Pongemia

Spectrum: Pongemia ZnO

Element	Series	Net un.	C norm.	Atom. C	Error (3 Sigma)
		[wt.%]	[wt.%]	[at.%]	[wt.%]
Oxygen	K-series	25944	22.28	53.80	2.12
Zinc	K-series	76074	62.23	36.77	5.72
Copper	K-series	20226	15.50	9.42	1.51
Total:		100.00	100.00	100.00	

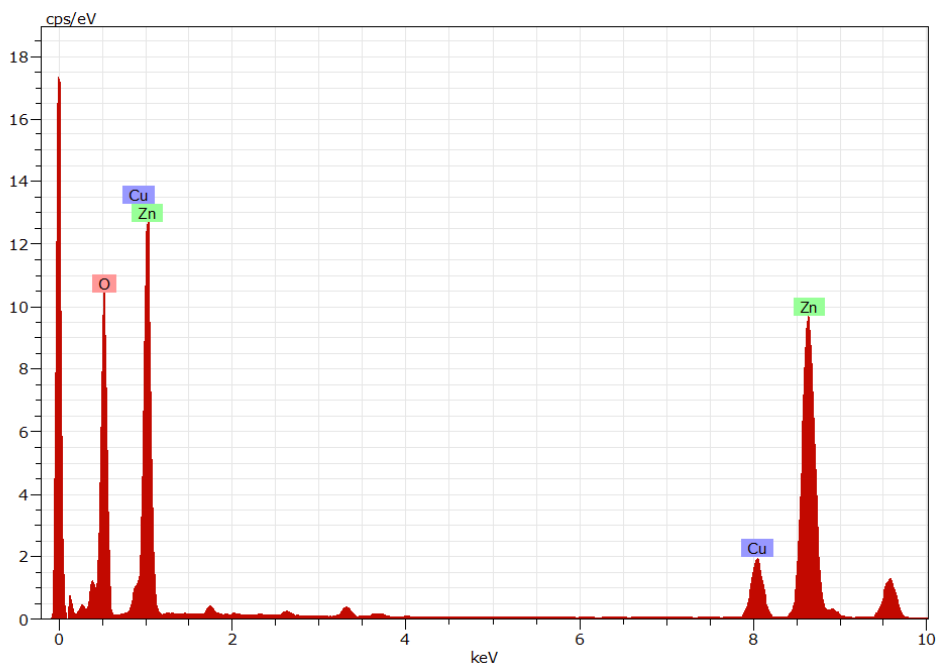


Fig. 8.3: EDAX-Clamatis

Spectrum: Clematis ZnO

Element	Series	Net un.	C norm.	Atom. C	Error (3 Sigma)
		[wt.%]	[wt.%]	[at.%]	[wt.%]
Oxygen	K-series	34619	27.14	60.26	2.56
Zinc	K-series	83004	62.00	33.67	5.70
Copper	K-series	15522	10.86	6.07	1.09
Total:		100.00	100.00	100.00	

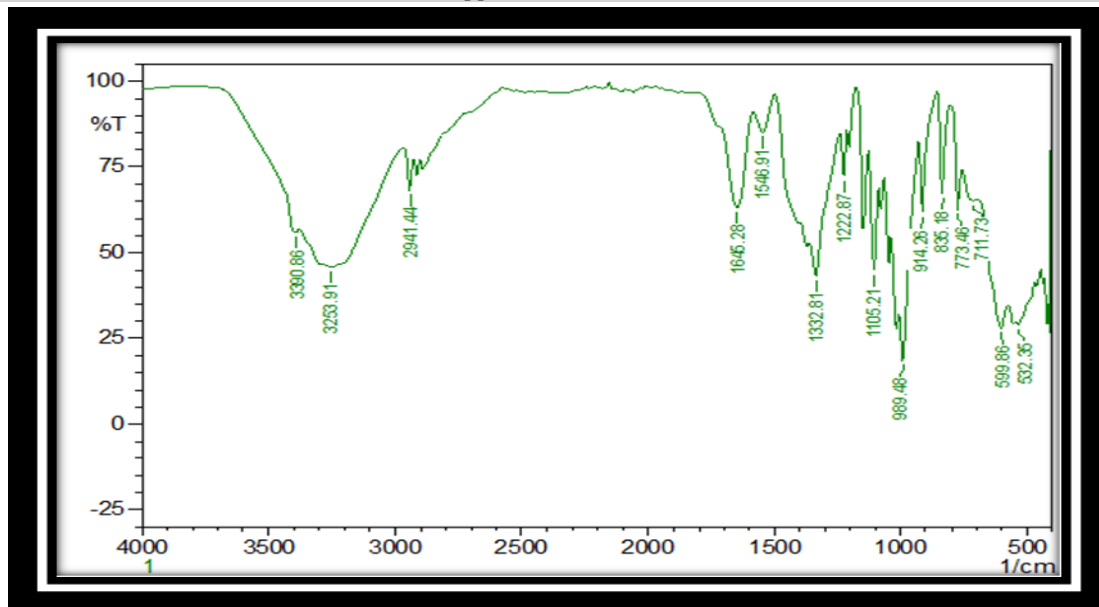


Fig. 9.1:FTIR-Parthenium

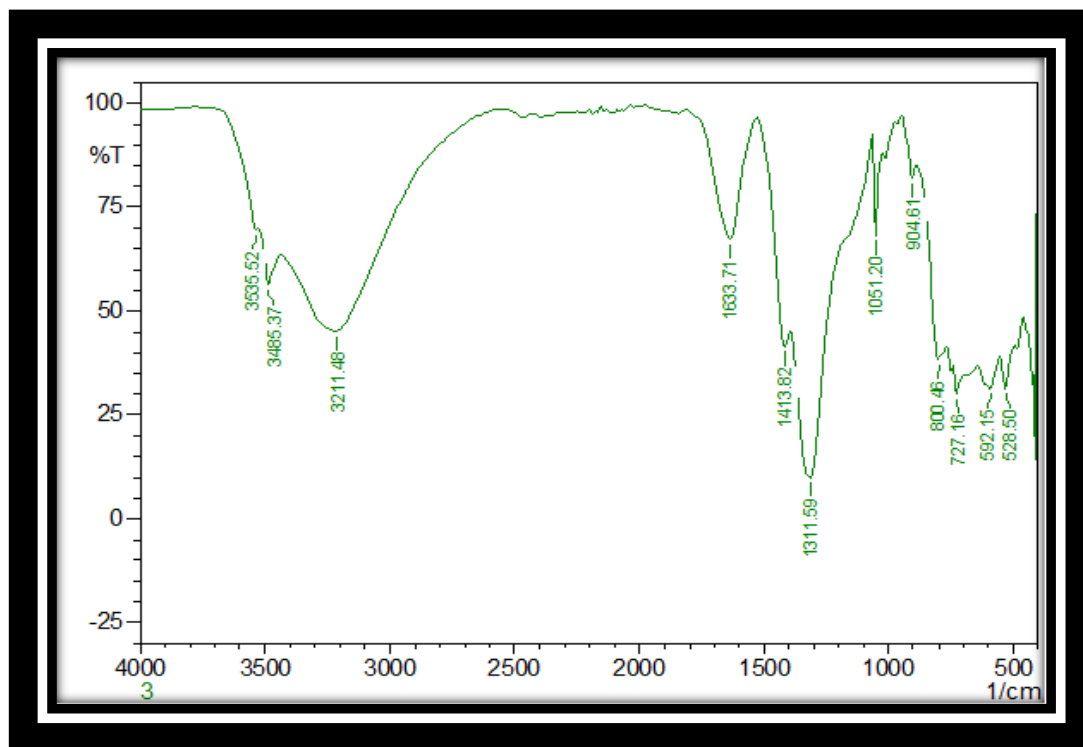


Fig. 9.2: FTIR- Pongemia

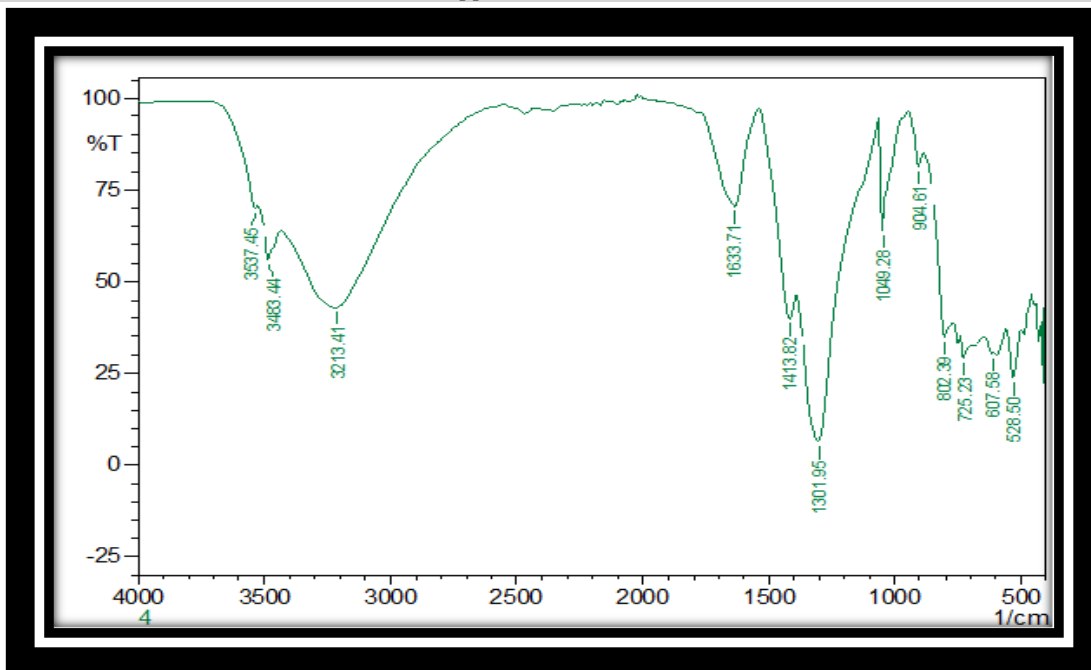


Fig. 9.3: FTIR- Clematis

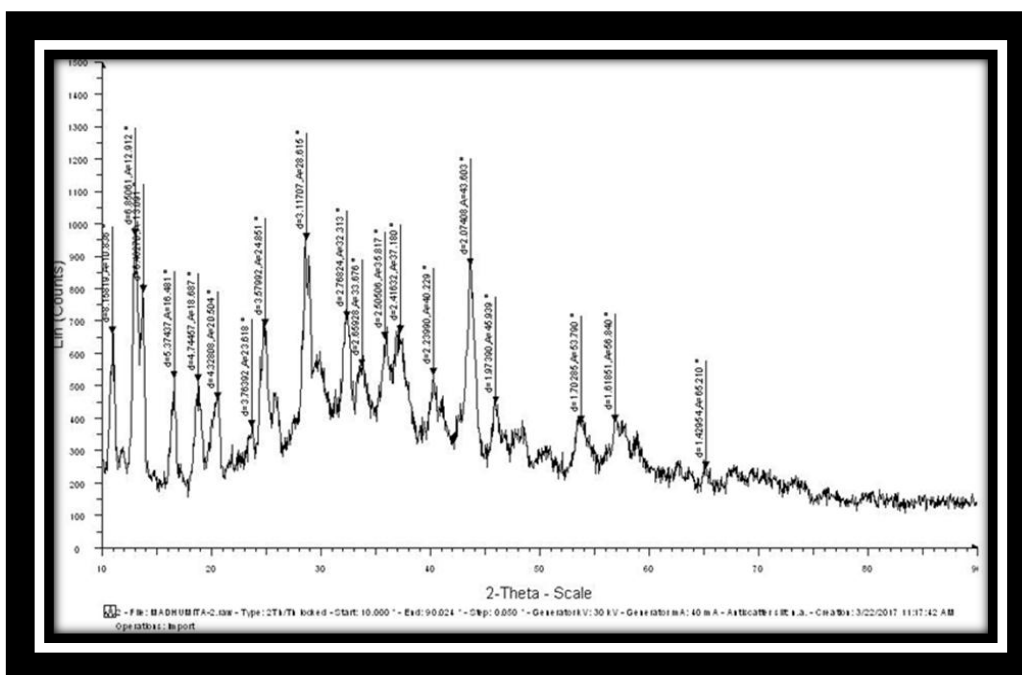


Fig. 10.1: XRD Pattern of ZnO NP synthesized from *P. hysterophorus*

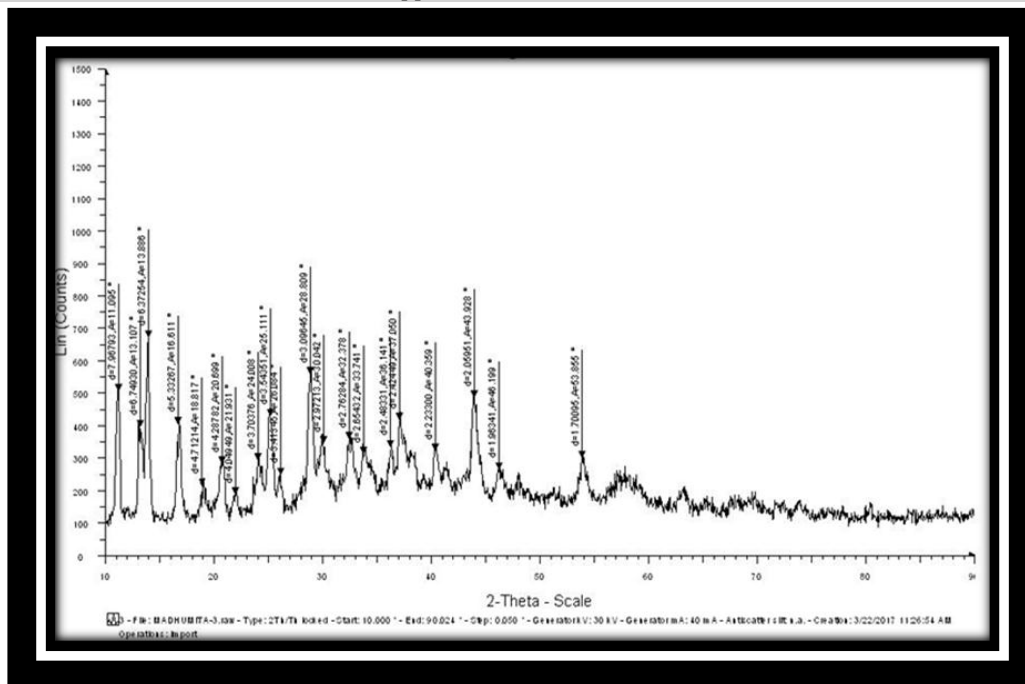


Fig. 10.2: XRD Pattern for ZnO NP synthesized from *P. pinnata*

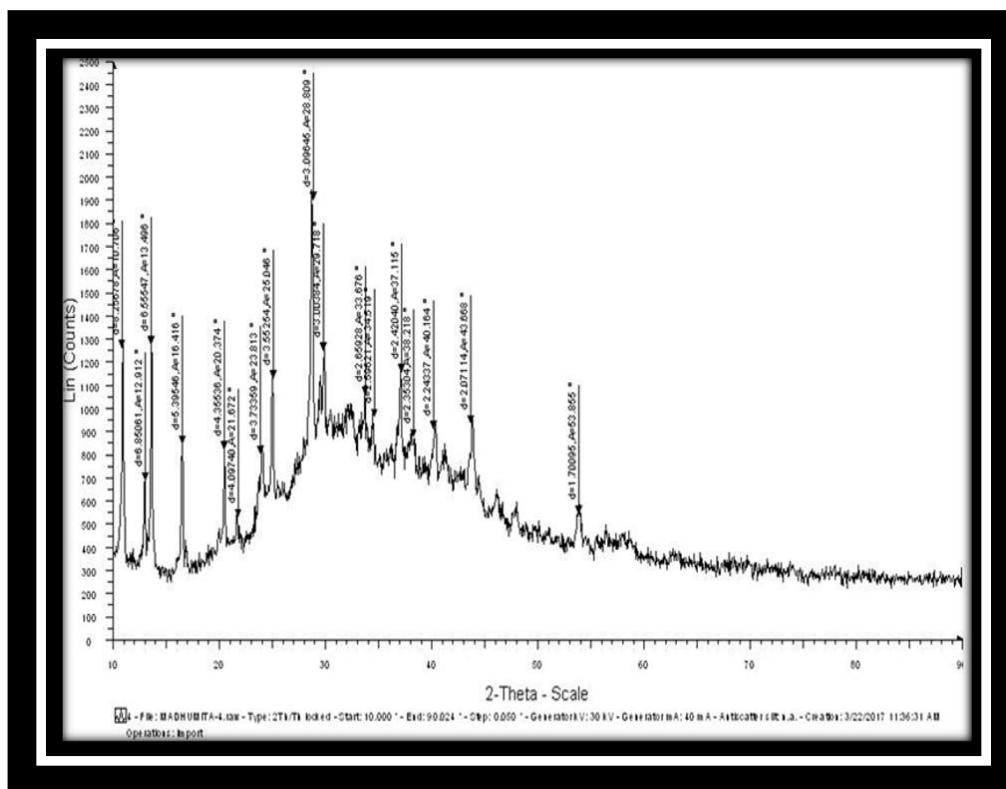


Fig. 10.3: XRD Pattern for ZnO NP synthesized from *C. montana*

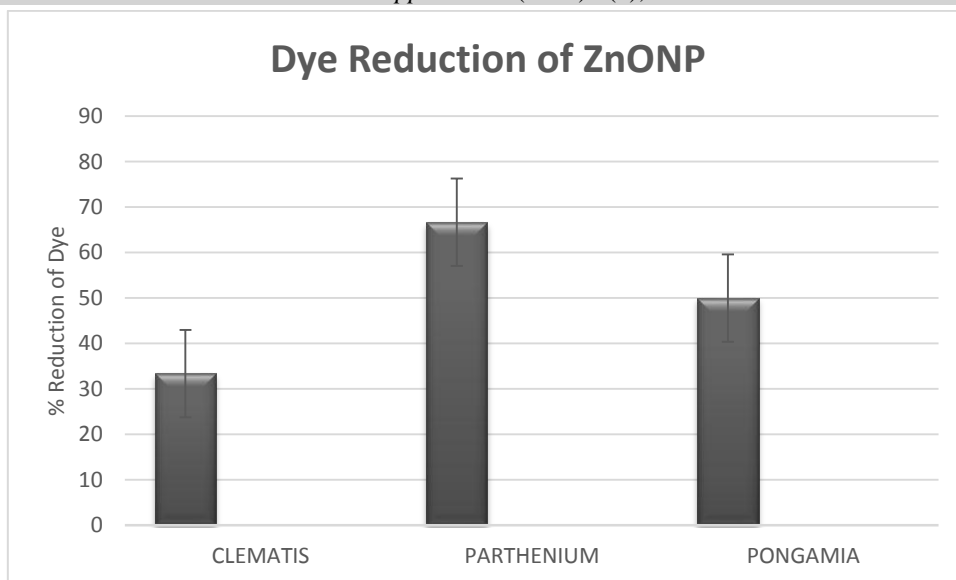


Fig. 11: Dye Reduction of ZnONPs

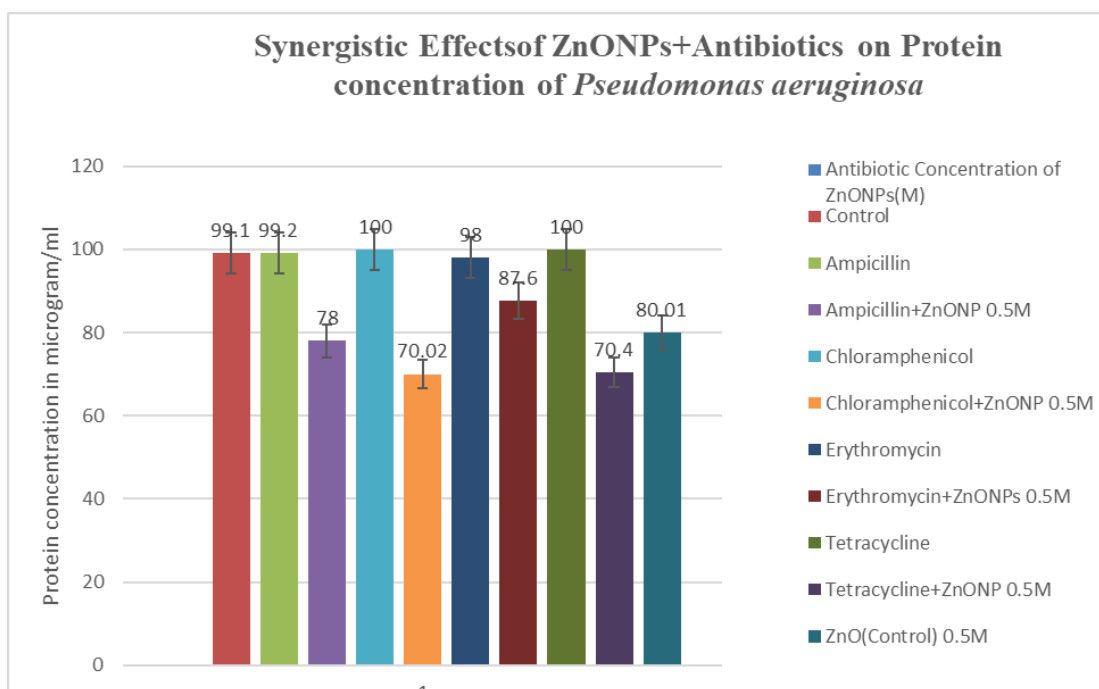


Fig. 12: Protein Concentration of P. Aeruginosa in Presence of ZnONPs and Antibiotics

**CONCLUSION**

There is a continuous need to discover new and effective antimicrobial compounds because there has been an increasing incidence of new and re-emerging infectious diseases and as well as the increasing development of resistance to the antibiotics in current clinical use. The multidrug-resistant (MDR) *Pseudomonas aeruginosa* is still a gigantic problem in the hospital setup. The infections are likely to affect critically ill patients who

require prolonged hospitalization. Infections with MDR *Pseudomonas aeruginosa* are also associated with adverse clinical outcome. The results led to conclude that Zinc Oxide Bio nanoparticles (ZnONPs) are important pharmacological substances which can be used for developing new and effective antimicrobial agents, especially against MDR *P. aeruginosa*. The combinations of antibiotics and ZnONPs proved to be effective to control such antibiotic-resistant bacteria. The synergistic

effect detected showed that the combined use of antibiotics with Bio Nanoparticles as eco-friendly molecules could be an interesting tool for antimicrobial therapy.

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