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### **Spectroscopic Investigation of Green and Chemically Synthesized Zinc Oxide Nanoparticles And Its Antimicrobial Activities**

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#### **ABSTRACT**

Green synthesis of metal nanoparticles using plant extract is a promising alternative to traditional method of chemical synthesis. In this paper, we report the synthesis of nanostructured zinc oxide particles by chemical and biological methods. Highly stable and spherical zinc oxide nanoparticles are produced using *Plumbago Zeylanica* leaf extract. Formation of zinc oxide nanoparticles has been confirmed by UV-Vis absorption spectroscopy, FT-IR Spectroscopy, Scanning Electron Microscopy (SEM), and X-Ray Diffraction. The findings indicated promising antioxidant activity of crude extracts of the above plants and needs further exploration for their effective use in both modern and traditional system of medicines. The antibacterial activity of green and chemically synthesized zinc oxide nanoparticles were tested against the widely used standard human pathogens *Staphylococcus aureus* and *Salmonella typhimurium* with efficient antibacterial potential is reported in this study.

**KEY WORDS:** *Plumbago Zeylanica*, Zinc oxide, nanoparticles, characterization, antioxidant , antibacterial activity.

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## INTRODUCTION

New technologies often create new tasks to science in addition to their benefits, increase concerns about health and various environmental problems. Recent nanotechnology grips a promise and a broad aspect towards wide applications of nanoparticles in a multiple way of developing fields of science and technology<sup>1</sup>. Recent inspiring improvements in the field of nanotechnology have been observed because of development of different and efficient methodologies to fabricate nanoparticles of particular shape and size depending on the specific requirements. Metal nanoparticles have various functions that are not observed in bulk phase<sup>2,3,4</sup> and have been studied extensively because of their exclusive catalytic, optical, electronic, magnetic and antimicrobial activities<sup>5,6,7</sup>. At present, there is a rising need to develop environmentally benevolent nanoparticles and synthesis routes, which can be proceeded by biological method instead of using toxic chemicals. The use of ecologically beneficial materials like plant leaf extract, bacterial cell extract, fungi and enzymes for the synthesis of nanoparticles proposes abundant benefits in terms of eco-friendliness and compatibility for a wide range of pharmaceuticals<sup>8</sup>. Zinc oxide nanostructure exhibits high catalytic efficiency, strong adsorption ability and are used more and more frequently in the manufacture of sunscreens<sup>9,10</sup> ceramics rubber processing, wastewater treatment, and as fungicides<sup>11</sup>. Chemical methods lead to the presence of some toxic chemicals adsorbed on the surface that may have opposing effects in medical application<sup>12,13</sup>. Currently, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity, eco-friendliness and extensive antimicrobial activities<sup>14</sup>. *Plumbago zeylanica* Linn. (Plumbaginaceae) is one of the well-known Ayurveda drug. It is commonly identified as chitramula and chitrack. The present study was carried out on the antibacterial activity of leaf of *Plumbago zeylanica* Linn. (Plumbaginaceae). It possesses multi-purpose medicinal property and is most commonly used in the traditional medicinal system of India. The plants contain various bioactive compounds like alkaloids, flavonoids, naphthoquinones, glycoside, saponins, steroids, triterpenoids, coumarins, phenolic compounds, tannins, carbohydrate, fixed oils, fats and proteins are present in different parts of the plant which have been described to show anti-bacterial, anti-plasmodial, anti-tumour, hepatoprotective, central nervous system stimulatory activity, anti-fungal, anti-inflammatory, anti-hyperglycemic, anti-cancer and anti-atherosclerotic activities<sup>15</sup>. In this study, ZnO nanoparticles are synthesized by the chemical and biological methods. The synthesized ZnO nanoparticles are characterized by the reduction of reaction mixture was initially investigated by UV-Visible spectroscopy. Spectral analysis was carried out from 300-600 nm. FTIR spectroscopy of the synthesized nanoparticles was recorded in the range of 400-4000 cm<sup>-1</sup>. The particle shape was recognized using Scanning Electron Microscopy<sup>16</sup>. The crystalline nature of the material was characterized from X-Ray Diffractometer (XRD). Antioxidant

based drugs and formulations for the prevention and treatment of complex diseases like Alzheimer's disease and cancer have appeared during last three decades<sup>17</sup>. Recent studies have focused on the analysis of antioxidant properties shown by the phytoconstituents extracted from the plant (including polyphenols, terpenes)<sup>18,19,20</sup> and also the estimation of antimicrobial activity against gram positive bacteria (*Staphylococcus aureus* and *Salmonella typhimurium*) of the synthesized samples using disk diffusion method.

## MATERIALS AND METHODS

The chemicals and materials used in this work, zinc nitrate Hexahydrate (99%), zinc acetate dihydrate, sodium hydroxide and ammonia were purchased from Merck. *Plumbago Zeylanica* plant (collected from Sitheri Hill, Dharmapuri (dt) Tamil Nadu) and deionised water was used for all experiments.

### *Preparation of plant extract*

Aqueous extract of *Plumbago Zeylanica* was prepared according to the method<sup>21</sup>. 10 g of thoroughly washed leaves was immersed in 100 ml of double distilled water heated at 60°C for 15 minutes. The extract was filtered using Whatman filter paper and stored at 4°C for further use.

### *Biosynthesis of ZnO nanoparticles*

Zinc oxide nanoparticles were synthesized using zinc acetate dihydrate  $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ <sup>22</sup>. Briefly, 0.01 M solution of zinc acetate was taken and leaf extract was added. The pH of the mixture was maintained at 12 and the solution was stirred continuously for 2 hrs. A yellowish precipitate resulted which was then dried at 60°C. Prior to drying, the precipitate was centrifuged at 15,000 rpm for 5 min and washed twice with sterile de-ionized water. Complete change to ZnO nanoparticles takes place during drying. The precipitate was used for further analysis<sup>23</sup>.

### *Chemical synthesis of ZnO nanoparticles*

2.28 g of zinc nitrate hexahydrate was dissolved in 75 ml of deionized water and then, 0.6 g of NaOH in 150 ml of deionized water was added dropwise under magnetic stirring. After the addition was completed, the stirring was continued for 30 min and then cooled with cold water. A white precipitate was filtered and washed by pure water several times. Then the obtained precipitates were dried at 60°C for 24 h and calcinated at 200°C for 2 h<sup>24</sup>.

## ***Characterization of ZnO nanoparticles***

### ***UV-Vis spectra analysis***

The sample was measured for its maximum absorbance using UV-Vis spectrophotometry. The optical property of ZnO nanoparticles was analyzed via ultraviolet and visible absorptionspectroscopy (spectrophotometer Cary E 500) in the range of 280–800 nm.

### ***Fourier transforms infra-red spectroscopy (FT-IR)***

The binding properties of ZnO nanoparticles using *Plumbago Zeylanica* extract were investigated by FTIR analysis. The characterization involved Fourier transform infrared spectroscopy (FTIR) analysis of the dried powder of the synthesized ZnO nanoparticles by Perkin Elmer Spectrum 1000 spectrum in attenuated total reflection mode and using the spectral range of 4000–400  $\text{cm}^{-1}$ .

### ***Scanning electron microscopy (SEM)***

The surface morphology of ZnO nanoparticles was examined by means of scanning electron microscopy (SU3500, Hitachi with spectral imaging system Thermo Scientific NSS (EDS), the type of detector (BSE-3D), acceleration voltage (15.0 kV), working distance (11.6 mm), pressure (in the case of variable vacuum conditions).

### ***X-ray diffraction (XRD)***

X-ray diffraction (XRD) studies of nanoparticles were carried out using BRUKER D8 ADVANCE brand  $\theta$ -2 $\theta$  configuration (generator-detector) X-ray tube copper S = 1.54 Å and LYNXEYE PDS detector. The estimation of the size of particles was calculated by Scherrer's formula.

### ***Antioxidant activity***

The ferric reducing ability was measured by the ferric reducing antioxidant power (FRAP) assay at low pH. 14,31. The stock solutions of  $10 \times 10^{-3} \text{ mol/dm}^3$  TPTZ in  $40 \times 10^{-3} \text{ mol/dm}^3$  HCl,  $20 \times 10^{-3} \text{ mol/dm}^3$   $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  and  $0.3 \text{ mol/dm}^3$  acetate buffer (pH 3.6) were prepared. The FRAP reagent contained 2.5 ml TPTZ solution, 2.5 ml ferric chloride solution and 25 ml acetate buffer. It was prepared freshly and warmed to 37°C. Then, 900  $\mu\text{l}$  of FRAP reagent was mixed with 90  $\mu\text{l}$  of distilled water and 30  $\mu\text{l}$  of test sample/methanol/distilled water/standard solutions. The reaction mixture was then incubated at 37°C for 30 min and absorbance was recorded at 595 nm. The concentration of  $\text{FeSO}_4$  was in turn plotted against concentrations of the standard antioxidants (L-ascorbic acid and Trolox)<sup>25</sup>.

### ***Antimicrobial susceptibility test***

The disc diffusion method<sup>26</sup> was used to screen the antimicrobial activity. *In vitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The concentration of extracts is 40 mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

## **RESULT AND DISCUSSION**

### ***FT-IR analysis***

FTIR analysis of the biosynthesized ZnO NPs resulted in various peaks at 1018.9cm<sup>-1</sup>, 1375.3cm<sup>-1</sup>, 1617.4cm<sup>-1</sup>, 2918.8cm<sup>-1</sup> and 3277.19cm<sup>-1</sup> **Fig-1**. Primary alcohols show a strong band near in the region 1018.9cm<sup>-1</sup>, the various C-H bending vibrations in alkene appear in the region 1375.3cm<sup>-1</sup>, nitrates (-O-N=O) can be readily recognized from the two strong bands in the region 1617.4cm<sup>-1</sup>, cyclohexane at 2918.8cm<sup>-1</sup> with increasing strain in the ring C-H bending also show in increase and hetero aromatic compounds containing N-H group show N-H<sub>str</sub> absorption in the region 3277.1cm<sup>-1</sup>, 1489 cm<sup>-1</sup>. FTIR analysis of the chemically synthesized ZnONPs revealed the presence of peaks at 1395.3cm<sup>-1</sup>, 1489.9cm<sup>-1</sup>, 1576.1cm<sup>-1</sup> and 3379.1cm<sup>-1</sup> **Fig-2**. The presence of nitro group in a compound is characterized by the presence of strong band at 1395.3cm<sup>-1</sup>, aromatic hydrocarbon compounds the most characteristic C=C stretching bands at 1489.9cm<sup>-1</sup>, 1576.1cm<sup>-1</sup> and the primary amides in dilute solution show the band 3379.1cm<sup>-1</sup>. FTIR analysis of the ZnONPs reveals the appearance of bonds that confirms the presence of certain enzymes that are responsible for the reduction, capping and stability of the nanoparticles. It can be stated that the nanoparticles are shielded by the enzymes and the proteins present in the extract so as to help prevent the formation of clusters<sup>27</sup>.

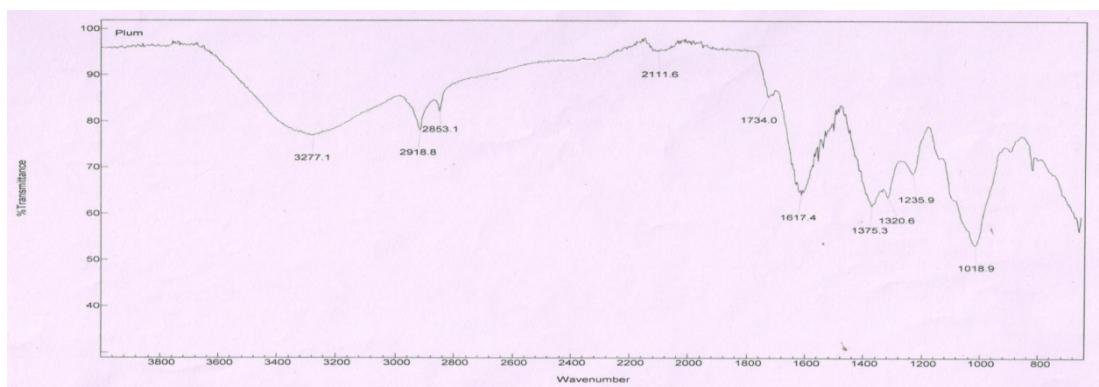


Fig-1 FTIR spectrum of biosynthesized Zinc oxide nanoparticle

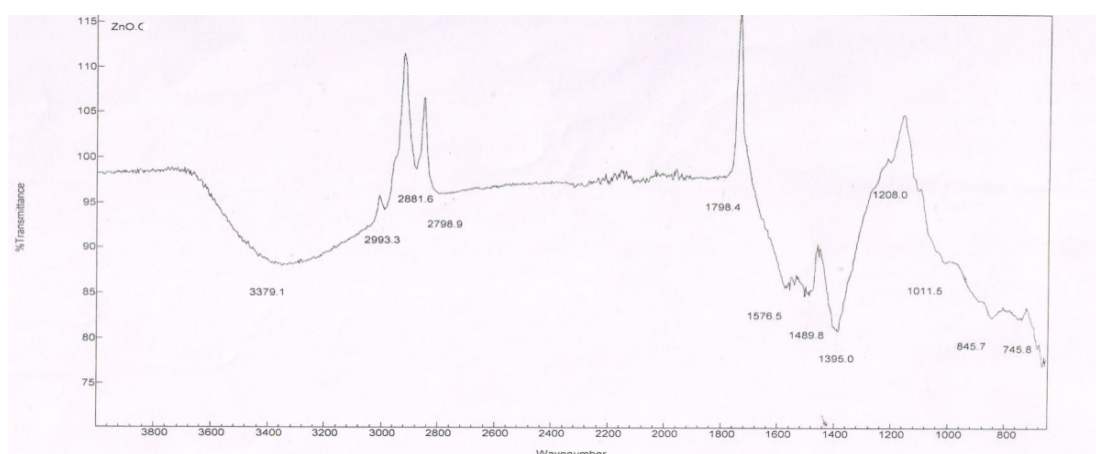


Fig-2 FTIR spectrum of chemically synthesized Zinc oxide nanoparticles

### UV-visible spectral analysis

Confirmation of presence of zinc nanoparticles in colloidal solution was done using UV-Spectral analysis. The samples were then studied by UV-spectral analysis (300-600 nm). It was observed that both samples showed an optical absorption band peaking at 400 nm and gradually decreasing at higher wavelengths **Fig 3**. Zinc oxide nanoparticles have been described to exhibit a characteristic broad absorption peak between 330-460 nm [26]. Thus optical absorption band peaking at 320 nm confirms the formation of zinc oxide nanoparticles by *Plumbago Zeylanica* leaf extract. It can be assumed from the above results that the biomolecules present in the plant extract induce the reduction of Zinc ions into zinc nanoparticles (ZnONP). This process of reduction is extracellular, fast and thus can be developed into an easy method for nanoparticle synthesis<sup>28</sup>. **Fig 4** shows the UV-Vis absorption spectrum of chemically synthesized zinc oxide nanoparticles. The absorption spectrum was recorded for the sample in the range of 280 - 420 nm. The spectrum exposed the absorbance peak at 340 nm corresponding to the characteristic band of chemically synthesized zinc oxide nanoparticles<sup>29</sup>.

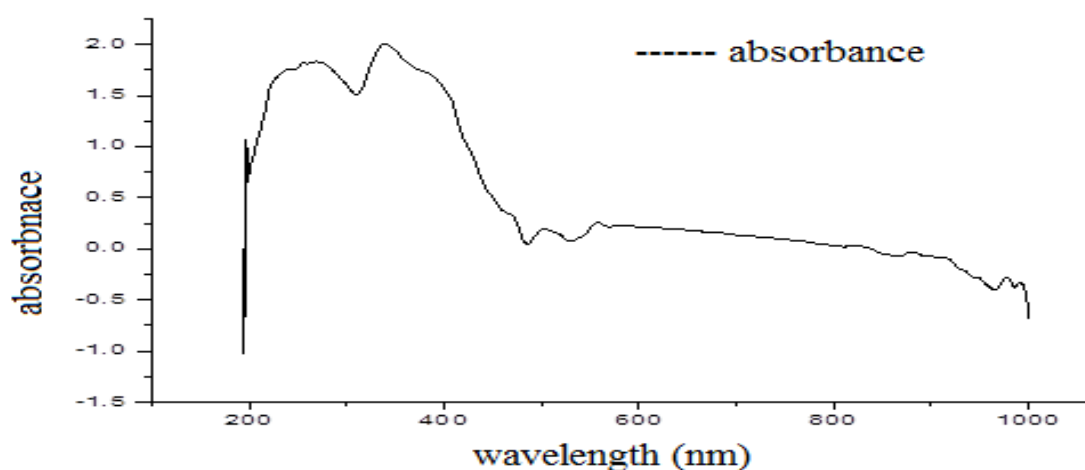


Fig-3 UV spectrum of biosynthesized Zinc oxide nanoparticles

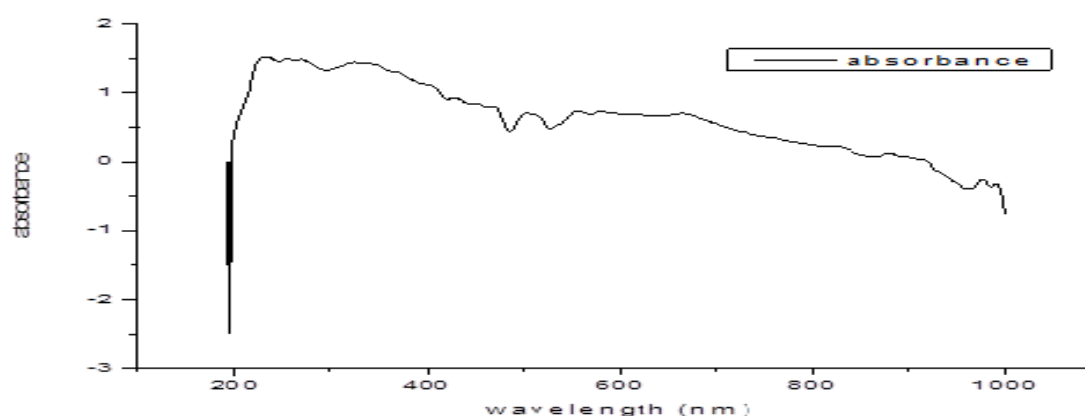


Fig-4 UV spectrum of chemically synthesized Zinc oxide nanoparticles

### Scanning Electron Microscopy (SEM) analysis

Scanning Electron Microscopy (SEM) performed has provided further insight into the morphology and sizedetails of the both synthesized nanoparticles. It is able to detect of their purity and particle size revealed on the formation of mono and poly dispersed nanoparticles and the biosynthesized ZnO NPs was appeared in the size range of 40-100nm (**Fig. 5**). The morphology of the nanoparticles is variable with majority of them being spherical. SEM studies clearly found that the reduction of Zn ions occurs extracellular, it would be important to identify the reducing agents responsible for this reaction<sup>30</sup>.The conformation of the nanostructure morphology of chemically synthesized ZnO nanoparticles comes from the analysis of SEM micrographs. The SEM image (**Fig. 6**) showed that nanoparticles were agglomerated with a particle size ranging from of 80-100nm<sup>31</sup>.



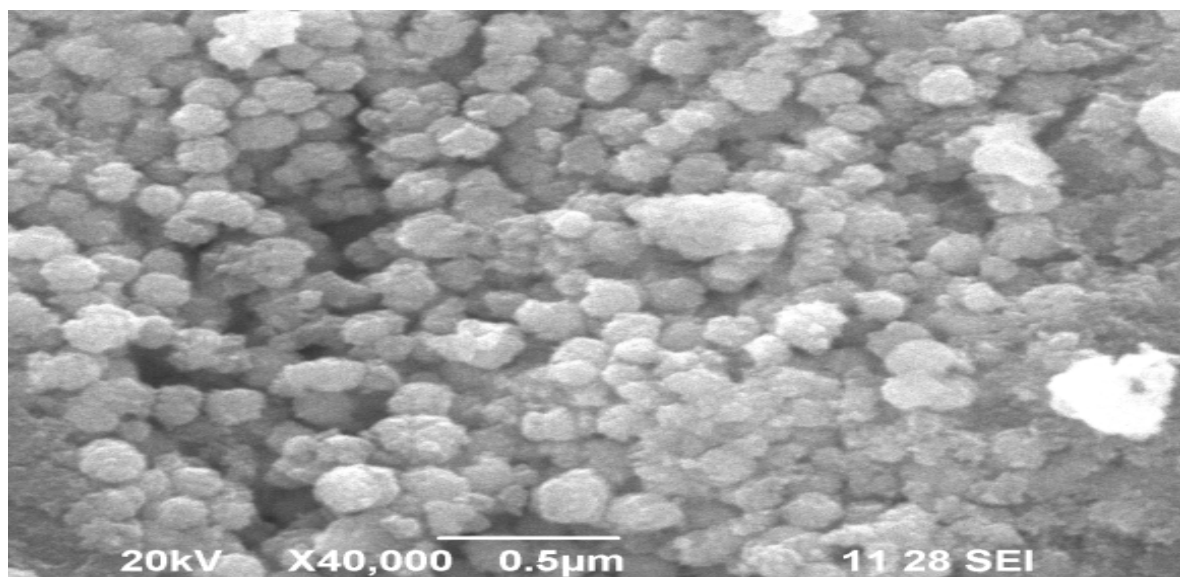


Fig-5 SEM image of biosynthesis of Zinc oxide nanoparticles

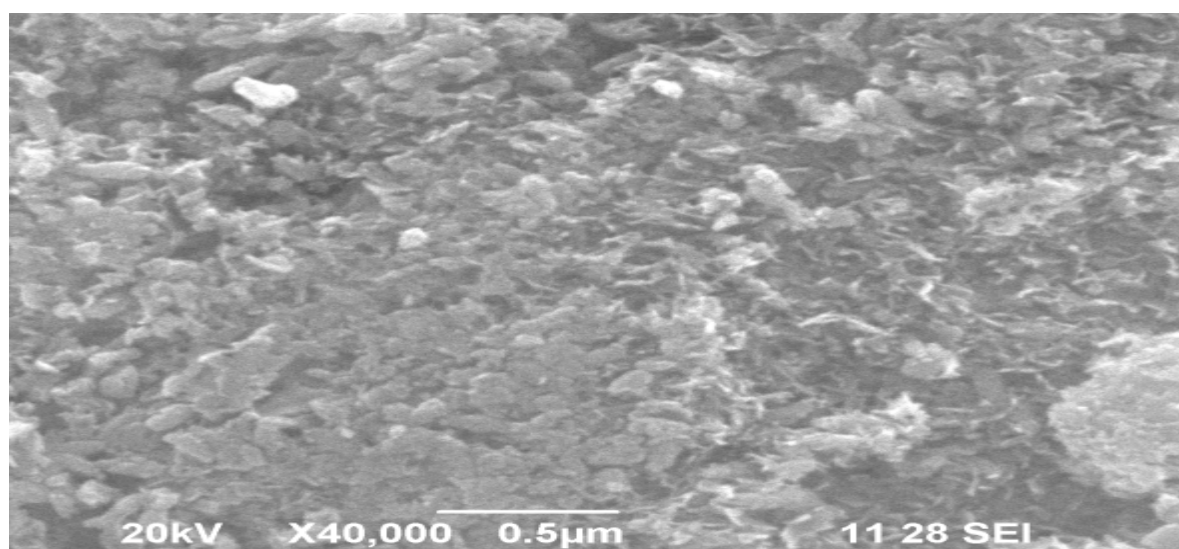


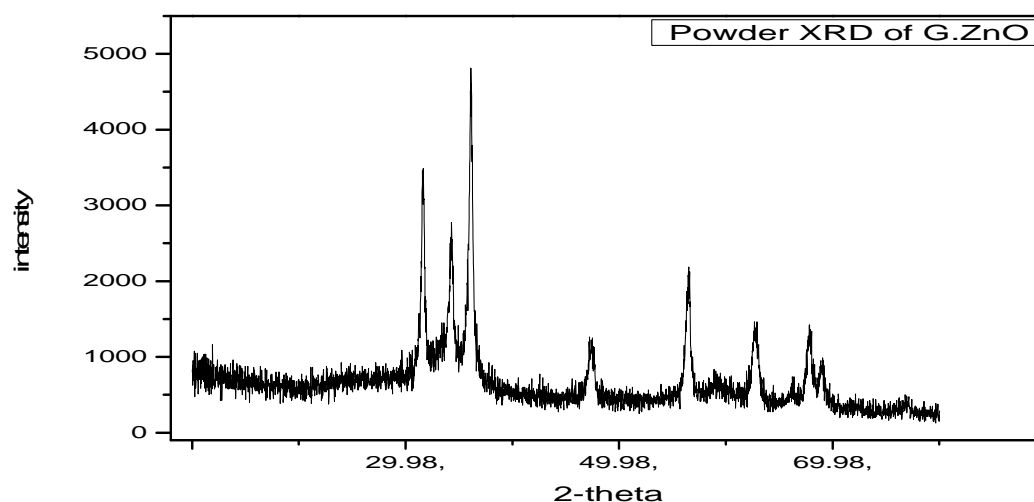
Fig-6 SEM images of chemical synthesis of Zinc oxidenanoparticles

### ***X-Ray Diffraction (XRD) analysis***

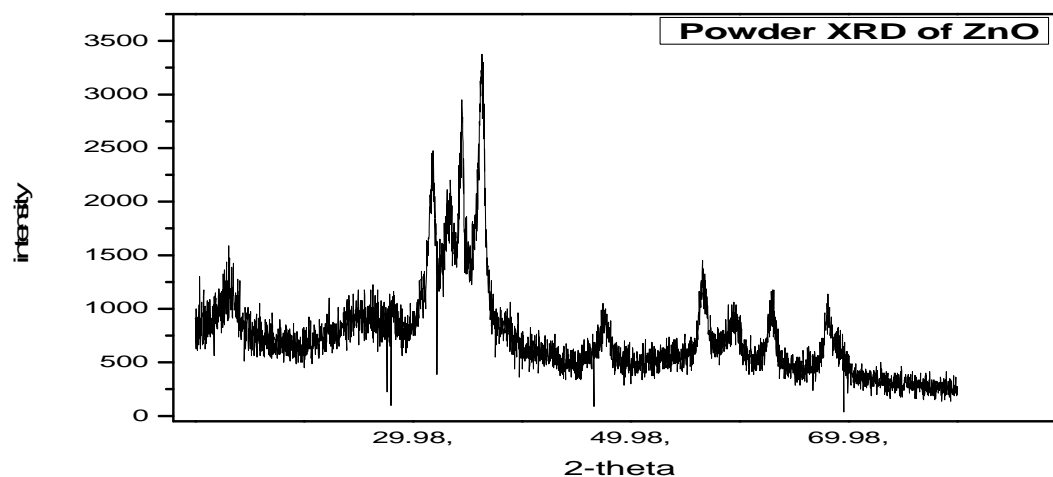
The obtainboth synthesized ZnO NPs were investigated to study their crystalline nature by XRD spectroscopy. From the results it can be incidental that the ZnO NPs were synthesized in their pure phase, without any impurities. The results also confirmed the biosynthesized ZnO nanoparticles 2θ values are 31.56°, 34.2°, 36.10°, 47.38°, 56.48°, 62.9°, 67.92° and 69.8° crystalline pattern. Furthermore, the crystalline structure was matched with the JCPDS data of 36-1451 and with the help of full-width and half-maximum data, with  $d = 18.031$  and  $2\theta = 36.1^\circ$ . The plane crystalline data were calculated by Scherrer's formula  $D = k\lambda/\beta\cos\theta$ <sup>32</sup>. The synthesized crystalline particles were said to be 36.1 nm in size, as illustrated in **Fig-7**. The X-Ray diffraction (XRD) pattern of chemically



synthesized zinc oxide nanoparticles is shown in **Fig-8**. X-Ray diffraction pattern shows  $2\theta$  values are  $31.72^\circ$ ,  $34.48^\circ$ ,  $36.32^\circ$ ,  $47.66^\circ$ ,  $56.64^\circ$  and  $63.04^\circ$ . The preferred orientation corresponding to the plane  $36.32^\circ$  is also observed. Zinc oxide crystallizes in two main forms, hexagonal wurtzite and cubic zinc blende. The wurtzite structure is most stable at ambient conditions and thus most common. It also confirms the synthesized nanopowder was free of impurities as it does not contain any characteristics XRD peaks other than zinc oxide peaks<sup>33</sup>.



**Fig-7 XRD of biosynthesized Zinc oxide nanoparticles**



**Fig-8 XRD of chemically synthesized Zinc oxide nanoparticles**

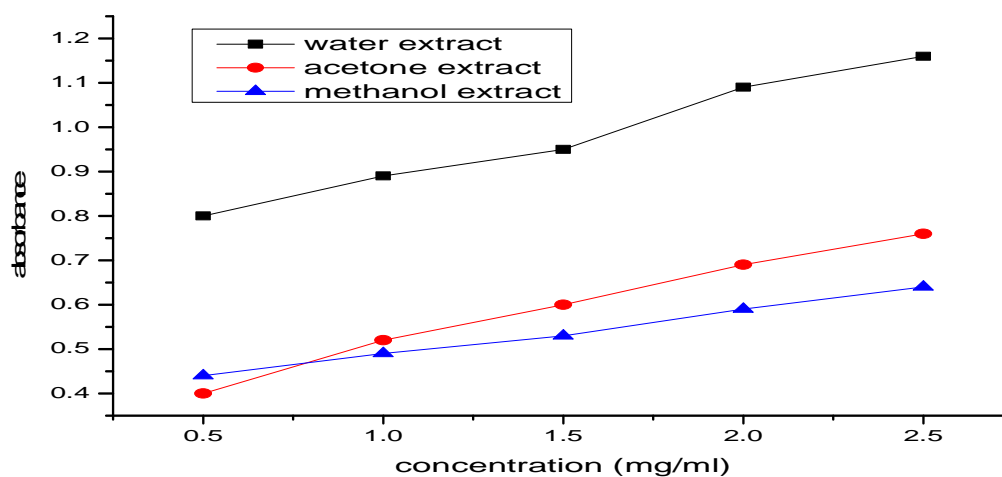
### ***Antioxidant activity***

Absorbance was measured at 580 nm. Higher absorbance value indicates higher reducing power. The reducing power was obtained as absorbance taken as 1.16 at 2.5mg/ml for water extract (*Plumbago zeylanica*) respectively. In *Plumbago zeylanica* Linn ingredient, plumbagin

(naphthoquinone) exhibit antioxidant property. The Antioxidant in- vitro testing that Reducing Power assay were performed with *Plumbago zeylanica* extracts using various solvents (methanol, acetone, water). Percentage of inhibition was calculated by plotting graph and ascorbic acid (Vitamin C) was used as standard and absorbance was taken at 580 nm<sup>34</sup>. Hence, the present study confirms that the water extract of *Plumbago zeylanica* Linn possess better antioxidant property in comparison to other extracts of *Plumbago zeylanica* Linn (Fig-9).

**Table-1 Antioxidant activity of various extracts of *plumbago zeylanica***

Concentration mg/ml	Absorbance (nm)					
	water extract		Acetone extract		Methanol Extract	
0.5	0.8		0.4		0.44	
1.0	0.89		0.52		0.49	
1.5	0.95		0.6		0.53	
2.0	1.09		0.69		0.59	
2.5	1.16		0.76		0.64	



**Fig-9 Antioxidant activity of various extracts of *plumbago zeylanica***

### ***Antibacterial activity***

The antimicrobial activity of green and chemical synthesized ZnO suspension of different concentrations (30mM, 40mM, 50mM, 60mM) towards various bacterial were tested by disc diffusion method and are shown in the Fig-11<sup>35</sup>. The inhibition zone clearly shows that the biocidal action of ZnO nanoparticles which involves distraction

of the membrane with high rate of generation of surface oxygen species and finally lead to the death of pathogens. Interestingly, the size of the inhibition zone was different rendering to the type of pathogens, synthesis method and the concentration of ZnO nanoparticles. As it was shown in the study of <sup>36</sup>, it has been found that by increasing the concentration of ZnO nanoparticles in wells and discs, the growth inhibition has also been increased dependably because of proper diffusion of nanoparticles in the agar medium. Both nano ZnO nanoparticles exposed antimicrobial activity against selected pathogens but maximum activity (15/16 mm) was observed in *S. aureus* followed by *S. typhi* (11/20 mm) (Fig. 10a & 10b). In our study, green ZnO nanoparticles showed a greater significant zone of inhibition when compared to chemically synthesized ZnO nanoparticles. However, low improvement of the antimicrobial activity was noted in the cases of ZnO at lower concentration (30 mM, 40 mM) but medium inhibition was noticed at higher concentrations (Fig. 11a & 11b). All the treatments namely, green and chemically synthesized nano ZnO particles showed significant difference on different organisms with varying concentration <sup>37</sup>. In the disc diffusion method the pathogens were more sensitive.

Table-2 Antibacterial activity of biosynthesized ZnO nanoparticles

Organisms	C	30	40	50	60
<i>S. typhi</i>	20	7	9	10	11
<i>S. aureus</i>	16	8	11	12	15

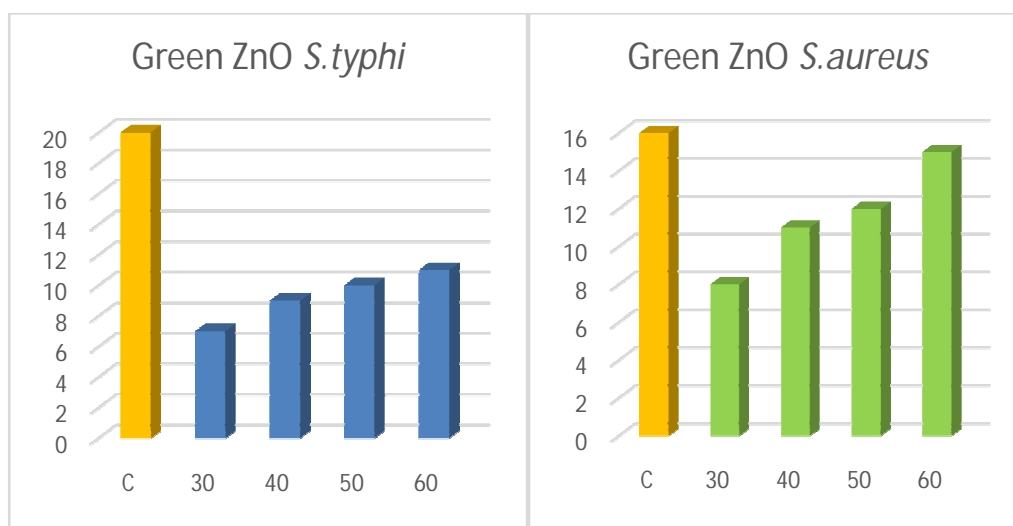


Fig-10a

Fig-10b

Fig- 10a,10b Antibacterial activity of biosynthesized ZnO nanoparticles

Table-3 Antibacterial activity of chemically synthesized ZnO nanoparticles

Organisms	C	30	40	50	60
<i>S.typhi</i>	19	0	7	9	11
<i>S.aureus</i>	18	0	9	10	11

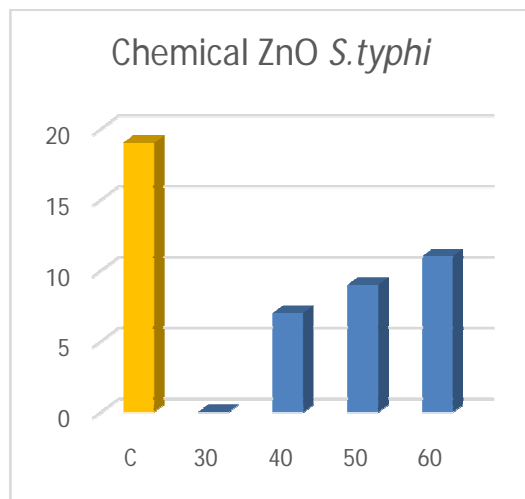


Fig-11a

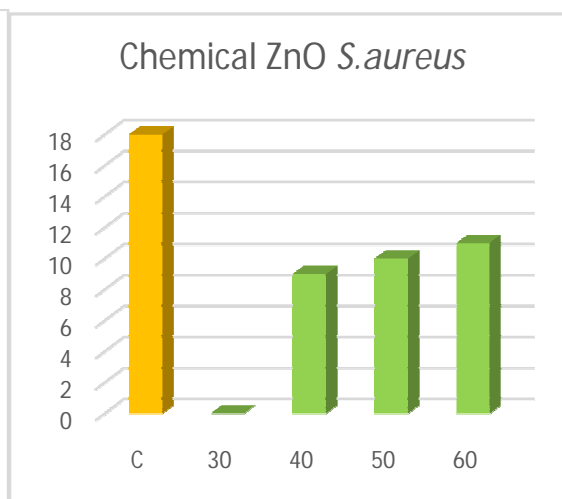


Fig-11b

Fig- 11a, 11b Antibacterial activity of chemically synthesized ZnO nanoparticles

## CONCLUSION

It is known that the synthesized by green route of ZnO nanoparticles is nontoxic and environmentally friendly as compared to chemical synthesis. In response to this assumption, this study proves the ZnO nanoparticles using *Plumbago Zeylanica* water extract possess high activities. The synthesized ZnO nanoparticles were characterized by UV-Vis absorption spectroscopy, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM). These studies confirmed the size of the synthesized ZnO nanoparticles in the range of 60–100 nm. The larger nanoparticles of ZnO resulted from the agglomeration of smaller nanoparticles. Moreover, the synthesized ZnO nanoparticles exhibited high activity against *S. aureus*, *S. typhi*. Also, the green synthesis of ZnO nanoparticles using *Plumbago Zeylanica* water extract can be a substitute to chemical methods. The present investigation revealed that the leaves, stem and root of *Plumbago zeylanica* contain significant amount of phenols and flavonoids and possess antioxidant property.

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