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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 ofdeveloping fields of science and technology [1]. 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 [2,3,4] and have been studied extensively because of their exclusive catalytic, optical, electronic, magnetic and antimicrobial activities [5,6,7]. 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[8]. Zinc oxide nanostructure exhibits high catalytic efficiency, strong adsorption ability and are used more and more frequently in the manufacture of sunscreens [9,10] ceramics rubber processing, wastewater treatment, and as fungicides[11]. Chemical methods lead to the presence of some toxic chemicals adsorbed on the surface that may have opposing effects in medical application [12,13]. Currently, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity, eco-friendliness and extensive antimicrobial activities[14] . *Plumbago zeylanica* Linn. (Plumbaginaceae) is one of the well-known Ayurveda drug. It iscommonly identified as chitramula and chitrack. The present study was carried out on theantibacterial activity of leaf of Plumbago zeylanica Linn. (Plumbaginaceae) Itpossessmulti-purpose medicinal property and is most commonly used in the traditional medicinal system of India. The plants contain of 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[15]. 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-1. The particle shape was recognized using Scanning Electron Microscopy[16].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 [17]. Recent studies have focused on the analysis of antioxidant properties shown by the phytoconstituents extracted from the plant(including polyphenols, terpenes)[18,19,20] andalso theestimation of antimicrobial activity against of 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 acetatedehydrate,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 [21]. 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 $Zn(CH_3COO)_2 .2H_2O$[22]. 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 yellowishprecipitate 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[23].

Chemical synthesis of ZnO nanoparticles

2.28 g of zinc nitrate hexahydrate was dissolve 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[24] .

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 rangeof 4000–400 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 tape 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 *-2* configuration(generator-detector) X-ray tube copper S= 1.54 A and LYNXEYE PDSdetector. 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 x 10–3 mol/dm^3 TPTZ in 40 x 10–3 mol/dm^3HCl, 20 x 10^{-3} mol/dm^3 $FeCl_3.6H_2O$ and 0.3 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 µl of FRAP reagent was mixed with 90 µl of distilled water and 30 µl 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 $FeSO_4$ was in turn plotted against concentrations of the standard antioxidants (L-ascorbic acid and Trolox)[25].

Antimicrobial susceptibility test

The disc diffusion method [26]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^{o}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 at1018.9cm^{-1} ,1375.3cm^{1},1617.4cm^{-1},2918.8cm^{-1} and 3277.19cm^{-1}**Fig-1**.Primary alcohols show a strong band near in the region 1018.9cm^{-1},the various C-H bending vibrations in alkene appear in the region 1375.3cm^{-1},nitrates (-O-N=O) can be readily recognized from the two strong bands in the region 1617.4cm^{-1},cyclohexane at 2918.8cm^{-1}with increasing strain in the ring C-H bending also show in increase and hetero aromatic compounds containing N-H group show N-H$_{str}$ absorption in the region 3277.1cm^{-1},1489 cm^{-1}.FTIR analysis of the chemically synthesized ZnONPs revealed the presence of peaks at 1395.3cm-1,1489.9cm-1,1576.1cm-1and 3379.1cm-1**Fig-2**. The presence of nitro group in a compound is characterized by the presence of strong band at 1395.3cm-1,aromatic hydrocarbon compounds the most characteristic C=C stretching bands at 1489.9cm-1,1576.1cm-1 and the primary amides in dilute solution show the band 3379.1cm-1 . 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[27].

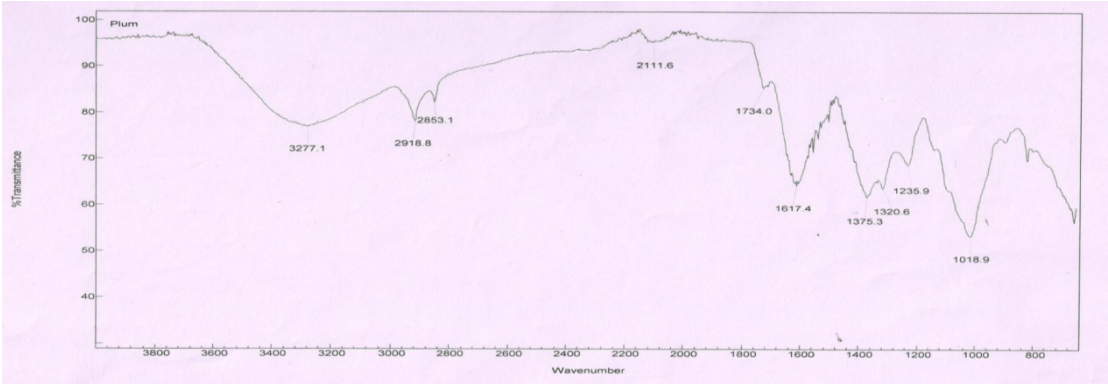


Fig-1 FTIR spectrum of biosyntheized Zinc oxide nanoparticle

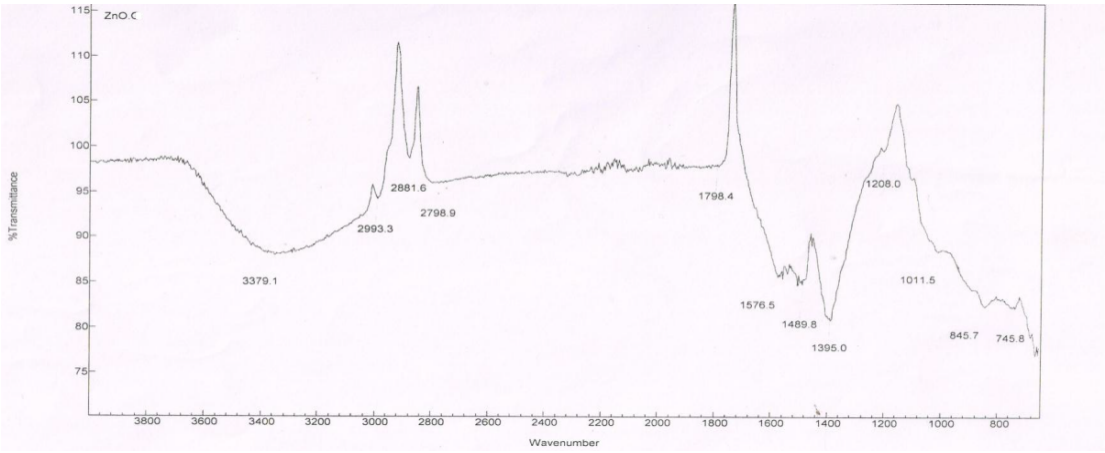


Fig-2 FTIR spectrum of chemically synthesized Zinc oxide nanoparticles

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*inleaf 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[28].**Fig 4**shows the UV-Vis absorption spectrum of chemically synthesizedzinc 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[29].

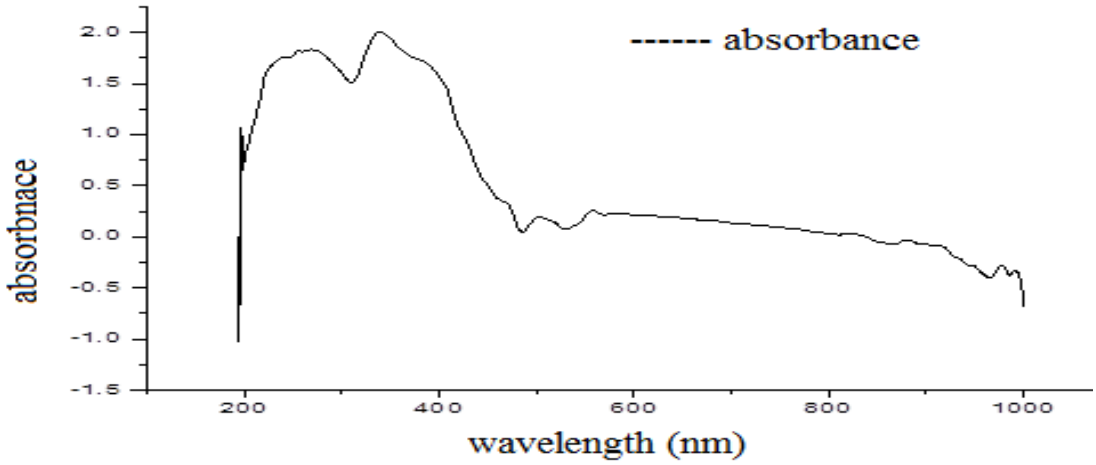


Fig-3 UV spectrum of biosynthesized Zinc oxide nanoparticles

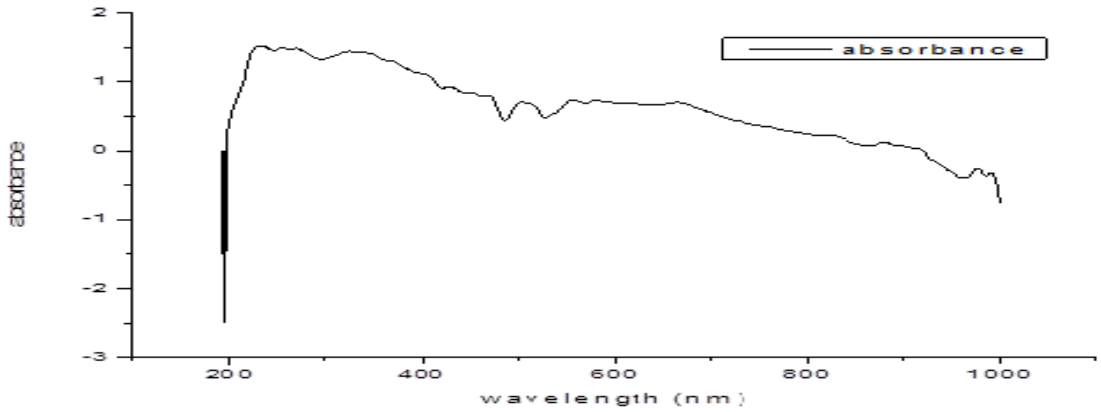


Fig-4 UV spectrum of chemically synthesized Zinc oxide nanoparticles

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[30].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[31].

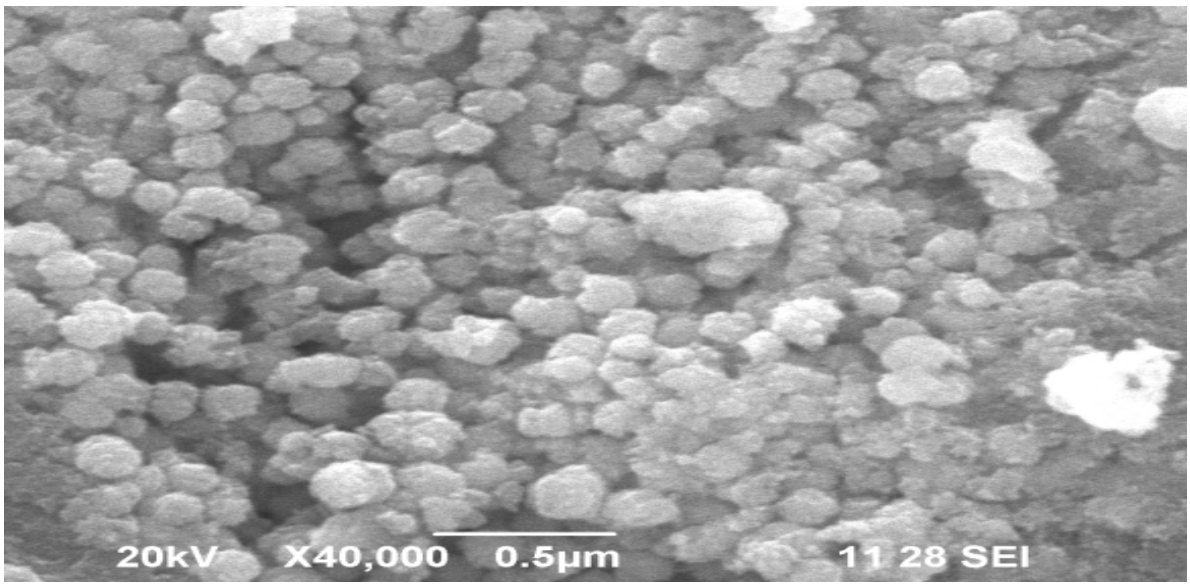


Fig-5 SEM image of biosynthesis of Zinc oxide nanoparticles

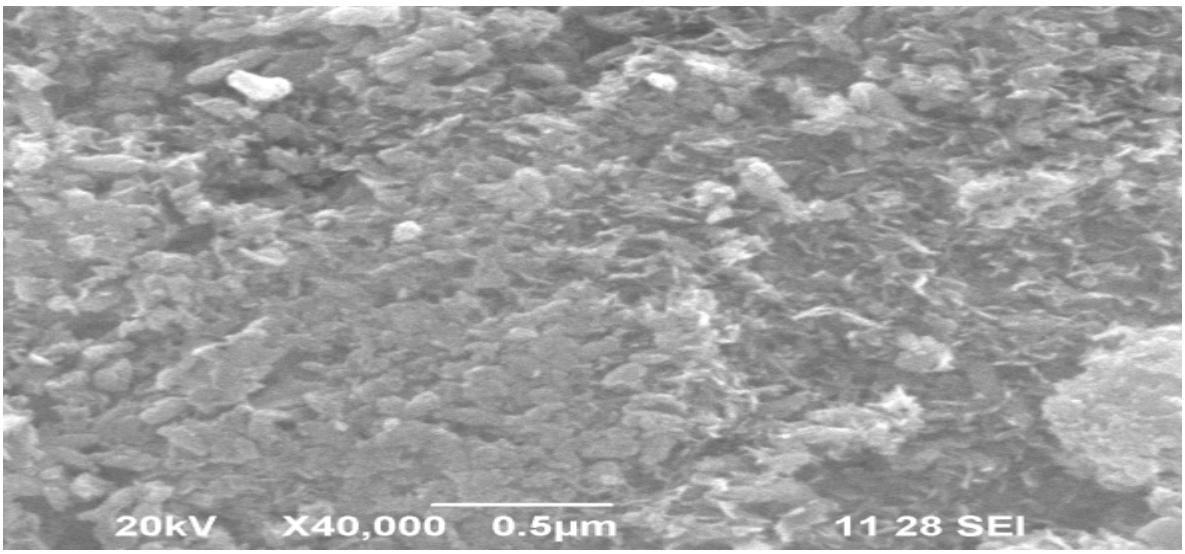


Fig-6 SEM images of chemical synthesis of Zinc oxidenanoparticles

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 36-1451and with the help of full-width and half-maximum data, with d =18.031and 2θ=36.1°The plane crystalline data were calculated by Scherrer's formula $D = k\lambda/\beta\cos\theta$[32]. 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 2θ values are 31.72˚, 34.48˚, 36.32˚, 47.66˚, 56.64˚and 63.04˚.The preferred orientation corresponding to the plane 36.32º 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 anycharacteristics XRD peaks other than zinc oxide peaks [33].

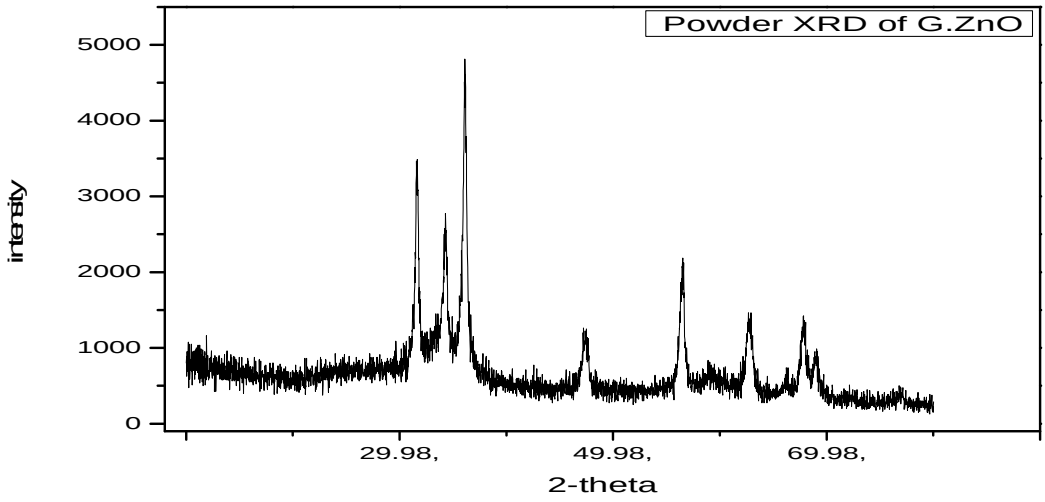


Fig-7 XRD of biosynthesized Zinc oxide nanoparticles

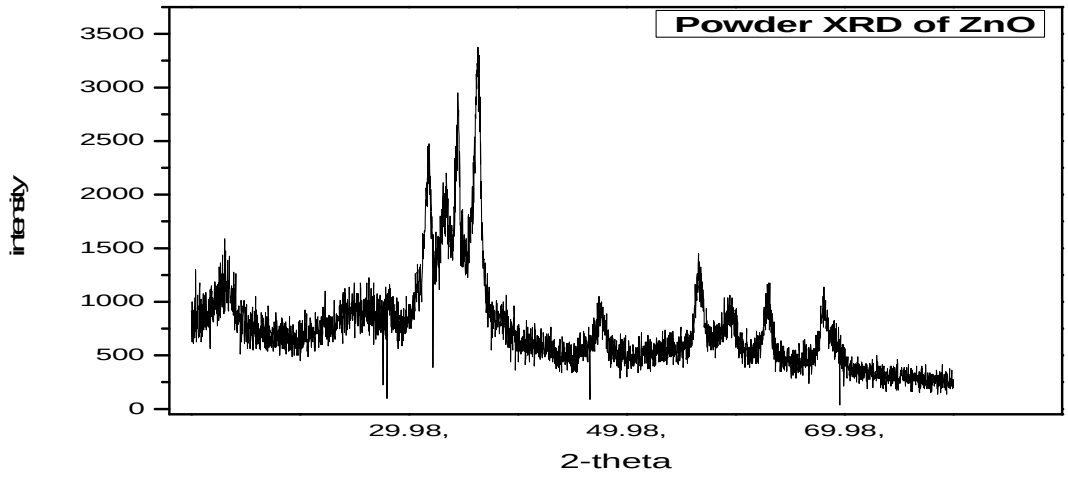


Fig-8 XRD of chemically synthesized Zinc oxide nanoparticles

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

(napthoquinone) 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[34].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

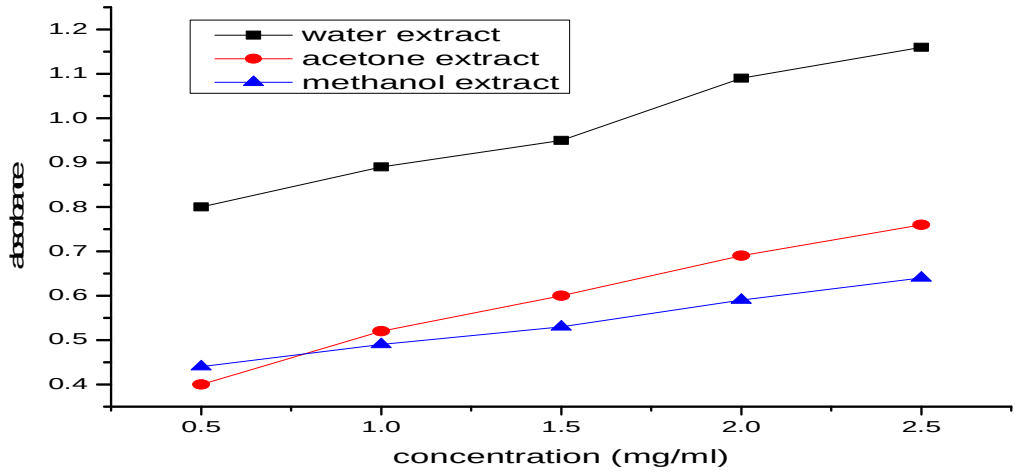


Fig-9Antioxidant activity of various extracts of *plumbago zeylanica*

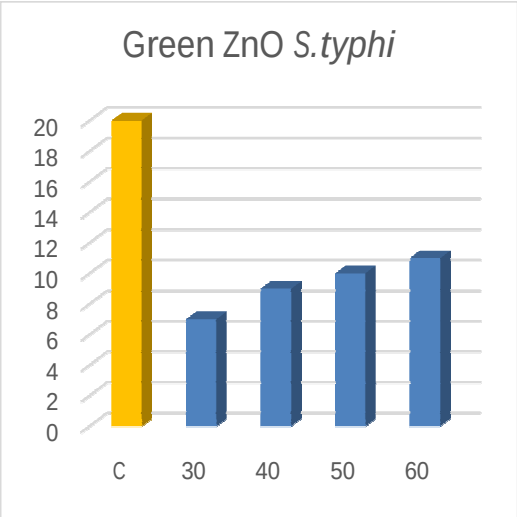
Antibacterial activity

The antimicrobialactivityofgreenandchemical synthesized ZnOsuspensionsofdifferentconcentrations (30mM,40mM,50mM,60mM)towardsvariousbacterialweretestedbydiscdiffusion agar methodandareshown inthe **Fig-11** [35]. Theinhibitionzoneclearlyshowsthatthe biocidalactionofZnOnanoparticleswhichinvolvesdistraction

ofthemembranewithhighrateofgenerationofsurface oxygen speciesandfinallyleadtothedeathofpathogens. Interestingly,thesizeoftheinhibitionzonewasdifferent renderingtothetypeofpathogens,synthesismethodand the concentrationsofZnOnanoparticles.Asitwasshownin the studyof [36] , ithasbeenfoundthatby increasingtheconcentrationof ZnOnanoparticlesinwellsand discs, thegrowthinhibitionhasalsobeenincreaseddependably because ofproperdiffusionofnanoparticlesintheagarmedium. Both nanoZnOnanoparticlesexposed antimicrobial activityagainstselectedpathogensbutmaximumactivity (15/16mm)wasobservedin *S. aureus* followed by S.typhi (11/20mm)(**Fig. 10a&10b**).In our study,greenZnOnanoparticles showedagreatersignificantzone of inhibitionwhencomparedtochemically synthesizedZnO nanoparticles.However,lowimprovementoftheantimicrobialactivitywasnotedinthecasesofZnOat lowerconcentration(30mM,40mM)butmediuminhibitionwas noticedathigherconcentrations(**Fig. 11a&11b**).Allthetreatments namely,greenand chemically synthesizednanoZnOparticlesshowedsignificant differenceondifferent organismswithvaryingconcentration [37].In the disc diffusion method the pathogens were more sensitive.

Table-2 Antibacterial activity of biosynthesized ZnO nanoparticles

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



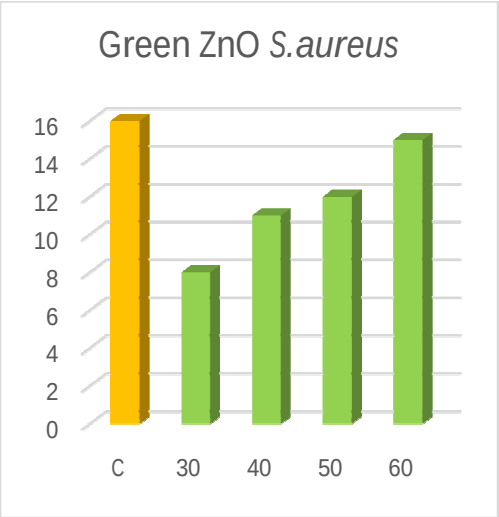
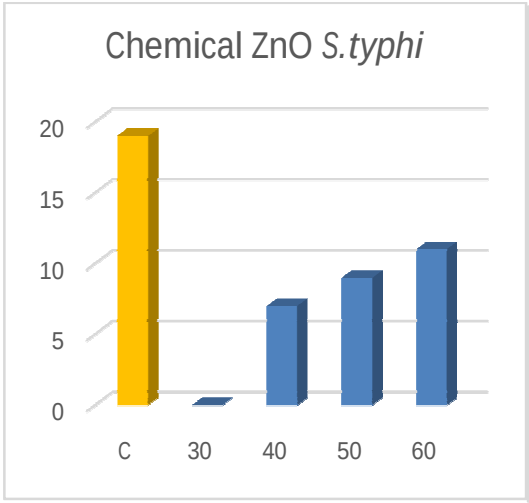


Fig-10a **Fig-10b**

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

Table-3 Antibacterial activity of chemically synthesized ZnO nanoparticles

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



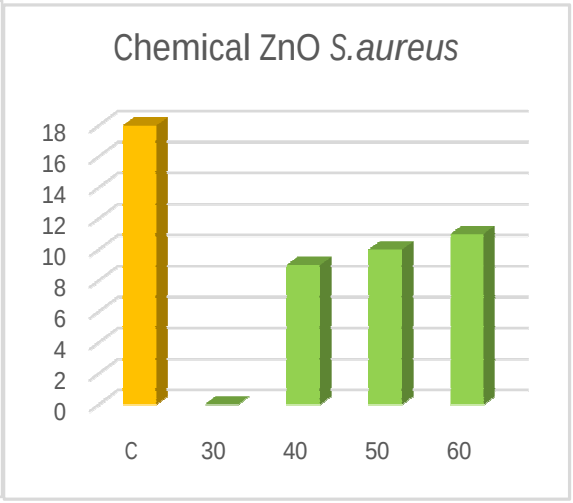


Fig-11a **Fig-11b**

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 tochemical synthesis. In response to this assumption, this study proves the ZnO nanoparticles using *Plumbago Zeylanica* water extractpossess 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 studiesconfirmed 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 an substitute to chemicalmethods.The present investigation revealed that theleaves, stem and root of *Plumbago zeylanica* contain significant amount of phenols andflavonoids and possess antioxidant property.

REFERENCES

1. Snelling, Eric Charles. Soft ferrites: properties and applications. Iliffe Books, (1969).
2. SosaI.O,Noguez.C, Barrera R.B, Optical properties of metal nanoparticles with arbitrary shapes, Journal of Physical Chemistry B 2003;107(62): 692–697.
3. Sunanda K, Kikuchi .Y, Hashimoto. K, Fujishima.A,Bactericidal and detoxificationeffectsofTiO2 thin filmphotocatalysts, Environmental ScienceandTechnology 1998;32: 726–728.
4. Sun Y.G, Mayers. B,Herricks. T ,Xia Y.N,Polyolsynthesisof uniform silvernanowires: a plausible growthmechanismandthe supportingevidence,NanoLetters(2003);3;955–960.
5. Ingle.A, Gade .A, Pierrat .P, onnichsen C.S, Rai.M, Myco-synthesis of silver nanoparticles using the fungus Fusarium acuminatum and its activity against some human pathogenic bacteria, Current Nanoscience 2008 ;4: 141–144.
6. Duran.N,Marcato P.D, Alves O.L, Souza .GMechanistic aspects of biosynthesis of silver nanoparticles by several Fusarium oxysporum strains, JournalofNanotechnology 2005;31–37.
7. Shankar S.S., Rai A, Ahmad A, and Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (Azadirachtaindica) leaf broth.Journal of Colloid and Interface Science 2004;275: 496–502.
8. Seshadri R, Rao C.N.R, MullerA, CheethamA.K(Eds.), The Chemistry of Nanomaterials, Wiley-VCH Verlag GmbH, Weinheim, 2004 ; 1: 94–112.
9. Shiv Shankar.S, Rai.A, Ankamwar .B, Singh.A ,Ahmad .A, Sastry .M Biological synthesis of triangular gold nanoprisms, Nature Materials 2004; 3: 482–488.
10. Wang .X, Lu .J , Xu. M, Xing. B , Sorption of pyrene by regular and nanoscaled metal oxide particles: influence of adsorbed organic matter, Environmental Science and Technology 2008; 42: 7267–7272.
11. Jain. D, Daima H.K, Kachhwaha S, Kothari K.L, Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities, Digest Journal of Nanomaterials and Biostructures 2009; 4:557–563.
12. Saxena .A, Tripathi R.M, Singh R.P, Biological synthesis of silver nanoparticles by using onion(Allium cepa) extract and their anti- bacterial activity, Digest Journal of Nanomaterials and Biostructures 2010;5: 427–432.
13. Khandelwal.N ,Singh.A,Jain.D, Upadhyay M.K, Verma H.N,Green synthesis of silver nanoparticles using Argimone mexicana leaf extract and evaluation of their antimicrobial activities, Digest Journal of Nanomaterials and Biostructures 2010;5: 483–489.

14. Kumar R, Kumar S, Patra A, Jayalakshmi S Hepatoprotective activity of aerial parts of Plumbago zeylanica linn against carbon tetrachloride-induced hepatotoxicity in rats. International Journal of Pharmacy and Pharmaceutical Sciences 1(Suppl 1): 2009; 171-175.
15. Arinjoy Datta*, Chandi Patra et al.,Green Synthesis of Zinc Oxide Nanoparticles Using Parthenium hysterophorus Leaf Extract and Evaluation of their Antibacterial PropertiesDatta et al., J Biotechnol Biomater 2017; 7: 1-3.
16. Aqil.F, Ahmad .I, Mehmood .Z.Antioxidant and Free Radical Scavenging Properties of Twelve Traditionally Used Indian Medicinal Plants. Turk. J. Biol. 2006 ;30: 177-183.
17. Zhou Y.C, Zheng R.L. Phenolic compounds and an analog as superoxide anion scavengers and antioxidants. Bioch. Pharm. 1991;2: 1177-1179
18. Quinn L.A, Tang H.H. Antioxidant properties of phenolic compounds in macadamia nuts. J. Am. Oil Chem. Soc. 1996; 73: 1585-1588
19. Seymour T.A, Li S.J, Morrissey M.T. Characterization of a Natural Antioxidant from Shrimp Shell Waste. J. Agri. Food.Chem.1996 ;44: 682-685.
20. Prasad K, Laxdal V.A, Yu M, Raney B.L. Evaluation of hydroxyl radicalscavenging property of garlic. Mol. C. Bioch. 1996;154: 55-63.
21. Datta A, Patra C, Bharadwaj H, Kaur S, Dimri N, et al. Green Synthesis of Zinc Oxide Nanoparticles Using Parthenium hysterophorus Leaf Extract and Evaluation of their Antibacterial Properties. J Biotechnol Biomater 2017 ; 7: 271.
22. Gnanasangeetha and Thambavani et al., 2013.
23. Pragati Jamdagni *, Poonam Khatri, J.S. Rana 'Green synthesis of zinc oxide nanoparticles using flower extract of Nyctanthes arbor-tristis and their antifungal activity" Journal of King Saud University – Science 2018; 30: 168–175.
24. Rudeerat suntako ,"Effect of zinc oxide nanoparticles synthesized by a precipitation method on mechanical and morphological properties of the CR foam" Bull. Mater. Sci., 2015;38: 1033–1038.
25. Jai C. Tilak1, Soumyakanti Adhikari, Thomas P.A. DevasagayamAntioxidant properties of Plumbago zeylanica, an Indian medicinal plant and its active ingredient, plumbaginRedox Report, 2004;9.
26. Bauer et al., 1966.
27. Sindhura KS, Prasad T.N, Selvam P.P, Hussain O.M,Synthesis,characterization and evaluation of effect of phytogenic zinc nanoparticles onsoil exo-enzymes. Appl Nanosci 2013; 1: 1-9.

28. Nagarajan S, Kuppusamy KA) Extracellular synthesis of zinc oxidenanoparticle using seaweeds of Gulf of Mannar, India. J Nanobiotechnol 2013; 11: 1-11.
29. Arinjoy Datta*, Chandi Patra, Himani Bharadwaj, Sukhjeet Kaur, Neha Dimri and Robinka Khajuria "Green Synthesis of Zinc Oxide Nanoparticles Using Parthenium hysterophorus Leaf Extract and Evaluation of their Antibacterial Properties" Datta et al., J Biotechnol Biomater, 2017; 7: 1-3.
30. Arinjoy Datta*, Chandra Maurya1, Prakash Mahanwar Biosynthesis of Zinc Oxide Nanoparticles UsingIxora Coccinea Leaf Extract—A Green approach" Journal of Synthesis Theory and Applications 2017 ; 5: 1-14.
31. Renata Dobrucka a,*, Jolanta Dugaszewska Biosynthesis and antibacterial activity of ZnO nanoparticles using Trifolium pratense flower extract. Saudi Journal of Biological Sciences 2016 ;23: 517–523.
32. Shamsuzzaman *, Ashraf Mashrai , Hena Khanam , Rezq Naji Aljawfi ,Arabian Journal of Chemistry 2017; 10: 1530–1536.
33. Elango, G, Roopan, S.M. ,Dhamodaran, K.I., Elumalai, K., Al-dhabi, N.A., Arasu, M.V. Spectroscopic investigation of biosynthesized nickel nanoparticles and its larvicidal, pesticidal activities. J. Photochem. Photobiol.2016; 162: 162–167.
34. Bigdeli, F., Morsali, A. and Retalleau, P. Synthesis and Characterization of Different zinc (II) Oxide Nano-Structures from Direct Thermal Decomposition of ID Coordination Polymers. Polyhedron,2010; 29: 801-806.
35. Tilak J.C, Adhikari S, Devasagayam T.P, "Antioxidant properties of Plumbago zeylanica, an Indian medicinal plant and its active ingredient, plumbagin." Redox Rep.2004; 9(4):219-227.
36. Sangeetha.G, Rajeshwari.S, Venckatesh.R et.al, Greensynthesisof zinc oxidenanoparticlesby aloe barbadensismiller leaf extract: structure andopticalproperties,MaterialsResearchBulletin 2011; 46: 2560–2566.
37. Sangeetha G, Rajeshwari S, Venckatesh R, Green synthesized ZnO nanoparticles against bacterial and fungal pathogens , Progress in Natural Science: Materials International 2012 ;22(6): 693–700.