GREEN SYNTHESIS,CHARACTERIZATION AND APPLICATIONS OF ZINC OXIDE NANOPARTICLES USING FRUIT EXTRACT OF TRIBULUS TERRESTRIS

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ABSTRACT
zinc oxide nanoparticles were successfully synthesized by using a simple and green synthetic route by using metal salt and fruit extract of Tribulus terrestris which act as reducing and stabilizing agent .The synthesized zinc oxide nanoparticles were characterized by using various analytical techniques such as UV-VISIBLE DRS, FT-IR, XRD, EDAX, FESEM, TEM.Biological test results exposed that synthesized zinc oxide nanoparticles have broad spectrum antimicrobial activities against Escherichia coli, Bacillus subtilis, Aspergillus niger and cytotoxic activity on MCF-7 cell line.

Keywords : Zinc oxide nanoparticles, Tribulus terrestris, green synthesis, antimicrobial activity.

INTRODUCTION

Zinc oxide is a unique and very key inorganic compound [1]. It has attracted extensive research because of its characteristic features and novel applications in wide areas of Science and Technology. The main aim is preparing zinc oxide nanoparticles in a short time by using laboratory methods [2-8]. Zinc oxide has various properties like semiconductors, pyroelctric, piezoelctric and optoelectronics [9]. Nanomaterials find a wide range of applications due to their exciting physical, chemical and catalytic properties because of their large surface to volume ratio[10-13].

Zinc oxide nanostructures can be synthesized by various approaches including vapour-liquid-solid (VLS) [14], hydrothermal synthesis [15], vapour phase deposition[16], chemical vapour deposition [17], metallorganic chemical vapour deposition [18], zinc oxidation [19], sol-gel method [20], microwave assisted thermal decomposition [21].The morphologies of Zinc oxide nano structures include nano particle [22], nanowire [23], nano belt [24], nano flower [25] respectively. Then conventional methods used for synthesis of Zinc oxide nanoparticles are expensive and harmful to environment due to involvement of various hazardous chemicals responsible for many health problems.

In recent, green synthesis of nano particles was achived by using plant extract due to its low cost, easily available, non toxic, biodegradable and environment friendly characteristics.

In this work, green synthesis of zinc oxide nanopaticles using fruit extract of Tribulus terrestris as reducing and capping agent.. Tribulus terrestris is found to be growing in subtropical areas around the world commonly known as Gokhru belonging to the family Zygophyllaceae widely distributed throughout India.

Fruits of T. terrestris.

Leaves and flowers of T. terrestris

Phytochemical studies have shown that this plant contains biologically rich compounds such as steroids, saponins, flavoniods, alkaloids and unsaturated acids which were involved in promoting numerous physiological responses [26].These saponin fractions of this plant find in contemporary medicine as a component of drugs effective in treating sexual dysfunctions and cardio vascular diseases.Tribulus terrestris has been shown to exhibit diuretic [27], anti-urolithiatic [28], CNS stimulant [29], antimicrobial [30],antifungal activities [31], antioxidant and antihypertensive activity in rat heart [32-33]. The use of non toxic materials like plant extract for synthesis of nanoparticles offers numerous benefits of pharmaceutical applications[34].

The effect of zinc oxide nanoparticles on antibiotics has been studied preservation in mind the fact that zinc oxide nanoparticles have an intrinsic bactericidal effect of their specis.The synthesized zinc oxide nanoparticles studied by UV-VISIBLE DRS, X-RD, FT- Infrared spectroscopy, SEM, TEM analysis. The antimicrobial activity of zinc oxide nanoparticles is well known [35-38]. Hence we create use of this property to inhibit the growth of Bacillus subtilis, Escherichia coli and Aspergillus niger using disc diffusion method. Zinc oxide nanoparticles are targeted as drug delivery, anticancer agents and antibacterial activity [39-42]. Bacillus subtilis, Escherichia coli and Aspergillus niger strains are selected as they are highly contagious, then we can assess the potential antimicrobial activity of zinc oxide nanoparticles.

EXPERIMENTAL METHOD

Reagents

Zinc nitrate hexahydrate, Sodium hydroxide (All the chemicals used in this were AR grade purchased from Sigma Aldrich, India) , double distilled water.

Preparation of Tribulus terrestris Fruit Extract

The fruits of Tribulus terretris were collected from Andhra university, Visakhapatnam. The fruits were rinsed with water several times followed by double distilled water to remove the dust particles and then the fruits were dried under direct sun light for two weeks to completely remove the moisture.

The dried fruits were pulverized well with mortar and pestle to make the powder. 10 gr of fruit powder was mixed in 100 ml of double distilled water and the mixture was heated at 80^0 c for 30 min. Then the boiled extract was cooled at room temperature and filtered using whatman no.1 filter paper and the filtrate was stored at 4^0c for further usage.

Green Synthesis of Zinc oxide Nanoparticles from the Fruit Extract of Tribulus terrestris

50 ml of Tribulus terrestris fruit extract was taken in a 250 ml beaker .Then 5 gr of $Zn(NO_3)_2.6H_2O$ and 100 ml of double distilled water were added to the extract and then boiled at 60-80^0c for 45 min by using a stirrer-heater. Followed by addition of 10ml of 1M NaOH drop wise to maintain alkaline pH(12). Then the mixture was boiled until it converted to a deep yellow colour paste. Then the paste is then taken out in a clean ceramic crucible and calcinated in an air heated furnace at 400^0c for 3 hours. A white coloured powder was obtained and this powder was collected carefully and then grind with mortar and pestle for uniformities of the powder.

CHARACTERIZATION

The synthesized zinc oxide nanoparticles were characterized by using a UV- VISIBLE DRS in the wavelength range between 200-800 nm. The FT-IR absorption spectra were recorded on a Perkin-Elmer GX FT-IR system used to obtain 16 cm^{-1} resolution spectra in the range 400 to 4000 cm^{-1}(absorbance mode).

The crystaline structure of the zinc oxide nanoparticles were measured by using a Bruker D8 Advance X-ray diffractometer with CuKα radiation of wavelength λ = 1.54056 Å. The X-ray diffraction (XRD) measurements were carried out in the locked coupled mode in the 2θ range of 20 to 80.

The surface morphology and composition of zinc oxide nanoparticles were investigated by FESEM (Field Emission Scanning Electron Microscopy) developed by Carl Zeiss. An accelerating voltage of 15 to 19 keV and probe current of ~800 pA. The shape and size of the nanoparticles were obtained by using Transmission Electron Microscope(TEM).

RESULTS AND DISCUSSIONS

UV-Visible Diffuse Reflectance Spectrum (UV-DRS)

UV-Visible Spectroscopy absorption peak means the electrons are absorbing the energy at 200-800 nm wavelength. Electrons are absorbing energy means the electrons are going to excited state from its ground state. Electrons are going to excited state from its ground state means the material is having band gap, thus which can be determine by absorption

wavelength. The electronic absorption spectra of metal oxide nanoparticles were recorded in DMF in the range 200–800 nm. Diffuse reflectance spectral studies in the UV-Visible-NIR region were carried out to estimate the optical band gap of the synthesized nanoparticles.

Fig.1 Shows the absorption spectrum of the zinc oxide nanoparticles excited at 357 nm which corresponds to the zinc oxide nanoparticles. The purity of the zinc oxide nanoparticles confirmed by the non existence of the other absorption peaks in the spectrum. The excitation peak corresponds to the band to band transition which also confirms the blue shift in the band gap of zinc oxide nanoparticles. The band gap estimated for this sample 3.47eV that is slightly higher than that of bulk zinc oxide 3.37eV. This blue shift may be attributed to quantum confinement effects.

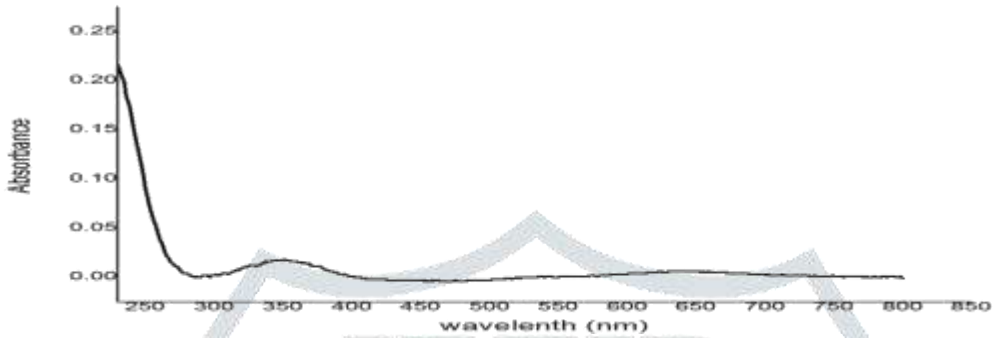


Fig.1 UV-Visible Diffuse Reflectance Spectrum of zinc oxide nanoparticles

X-Ray Diffraction (XRD)

The XRD results are shown in fig.2 The XRD peaks match with that reported for zinc oxide. The peaks are indexed as (100), (002), (101), (102), (110), (200), (112), (201), (004) when 2θ is varied from 20° to 80° (23.90^0, 30.9^0, 33.00^0, 36.1^0, 39.2^0, 45.5^0, 49.99^0, 50.1^0, 55.5^0, 66^0 and 69^0). All the peaks in the figure match well with the standard JCPDS pattern for zinc oxide (JCPDS card 36-1451).

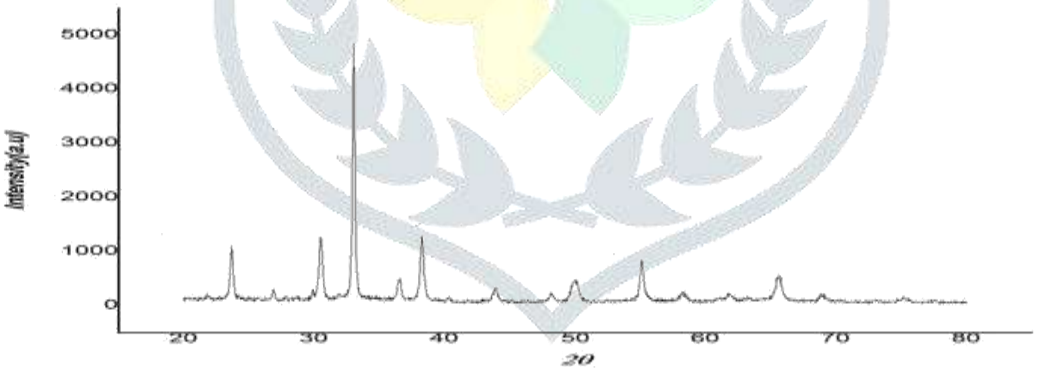


Fig.2 The X-Ray Diffraction pattern of zinc oxide nanoparticles

The peaks are sharp indicating good nanoparticles of the product. The broadness at the bottom of the peaks shows that the product is of size 76 nm. No other peak other than zinc oxide is observed indicating that the product is pure zinc oxide without any impurities or unreacted precursor. Average particle size of zinc oxide nanoparticles is found to be 76 nm using the Debye–Scherrer's formula. Diffraction pattern corresponding to impurities are found to be absent. This proves that zinc oxide nanoparticles are in amorphous nature.

$$D = \frac{k\lambda}{B\cos\theta}$$

where

k is Sherrer constant,
B is the full width at half-maximum,
λ is the X-ray wavelength,
θ is the Bragg diffraction angle.

Fourier-Transform Infrared Spectrum(FT-IR)

Fig.3 Shows the FT-IR spectrum of the zinc oxide nanoparticles synthesized by green method, which is acquired in the range of 400-4000 cm^{-1}. The band between the 500-700cm^{-1} correlated to metal oxide bond (zinc oxide).

The peaks in the range of 581 cm^{-1} indicates the presence of ZnO(Zn-O bond). The peaks located at 1074 cm^{-1} represents C-N Stretching vibrations of amines.The peaks located at 1607 cm^{-1} represents the aromatic ring **(c=c)** stretching. The absorption peak at 2115 cm^{-1} is due to (C≡C) stretching. The broad and intense peak at 3423 cm^{-1} is due to O-H Stretching.

The presence of hydroxyl, amines and aromatic ring confirms that the fruit extract could possibly enhance the stabilization of zinc oxide nanoparticles.

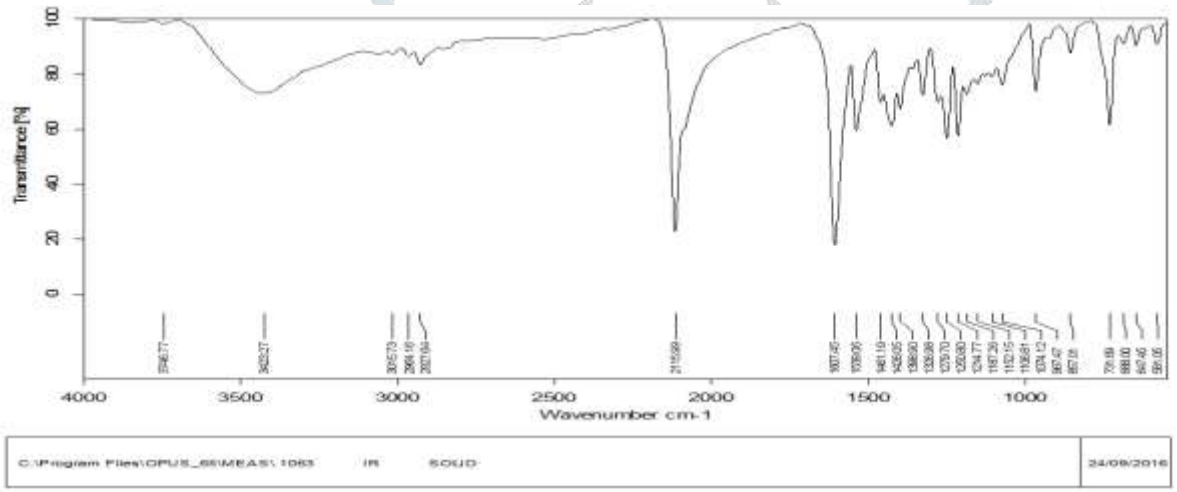


Fig.3 FT-IR Spectrum of zinc oxide nanoparticles

Field Emission Scanning Electron Microscope (FESEM) Analysis

FESEM analysis was used for the morphological study of zinc oxide nanoparticles calcined at 400°C is shown in Fig.4 It can be seen that the particles adopt irregular morphology with different sized particle. In addition zinc oxide nanoparticles show rod shape with smooth surface. It clearly indicates the fine rod like particles adsorbed on the surface due to the agglomerates. Nanoparticles size is in the range of 20-100 nm in diameter. The FESEM image shows zinc oxide nanoparticles are rod shape with smooth surface and the size of the particles around 100 nm.

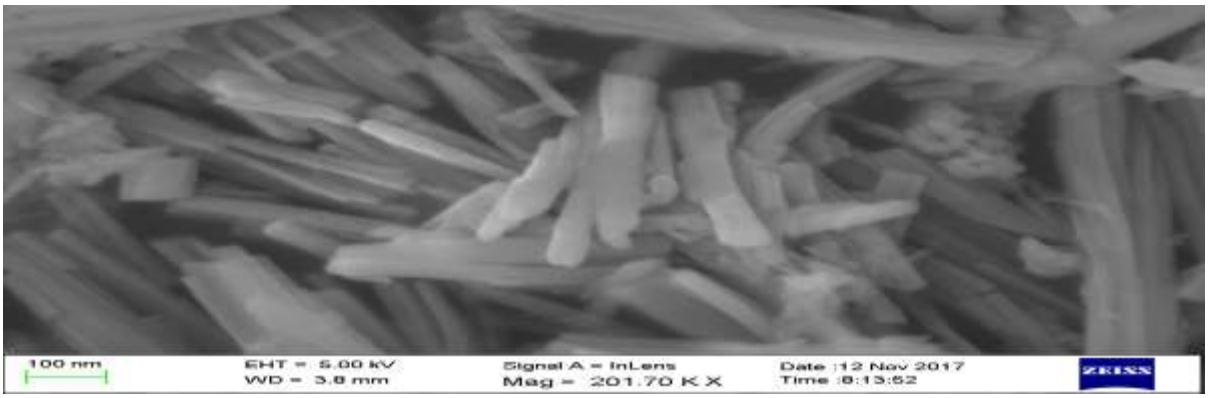
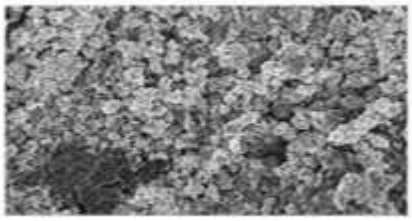


Fig.4 FESEM image of zinc oxide nanoparticles

EDAX Analysis

Element	Weight%	Atomic%
O K	35.10	68.85
Zn K	64.90	31.15
Totals	100.00	



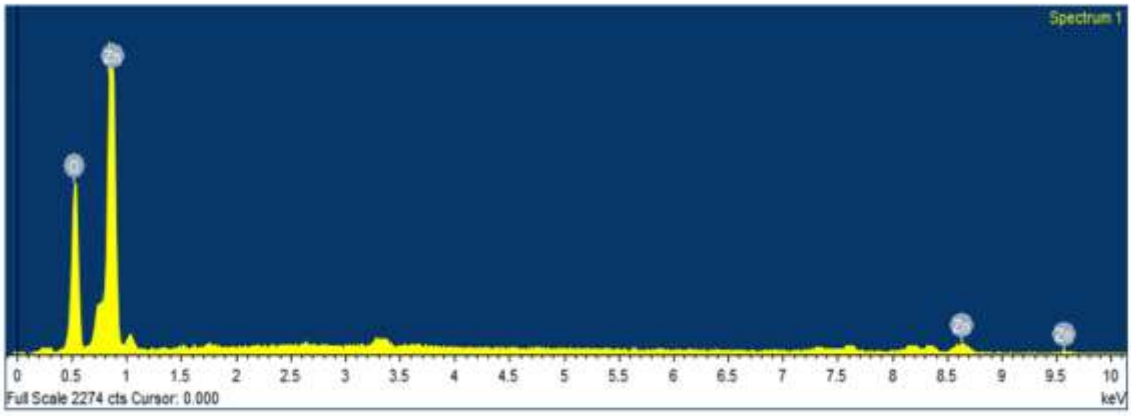


Fig.5 EDAX image of zinc oxide nanoparticles

The quantitative compositional analysis of zinc oxide nanoparticles synthesized from Tribulus terrestris fruit extract is carried out using Energy Dispersive X-ray (EDAX) Spectroscopy measurement. The EDAX analysis also confirms the presence of zinc oxide nanoparticles (Fig.5). From EDAX Spectrum the composition of elements such as a zinc (64.90%)and oxygen(35.10%) are identified.

Transmission Electron Microscope (TEM) Analysis

Fig.6. Shows the TEM image of zinc oxide nanoparticles synthesized from Tribulus terrestris fruit extract. TEM analysis is carried out to confirm the actual size of the particles, TEM images gives nanoparticles nature, morphology and orientation of the zinc oxide nanoparticles and illustrated in fig. The size of the particle is around 20 nm.

Fig.6 TEM image of zinc oxide nanoparticles

Antimicrobial screening of zinc oxide nanoparticles

The Zinc oxide nanoparticles is screened in vitro for antibacterial activity against Escherichia coli, Bacillus subtilis and antifungal activity against Aspergillus niger by Agar-well disc diffusion method. The antibacterial and antifungal activities of zinc oxide nanoparticles are listed in table.1.

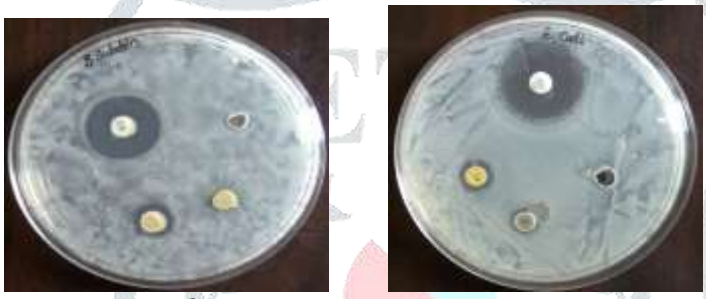


Fig.7 Inhibition zones for zinc oxide nanoparticles against B.subtilis, E.coli

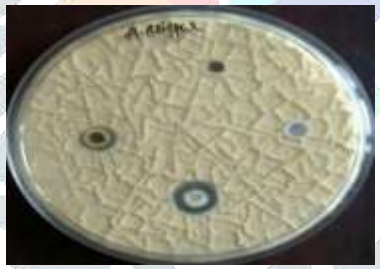


Fig.8 Inhibition zones for zinc oxide nanoparticles against A.niger

TABLE.1Antimicrobial activities of zinc oxide nanoparticles

Bacteria	**Inhibition zone (mm)**
E.coli	8
B.subtilis	8
Fungi	**Inhibition zone (mm)**
A.Niger	6

The zinc oxide nanoparticles showed good antibacterial activity against E.coli, B.subtilis and Anti-fungal activity against A.niger.

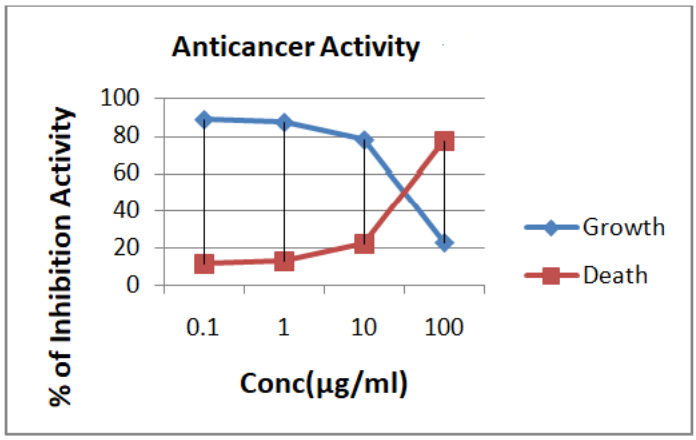
Cytotoxic studies of zinc oxide nanoparticles

The synthesized nanoparticles is screened for their cytotoxicity (MCF-7, cell lines). From the data, it is observed that the nanoparticles displayed their cytotoxic activities as IC_{50} (μg/mL) against breast cancer MCF-7 cell line.

TABLE.2.Dose response of nanoparticles on MCF-7 cell line

Conc (µg/ml)	% cell survival	% cell inhibition
0.1	89.1	10.9
1	87.50	12.50
10	79.45.	20.55
100	20.68	79.32

The above table details the high potent nature of zinc oxide nanoparticles. The cell survival is decreasing and percentage of cell inhibition is increasing with the increasing concentration of the zinc oxide nanoparticles. The IC_{50} value for zinc oxide nanoparticles is about 51.45µg/mL.



IC50	51.45µg/mL

Fig.9.. Effect of zinc oxide nanoparticles on MCF7 cell viability for 24hr incubation time

CONCLUSIONS

Synthesis of Zinc oxide nanopaticles is carried out using fruit extract of Tribulus terrstris. Very versatile, nontoxic and eco-friendly approach for the synthesis zinc oxide nanoparticles is presented in this work. Zinc oxide nanoparticles have been synthesized successfully by using a green method. The nanoparticles are characterised for structure and morphology using UV-VISIBLE, FT-IR, SEM, XRD, EDAX,TEM.

UV-Visible Diffuse Reflectance Spectrophotometer gives absorption maximum at 357 nm and having band gap 3.47 eV for Zno nanoparticles. FT-IR spectra gives the peak in the range of 581 cm^{-1} indicates the presence of ZnO(Zn-O bond). FESEM shows zinc oxide nanoparticle size is below 100 nm. EDAX analysis of the samples showing Zn and O present in the samples . XRD pattern shows the phase nature and crystalline size. Which is done by particle size is calculated by using by Debye-Scherrer's formula. TEM image exhibits as synthesized zinc oxide nanoparticles prepared by green method with an average diameter of 20 nm.

Zinc oxide nanoparticles are screened in vitro for antibacterial and antifungal activity by disc diffusion method. The bacterial organisms used in this study are Escherichia coli, Bacillus subtilis and fungal organism is Aspergillus niger. The observed inhibition zones for zinc oxide nanoparticles are in the range of 8-10mm for E.Coli, 8-10mm for Bacillus subtilus and 6-8mm for A.Niger. The screened data in these reports are in good agreement with the previous data and the inhibition zone images are pictorially recorded. The cytotoxicity activities of zinc oxide nanoparticles screened by MTT assay. We have screened for one type of cancer cell line, viz., MCF-7 (breast cancer), zinc oxide obtained IC_{50} values in the range of 45- 55µg/ml for MCF-7 cell line most of these nanoparticles are in cytotoxic activity. In some cases, the IC_{50} values are less than the first metal based drug cisplatin.

ACKNOWLEDGMENTS

The corresponding author is greatful to UGC, New Delhi for supporting through fellowship (JRF&SRF) and Department of Engineering chemistry , AUCE, Andhra University, Visakhapatnam for providing general lab facilities. The author is also thankful to Prof.B.Venkateswara Rao for his valuable and constant support.

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