GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES BY *CELOSIA ARGENTEA* AND ITS CHARACTERIZATION

J. VAISHNAV[a], V. SUBHA[a], S. KIRUBANANDAN[a], M. ARULMOZHI[b], S. RENGANATHAN[a*]

[a]*Department of Biotechnology, A.C.Tech., Anna University, Chennai-600 025, India*

[b]*Department of Petrochemical technology, BIT Campus, Anna University, Tiruchirappalli – 620024, India*

The nanoparticles present a range of characterization challenges that affect the detailed and appropriate characterization of nanoparticles. Thus understanding the problems faced during characterization of nanoparticles and selecting a suitable characterization technique are of utmost importance. Specifically, nanoparticle characterization is performed to assess the surface area and porosity, pore size, solubility, particle size distribution, aggregation, hydrated surface analysis, zeta potential, adsorption potential and shape, size of the interactive surface, crystallinity, fractal dimensions, orientation, and the intercalation and dispersion of nanoparticles and nanotubes in nanocomposite materials. Several techniques can be used to determine nanoparticle parameters, including ultraviolet- (UV-) visible spectroscopy, scanning electron microscopy (SEM), dynamic light scattering (DLS), thermogravimetric analysis (TGA), powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR). The green synthesized zinc nanoparticles act as a drug carrier for developing drug delivery systems.

(Received December 28, 2016; Accepted March 23, 2017)

Keywords: Zinc nanoparticles, Antimicrobial activity, Drug Carrier and *Celosia argentea*

1. Introduction

Zinc oxide (ZnO) nanopowders are available as powders and dispersions. These nanoparticles exhibit antibacterial, anti-corrosive, antifungal and UV filtering properties. Some of the synonyms of zinc oxide nanoparticles are oxydatum, since oxicum, permanent white, ketozinc and oxozinc. Few features of ZnO nanoparticles are given below, Large surface to volume ratio, High UV absorption, Anti-bacterial, Anti-fungal, Anti-corrosive and UV filtering properties, Anti-oxidant activity. Reddy *et al* ., reported that the toxicity of ZnO nanoparticles to gram-negative (*Escherichia coli),* gram-positive *(Staphylococcus aureus)* bacterial systems, and primary human immune cells. Zinc oxide nanoparticles are presently under intensive study for applications in the field of optical devices, sensors, catalysis, biotechnology, DNA labeling, drug delivery, medical, chemical and biological sensors and as a catalyst. Nanosized ZnO has been used in sunscreen coatings and paints because of its high UV absorption efficiency and transparency to visible light(Fan Z. and Lu J.G,2005). As biomolecules are very sensitive to the solution pH and temperature, there is a general need to synthesize metal oxide semiconducting nanoparticles for possible applications in biological sensing, biological labeling, drug and gene delivery, and nanomedicines. In particular, due to their easy fabrication, environmentally friendly nature, and non-toxic synthesis route, ZnO nanoparticles can provide a better option for various biological applications. However, water solubility and biocompatibility of ZnO nanoparticles are the main requisites for biological applications.

ZnO nanostructures have a great advantage to apply to a catalytic reaction process due to their large surface area and high catalytic activity [Huang *et al*. 2006]. One-dimensional nanostructures exhibit interesting electronic and optical properties due to their low dimensionality

*Corresponding author: rengsaha@gmail.com

leading to quantum confinement effects [Baruah, S. and Dutta, J. 2009]. Cancer cells frequently contain a high concentration of anionic phospholipids on their outer membrane and large membrane potentials [Abercrombie M and Ambrose EJ. 1962; Bockris JOM and Habib MA. 1982 and Papo N *et al* ., 2003], interactions with positively charged ZnO nanoparticles are expected to be driven by electrostatic interactions, thereby promoting cellular uptake, phagocytosis, and ultimate cytotoxicity. *Celosia argentea* is a growing **s**hrub of the genus Celosia and commonly known as plumed cockscomb, or the silver cock's comb, is a herbaceous plant of tropical origin. It is known for its very bright colors. In India and China, it is known as a troublesome weed. It is an annual plant and also used as an ornamental plant. It can grow in mediterranean, desert, subtropical, temperate or tropic climate. The leaves are oval, alternate, and linear to lanceolate and can be green, red or purple or something together in color. Flowers are small grown in groups on inflorescence stalk in a shape of a cone, flower color can be: red, yellow, orange, pink, purple, white. It is used as an ornamental flower and its Leaves and the flowers of this plant are edible**.** Leaves are eaten as a vegetable; a good source of protein and carbohydrate. Considered an excellent pot-herb and a slightly bitter spinach alternative, rich in protein and vitamins. In Indian folk medicine, used for diabetes. Seeds traditionally used for the treatment of jaundice, diarrhea, gonorrhea, wounds and fever. In Sri Lanka, leaves used for inflammations, fever, and itching. Seeds used for fever and mouth sores. In China, flowers, and seeds used in the treatment of gastroenteritis and leucorrhea. In the Antilles, decoction of flowers used for phthisis. The curative properties of medicinal plants are due to the presence of various complex chemical substances of different composition which occur as secondary metabolites. They are grouped as alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrate and essential oils. The leaves are used for the treatment of inflammations, fever, and itching. The seeds are bitter, useful in blood diseases, mouth sores. They are an efficacious remedy in diarrhea. The plant was investigated for anti-inflammatory, antipyretic, anti-diabetic, antibacterial and diuretic properties. Bioactive compounds found to be present in the leaves includes: Alkaloids, Flavonoids, Phenols, Terpenoids, Starch, Cellulose, etc.

2. Methods

Preparation of Aqueous leaf Extract of *Celosia argentea*

5 gm of Leaf powder in 100 ml sterile water is heated using magnetic stirrer at 60˚C and 700rpm for 30 minutes and left on overnight shaking. This Solution is filtered using Whatman Filter paper and filtrate is collected and stored at 4˚C for further use.

Fig. 1. Preparation of leaf powder

Synthesis of Zinc oxide nanoparticles

100ml of 100mM Zinc Sulphate heptahydrate solution is prepared and kept on a magnetic stirrer set at 60˚C and 750 rpm.15 ml of leaf extract is added in a dropwise manner and the color change is observed. pH is checked and adjusted to 12 by the addition of a 1M solution of NaOH. Observation of white cloudy appearance marks the formation of ZnO nanoparticles formation. The solution is left for two hours in same condition. Overnight incubation at room temperature. Centrifugation at 5000 rpm and 20 minutes, the White pellet is collected and dried in an oven at 150˚C. White dried powder is obtained, which was collected for further use.

Plant Constituent	Test	Observance
Alkaloids	Wagner's test (extract with 1.27gm of Iodine & 2gm of KI in 100 ml of water)	Reddish brown color
Flavonoids	2ml of extract + few drops of 20% NaOH	The intense yellow color which becomes colorless upon addition of dil.HCl
Terpenoids	Salkowki's test (1ml leaf extract + 0.5 ml $CHCl_3$)	Color lightens, followed by Reddish brown ppt. on the addition of few drops of conc. H_2SO_4
Carbohydrates	Color lightens, followed by Reddish brown ppt. on the addition of few drops of conc. H_2SO_4	Formation of red or dull violet color at the interphase of two layers.
Saponins	Shaken well with Leaf extract + water	Observation of stable froth for 15 minutes
Phenols	Extract with 5% aq. $FeCl_3$	Formation of deep blue or black color

Anti-oxidant analysis

An antioxidant is a biomolecule that reduces the oxidation of other molecules. Oxidation is a biochemical reaction that can produce enormous free radicals, leading to chain reactions that may result in cell damage. Antioxidants such as thiols or ascorbic acid (vitamin C) etc., could terminate these chain reactions. To test the Anti-oxidant activity, DPPH assay was used, which can be performed on the plant extract as well as on synthesized nanoparticles. 50 mg/ml of methanolic leaf extract was used for the analysis. Ascorbic acid solution (50 mg/ml) is taken as standard. 100µl, 70 µl, 40 µl and 10 µl of the above-prepared stock solution is taken in different test tubes and the volume is made up to 100 µl in all the test tubes using methanol. DPPH solution-0.24mg/ml in methanol was freshly prepared, methanol was used as a blank and DPPH with methanol used as a control. Sample 10ul sample of different concentration with 190ul of prepared DPPH solution was used as testing samples. Absorbance is measured after 30 minutes of incubation at 517nm. Absorbance is denoted by Ab. Percentage of Inhibition could be calculated by following equation; [(Control sample(Ab)-test sample(Ab)) / control sample(Ab) x 100].

Antimicrobial studies

Antimicrobial studies of synthesized nanoparticles were studied on three bacterial strains viz. *E. coli*, *Salmonella*, *Acetobacter* by using disc diffusion method as described in following steps: **Preparation of Inoculum:** Nutrient broth and L.B broth were prepared, autoclaved and inoculated from mother culture inside laminar air flow chamber for inoculation preparation.

Preparation of Different concentration of Zinc oxide nanoparticles:

ZnO Nanoparticles are taken for the antibacterial activity at different concentration as follows; 50mg/ml - 100%, 37.5mg/ml- 75%, 25mg/ml- 50%, 12.5mg/ml- 25%,

Preparation of plates

Three L.B. agar plates were prepared and inoculated with *E.coli*, *Acetobacter aceti*, *Salmonella typhimurium* by spread plate method. Sterilized Discs from Whatman filter paper were used to carry nanoparticle preparation and kept on plates on four different quadrants of plates for four different concentrations (100%, 75%, 50%, 25%) to study the antimicrobial effect of different concentration of zinc oxide nanoparticles. These plates are incubated at 37˚C for overnight and checked next morning.

Drug Delivery Studies:

I X PBS with pH 7.4 was used as an *invitro* drug delivery medium equivalent to body fluid- blood and egg membrane was chosen as equivalent to biological membrane. Metronidazole benzoate drug profile was checked. Gelatin used as the binding polymer.

Preparation of Egg membrane:

An egg is kept immersed in diluted Acetic acid for overnight. The outer shell dissolves and inner membrane is taken out carefully.

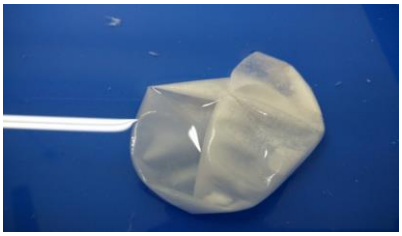

Fig. 2. Egg Membrane

Loading of Drug onto nanoparticles

10 mg of drug is dissolved in 2 ml of methanol and 100 mg of freshly prepared nanoparticles are added to it and left for overnight incubation. Next day, it is centrifuged at 6000 Rpm for 15 minutes. Pellet is collected and dissolved in a gel made of gelatin. The supernatant is kept for UV –Vis analysis to test drug loading capacity of nanoparticles.

Packing of drug-NP-gel inside egg membrane

Gel containing drug and nanoparticles is packed inside egg-membrane as shown in figure as follows

Fig: 3 Egg membrane containing Drug-NP-gel

Setting up of apparatus for drug-release

The egg-membrane containing drug is tied using thread and hanged into a beaker containing 200 ml of PBS set at constant slow stirring as shown in the figure. 2 ml of this PBS from above set up is taken out and O.D was taken at 232 nm at 0 min,15 min., 30 min. 45 min. 60 min. 12 ours, 24 hours, 42 hours, etc. Every time 2 ml of PBS was taken out, it is replaced by adding the same amount of freshly prepared PBS to the setup. λ max of the drug was found to be 241 nm by taking UV-Vis scan for 200-500 nm. So, all O.D were taken at 241 nm

Fig. 4. Apparatus set-up for drug release study

A similar arrangement is set up for the study of drug release without the presence of nanoparticles which is taken as standard and to study the role of nanoparticles in sustained drug-delivery. The difference in the rate of drug release of the two systems can be attributed to the role of ZnO nanoparticles in sustained drug release.

3. Results and discussion

Characterization of synthesized Zinc oxide nanoparticle

UV-Vis Absorbance

UV-Vis spectrophotometric analysis of synthesized ZnO nanoparticles is shown in the figure. An absorbance peak at 351 nm indicates the presence of ZnO nanoparticles, which is in accordance of UV absorbance result shown by previous studies, Akl M. Awwad *et al.*, 2014 obtained peak at 377nm and Sivakumar *et al.* , 2014 work has shown absorption peak at 325 nm for Zinc oxide nanoparticles.

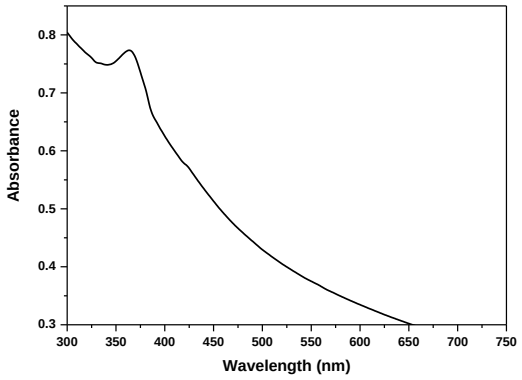

Fig. 5: UV-Vis Analysis of synthesized ZnO nanoparticles

X-Ray Diffraction:

Every Crystalline Substance has a characteristic atomic structure, it will diffract x-ray in a characteristic unique diffraction pattern. XRD result obtained for synthesized zinc oxide nanoparticle is shown as:

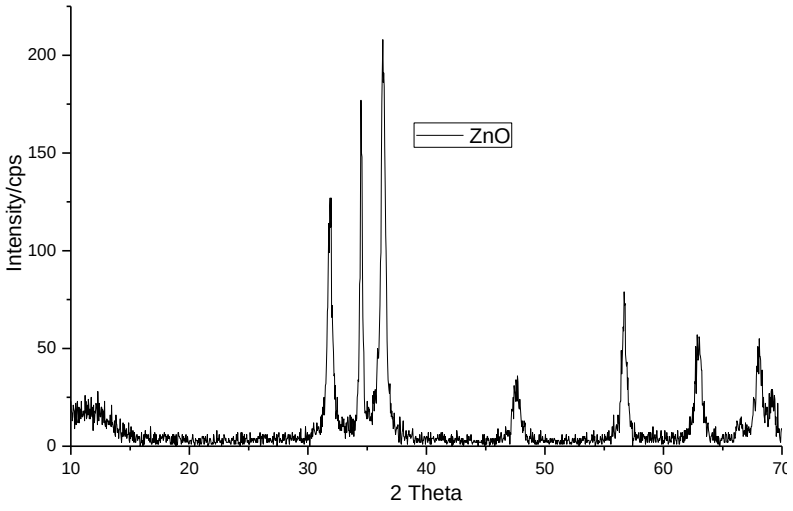

Fig. 6: XRD pattern for synthesized ZnO NPs

The obtained peaks for this sample are indexed as 31.88 (100), 34.50(002), 36.33 (101), 47.69(102), 56.70 (110), 63.03(103), 68.10(112), 69.13 (201). Similar X-Ray diffraction patterns are obtained by earlier studies also for green synthesis of Zinc oxide nanoparticles by Harish Kumar and Renu Rani(2013), S.R. Senthilkumar, T. Sivakumar (2014), Sangeetha 2013). All diffraction peaks of sample correspond to the characteristic hexagonal wurtzite structure of zinc oxide nanoparticles. The average particle size (D) of synthesized nanoparticles was 22 nm which was calculated using the well known Debye-Scherrer equation, $D = (0.9\ \lambda)/(\beta \cos \Theta)$. Where, λ= wavelength of X-ray, β=Full width at half maximum (in radians), Θ= Bragg's diffraction angle, Peak broadening shows the smaller size of nanoparticles. X-Ray Diffraction result obtained with synthesized ZnO nanoparticles confirms its presence and reveals about structure and size information as follows, Synthesized ZnO NPs have characteristic hexagonal wurtzite structure. The average size of crystallite was 22nm.

EDX (Energy dispersive X-Ray diffraction)

Synthesized ZnO nanoparticles were given for EDX analysis to know about its elemental composition. Figure 5.3 illustrates the obtained result which confirms the presence of Zinc and oxygen signals and hence the presence of Zinc oxide nanoparticles. The result obtained is also in agreement with earlier studies (Anand Raj and Jayalakshmi, 2015, Nagarjan and Kuppusamy, 2013).

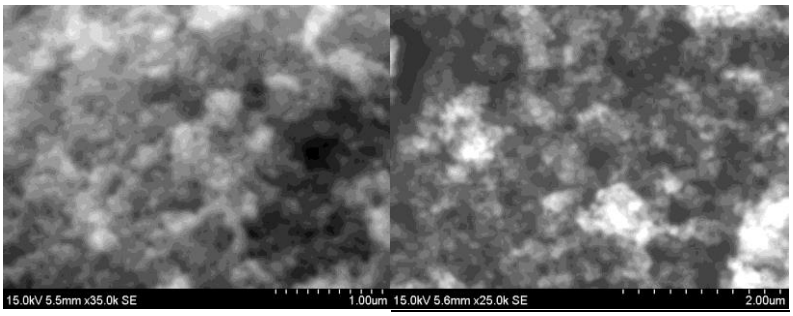

Fig. 7 SEM image and EDX Result of synthesized ZnO nanoparticles

SEM (Scanning Electron Microscope)

SEM analysis of synthesized ZnO nanoparticles revealed about its surface morphology and to estimate obtained structural shape. From SEM images it is interpreted that shape of synthesized zinc oxide nanoparticles is spherical. The average size of synthesized nanoparticles was 25 nm.

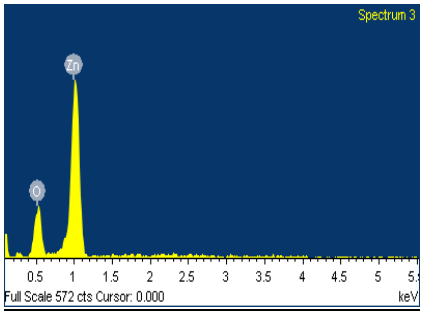

Fig. 8. EDX Spectrum of Zinc Oxide nanoparticles.

FT-IR Spectroscopy

FTIR of synthesized ZnO NPs and aq. Leaf extract was obtained and studied which ranges from 400 to 4000nm. FTIR helps to identify possible biomolecules present in leaf extract which acted as reducing agent for synthesizing Zinc oxide nanoparticles and responsible for their capping

and stabilization. Graphs obtained for FTIR are shown in above figure. The bands of biosynthesized ZnO NPs were seen at 509cm^{-1}, 1116 cm^{-1}, 1430cm^{-1}, 2362cm^{-1}, 2991 cm^{-1}, 3409 cm^{-1} while those for Celosia argentea leaf extract were obtained at 676, 1414, 2085, 2352, 2544, 2922, 3428 cm^{-1}. The bands of FTIR of leaf extract at 3428, 2922 corresponds to O-H (belonging to alcohols, phenols) and C-H (belonging to alkanes) stretching respectively. The band at 1414 corresponds to C-C stretch of aromatics. The band at 676 cm^{-1} corresponds to N-H wagging of 1˚ or 2˚ amines. The band at 1116 corresponds to C-H stretch belonging to alcohols, carboxylic acids, esters or ethers. The FTIR band of ZnO NPs at 1116 cm^{-1} indicates Zn-O bond stretching. While that at 509 cm^{-1}, 1430 cm^{-1}, 2991cm^{-1}, 3409 cm^{-1} suggests the presence of biomolecules present in leaf extract on ZnO NPs which acted as reducing agent and also acted as capping and stabilizing agents. The presence of FT-IR peaks for ZnO nanoparticles and other metal oxides are well supported by previous literature (Nagarjan and Kuppusamy, 2013; Raj and Jayalakshmi, 2015).

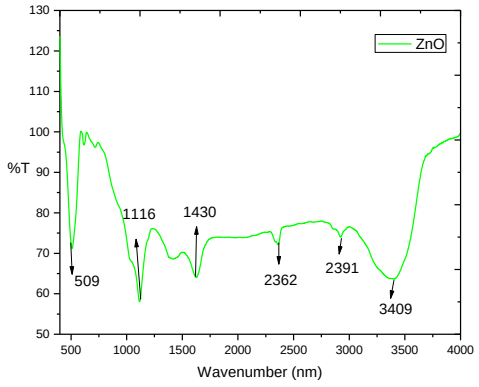

Fig. 9. (a) FTIR Spectrum of synthesized ZnO NPs

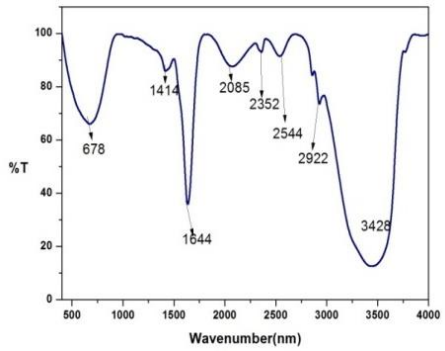

Fig. 9: (b) FTIR Spectrum of Leaf extract of Celosia argentea

Particle size analysis

There are different techniques for the analysis of particle size and its distribution. Among them, Dynamic light scattering is widely used for the size distribution of nanoparticles. Dynamic light scattering is a non-invasive technique for measuring the size of particles and molecules in suspension.

PRINCIPLE: Particle in suspension undergo Brownian motion, induced by bombardment due to solvent molecules moving due to their thermal energy. If particles are illuminated with a laser, the intensity of scattered light fluctuates at a rate that is dependent on the size of particles (as smaller particles are kicked further by solvent molecules and move more rapidly).

Analysis of these intensity fluctuation yields velocity of the Brownian movements and hence the particles size using stoke's eintein's relationship:

Table 5.1: DLS Result for synthesized ZnO NPs

	SIZE (d.nm)	**% Intensity**
Z average (d. nm) = 571.2 nm	Peak 1= 165.6 nm	22.5
PDI = 0.577	Peak 2= 581.9 nm	77.5

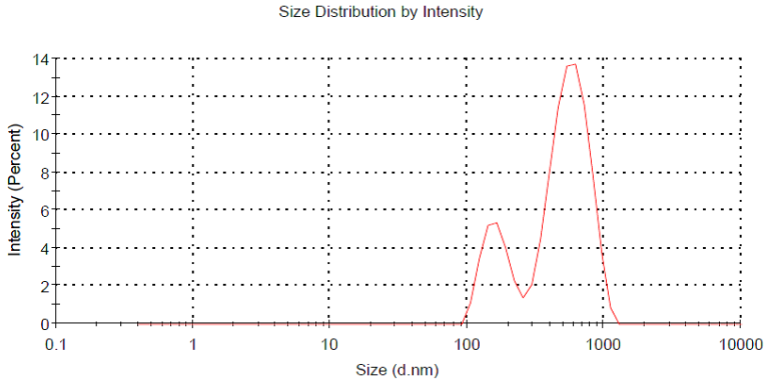

Fig. 10: DLS graph for synthesized ZnO nanoparticles

The two peaks obtained for DLS of synthesized ZnO NPs gives two average sizes viz. 165.6 nm and 581.9 nm which might be resulted from the agglomeration of particles.

Phytochemical analysis of leaf extract

The phytochemical analysis performed on aqueous leaf extract of Celosia argentea has yielded following results:

Table 5.2 Result of Phytochemical analysis of aq. leaf extract of Celosia argentea

S.No.	**Phytochemical constituents**	**Result**
1	Alkaloids	Present
2	Flavonoids	Present
3	Carbohydrates	Present
4	Saponins	Present
5	Terpenoids	Present
6	Phenols	Present

Table 5.3 Result of anti-oxidant activity of Celosia argentea leaf extract

S. No.	**Standard solution**	**% Inhibition**	**Sample solution**	**%Inhibition**
1	5 mg/ml	96.66 %	5 mg/ml	64.90
2	2.5 mg/ml	96.46%	2.5 mg/ml	64.15
3	2 mg/ml	96.43 %	2 mg/ml	39.71%
4	0.5 mg/ml	96.40%	0.5 mg/ml	11.47%

Antimicrobial activity of synthesized Zinc oxide nanoparticles

The anti-microbial activity of synthesized Zinc oxide nanoparticles is studied against three microbial strains- *E.coli, Salmonella, Acetobacter* . The zone of Inhibition obtained at a different concentration of zinc oxide nanoparticles are shown in the table given below:

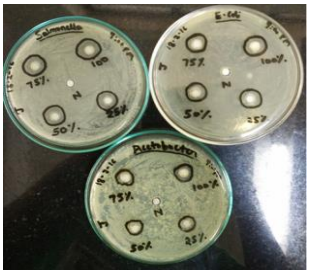

Fig. 11: Antimicrobial Potential of Zinc nanoparticles

Microbial strains	Diameter of Zone of inhibition observed at different dilutions of ZnO NPs (in mm)			
	25%	50 %	75 %	100 %
Acetobacter	9	10	11	12
E.coli	11	11	12	13
Salmonella	11	12	15	17

Application of synthesized ZnO nanoparticles in drug delivery

FTIR of ZnO Nanoparticle, Drug, gelatin, ZnO-Drug and ZnO-Drug-Gelatin are shown in the following figure**:**

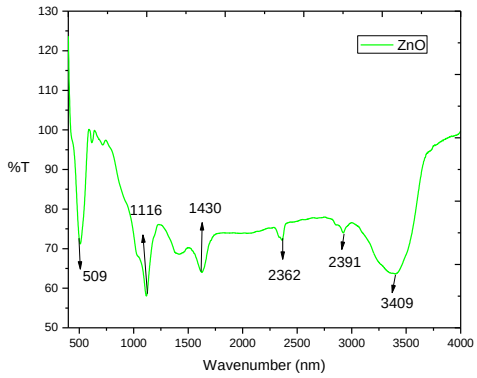

Fig. 12 (a): FTIR Spectrum of Zinc nanoparticles

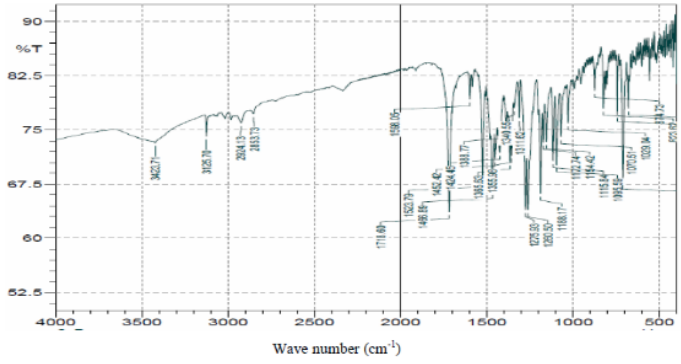

Fig. 12 (b): FTIR Spectrum of Drug- Metronidazole benzoate

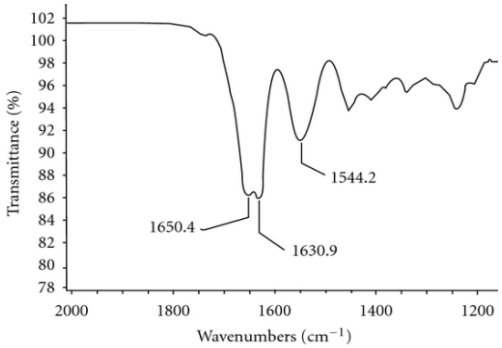

Fig. 12 (C) FTIR spectrum of Gelatin

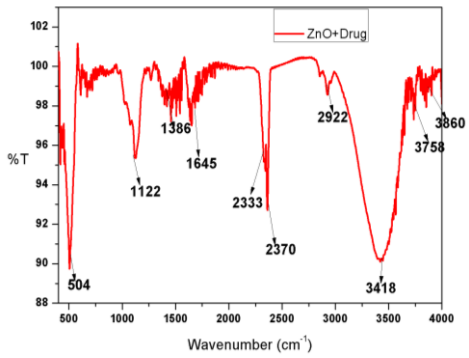

Fig. 12 (D): FTIR Spectrum of ZnO + Drug

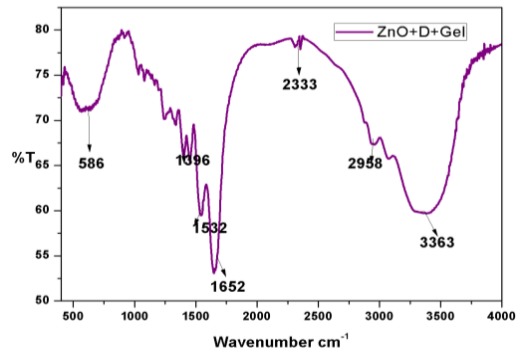

Fig. 12 (e): FTIR analysis of (ZnO+Drug+Gelatin)

The FTIR analysis of ZnO-Drug shows that both are interacting which suggest that there might be the complex formation of these two, while by analyzing FTIR of Gelatin and that of ZnO-Drug-Gelatin and others, it might be inferred that Gelatin is forming a monolayer over ZnO-Drug complex.

A standard UV absorbance curve of Metronidazole benzoate for concentration 1µg/ml to 10µg/ml is plotted as given in the figure below:

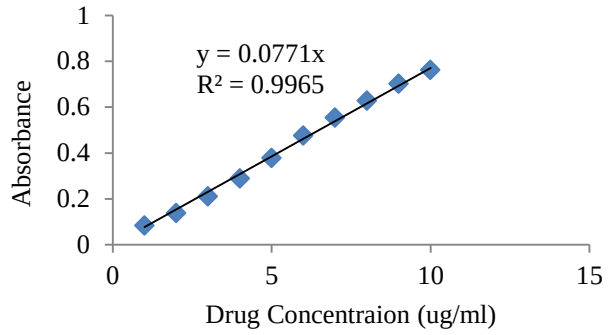

Fig. 13 (a) Standard curve for UV absorbance of Metronidazole benzoate

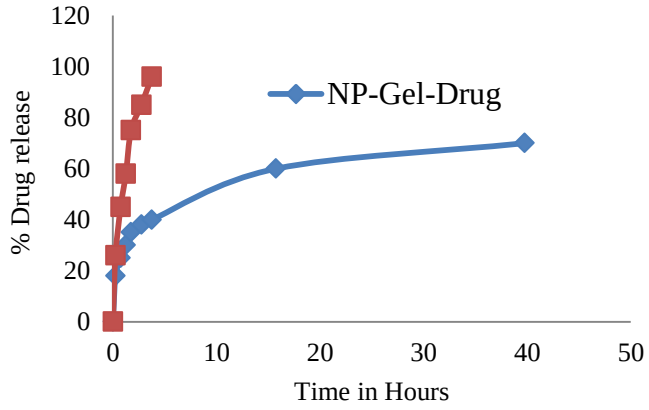

Fig. 13 (b) Graph showing percentage drug release with time

Percentage Drug release is calculated by using following formula:

% of drug release = (Amount of drug released from NP-gel-Drug/ Total amount of Drug-loaded) x 100

Till 40 hours, 70 % of drug from setup having drug-nanoparticle-gel is found to be released while the nearly whole of the drug from another set having drug-gel (but not nanoparticle) was observed to be released in 5 hours.

4. Conclusions

The successful formation of ZnO NPs using leaf extract of *Celosia argentea* presents a new method of synthesis of ZnO nanoparticles which has merits over other reported methods as this is a low-cost, unreported and simple procedure. UV-Vis Spectroscopy results- An observed absorbance peak at 351nm confirms the presence of ZnO nanoparticles. While XRD, SEM, EDX, DLS, FTIR results have are also in accordance with previous literature serving as a confirmatory evidence for the synthesis of ZnO NPs using aq. leaf extract of *Celosia argentea*. . Phytochemical analysis of aq. leaf Extract of *C.argentea* has shown the presence of alkaloid, flavanoids, carbohydrate, saponins, which might be responsible for the reducing property for the formation of Zinc oxide nanoparticles and also acting as capping and stabilizing agent for the synthesized nanoparticles. The leaf extract of *Celosia argentea* was also found to exhibit good anti-oxidant potential using DPPH assay, which can have medicinal use and which in combination with ZnO nanoparticle can further enhance the anti-oxidant potential of the two as already present literature reveals that ZnO nanoparticles also exhibit anti-oxidant potential.

Antimicrobial activity- Synthesized ZnO nanoparticles have shown antibacterial activity against *E.coli, Salmonella, Acetobacter* with different efficacies. They exhibit comparatively good

activity against *Salmonella*, while moderate activity against *E.coli* and *Acetobacter*. The role of synthesized Zinc oxide nanoparticles in sustained drug release by using drug metronidazole benzoate was studied by observing its diffusion through egg membrane (biological membrane) in I X PBS media. Results revealed that presence of ZnO nanoparticles with the drug have much effect on its sustained release as there was a significant time lag between the complete release of drug-gel through that biological membrane and drug-NPs-gel through the similar biological membrane.

Bio-synthesized ZnO NPs may be used for sustained drug release for various drugs. It can open up new avenues and play a significant role in medicinal biology apart from its already well-established uses in cosmetic, food, textile, pharma industry. Already existing literature suggests that Zinc oxide nanoparticles have anti-inflammatory, anti-cancerous properties and other such properties which can be very significant for future research work and may come out with promising outcomes.

References

[1] S. Li, Y Shen, A Xie, X Yu, L Qui, L Zhang, Q. Zhang, Green Chem. **9**, 852 (2007)
[2] D Mandal, ME Bolander, D Mukhopadhyay, G Sarkar, P. Mukherjee, Appl Microbiol Biotechnol. **69,** 485 (2006).
[3] D Cui, H. Gao, Biotechnol Progr .**19,** 683 (2003).
[4] S. Iravani, Green Chem. **13,** 2638 (2011).
[5] O Collera Zuniga, FG Jimenez, RM. Gordillo, Food Chem . **90,** 109 (2005).
[6] BH Jagadeesh, TN Prabha, K. Srinivasan, Indian J Plant Physiol . **9,** 164 (2004).
[7] JL Gardea-Torresdey, E Gomez, JR Peralta-Videa, JG Parsons, H Troiani, M. Jose-Yacaman Langmuir . **19**, 1357 (2003)
[8] SS Shankar, A Ahmad, R Pasricha, M. Sastry, J Mater Chem. **13,** 1822 (2003).
[9] JL Gardea-Torresdey, KJ Tiemann, G Gamez, K Dokken, NE Pingitore. Adv Environ Res . **3,** 83 (1999).
[10] V Armendariz, I Herrera, JR Peralta-Videa, M Jose-Yacaman, H Troiani, P. Santiago, et al. J Nanopart Res **6,** 377 (2004).
[11] A Rai, A Singh, A Ahmad, M. Sastry, Langmuir . **22,** 736 (2006).
[12] Y Park, YN Hong, A Weyers, YS Kim, RJ. Linhardt, IET Nanobiotechnol **5,** 69 (2011).
[13] RG Haverkamp, DV Agterveld, AT. Marshall, J Nanopart Res **9,** 697 (2007).
[14] MK. Brakke, Density gradient centrifugation and its application to plant viruses. Advances in Virus Research New York: Academic Press;1961.
[15] VL Jimenez, MC Leopold, C Mazzitelli, JW Jorgenson, RW. Murray, Anal Chem . **75**, 199 (2003).
[16] M Hanauer, S Pierrat, I Zins, A Lotz, C. Sonnichsen, Nano Lett .**7,** 2881 (2007).
[17] B Kowalczyk, I Lagzi, BA. Grzybowski, Curr Opin Colloid In . **16,** 135 (2011).
[18] A. G. Ingale, A. N. Chaudhari, Journal of Nanomedicine and Nanotechnology, **4(**2), 2013.
[19] G. B. Khomutov, S. P. Gubin, Materials Science and Engineering C, **22**(2), 141 (2002).
[20] Y. Choi, N.-H. Ho, C.-H. Tung, Angewandte Chemie, **46**(5), 707 (2007). View at Publisher ·
[21] V. Gupta, A. R. Gupta, V. Kant, Science International, **1(**5), 167 (2013).
[22] A. G. Ingale, A. N. Chaudhari, Journal of Nanomedicine and Nanotechnology, **4**(2), 2013.
[23] M.-C. Daniel, D. Astruc, Chemical Reviews, **104(**1), 293 (2004).
[24] P. V. Asharani, Y. L. Wu, Z. Gong, S. Valiyaveettil, Nanotechnology, **19**(25), Article ID 255102, 2008.
[25] M. Linga Rao, N. Savithramma, Journal of Pharmaceutical Sciences and Research, **3(**3), 1117 (2011).
[26] N. N. Devi, P. D. Shankar, W. Femina, T. Paramasivam, International Journal of Pharmaceutical Sciences Review and Research, **12**(1), 164 (2012).
[27] V. Gupta, A. R. Gupta, V. Kant, Science International, **1(**5), 167 (2013).
[28] K. Tiede, A. B. A. Boxall, S. P. Tear, J. Lewis, H. David, M. Hassellov, Food Additives and Contaminants A, **25**(7), 795 (2008).
[29] K. S. Prasad, D. Pathak, A. Patel et al., African Journal of Biotechnology,

10(41), 8122 (2011).
[30] D. Mubarak Ali, M. Sasikala, M. Gunasekaran, N. Thajuddin, Digest Journal of Nanomaterials and Biostructures, **6**(2), 385 (2011).
[31] S. L. Pal, U. Jana, P. K. Manna, G. P. Mohanta, R. Manavalan, Journal of Applied Pharmaceutical Science, **1(**6), 228 (2011).
[32] V. Gupta, A. R. Gupta, V. Kant, Science International, **1**(5), 167 (2013).
[33] M. Linga Rao and N. Savithramma, Journal of Pharmaceutical Sciences and Research, **3**(3), 1117 (2011).
[34] N. N. Devi, P. D. Shankar, W. Femina, T. Paramasivam, International Journal of Pharmaceutical Sciences Review and Research, **12**(1), 164 (2012).
[35] E. Suzuki, Journal of Microscopy, **208**(3), 153 (2002).
[36] K. Jores, W. Mehnert, M. Drechsler, H. Bunjes, C. Johann, K. Mäder, Journal of Controlled Release, **95(**2), 217 (2004). View at Publisher · View at Google Scholar · View at Scopus
[37] S. Prashanth, I. Menaka, R. Muthezhilan, N. K. Sharma, International Journal of Engineering Science and Technology, **3(**8), 6235 (2011). View at Google Scholar
[38] M. M. Priya, B. K. Selvi, J. A. J. Paul, Digest Journal of Nanomaterials and Biostructures, **6**(2), 869 (2011).
[39] R. P. S. Chauhan, C. Gupta, D. Prakash, International Journal of Bioassays, **1(**7), 6 (2012).
[40] V. Gupta, A. R. Gupta, V. Kant, Science International, **1**(5), 167 (2013).
[41] M. Faraji, Y. Yamini, M. Rezaee, Journal of the Iranian Chemical Society, **7**(1), 1 (2010).
[42] K. S. Prasad, D. Pathak, A. Patel et al., African Journal of Biotechnology, **10**(41), 8122 (2011).
[43] M. M. Priya, B. K. Selvi, J. A. J. Paul, Digest Journal of Nanomaterials and Biostructures, **6**(2), 869 (2011).