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Ficus Benjamina leaf extract Mediated Zinc oxide nanoparticle and its Anticancer Activity

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Abstract

Phyto assisted synthesis of metal oxide nanoparticles have been identified as key research area in nanotechnology due to its wide applicability of the nanoparticles in various biomedical fields. The present work explores the biosynthesis of Zinc oxide nanoparticles (ZnO-NPs) using *Cassia fistula* leaf extract as reducing agent. The synthesized ZnO-NPs was characterized by UV–Vis Spectroscopy (UV–Vis), Scanning Electron Microscopy (SEM), Energy Dispersive X-ray spectroscopy (EDX), Fourier Transform Infrared spectroscopy (FT-IR), X-ray diffraction (XRD) studies, Transmission Electron Microscopy (TEM) and Selected area electron diffraction (SAED) studies. Biosynthesised ZnO-NPs was found to have Wurtzite hexagonal structure with particle size distributed in the range of 50-200 nm as confirmed by TEM studies. The *invitro* cytotoxicity studies of biosynthesised ZnO-NPs against DLA cells reveals better antitumor activity of 92% inhibition with concentration of 200 µg/ml of ZnO-NP's and as the concentration of the ZnO-NPs is increased The anticancer efficiency as well increased.

I. KEYWORDS: NANOSTRUCTURED MATERIALS, CHARACTERISATION, ANTICANCER ACTIVITY

II. INTRODUCTION

In the current research area of material science, nanoparticles are found to be main building blocks. Various physical properties like size, shape and surface morphology of the synthesised nano sized particles plays a vital role in various fields of its application [1]. Among various metal oxides, Zinc oxide is an n-type semiconductor and found to have band gap of 3.3 eV [2] and excitation binding energy of 60 meV [3], Because of these characteristic properties ZnO finds application in biomedical applications [4-6]. Various strategies have been applied for ZnO-NPs synthesis which includes physical and chemical methods [7-8]. Physical methods include pulsed layer deposition; Thermal deposition etc. [9] and chemical methods such as spray pyrolysis, wet chemical, chemical micro emulsion and electro deposition are widely used

[10]. But the reported physical and chemical methods are not eco-friendly and associated with several disadvantages for biomedical application of synthesised nanoparticles. Hence there is a urge to develop environmentally beningn methods for nanoparticle synthesis which includes usage of various medicinal plants [11]. *Ficus benjamina* contains essential phyto constituents responsible for reduction of metal. *Cassia fistula* finds use as a fodder for livestock, eco-friendly manure and also for biomass production. Zinc oxide nanoparticles are reported to possess antifungal activity, ant bactericidal activity and anticancer activity [13-17].

III. Materials and Methods

A. Preparation of Ficus benjamina Leaf extract

The fresh leaves of *Ficus benjamina* were collected from Karpagam university campus, Coimbatore and used for the preparation of leaf extract. The leaves were first washed with triple distilled water to remove dirt and cut into small pieces. The freshly cut leaf pieces (50g) were added to 500 ml deionized water and boiled for 5 minutes. The resultant pale yellow solution was cooled to room temperature (approximately 28^{o}C). Then the solution was filtered through Whatman filter paper and the resultant filtrate was refrigerated for further use.

B. Biosynthesis of Zinc Oxide Nanoparticles

The Zinc nitrate was purchased from S.D.Fine chemicals (Bangalore, India). The freshly prepared leaf extract and 0.01 M Zinc nitrate solution in the ratio (1:4) was mixed in a conical flask and stirred at room temperature for 4 hours. The resultant reaction mass was dried in hot air oven at 100°C. The yellow coloured paste thus obtained is finally calcined at 400°C to get fine crystals and stored in air tight containers for further characterisation.

C. Analysis of Ficus benjamina mediated ZnO-NPs

Biosynthesised ZnO-NPs was confirmed by UV-Vis spectra and the spectrum was recorded using UV-1601 Shimadzu spectrophotometer. FT-IR spectra were recorded for biosynthesised ZnO-NPs with frequency ranging from 400 to 4000cm^{-1} in KBr matrix using BRUKER- FTIR- TENSOR-27 spectrophotometer instrument. The surface morphology of the nanoparticles was examined by JEOL JSM 6390 Scanning electron microscope (SEM) instrument operated at an accelerating voltage at 10kV. For energy dispersive X-ray (EDX) analysis, the particles were dried on a carbon coated copper grid and performed on SEM instrument equipped with thermo EDX attachment. The synthesized ZnO-NPs were characterized by HR-TEM techniques with the help of JEOL JEM 2100 instrument. The size of ZnO-NPs in HR-TEM image was measured by using the image J software. *Leucaena* mediated ZnO-NPs was analyzed with XRD analysis by X-ray diffractometer (PANanalytical X-Pert PRO) operated at 40 kV and 30 mA. The scanning was done in the region of 2θ between 20° - 90° at 0.05°/Min and the time constant was 2s.

D. In vitro cytotoxicity assay

The *invitro* cytotoxicity assay of the *cassia* mediated ZnO-NPs was done against DLA cells. The tumour bearing cells isolated from peritoneal cavity of tumour bearing mice was washed thrice with PBS or normal saline. The anticancer activity of the biosynthesised ZnO-NPs was determined based on trypan blue exclusion method by adding viable tumour cell suspension of concentration (1×10^{6} cells in 0.1 ml) to tubes containing different concentrations of Zinc oxide nanoparticles ranging from 10-200µg/ml and then the volume of the tubes was adjusted up to 1 ml using phosphate buffered saline (PBS) along with the control tube containing only viable tumour cell suspension. After addition the assay mixture was incubated at 37°C for 3 hours. Then 0.1 ml of 1% trypan blue dye solution was added to the tubes containing cell suspension and kept for 2-3 minutes and loaded on a haemocytometer. Dead cells take up the blue colour of trypan blue while live cells do not take up the dye. The number of stained and unstained cells was separately counted.

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$$\% \text{ of cytotoxicity} = \frac{\text{Number of dead cells}}{\text{Number of live cells} + \text{Number of dead cells}} \times 100$$

IV. Results and Discussion

A. Characterisation of biosynthesised ZnO-NPs

UV–Vis spectral analysis confirms the formation of ZnO-NPs Fig.1 shows that the maximum absorbance of the cassia leaf extract falls in the range of 280-300 nm, which is due to the presence of complex organic molecules carrying different charge centres. Biosynthesised ZnO-NPs has the absorption peak at 370 nm, which is in agreement with the reported studies (absorption peak at 374 nm) using various plant extracts, which confirms the presence of ZnO nanoparticles.FT-IR spectra of the Cassia leaf extract and biosynthesised Zinc oxide nanoparticles were shown in Fig.2a & b. FT-IR spectra *depicted* several absorption bands at 3313, 2922, 2358, 1612, 1446, 1066, 898 and 596cm^{-1} along with other small bands. These bands indicative of –OH and/or –NH, C-H bending modes in the hydrocarbon chains, C=O group, C=C stretching, C-OH stretching vibrations, C-O stretching and C-H bend of alkynes. FT-IR spectra of Zinc oxide nanoparticles reveals a broad peak located at 3444 cm^{-1} reveals the stretching vibrations of hydroxyl groups (-OH) The peak at 1793 cm^{-1} corresponds to C= O stretching vibrations of carbonyl group and peak at 1745 cm^{-1} can be assigned to C-O-C stretching of polysaccharides present in the leaf extract. The peaks at 1164 cm^{-1} and 1382 cm^{-1} may be due to C=N stretching vibrations of amide bonds. Moreover absorption peak at 1018 cm^{-1} corresponds to C-O-C stretching vibrations. The peak at 713 cm^{-1} is due to presence of R-CH group and peak at 669 cm^{-1} indicates the stretching vibrations of ZnO nanoparticles consistent with the reported data . the characteristic peak at 439 cm^{-1} could be attributed to the metal oxygen [Zn-O] bond.Fig.3 SEM analysis shows that synthesised nanoparticles have spherical shape. The EDX spectrum of biosynthesised ZnO-NPs as shown in Fig 3b confirms the elemental composition of ZnO nanoparticles having 49 % Zn and 51 % O and also reveals the high purity of synthesised ZnO-NPs without any other impurities.HR-TEM Fig.4 shows biosynthesised ZnO-NPs has the particle size in the range of 50-200 nm. SAED pattern confirms the crystalline nature of the prepared ZnO-NPs.XRD analysis shown in Fig.5 explains the obtained diffraction peaks at 31.72, 34.38, 36.20, 47.48, 56.52, 62.81, 67.89 and 69.01 corresponds to miller indices of 100, 002, 101, 102, 110, 103, 112 and 201 planes. The results obtained were in good agreement with JCPDS file no 89-7102 [and synthesised ZnO-NPs was found to have high crystallinity and found to have hexagonal wurtzite structure.

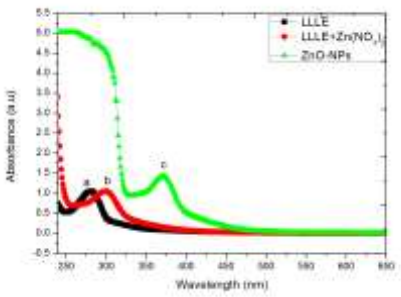

Fig. 1 UV-Visible spectrum of *Ficus benjamina* leaf extract (a), *Ficus benjamina* leaf extract with zinc nitrate (b) and ZnO-NPs (c)

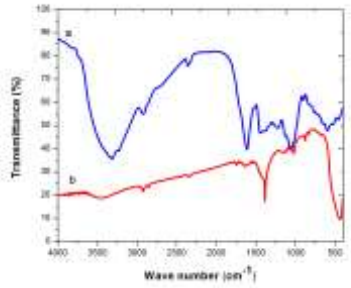

Fig. 2 FT-IR spectrum of *Ficus benjamina* (a) and ficus mediated ZnO-NPs (b)

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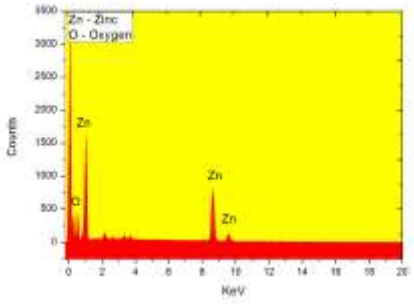


Fig.3. (a) SEM image of ZnO-NPs

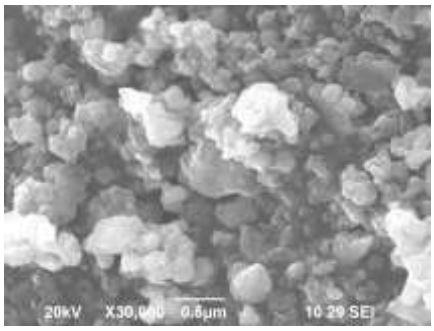


Fig.3. (b) EDX spectra of pure ZnO-NPs.

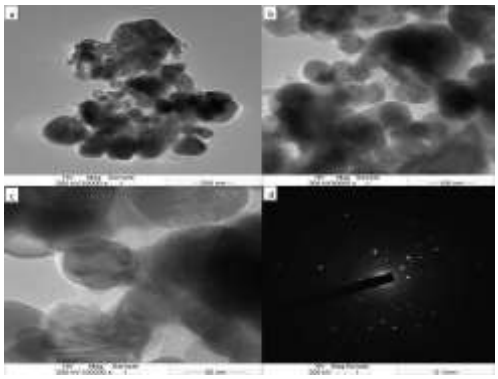
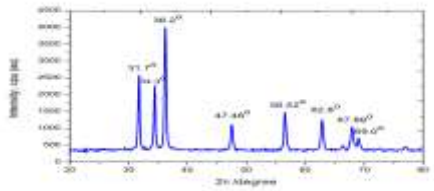


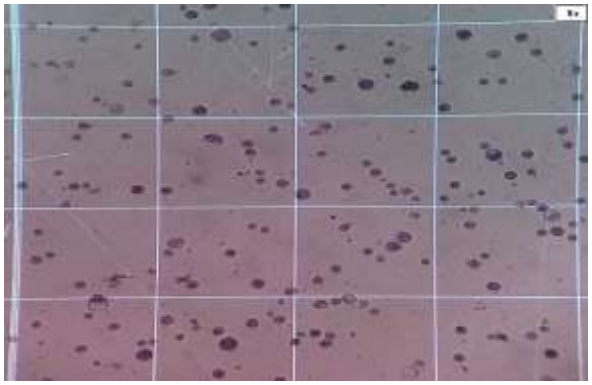
Fig.4.HR-TEM image of ZnO-NPs (a) 200nm (b) 100nm (c) 50nm (d) SAED patterns of ZnONPs

Fig.5. XRD patterns of *Ficus benjamina* mediated ZnO-NPs.



B. Short term invitro cytotoxicity against DLA cells

Literature reports reveals anticancer activity of various plant mediated ZnO-NPs. Trypan blue dye exclusion method is used to assess the cytotoxicity assay of biosynthesised ZnO-NPs. Viable cells which remained unstained by trypan blue are counted by haemocytometer. Cytotoxicity of ZnO-NPs synthesized using Cassia fistula leaf extract and aqueous zinc nitrate solution. The percentage inhibition of DLA cells increases in a dose dependent manner with varying concentration of ZnO-NPs from 10µg/ml to 200µg/ml. This study reveals that the maximum inhibition of DLA cells takes place at 200µg/ml as shown in the Fig.6b. Curcumin was used as a reference drug and the percentage cytotoxicity of as prepared ZnO-NPs was compared against the standard drug as shown in Table 1 (Fig.6a). This study provides evidence that Cassiae mediated ZnO-NPs show 92 % activity at 200 µg/ml as compared with the standard drug Curcumin [14].



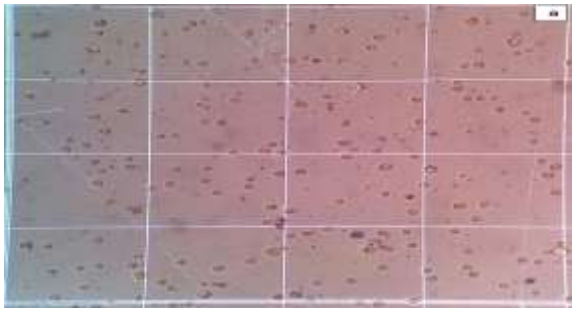


Fig.6. In vitro cytotoxicity of ZnO-NPs against DLA cells (100% live cells: (a) White transparent cells and 92% dead cells: (b) Dark opaque cells)

Table-1 Invitrocytotoxicity of ZnO-NPs

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Samples	Zinc oxide nanoparticle concentration/(µg-ml $^{-1}$)	Percentage cytotoxicity of DLA cell lines	Curcumin concentration/(µg-ml $^{-1}$)	Percentage cytotoxicity of DLA cell lines
1	10	21.54	10	93.66
2	20	31.46	20	96.19
3	50	43.10	50	98.07
4	100	76.32	100	100.00
5.	200	92.05	200	100.00

V. CONCLUSIONS

In the present work, we report the biosynthesis of ZnO from *Ficus benjamina* leaf extract. The biosynthesised ZnO-NPs has Wurtzite hexagonal structure with particle size in the range of 50-200 nm. The invitro cytotoxicity studies against DLA cells reveals dose dependent cytotoxicity with the increasing concentration of ZnO-NPs and concentration of 200 µg/ml of the biosynthesised ZnO-NPs shows 92% inhibition as compared with the standard drug curcumin.

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