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Green synthesis of zinc oxide nanoparticle using *Pentatropis capensis* and its anti-proliferative activity

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The ZnO nanoparticle synthesised by green synthesis method using aerial parts of *Pentatropis capensis* was characterised using UV-Vis spectroscopy, FTIR, SEM, and EDAX. The antiproliferative activity was tested against HepG2 and MCF-7 cell lines by MTT assay and the IC_{50} value was calculated.The absorption peak at 330 nm confirms the presence of ZnO nanoparticle. The FTIR spectra of synthesised ZnO nanoparticle confirm the various groups present in it. The SEM image reveals the size of ZnO nanoparticle, which ranged from 73.4-398.3 nm. The elemental composition of ZnO nanoparticles consists of zinc, carbon, oxygen, potassium, calcium, and chlorine which was determined in EDAX analysis. The IC_{50} value of anti-proliferative activity was found to be 23.277 µg/mL for HepG2 cell line and 12.926 µg/mL for the MCF-7 cell line. The green synthesised ZnO nanoparticle showed an effective anti-proliferative activity on both HepG2 and MCF cell line.

Keywords: Anti-proliferative, EDAX, FTIR, Green synthesis, SEM, UV-Vis, ZnO nanoparticle.
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Introduction

Green synthesis is a methodology that is used to synthesise nanoparticles by using natural resources and eco-friendly chemicals[1,2]. Though there are some physical and chemical techniques for nanomaterial synthesis, this method is preferred since it releases lesser pollutants comparatively[3,4]. Moreover, in chemical synthesis more frequently toxic chemical substances get adsorbed on the surface of the nanoparticles resulting in harmful effects to individuals when used in medical applications[5].

The need for nanoparticles arise due to their smaller size and a larger surface to volume ratio, and these properties help to play a remarkable role in the field of biotechnology, sensors, medical, catalysis, optical devices, DNA labelling, drug delivery[6].The higher surface to volume ratio contributes to increased interaction between the nanoparticle and the surrounding molecules[7-12]. One such nanoparticle is ZnO which has numerous applications in biomolecular detection, diagnostics, etc[8].

One of the natural sources used to synthesise zinc oxide nanoparticle is plants, which are economical, and moreover, it can be scaled up to larger scale without much difficulty of using toxic chemicals, high pressures and temperatures[9]. *Pentatropis capensis* is an ethnobotanical plant which has anti-inflammatory properties in its aqueous extracts. The above plant also played a significant role in the synthesis of silver nanoparticle[10]. The nanoparticle synthesised from *P. capensis* had appreciable anti-cancer activity[11]. But a limited number of works have been done on this particular plant.

Zinc oxide nanoparticle has gained significant importance in synthesis due to their advantages such as high catalytical and phytochemical activities and they possess effective anti-microbial and anti-fungal activity at a lower concentration and thereby plays a significant role in thin coating application[12, 13]. To the best of our knowledge, the leaf extract of *P. capensis* had been used for the first time as a reducing agent for the synthesis of ZnO nanoparticle in this present work. The structure, phase and morphology of the nanoparticle have been used to investigate the standard characterisation technique.

Materials and Methods

Collection and identification of of plant material

The medicinal plant *P. capensis* was collected during October 2015 from Erode district (Latitude: 11.3649089, Longitude: 77.7480554) Tamil Nadu (India). The plant sample was authenticated by Prof. (Dr) C. Livingstone, Former Professor and Head of the Department of Botany, Madras Christian College,

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Chennai and the voucher specimens were deposited in the herbarium of the institute.This plant is a twinning perennial climber found all over India. The aerial parts were washed with distilled water, and the plant material was shade dried for around 10 days.

Synthesis of zinc oxide nanoparticle

The shade dried aerial parts was ground using a blender and made into coarse powder. About 5 g of dry powder was mixed with 100 mL of deionised water and kept in magnetic stirrer for 20 mins. The extract was cooled to room temperature and filtered using filter paper. 70 mL of 0.2 M zinc acetate (90 % pure) solution was added to 30 mL of extract, and then it is homogeneously mixed. The mixture was concentrated by keeping at 60 ºC. Pale yellow zinc oxide nanoparticle is calcined at 100 ºC in a muffle furnace to remove moisture[14].

UV-Vis spectrum analysis

The pale yellow colour mixture was analysed using Syatronics UV double beam spectrophotometer (model 2201), at a resolution of 1 nm, between 200 and 600 nm using 10 mm optical path length quartz cuvette.

Fourier transform infrared spectroscopy analysis (FTIR)

The synthesised ZnO nanoparticle and Zinc acetate salt were studied using Nicolet Impact 400 FTIR spectroscopy to find the functional groups present in the samples.

SEM and EDAX analysis

A scanning electron microscope (JEOL 6380A; Tokyo, Japan) was used to record the micrograph images of synthesised ZnO nanoparticle, and elemental composition was studied using EDAX.

Cytotoxicity assay

Chemicals and reagents

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) Invitrogen, USA. Acridine orange was obtained from Sigma, USA. All other fine chemicals were obtained from Sigma–Aldrich, St. Louis.

Cell culture

HepG2 and MCF-7 cells obtained from NCCS (National Centre For Cell Science, Pune) were cultured in Rose well Park Memorial Institute medium (RPMI), supplemented with 10 % fetal bovine serum, penicillin/streptomycin (250 U/mL), gentamycin (100 µg/mL), and amphotericin B (1 mg/mL) were obtained from Sigma Chemicals, MO, USA. All cell cultures were maintained at 37 ºC in a humidified atmosphere of 5 % CO_2. Cells were allowed to grow to confluence over 24 h before use.

Cell growth inhibition studies by MTT assay

Cell viability was measured by the conventional MTT reduction assay method as described previously with slight modification[15]. Briefly, HepG2 and MCF-7 were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h, in 200 µL of RPMI with 10 % FBS. Then, the culture supernatant was removed, RPMI containing different concentrations (50-250 µg/mL) of ZnO nanoparticle was added and incubated for 48 h. After treatment, cells were incubated with MTT (10 µL, 5mg/mL) at 37 ºC for 4 h and then, with DMSO at room temperature for 1 h. The plates were read at 595 nm on a scanning multi-well spectrophotometer. The data represent the mean values for six independent experiments.

Cell viability (%)= (Average test OD/Control OD) x 100
Cell death (%) = 100 - cell viability %

Results

Synthesis of ZnO nanoparticle

The colour change of zinc acetate solution of white colour to yellow indicates the formation of zinc oxide nanoparticle. This colour change is due to surface Plasmon resonance property.

UV-Vis spectroscopy analysis

The absorption peak between 325-385 nm confirms the presence of ZnO particle. The UV-Vis spectroscopy analysis of sample shows maximum absorption peak around 330 nm.

FTIR analysis

FTIR analysis measures the absorption of infrared radiation by the sample. The graph was plotted against wavenumber (cm^{-1}) and absorption. The peaks determine the functional group present in the given sample. Fig. 1a shows the absorption spectrum of zinc acetate salt, which was used as a precursor for synthesising zinc oxide nanoparticle. The band at 3116 cm^{-1} is due to the aromatic =C-H bond. The peak at 1559 and 1445 cm^{-1} is due to the stretching vibration of the aromatic C=C bond. C-O and C-N stretch appear at 1053 and 1019 cm^{-1} respectively. The absorption peak at 953, 843, 695 and 622 cm^{-1} is due to the presence of an aromatic C-H bond, carbonate ion and alkyne C-H bend respectively. Fig. 1b shows the absorption spectrum of synthesised zinc oxide nanoparticle. The band at 3430 cm^{-1} is due to the stretch in OH group. The alkenyl, C=C bond stretch and methylene group appears at 1633 and 1383 cm^{-1}

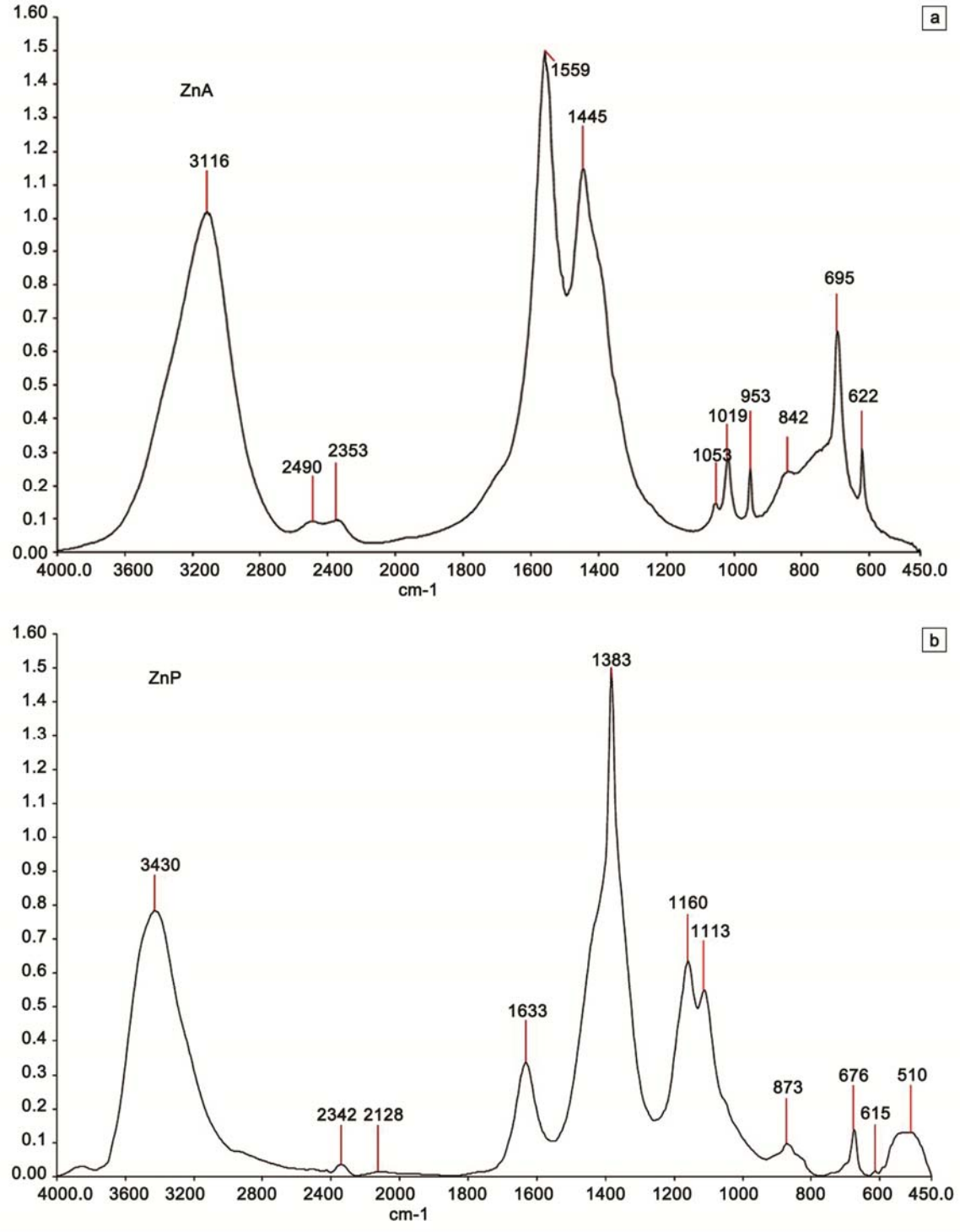

Fig. 1 — FTIR Spectra of a) Zinc acetate solution, and b) ZnO nanoparticle.

respectively. The absorption peak at 1160 and 1113 cm^{-1} is due to the presence of bend in the aromatic C-H group and C-N respectively. Other peaks at 873, 676 and 615 cm^{-1} are mainly due to the presence of carbonate ions and alkyne C-H bend.

SEM analysis

SEM analysis reveals the size of the nanoparticle. The sizes of nanoparticles are viewed in different magnifications (Fig. 2). The size ranges from 73.4-398.3 nm. The clumps found are due to aggregation of nanoparticles.

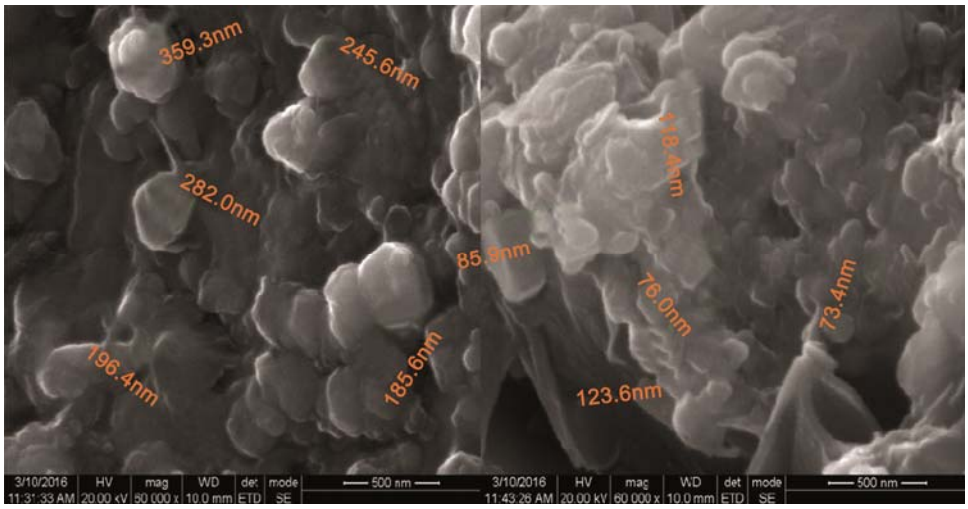

Fig. 2 — SEM image of ZnO nanoparticles reduced by the extract of *P. capensis.*

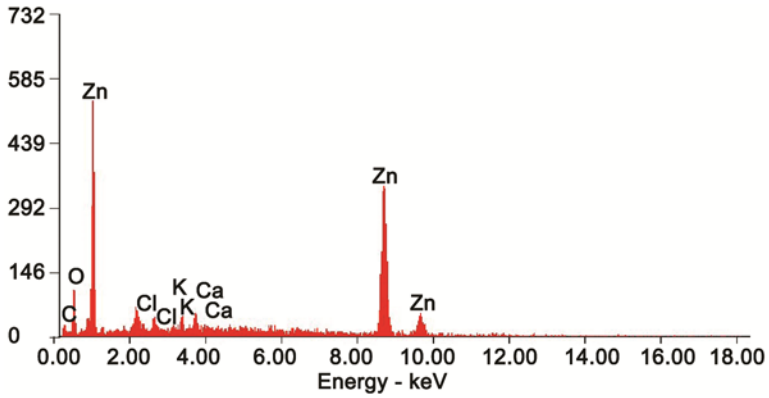

Fig. 3 — EDAX analysis of ZnO nanoparticles.

EDAX analysis

Energy dispersive X-ray spectroscopy determines the elemental composition of the given sample. The elemental composition of ZnO nanoparticles consists of zinc, carbon, oxygen, potassium, calcium and chlorine (Fig. 3). The percentage of elemental composition showed Zn (80.2 %) and Oxygen (7 %) to be at maximum and other elements at a very less percentage.

Cytotoxicity assay

The synthesised zinc oxide nanoparticle from aerial parts of *P.capensis* showed a cytotoxic effect against HepG2 (Plate1a) and MCF-7 (Plate 1b) cell line. The increase in cell death with an increase in the concentration of nanoparticle signifies that the ZnO nanoparticle is effective. IC_{50} value in HepG2 cell line was calculated as 23.277 µg/mL, and the IC_{50} value for the MCF-7 cell line was calculated as 12.926 µg/mL.

Discussion

The eco-friendly synthesis of ZnO nanoparticle by using aerial parts of *P. capensis* was investigated in the present study, where the aerial parts of this plant extract act as reducing and capping agent. The synthesised nanoparticles were characterised and confirmed using UV-Vis spectroscopy, FTIR, SEM, and EDAX. The reduction Zn^{2+} ions in zinc acetate solution to zinc oxide are due to the bioactive principles present in *P. capensis*, the absorption peak at 330 nm confirms the presence of ZnO nanoparticle. FTIR was used to study the details about the compounds present in the plant sample. The typical FTIR peaks in the nanoparticle may be due to the presence of alkaloids and flavonoids which was the major constituent in this plant[16]. The SEM image reveals the round shape of ZnO nanoparticle which ranges from 73.4-398.3 nm. EDAX analysis confirms the presence of Zn and Oxygen along with other elements, which need to be removed before using ZnO nanoparticle as a drug.

The application of ZnO nanoparticle against cancer cell was determined by testing the samples in

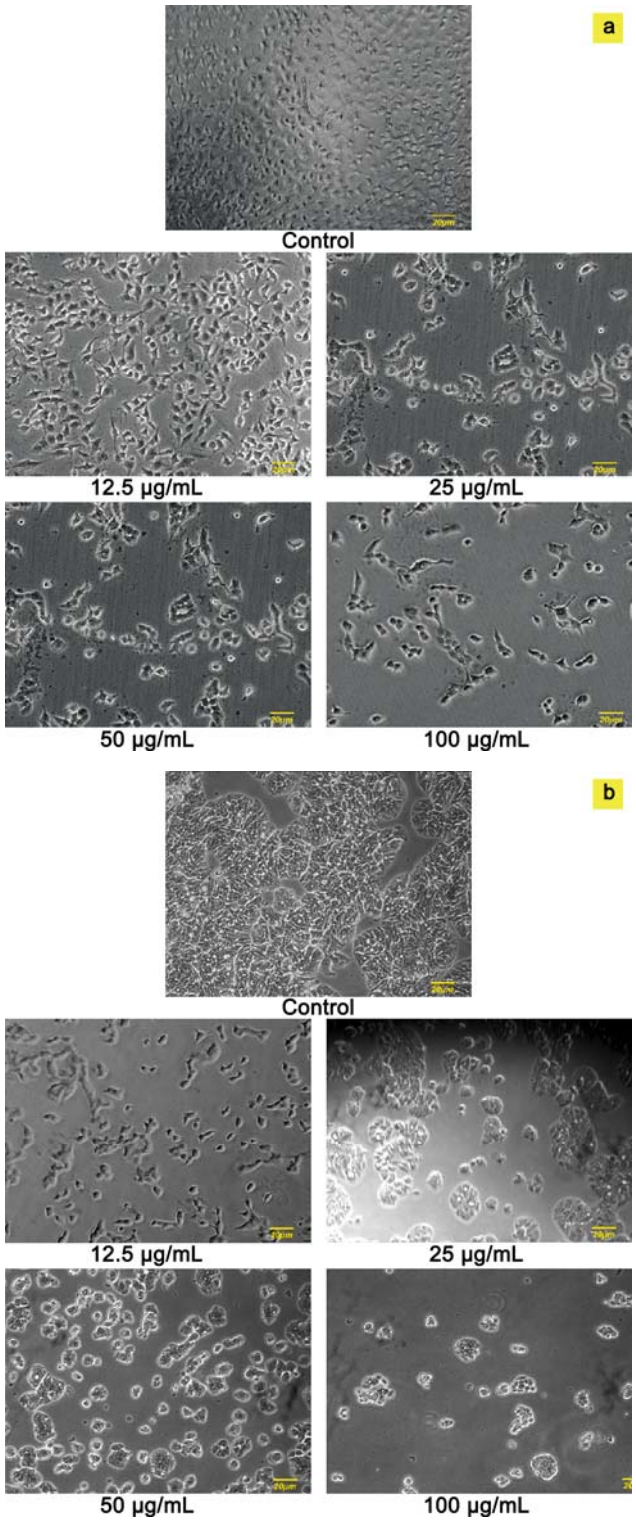

Plate 1 — a) HepG2, and b) MCF7 Cells after ZnO nanoparticle treatment.

cancer cell lines such as HepG2 and MCF-7 cell line. Significant killing effect of ZnO was observed on HepG2 and MCF-7 cells. The synthesised ZnO nanoparticle showed better results in MCF-7 cell line when compared to HepG2 cell line. The antiproliferative activity may be due to the availability of phytoconstituents present in *P. capensis*, which is associated along with the ZnO nanoparticle. The above constituents might have been involved in the generation of ROS, which is responsible for the killing of cancer cells. Therefore, ZnO nanoparticle reduced by the extract of *P.capensis* may be used as an efficient anticancer agent against MCF cells.

Conclusion

The present study documented that an aerial part extract of *P. capensis* may act as a reducing and capping agent for the synthesis of ZnO nanoparticles. The characterisation study confirms the size of the nanostructures. Based on the antiproliferative study using HepG2 and MCF-7 cells, it is concluded that ZnO nanoparticles prepared using an extract of *P. capensis* may be used as an alternative therapeutic option against MCF-7 cell line and related cancer treatment.

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