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Biological synthesis of stable Zinc oxide nanoparticles and its role as anti-diabetic and anti- microbial agents.

Aakash Koli, Department of Biotechnology, H. P. T Arts and R. Y. K Science College. Nashik-422005.

Abstract: *Nanotechnology is a fast emerging field which deals with synthesis of nanoparticles having variety of applications in various fields. Metallic nanoparticles have many applications in fields such as medicine, drug delivery, defence etc. Zinc oxide nanoparticles were synthesized biologically using plant leaf extract of Cheilocostus speciosus (Costusspeciosus). The reduction of metallic zinc was carried out by the plant extract itself. Synthesis of nanoparticles was confirmed by the U.V- Vis peak at a specific wavelength. The presence of zinc oxide nanoparticles was confirmed by Surface Plasmon behaviour seen using UV-Visible spectroscopy and the nanoparticles were characterized using XRD and FTIR. The bio stabilised nanoparticles were studied as a potential antimicrobial and anti-diabetic agent. Furthermore, this green synthesis approach is rapid and effective method for synthesis of nanoparticles.*

Key words: Anti-diabetic, *Cheilocostus speciosus,* green synthesis, nanoparticles, Surface Plasmon.

Introduction

Nanotechnology is the alteration of material at atomic, molecular and supramolecular level. Nanotechnology literally means the construction of materials from bottom up, using techniques developed to synthesise such material. These nanoparticles have been demonstrated in four generations namely: active nanostructures, passive nanostructures, systems of nano systems and molecular nano systems. Active nanosystems consist of nanostructures with bio- active properties and are targeted drugs and bio devices. Nanoparticles can be synthesised using various elemental materials, namely gold, silver, zinc, platinumetc. [(1)].The major products of nanotechnologies are nanomaterials such as nanoscale particles, rods, tubes or fibres. The description of nanoparticles includes their size, shape their surface structure and their ability to dissolve. Nanoparticles synthesised from plants or microbes (biological means) find potent implications as anti-bacterial agents [(2, 3)]. The reducing agents present in the plant extracts help the reduction of elemental forms to nanoscaleparticles[(4)]. The ongoing study focuses on the anti-bacterial and anti-diabetic properties of synthesised zinc oxide nanoparticles.

Materials and methods

1. Reagents: All the reagents were of analytical grade. Zinc nitrate was purchased from Fisher Scientific; U.S.A. Nutrient agar medium was purchased from HiMedia, India. All aqueous extracts were prepared in autoclaved double distilled water [(5)]. HiMedia tissue culture media (DMEM) was used for the experiment.

2. Instruments: UV visible spectrophotometer (Shimadzu, UV Vis- 1800) was used for UV Visible spectroscopy analysis. FTIR spectra were measured on Jasco FT/IR- 16100. The X-ray diffraction (XRD) of obtained nanoparticle powder was performed using P W1840 diffractometer control.

3. Screening of plant material: In order to find the most rapid and economized synthesis of ZnONps, initially various plants were screened to find out the plants that give a specific peak when reacted with zinc nitrate solution [(6)]. The list of the plants screened is given as follows:

Table no. 1. Plants screened for positive reaction

Sr. No	Common name of plant	Botanical name	Result
1	Costus speciosus	*Cheilocostus speciosus*	Positive
2	Tulsi	*Ocimum tenuiflorum*	Positive
3	Curry leaves	Murrayakoenigii	Negative
4	Purple cabbage	*Brassica oleracea*	Positive
5	Red capsicum	Bell pepper	Positive
6	Yellow capsicum	Bell pepper	Negative

4. Plant Material and preparation of extract: Fresh leaves of *Cheilocostus speciosus* (Costus speciosus) were collected from a nursery in Urali kanchan, Pune. The leaves were washed with distilled water. The leaves were crushed using mortar and pestle and then filtered to prepare 10% (w/v) aqueous extract by physical crushing method. The extract prepared was stored in clean bumper tubes. This leaf extract was then used for further synthesis of zinc oxide nanoparticles.

5. Biological Synthesis of ZnONps

The synthesis of nanoparticles was done using the leaf extract, dissolved in equal volume of zinc nitrate, under constant stirring. The solution was then kept in hot air oven at constant temperature of 120°C for 5 hr. After complete dissolution, the solution was centrifuged at 5000rpm for 15 min and the supernatant was discarded. The pellet was dried in hot air oven at 80° C for 2hrs to obtain dry powder of nanoparticles [7]. This powder was collected in clean, autoclaved eppendorf tubes and stored.

6. Anti- bacterial activity: The antimicrobial effect of nanoparticles was studied against gram positive as well as gram negative organisms namely *Staphylococcus aureus* and *Escherichia coli*. The antagonistic activity of zinc oxide nanoparticles was studied against bacteria by using the agar well diffusion method [8, 9]. Sterile nutrient agar plates were prepared and incubated to check overnight sterility. Contamination free plates were used for study. 0.1ml of test bacterial culture was spread on the Nutrient agar plates. Using a borer neat 6mm wells were punched on agar plates. To these wells 20µl of nanoparticle solution of (20mg/ml) concentration was added. The plates were incubated at 37°C overnight. After 24hr the zone of inhibition was measured.

7. Anti-diabetic (Glucolipotoxicity assay): The cultured cells of INS 1 E diabetic cell line were observed under light microscope. At 60% confluency, the cells were used for further experimentation. The cell count was taken using a haemocytometer. The cell counting was done by removal of the culture media and adding 2ml trypsin followed by its removal after 1min. The addition of trypsin helps the removal of the cells adhered to the surface of the culture flask. 10µl of trypan blue stain was added to 10µl these cells and counted in the haemocytometer. The total number of cells was divided by the number of large squares and multiplied by 10^5 i.e. the total number of cells.

These cells were then seeded into a 96 well plate followed by addition of 100µl media into each well for multiplication of cells overnight. After 6h, the used media was removed from each well and varying concentrations of palmitic acid and glucose were added where one well was maintained as control and one column was maintained as solvent control (BSA). BSA is used as a solvent control because palmitic acid is prepared by its dissolution in BSA. Glucose is prepared by its dissolution in distilled water and hence does not require the need of any control. The last column was treated with the synthesized zinc oxide nanoparticles whose toxic effects on the cell growth were to be observed. 10% MTT (3-4, 5-dimethylthiozol-2-yl) - 2, 5- biphenyltetrazolum bromide was added in dark conditions and incubated for 4h. MTT was removed and 100 µl DMSO was added and kept on shaker for 1 hr. The plate is read on ELISA plate reader at 540nm.

Results and discussion

1) Synthesis of zinc oxide nanopartilces

The zinc oxide nanoparticles were synthesized from plant extract using mortar and pestle. Zinc nitrate was added in equal amount to the extract and kept on the magnetic shaker for homogeneous mixing.

Fig No 1: *Costus speciosus* on magnetic stirrer after addition of zinc nitrate.

2) Characterization of synthesised nanoparticles

2.1. U.V-VIS spectroscopy for synthesized zinc oxide nanoparticles:

Fig No. 2. U. V-Vis spectroscopy.

The peak for the plant extract solution containing ZnONps was observed at **580nm.**

2.2 XRD pattern for zinc oxide nanoparticles sample

The XRD pattern shows intense peaks at 2 theta (2θ) scale confirming the crystalline nature of the nanoparticles. Using the Scherrer's formula we calculate the size of our nanoparticles.

Scherrer's Formula:

$$D = k\lambda/\beta cos\theta$$

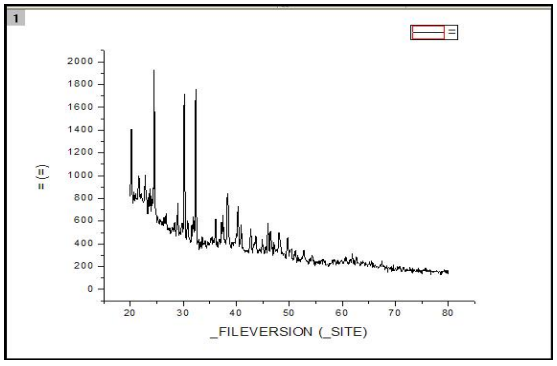

Fig No. 3 XRD pattern of zinc oxide nanoparticles.

2.3 FTIR (Fourier Transform Infra Red) analysis for zinc oxide nanoparticles sample

FTIR is a type of spectroscopy that measures absorption, emission and and photoconductivity of solids, liquids and gases. The peaks visible in the FTIR graph are due to the amide linkages between amino acid residues in the proteins

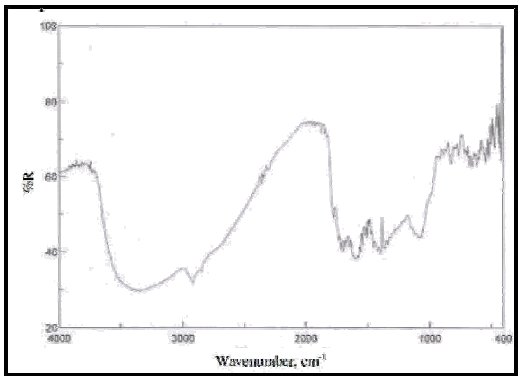

Fig no. 4 FTIR analysis of zinc oxide nanoparticles using *Costus speciosus* leaf extract.

Antagonistic activity against bacteria

The growth of microbes such as gram negative *Escherichia coli* and gram positive

Staphylococcus aureus is inhibited by the zinc oxide nanoparticles. The zone of inhibition i.e. the zone around the wells in which the microbes show no growth of was observed and recorded. The zones of inhibition are represented in table 1.

Table no. 2. Antagonistic activity of zinc oxide nanoparticles on bacteria

Experimental organism	Zone of inhibition (mm)
Escherichia coli	7.5
Staphylococcus aureus	6

Anti diabetic (Glucolipotoxicity assay): The comparative study of effect of zinc oxide nanoparticles and glucolipotoxicity was done using a 96 well plate. The graph clearly shows that the number of viable cells after treatment with ZnO nanoparticles has reduced gradually.

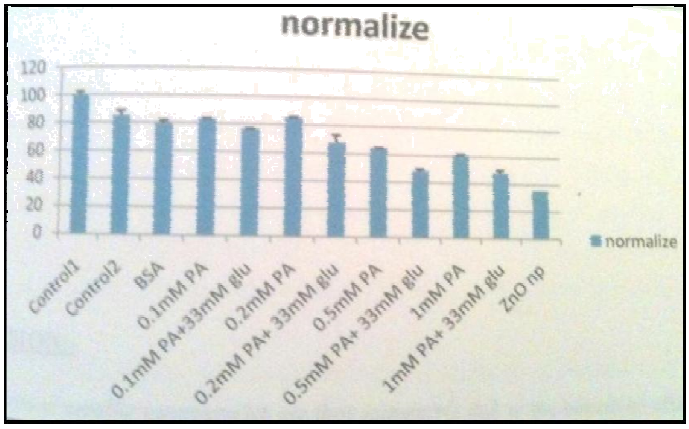

Fig No. 5 Effect of ZnO nanoparticles on INS 1 E cell line.

The percent viability of cells after treatment with ZnONp is approximately 35%. This shows that the ZnONp synthesised from Costus speciosus on leaf extract possess anti diabetic property.

Conclusion

The applications of zinc oxide nanoparticles are thus imperative due to the beneficial effects of the noble metal. Theproduction of the zinc oxide nanoparticles using chemical synthesis method poses a potential threat to the environment as a lot of toxic chemicals are used. We have experimented on a sustainable nanoparticles synthesis method that utilises the reducingproperties and enzymes of plant extracts.

The *Cheilocostu sspeciosus (Costus speciosus)* leaf extract was used effectively for zinc oxide nanoparticles synthesis. The zinc oxide nanoparticles produced by this biological method were stable. This was due toplant peptides, proteins and phytochemicals. These nanoparticles are known to lyse the bacterial cells and thus have potent antagonistic effects. The anti-microbial effect was observed against *Escherichia coli* and *Staphylococcus aureus*. The anti-diabetic activity was checked using the INS 1 E cell line, proving that these particles possess potent activity against diabetic cells.

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