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Mycosynthesis of Zinc oxide Nanoparticle from Mycelial Biomass of *Macrocybe crassa* (Sacc.) Pegler & Lodge and its Bio- applications

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Abstract

Myconanotechnology has gained a great attention now a days due to increased Bio-medical application , eco- friendly nature, cost – efficient without side effects. The present study was focused on mycosynthesis of Zinc Oxide nanoparticle using the mycelium of *Macrocybe crassa* with zinc nitrate as precursor. The synthesized nanoparticle was further characterized by UV-VIS spectroscopy, FTIR spectroscopy and SEM anlaysis which proved the presence of spherical shaped nano structure. The Anti-bacterial activity of synthesized nanoparticle was studied using four bacteria, a high zone of inhibition was obtained in *Pseudomonas florescens* followed by *B. subtilis, S. aureus, E. coli*. The Anti-tumor effect of ZnO nano particle was also analysed against DLA cell line using tryphan blue exclusion method. The synthesized ZnO nanoparticle could be a excellent supplement when used along with anti-cancer drug in the medicinal field after proper *in vivo* analysis.

Key words : Mycosynthesis, *Macrocybe crassa*, DLA cell line, Anti-tumor, SEM.

Nano particles can be synthesized by Physical, Chemical and Biological methods. The synthesis of nano particles using biological method is inexpensive,eco friendly and easier[13]. The biosynthesis of nanoparticle using microbial extract / plant extracts mainly contains various reducing agents which reduces the metal compounds to elemental metal nanoparticles (or) metal oxides nanoparticles[3]. There are various NPs that can be prepared as their oxides, including Tio2, tin (IV) oxide, zinc oxide, silicon dioxide (Sio2)[15]. The zinc nanoparticles have gained focus on research due to their excellent thermal, chemical stability[4] conductivity,

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catalytic properties, photonics, optoelectronics, anti bacterial and anti fungal properties[1]. The green synthesis is safe stratergy for the production of ZnO np because of the least amount of chemicals used, natural moieties such as plants and micro organisms is used in this method[9]. Several researchers have successfully reported the eco friendly nature of green synthesized nanoparticle from edible mushrooms extracts[8] and truffles[7], few studies which includes *P. florida, V.volvaceae, P. sajor caju, L. edodes*[2,10,14,18] have positively synthesized zinc oxide nanoparticle.However, there is no report on mycosynthesized ZnONPs from the mycelium of *Macrocybe crassa*. Thus, the present analysis was chiefly concentrated on the extraction, characterization and its applications (anti bacterial activity, cytotoxic effect).

Macrocybe crassa is a wild edible mushroom native to India, Thailand, Sri lanka *etc*., with long stipe and broad gap and the fruitbody weighing upto 200-400g has high medicinal property *Macrocybe crassa* which has been recently explored for its nutritional, medicinal prospect and identification of bioactive component. This wild mushroom grows mainly in tropical region and commonly known as milky mushroom due to its brilliant white colour along with sweet flavour. It also possesses satisfactory nutritional value which is much promising for commercialization[5,16].

Collection of sample and Biomass extraction:

Fresh fruit body of *Macrocybe crassa* was obtained from the Mycology laboratory of S.D.N.B. Vaishnav college, Chennai,Tamil Nadu, India. Basidiomycetes were produced on Potato Dextrose Agar medium at 25-30^{o}C by tissue culture method after 5-8 days from the active culture (6 mm in diameter) transferred to PDB medium (Potato dextrose broth) to obtain the fungal bio-mass and regular sub culturing was done to maintain the active sporulation (Figure 1A). All the chemicals needed for the study are purchased from Sigma-Aldrich.

Intracellular Nano particle synthesis :

The initial step in biological synthesis was prepared by taking approximately the 10g of the washed and dried fungal biomass in a 250 ml Erlenmeyer flask along with 100 ml of sterile double distilled water for 48 hours at 22^{o}C after incubation[17], the extract is filtered through whatman no. 1 filter paper to get the cell free culture filtrate (Figure 1B). To this 20 ml of filtrate added 80 ml of Zinc nitrate 5mM ($Zn(NO_3)_2,.5H_2O$) at room temperature in a shaker for 24 h and the mixture turned pale white indicating the presence of nanoparticle (Figure 1C). Through centrifugation the pellets were separated (8,000 rpm for 10 min) rinsed with double distilled water two times and dessicated by lyophilization process then, the dry powder (extracted nanoparticle)was stored in polypropylene tubes (Figure 1D)in dark condition at room temperature for future analysis.

Characterzation :

ZnO NPs were characterized by ELICO SLIS9 UV VISIBLE spectroscopy with spectral range between 200-800 nm and FTIR (Fourier transform infrared spectroscopy) to find the functional groups with IR range

between 4000 and 400 cm^{-1} using SHIMADZU-IR Spectrophotometer further, the morphology of the nanoparticle is determined using Scanning Electron Microscope (SEM, Hitachi –S3400, Singapore) with an acceleration voltage of 25.0 kV and 15.0 kV.

Bio-applications :

Anti-bacterial assay :

The pure culture of both gram positive and gram negative bacterias *S. aureus, B. subtilus, E. coli* and *P. fluorescens* where obtained from Plant Biology and Plant Biotechnology department of S.D.N.B. Vaishnav College, Chennai, Tamil Nadu. Extracted ZnO NPs where assessed against the above bacteria at different dose to check the higher growth inhibition by bacterial disk diffusion method.Streptomycin as positive control and Distilled water as negative control were placed.

In vitro Short-term Cytotoxicity analysis [12]*-Tryphan blue exclusion method.*

Dalton's lymphomatic ascites (DLA) cell lines were maintained as ascites tumors in Swiss albino mice. The cells were extracted and washed three times in phosphate buffer saline (PBS). The 1 million cell/ml cell slurry was prepared. The addition of a live suspension to the synthesized zinc oxide nanoparticle at different concentrations (200, 100, 50, 20 and 10 µg) and finally made the volume upto1 ml with PBS and incubated for 3 h at 37°C. Each concentration received 0.1 ml of tryphan blue dye (1%), non-viable cells gets stained is being counted by haemocytometer while the viable cells remains as such. The percentage of cytotoxicity is calculated by the following formula,

Cytotoxicity % = [No.of dead cells / No.of live cells + No. of dead cell] x 100

Nanoparticle from the mycelial extract was pale in colour (Figure 1) the nanoparticle thus concentrated through oven drying which is used for characterization.

UV-VIS Spectroscopy : ZnO NPs thus obtained from the *M.crassa* mycelium extract shows a strong peak between the spectral ranges from 200-800nm was investigated at 365nm, proves the presence of nano particle. This report founds to be in agreement with the earlier findings[6]. (Figure 2).

FTIR- Depicts the different functional group responsible for ZnO NP synthesis showed peaks at 3428.7, 2843.6, 1685.4, 1166.9, 603.7, 498.6, 471.9 cm^{-1} were relating with O-H stretching of alcohol and phenols, C=C stretch due to aromatic ring, C-OS stretch, Zn-OH stretch, ZnO vibration respectively (Figure 3), this findings is in agreement with earlier investigations[6,11]. (Figure 3).

SEM : The Morphology of the nano particle shows spherical shape and well-distributed without agglomeration[6] with size 30.2 nm in SEM analysis (Figure 4).

Anti-bacterial assay : showed good inhibition zone for both the gram positive and gram negative bacterias *S. aureus, B. subtilus, E. coli* and *P. fluorescens* with highest zone 21.3 ± 0.5 mm, 22.3 ±0.3 mm, 20.3 ± 0.7mm and 24.6 ±1.7 mm at 50 µl dose and a least zone of 8.0.±0.5mm, 5.0 ± 1.5mm, 5.0 ±0.5

and 7.0 ±1.7 at 10 µl respectively (Figure 5, Table-1).

Short term cytotoxicity study: As the earlier investigations[5] about the high content of anti oxidant presence in *Macrocybe crassa* could be a reason to develop the anti cancer property in the mushroom, thus *invitro* short term cytotoxicity study using DLA cell line which shows excellent anti proliferative property at 200 µg/ml concentration the cell inhibition percent was high (46.6 ± 1.5%) whereas, at 10 µg/ml concentration the least percent was (4.5 ± 0 %) recorded, this findings relates with earlier investigation[12]. (Figure 6, Table-2).

A)Mycelium on PDB medium

B)Mycelial extract

C) Mycelial extract+ Zinc nitrate

D) ZnO NPs

Figure 1: Synthesis of ZnO nano particles.

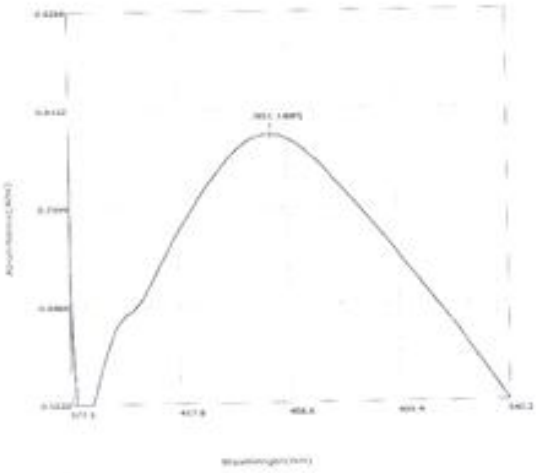


Figure 2: UV-Vis spectrum showing peak

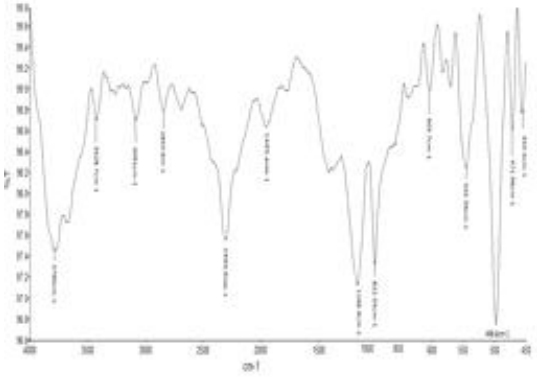


Figure 3: FTIR-spectrum

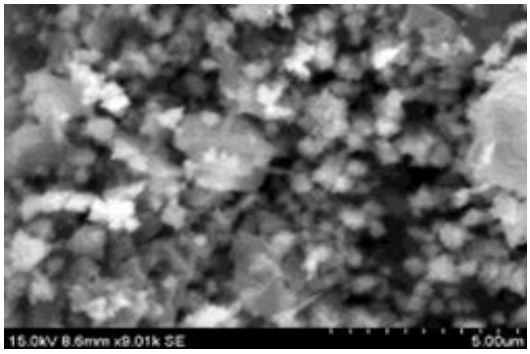
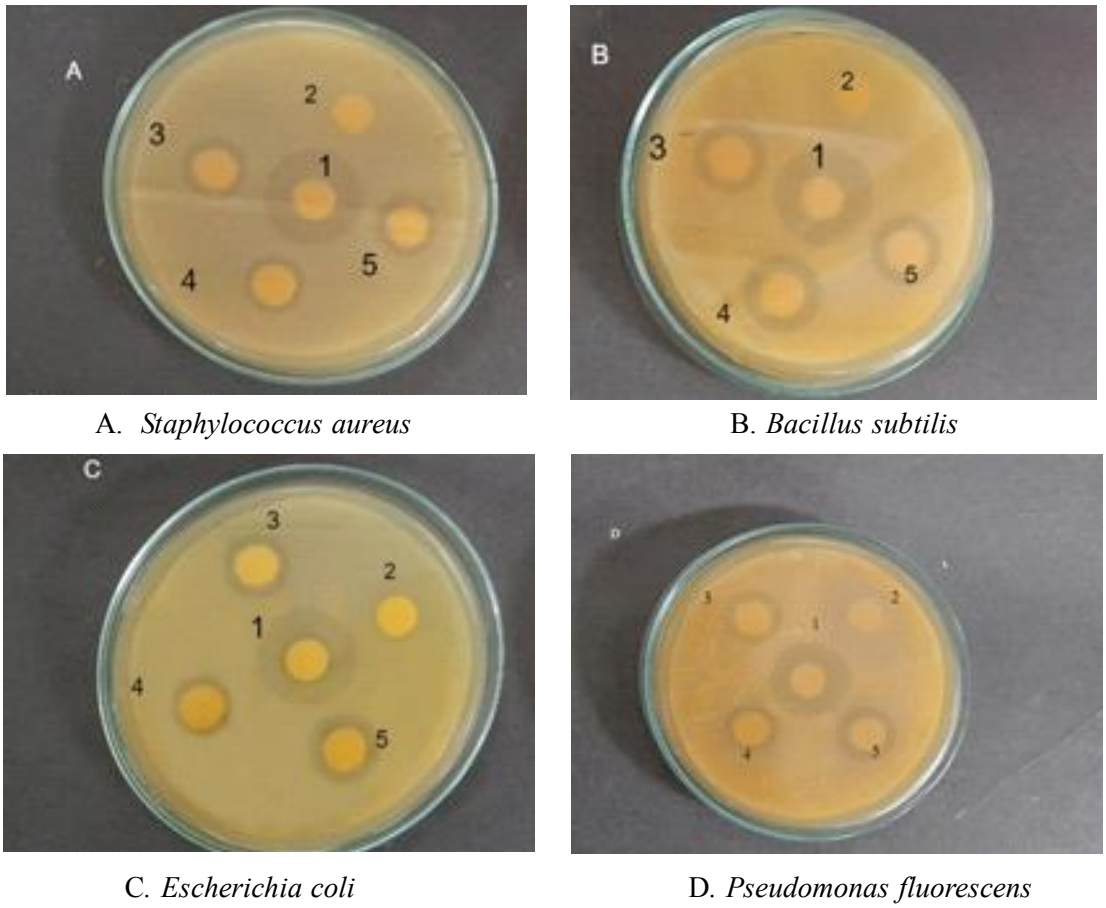


Figure 4: SEM image



A. *Staphylococcus aureus*

B. *Bacillus subtilis*

C. *Escherichia coli*

D. *Pseudomonas fluorescens*

Figure 5: Antibacterial assay. 1. Positive control 2. Negative control 3. 10 µL 4. 20 µL 5. 50 µL

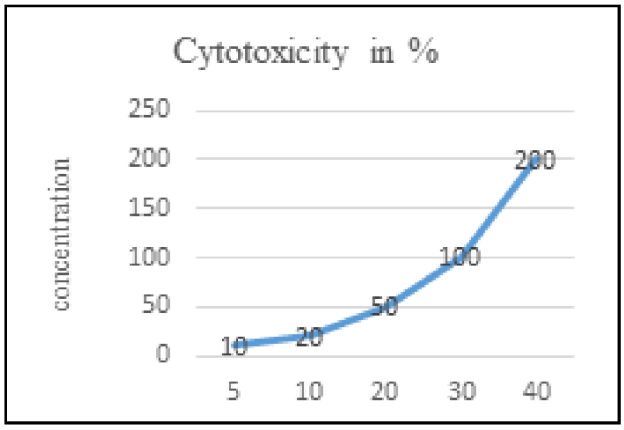


Figure 6: *in vitro* short term cytotoxicity study

Table-1. Antibacterial assay of mycelium extract of *Macrocybe crassa*

	Bacteria	Type	Positive control 20 µL	Negative control	Drug dose		
					10 µL	25 µL	50 µL
A	*Staphylococcusaureus*	Gram positive	30.3 ± 0.3	-	8.0± 0.5	18.3 ± 0.5	21.3 ± 0.5
B	*Bacillus subtilis*	Gram positive	25.6 ± 0.5	-	5.0± 1.5	17.0± 1.5	22.3± 0.3
C	*Escherichia coli*	Gram negative	24.6 ± 1.7	-	5.0± 0.5	15.0± 1.5	20.3± 0.7
D	*Pseudomonas fluorescens*	Gram negative	30.3 ± 1.7	-	7.0 ± 1.7	20.0 ± 1.3	24.6 ± 1.7

Data (mean ± SE) represents the zone of inhibition (mm) positive control -streptomycin, negative control-Distilled water

Table -2: *in vitro* Short-term cytotoxicity activity-DLA cell line

Drug concentration µg/ML	Cytotoxicity % ZnO NPs
10	4.4 ± 0
20	4.5 ± 0
50	6.42 ± 0
100	34 .7± 1.2
200	46.6 ± 1.5

Data in mean ± SE

The present study is concluded by the successful extraction of Zinc oxide nanoparticle from the mycelium of wild edible macro fungus *Macrocybe crassa* confirms the presence of nano particle showing peak at 365 nm and FTIR proves the zinc oxide stretch at 471.9cm^{-1} and the SEM analysis proved the morphology as smooth edged spherical shaped nanoparticles of size 30.2 nm without agglomerations.The anti bacterial assay showed excellent zone of inhibition at higher drug concentration (50 µl) for both gram positive and gram negative bacterias *S. aureus, B. subtilis*, *E. coli* and *P. fluorescens.* The mycosynthesized Zinc oxide NPs showed good anti cancer efficiency in dose dependant manner at 200 µg/ml concentration higher the cytotoxic effect (46.6 ± 1.5%), while the least percent was seen in 10µg/ml concentration.Thus,Our study proves the green synthesized Zinc oxide NPs can be a suggestion in the future therapeutical field for combating cancer with further *in vivo* analysis.

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