BIOSYNTHESIS AND CHARACTERIZATION OF ZINC NANOPARTICLES USING CALOCYBE INDICA AND ANALYSIS OF THEIRANTIMICROBIAL ACTIVITY

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ABSTRACT

Recently, the use of nanotechnology has been expanding very rapidly in diverse areas of research, such as consumer products, energy, materials, and medicine. This is especially true in nanomedicine, due to physicochemical properties, such as mechanical, chemical, magnetic, optical, and electrical properties, compared with bulk materials. The first goal of this study was to produce Zinc oxide nanoparticles (ZnONPs) using two different biological resources as reducing agents, *Calocybe indica* fruiting bodies. The second goal was to investigate the antimicrobial potential of the as-prepared ZnONPs in lung cancer cells. The final goal was to investigate the role of p53 in the cellular response elicited by ZnONPs. The synthesis and characterization of ZnONPs were assessed by various analytical techniques, including ultraviolet-visible (UV-vis) spectroscopy, X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, and transmission electron microscopy (TEM).The presentfindings could provide insight of the future development ofgreen synthesis inZnO nanoparticle,along with their antimicrobial activity.

Keywords: ZnO nanoparticles; *Calocybe indica;*Characterization studies; Antimicrobial assay; Green synthesis;Environmentally friendly.

INTRODUCTION

Nanoparticles can be synthesized by physical, chemical, and biological routes. The conventional physical and chemical methods for nanoparticle synthesis have some adverse aspects like critical conditions of temperature and pressure, use of expensive and toxic chemicals, long reflux time of reaction, toxic by-products etc. Biosynthesis of nanoparticles employs a biological system or its components for the formation of nanoparticles.The major applications of nanoparticles in biology are due to their excellent antibacterial activities on several Gram-positive and Gram-negative bacteria. The bactericidal potential of nanoparticles depends on their size, shape, size distribution, morphology, and stability.

Zinc oxide (ZnO) is a well-engineered compound that has received remarkable interest globally due to its distinctive properties and usages in various applications, such as pharmaceuticals, cosmetics, photonics, and photocatalysis. ZnO nanoparticles (NPs) can be synthesized through many physiochemical routes, such as sol-gel processes, co-precipitation, laser vaporization, microemulsion, and ball milling. Commonly, these preparation methods face several limitations, such as the high cost of equipment, the large area required for equipment set up, and additional use of capping agents, stabilizers and toxic chemicals. According to the literature, mushroom extract have been proposed as novel alternatives to chemical methods for synthesis of NPs. Furthermore, ZnO NPs have shown promising properties in photocatalytic applications; it has been reported that *Parkia roxburghii* extract/ZnO NPs showed excellent degradation of methylene blue (MB) and Rhodamine B dyes, reaching about 98%.

Zinc is an important nutrient in living organisms. Evidence has indicated that ZnO NPs have a great potential in biological applications, particularly as the antimicrobial agents. Moreover, numerous studies have been reported on the efficiency of ZnO NPs in inhibiting

the growth of broad-spectrum of pathogens, which potentially could replace the conventional antibiotic. Furthermore, zinc is an important trace mineral that plays a vital role in many physiological functions in the body. As such, the integration of NPs in feed would increase the absorption and efficient use of zinc in the body, hence, result in improved health and productivity.

Milky mushroom (*Calocybe indica* fruiting bodies) is second tropical mushroom after paddy straw mushroom, suitable for cultivation in tropical and subtropical regions of the country. This variety is new introduction to world mushroom family from India. During last decade it has become a major variety for cultivation in South India and during last 2-3 years its cultivation has become popular in North India, particularly in Haryana. Its high biological efficiency, better keeping quality, simple cultivation process and white attractive colour are factors for its popularity.

MATERIALS AND METHODS

SAMPLE COLLECTION

Fresh fruiting bodies of *Calocybe indica* fruiting bodies were cultivated in the mushroom units maintained at Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India.

PREPARATION OF EXTRACT

The fresh fruiting bodies of the mushroom were shade dried after washing and powdered in a mixer grinder. For hot extract preparation, 10g of mushroom powder was dissolved in 100 ml of distilled water. The extract was boiled for 1 hr and the supernatant was filtered. The extract was stored at 2-8 °C for further usage. Similarly, cold aqueous, ethanol, methanol extract was prepared.

Green synthesis of ZnO Nanoparticles

Zinc acetate dihydrate with 90% purity was obtained from Himedia and distilled water was used throughout the experiments. 0.2 M of zinc acetate dihydrate was dissolved in 70 mL of distilled water and stirred for few minutes. 5 g of sample, in dried form, was added to 100 mL of distilled water and magnetically stirred for 2 h 58 min at 80qC. After cooling to room temperature and filtering through Whatman No. 1 paper. 30 mL of the extract was mixed homogenously with the already prepared zinc acetate solution. The reacted solution was dried at 60qC overnight to yield pale white ZnO nanoparticles, which were finally calcined at 100qC for 1 h and preserved in air-tight vials for further studies. The green synthesized ZnO NPs were characterized by UV-Vis, FT-IR, TEM, XRD and FE-SEM techniques.

ANTIMICROBIAL ACTIVITY FOR SYNTHESIZED NANOPARTICLES

TEST MICROORGANISMS

The following five clinical isolates of bacteria and fungi were used for the study: Tetracycline was used as positive control for *Escherichia coli, Pseudomonas aeruginosa, Salmonella aureus, Proteus penneri, Bacillus subtilis.* Amphotricin for*Aspergillus niger, Fusarium solani, Bipolarisoryzae, Curvularialunata.* Sterilized distilled water was used as negative control.

ANTIBACTERIAL ACTIVITY

The pathogens selected for antibacterial activity of zinc nanoparticles synthesized using fresh extract was determined on Muller & Hinton Agar (Hi-Media Pvt. Ltd. Mumbai, India) using well diffusion method (Yan zohu*et al.,* 2012).

ANTIFUNGAL ACTIVITY

The pathogens selected for antifungal activity of zinc nanoparticles synthesized using fresh extract was determined on potato dextrose Agar (Hi-Media Pvt. Ltd. Mumbai, India) using well diffusion method (Yan zohu*et al.*, 2012).

CHARACTERIZATION STUDIES FOR SYNTHESIZED NANOPARTICLES

VISUAL INSPECTION

The colour change in reaction mixture (metal ion solution+ *Calocybe indica* fruiting bodiesextract) was recorded through visual observation.

UV-VIS SPECTRA ANALYSIS

The reduction of nanoparticle was monitored by measuring the UV-Vis spectrum the most primary confirmatory tool for the detection of surface Plasmon resonance property (SPR) of ZnO Nanoparticles. Measurements were carried out on a Cary 100 BIO UV–vis spectrophotometer (Varian, Palo Alto, CA, USA).

FTIR MEASUREMENT

The synthesized nanoparticles after 20 min of reaction mixture were centrifuged at 10,000 rpm for 15 min at room temperature, following which the pellet was re-dispersed in sterile distilled water to remove any uncoordinated biological molecules. The chemical composition of the Zinc oxide nanoparticles was characterized by FT-IR (Bruker Tensor 27).

The phase formation of bio-reduced nanoparticles was studied with the help of XRD. Bio reduced zinc chloride solution was air dried. XRD patterns were recorded by a SEIFERT X-ray diffractometer with Cu Kα radiation. The samples were scanned in the 2θ range of 10 °C-70 °C.

SCANNING ELECTRON MICROSCOPY AND EDX ANALYSIS

The morphology of the prepared nanoparticle products was examined by SEM to examine the shape of the nanoparticles. SEM micrographs image show Zinc oxide nanoparticles were spherical in shape. EDX (energy-dispersive X-ray) report confirms a chemical composition or contaminants for the synthesized Zinc oxide nanoparticles. The EDX spectrum gives the number of signals for contaminants in nanoparticles. The spectrum shows two types of signal peaks, one was zinc atom and other peak was elemental oxygen. The signals were likely due to X-ray emission from carbohydrates/proteins/enzymes present in the cell wall of the biomass (YiZhong*et al*., 2012).

XRD ANALYSIS

The phase formation of bio reduced Zinc oxide nanoparticles was studied with XRD. The size of the particles was determined using the X-ray diffraction technique. The crystallite size was calculated using Scherrer's formula

$$d = 0.9\lambda / \beta \cos\theta$$

Here 0.9 is the shape factor, generally taken for a cubic system, λ is the x-ray wavelength, typically 1.54 Å, β is the full width at half the maximum intensity (FWHM) in radians, and θ is the Bragg angle.

Transmission electron microscopy (TEM) measurements

The shape and size of nanoparticles were characterized by transmission electron microscopy (TEM). The samples were prepared by mounting a drop of solution on a carbon coated Cu grid and allowing it to dry in air. The samples were observed with the help of a Philips CM10 transmission electron microscope operating at 120kV. The system was fitted an intensified video camera to assist the alignment and a slow scan CCD (charge-coupled device) camera.

RESULTS AND DISCUSSION

The zinc ions were reducedto zinc nanoparticles within few minutes, by the addition of *calocybe indica*fruiting bodies extract. The observed quickconversion of solution color from pale yellow to dark honey color (indicating the formation of zinc nanoparticles). The zinc nanoparticles exhibited dark honey colorinaqueous solution, due to the surface plasmon resonance phenomenon, which is the collection ofoscillation of electrons.

Characterization of zinc nanoparticles

Ultraviolet-visible Spectroscopic analysis

The optical absorption spectrum of zinc nanoparticles was recorded using Ultraviolet visibledouble beam spectrophotometer, Schimadzou 4650 in the wavelength rangeof200 - 800 nm. The reduction of zinc nitrate to zinc in aqueous colloidal solution was monitoredusing optical absorption spectroscopy.

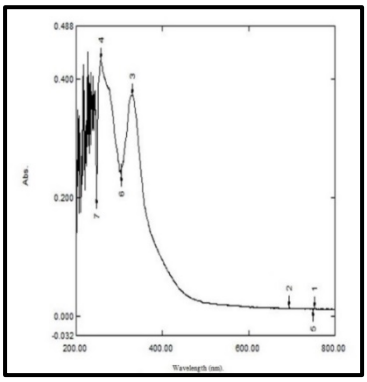

Figure 1. Absorption spectrum of zinc nanoparticles synthesized using *Calocybe indica* fruiting bodiesextract.

Fourier transform infrared spectroscopic analysis.

The infrared spectra of *Calocybe indica* fruiting bodiesextract and zincnanoparticlessynthesized using *Calocybe indica* fruiting bodiesextract are shown in Fig.

1.2a and 1.2b.Theinfrared spectra were recorded using Fourier transform infraredspectrometer (FTIR), BrukerTensor 27. Infrared spectroscopy gives the vibrational state of particles. The FTIR spectrum of*Calocybeindica* fruiting bodiesextract showed thepresence of functional groups of alcohols,phenols, alkenes, alkanes, carbonyls, aromatics, alkyl halides and alkynes. The spectrum showedthe bands for the functional groups located at 3779, 3691, 2910, 2851, 1672, 1417, 1275, 1155and 583 cm·1. The strong band of -C=O- stretch (carbonyls) was recorded at 1672 cm-1. Themedium bands of -C-C- (aromatics) and -CH2-X (alkyl halides) were recorded at 1417 and1155 cm-1.

The FTIR spectrum of zinc nanoparticles synthesized using the *calocybe indica*fruiting bodiesextract of*Calocybe indica* fruiting bodiesshowed the presence of additional functional groups, whencompared to the spectrum of extracts. The presence of additional functional groups in thespectrum of zinc nanoparticles was due to the bio-reduction and stabilization of metal group -Zn. The bio-molecules like flavonoids and terpenoids present in the *calocybe indica*fruiting bodiesextract binds with themetal group zinc resulting in the stretching of functional groups and reduction of the metal zincto zinc nanoparticles.

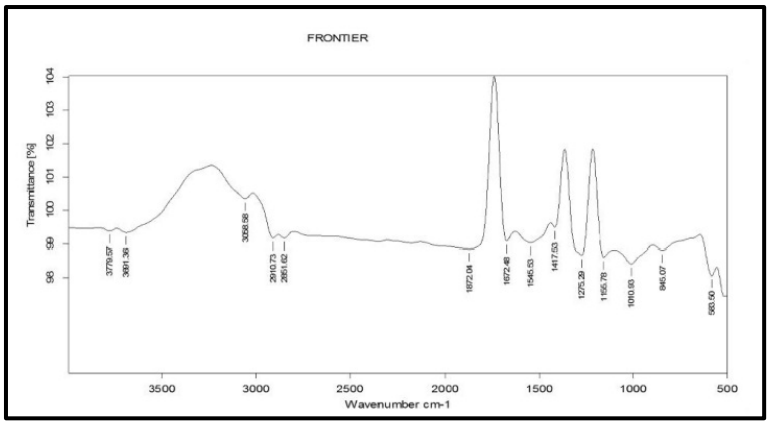

Figure 1.2. FTIR spectrum of *Calocybe indica* fruiting bodiesextract

X-ray Diffraction analysis

The X-ray diffraction pattern of zinc nanoparticles synthesized using the leaves extractsof *Calocybe indica* fruiting bodiesis shown in Fig. 1.3. Here, the X-Ray diffraction pattern isrecorded from 10 degrees to 60 degrees using Siefert X-ray diffractometer, model 3003. Thediffraction pattern exhibited hexagonal closely packed (hep) structure of crystalline zincnanoparticles. The XRD pattern showed characteristic Bragg peaks with diffraction intensities at19°, 36°, 39°, 43° and 54° (28 angles) corresponding to (hkl) values of (1 0 1), (2 0 1), (2 0 2),(2 0 3) and (3 0 0). The data is in agreement with JCPDF 011238 data. Broadening of peaksobserved from the XRD pattern was due to the presence of nano-sized particles (zincnanoparticles). The average particle size of the zinc nanoparticles was estimated as 20 nm usingDebye Scherer formulae, corresponding to (2 0 3) plane.

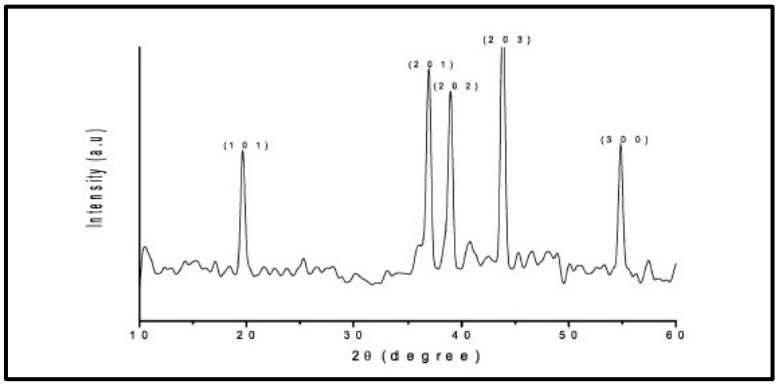

Figure 1.3. X-ray diffraction pattern of zinc nanoparticles synthesized using theextract of *Calocybe indica* fruiting bodies

Scanning electron microscopic analysis (SEM)

The structural morphology of *Calocybe indica* fruiting bodies extracted zincnanoparticles was studied by scanning electron microscopic analysis. The scanning electronmicrographs of zinc nanoparticles synthesized from the *calocybe indica*fruiting bodiesextract of *Calocybe indica* fruiting bodiesrecorded at 3 µm, 1 µmare shown in Fig. 1.4a, Fig. 1.4b. The SEM micrographs revealed spherical shaped and poly-dispersed zincnanoparticles, with an average particle size of 20 nm.

SEM analysis is considered to be "non-destructive i.e., the interactions do not lead tovolume loss of the sample, so it is possible to analyse the same materials repeatedly.

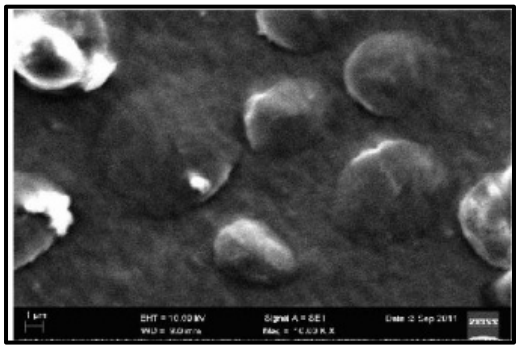

Figure 1.4b. Scanning electron micrograph of zinc nanoparticles synthesized using the extract of *Calocybe indica* fruiting bodies.

Energy dispersive X-ray spectroscopy (EDAX)

The Energy dispersive X-ray analysis was performed using EDAX, Oxford Systems.Figure 1.5. Shows the EDAX spectrum of zinc nanoparticles. The EDX reading proved thattherequired phase of zinc (Zn) is present in the sample. The graph also showed the elements ofcarbon (C) and Aluminum (Al), which may be probably due to the presence of substrate overwhich the zinc nanoparticles sample was held during the spectral analysis.

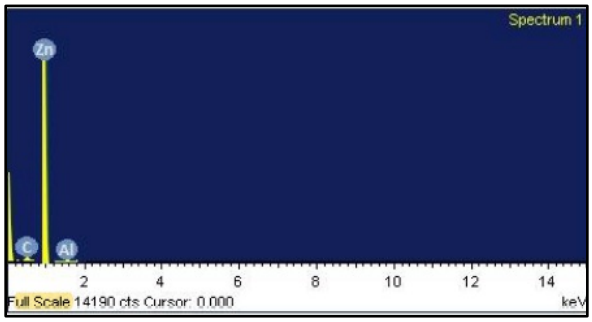

Fig. 1.5. EDAX spectrum of zinc nanoparticles synthesized using the extract of *Calocybe indica* fruiting bodies.

Element	Weight%	Atomic%
O	4.80	7.88

Al	0.74	0.30
Zn	94.36	91.82
Totals	100.00	

Table 1 - Elemental analysis

Transmission electron microscopy

A HITACHI H-7500, transmission electron microscope was used to obtain TEMmicrographs. Fig.1.6 shows the TEM micrographs (100 nm) shows the selected area electrondiffraction patterns of *Calocybe indica* fruiting bodiesextracted zinc nanoparticles.

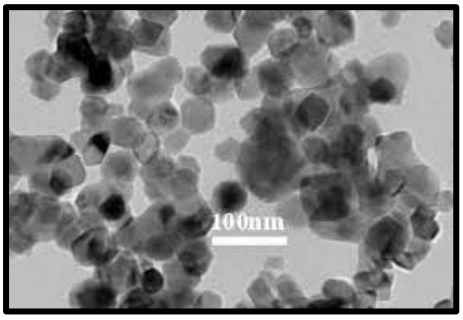

Figure 1.6- TEM micrograph of zinc nanoparticles synthesized using the extract of *Calocybe indica* fruiting bodies(100 nm).

Antimicrobial activity

The results of the antibacterial activity of green synthesized ZnO nanoparticle against the bacterial isolates.The result of the study showed that ZnO nanoparticle formed a zone whose size ranged from 16±0.8 mm to 29.6±1.2 mm in diameter. The maximum zone of 29.6±1.2 mm was exhibited toward *Pseudomonas aeruginosa*and the least zone of 16±0.8 mm in diameter was shown by *Escherichia coli*. The values were significant at 0.1% level.

Antimicrobial	***Calocybe indica* fruiting**	**Standard**

	bodiesextract	
Escherichia coli	16±0.8	10 cm
Pseudomonas aeruginosa	29.6±0.6	0.2 cm
Salmonella aureus	10±0.4	0.3 cm
Proteus penneri	12±0.9	0.4 cm
Bacillus subtilis	9±0.1	1.8 cm

Table 2 –Antibacterial activity

The maximum zone of 21.6±1.2 mm was exhibited *Aspergillus niger*and the least zone of 10±0.8mm in diameter was shown by *Curvularialunata*. The values were significant at 0.1% level.

Antifungal	***Calocybe indica* fruiting bodies extract (cm)**	**Standard(cm)**
Aspergillus niger	21±0.8	1.0 cm
Fusarium solani	11±0.8	0.2 cm
Bipolarisoryzae	16±0.8	0.3 cm
Curvularialunata	10±0.8	0.4 cm

Table 3–Antifungal activity

CONCLUSION

Thus, from the results of the above study, it can be concluded that green synthesis of ZnO nanoparticle using several sources has several merits such as simple, inexpensive, good stability of nanoparticles, less time consumption, non-toxic byproducts, ecofriendly, and large-scale synthesis.In Antimicrobial studies, Antibacterial activityreveals the maximum zone exhibited toward *Pseudomonas aeruginosa* and least

zone to E.coli and Antifungal activity reveals the maximum zone exhibited towards *Aspergillus niger*least zone to *Curvularialunata.*

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