

Original Article

GREEN TEA (CAMELLIA SINENSIS) MEDIATED SYNTHESIS OF ZINC OXIDE (ZNO) NANOPARTICLES AND STUDIES ON THEIR ANTIMICROBIAL ACTIVITIES

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ABSTRACT

Green synthesis of Zinc oxide nanoparticles (ZnO Nps) was carried out using the aqueous extract of green tea (*Camellia sinensis*) leaves. The UV-Vis spectrum was recorded to monitor the formation of the nanoparticles, which exhibited a blue shifted absorption peak at 325 nm. The XRD pattern revealed well-defined peaks appearing at 2θ positions corresponding to the hexagonal wurtzite structure of ZnO nanoparticles. The average size of the nanoparticles calculated using XRD data was 16 nm. FT-IR spectra were recorded for the green tea extract and for the ZnO nanoparticles to identify the biomolecules involved in the synthesis process. The higher percentage of phenolic compounds, with antioxidant potential, provided the reducing action on the metal oxides and significantly present amino acid, protein and lipids helped to stabilize the growth of the nanoparticles. Agar well -diffusion method was used to study the antibacterial and antifungal activities on selected pathogenic species. The synthesized ZnO Nps showed better and comparable antimicrobial activities with respect to the activities of synthetic drugs.

Keywords: Green synthesis, Green tea, ZnO Nanoparticles, Antimicrobial activity, FT-IR, XRD.

INTRODUCTION

Nanoparticles possess the unique size dependent property known as high 'aspect ratio', which is the ratio of the surface area to volume. Smaller the size of the particles greater will be the aspect ratio i.e., greater surface area compared to their volume. This increased surface area of the smaller nanoparticles enhances the reactivity of the nanoparticles with the surrounding molecules. The synthesis of nanoparticles by conventional physical and chemical methods has some adverse effects like, critical conditions of temperature and pressure, expensive and toxic chemicals, long reflux time of reaction, toxic byproducts etc. Green synthesis of nanoparticles has gained significant importance in recent years and has become one of the most preferred methods. Green synthesis procedures have several merits such as, simple, inexpensive, good stability of nanoparticles, less time consumption, non-toxic byproducts and large-scale synthesis [1, 2, 3, 4, 5, 6].

Extensive studies have been made on the green synthesis of nanoparticles of noble metals gold (Au) and silver (Ag) and their antimicrobial activities. However relatively fewer works were reported on the green synthesis of some metal oxide nanoparticles such as TiO₂, MgO, CuO, FeO₂, AlO, ZnO. All these works were carefully collected and presented in the form of 'review' articles by some authors [7, 8, 9, 10, 11].

Zinc oxide (ZnO) nanoparticles have received considerable attention due to their antimicrobial, UV blocking, high catalytic and photochemical activities [12]. Sharma et al. [13] have reported that ZnO nanoparticles possess antibacterial and antifungal activities even at lower concentrations hence suitable for thin coating applications. Further antifungal activity of ZnO nanoparticles does not affect soil fertility compared to the conventional antifungal agents. Feris et al. [14] have concluded that the bacterium and fungal lipid bilayers get ruptured due to cytotoxic behavior of ZnO nanoparticles resulting in the drainage of the cytoplasmic contents. Raghupathi et al. [15] have investigated the antibacterial effect of ZnO nanoparticles and developed antibacterial agents against a wide range of microorganisms to control the bacterial infections. Jayaseelan et al. [16] have reported significant antimicrobial activity of biosynthesized ZnO NPs. The green synthesis of ZnO nanoparticles and the studies on their antimicrobial activities are still in the infancy stage and limited number of works have been reported [17, 18, 19, 20, 21]. Fresh tea leaf is unusually rich in the flavonol group of polyphenols known as catechins (approximately 30% of the dry

leaf weight). Other polyphenols present are flavonoids and their glycosides, chlorogenic acid, gallic acid, coumarylquinic acid and theogallin. Green tea is usually prepared without fermentation so as to preclude the oxidation of green leaf polyphenols. Green tea chemical composition is very similar to that of fresh leaf except for a few enzymatically catalyzed changes which occur with extreme rapidity following plucking. Some new volatile substances are produced during drying. The commonly measured approximate compositions of green tea leaf are: i) phenolic compounds (30%), ii) proteins (15%), iii) amino acids (4%), iv) carbohydrates (7%), v) lipids (7%) and vi) vitamins C and E. [22,23]

Phenolic compounds exhibit higher antioxidant potential and antioxidants are very good reducers of metal ions, thus favoring the green synthesis of nanoparticles. Further higher contents of proteins, lipids and amino acids help to stabilize the growth of nanoparticles and inhibit particle agglomeration. The present work was aimed at the green tea mediated synthesis of ZnO nanoparticles and to evaluate their antimicrobial efficiency against some pathogenic bacteria and fungi.

MATERIALS AND METHODS

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 green tea leaf powder, in dried form, was added to 100 mL of distilled water and magnetically stirred for 2 h at 80 °C. After cooling to room temperature and filtering through Whatman No. 1 paper, 30 mL of this green tea extract was mixed homogeneously with the already prepared zinc acetate solution. The reacted solution was dried at 60 °C overnight to yield pale-white ZnO nanoparticles, which were finally calcined at 100 °C for 1 h and preserved in air-tight vials for further studies.

Antimicrobial studies

The agar well diffusion method was used to screen the antimicrobial activity of the green synthesized ZnO NPs. The organism was introduced on the plates of Muller-Hinton agar and spread uniformly. Wells were made on the agar plates with the help of a sterile polystyrene tip (4 mm). Different concentrations of ZnO NPs (5, 10, 20 µg mL⁻¹) had been prepared separately and used in the assays. The antimicrobial activity was determined by measuring the diameter of zone of inhibition around the wells. All the bacterial and fungal strains used in this study were obtained from the Department

of Microbiology, Raja Muthiah Medical College, Annamalai University, Tamil Nadu.

Regarding the antibacterial assay, the following pathogenic bacterial species were used. Gram-negative bacteria: *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and Gram-positive bacteria: *Staphylococcus aureus* were included in the study. Imipenem 10 µg mL⁻¹ was used as a positive control to compare the antibacterial activity of ZnO NPs.

In the antifungal assay, pathogenic fungi *Aspergillus fumigatus*, *Aspergillus flavus*, *Penicillium* sp. and *Aspergillus niger* were used in this study. Spores of the fungi were harvested from fresh culture on sabouraud dextrose agar (SDA) plates and mixed with sabouraud dextrose broth. This was adjusted to McFarland opacity (0.5 = 10⁶ cells mL⁻¹). The above mentioned standardized fungal spore inoculums were spread uniformly on SDA plates.

Characterization techniques

The UV-Vis spectrum of ZnO NPs was recorded using LAMBDA 25-PERKIN ELMER spectrometer. The FT-IR spectra of green tea powder and that of ZnO NPs were recorded in SHIMADZU-8400 spectrometer using KBr pellet method. X'PORT PRO X-ray diffractometer was used to record the XRD pattern of the synthesized ZnO NPs.



Fig. 1: a) Green tea plant, b) leaves and c) synthesized ZnO NPs.

RESULTS AND DISCUSSION

The photographs of the green tea plant, leaves in dried form and the synthesized ZnO NPs are shown in Fig. 1. The pale-white colour of the ZnO NPs arise due to capping action of biomolecules of green tea extract on the surface of the nanoparticles.

UV-Vis spectrum

The UV-Vis spectrum of ZnO Nps is shown in Fig. 2. Confirmation of the synthesized ZnO product in nano-scale was exhibited by the highly blue-shifted absorption maximum occurring around 325 nm. For bulk ZnO the absorption maximum usually occurs around 385 nm approximately.

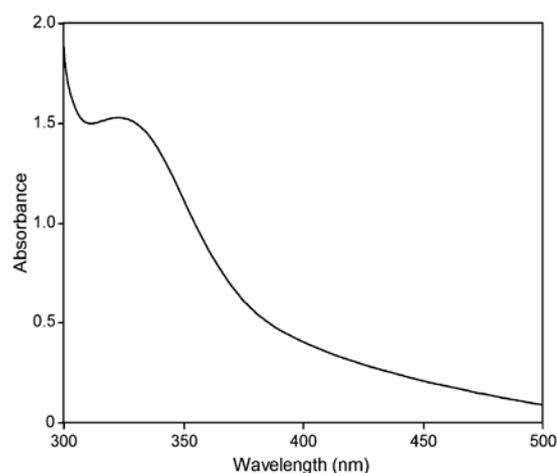


Fig. 2: UV-Vis spectrum of synthesized ZnO NPs.

XRD studies

The XRD spectra of the 'as prepared' and 'calcined' ZnO NPs are shown in Fig. 3. Calcination at 100 °C is essential for complete removal of water and to obtain higher crystallinity. The prominent peaks corresponding to the diffraction planes (100), (002), (101), (102), (110), (103) and (112) agree well with the JCPDS Card No. 36-1451, confirming the hexagonal wurtzite structure of the ZnO NPs. The average particle size (D) of synthesized nanoparticles was calculated using the well known Scherrer formula [24] $D = 0.9 \lambda / \beta \cos \theta$, where λ is the wavelength of X-ray source (CuK α line - 0.1541 nm), β is the full width at half maximum (FWHM) in radians and θ is Bragg's diffraction angle. The calculated value of D was 16 nm.

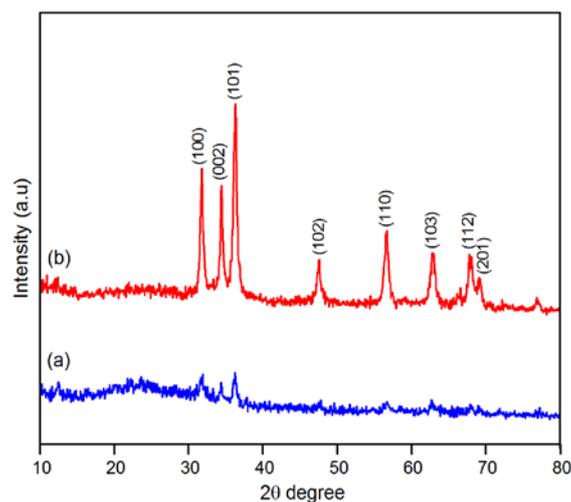


Fig. 3: XRD spectra of a) 'as prepared' and b) 'calcined' (100 °C) ZnO NPs.

FT-IR analysis

FT-IR spectroscopy is the measurement of absorption of IR radiations by a sample plotted against the wavelength. The interpretation of the IR spectrum involves the correlation of the absorption bands (vibrational bands) with the chemical compounds in the sample. In this way, the biomolecules present in plant extracts that are responsible for the reduction and stabilization processes of the green synthesis of nanoparticles can be identified. The FT-IR spectrum of the green tea extract and that of the synthesized ZnO NPs are shown in Fig. 4.

In the IR spectrum of green tea, the band at 3394 cm^{-1} is due to stretching vibrations of O-H groups in water, alcohol and phenols and N-H stretching in amines. The C-H stretch in alkanes and O-H stretch in carboxylic acid appear at 2926 and 2864 cm^{-1} respectively. The strong band at 1627 cm^{-1} is attributed to the C=C stretch in aromatic ring and C=O stretch in polyphenols. The C-N stretch of amide-I in protein gives the band at 1396 cm^{-1} . The C-O-C stretching in polysaccharides gives a band at 1741 cm^{-1} and C-O stretching in amino acid causes a band at 1037 cm^{-1} . Finally the weak band at 819 cm^{-1} is the result of C-H out of plane bending. Thus from the IR spectrum it can be observed that green tea sample is rich in polyphenols, carboxylic acid, polysaccharide, amino acid and proteins. The involvement of these biomolecules in the reduction and stabilization (capping) actions are clearly evident from the IR spectrum of the synthesized ZnO NPs. In addition to the absorption bands of these biomolecules, two new peaks appearing at 682 and 457 cm^{-1} in the IR spectrum of the ZnO NPs are the characteristic peaks of ZnO molecules. It may be concluded that the presence of higher percentage of phenolic group of molecules are responsible for the reduction process and the amino acids and amide linkages in protein are responsible for the stabilization of the ZnO nanoparticles.

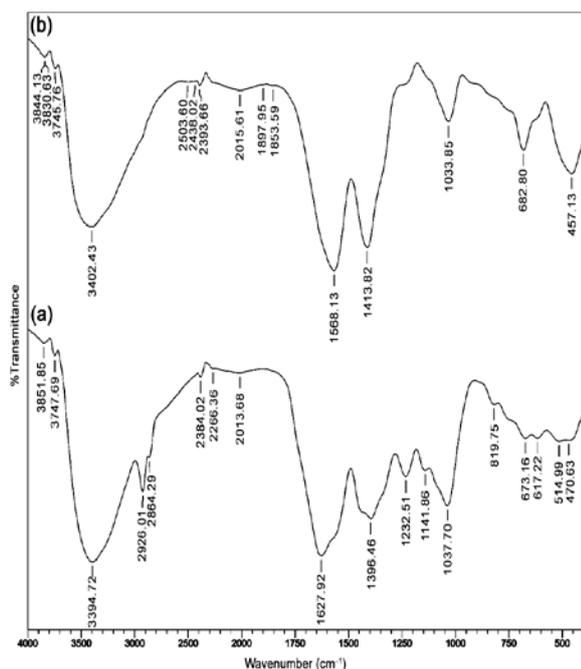


Fig. 4: FT-IR spectra of a) green tea extract and b) synthesized ZnO NPs.

Antimicrobial studies

The characteristic features of nanoparticles namely the larger aspect ratio renders greater surface area of contact with the microbial pathogens and provides enhanced reactivity. Additionally the smaller size of NPs facilitates easy entry into the microbial cell membrane and enables inhibition mechanisms to occur inside the

cell. ZnO NPs generate hydrogen peroxides which chemically interact with membrane proteins and lipid bilayers [25]. The antimicrobial activity of these NPs may involve both the production of reactive oxygen species (ROS) and the accumulation of NPs in the cytoplasm on the outer membranes. ROS causes membrane dysfunction [26] and cell death by oxidizing the membrane lipids [27]. Xia et al. [28] have suggested that smaller sized NPs can enter the mitochondria of cells through various pathways and thereby induce oxidative stress and cell death via apoptosis. It had been indicated by Liu et al. [29] that the ZnO NP may distort and damage bacterial cell membrane, causing leakage of intracellular contents leading to cell death. However, according to Jieng et al. [30] knowledge of exact mechanism of nanoparticle interaction with bacterial cell is still lacking.

Antibacterial assay

Pathogenic bacteria isolated from the clinical specimens were used in this study. The Gram-negative bacterial species, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli* and the Gram-positive *Staphylococcus aureus* had been used in the assay. 24 h fresh cultures were prepared and the standardized (McFarland No. 0.5) inoculum was made and used for the antibacterial assay.

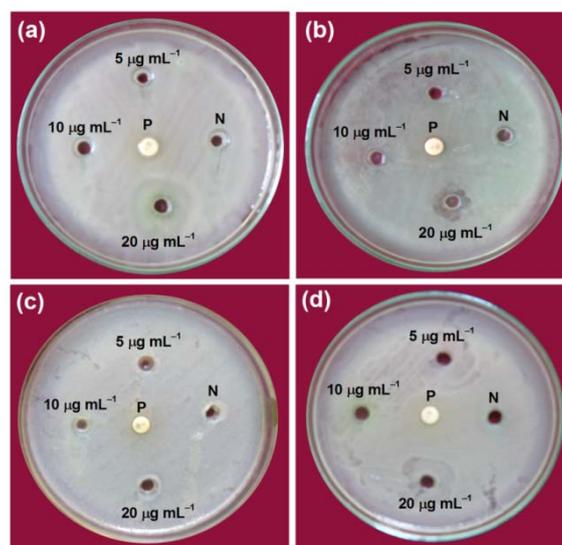


Fig. 5: Antibacterial activity of ZnO NPs against a) *K. pneumoniae*, b) *P. aeruginosa*, c) *E. coli* and d) *S. aureus* at different concentrations. Positive control: P, Negative control: N

The antibacterial activities of ZnO NPs against the studied pathogenic strains are shown in Fig. 5. The values of zone of inhibition obtained from the assay are presented in Table 1. All Gram-negative bacteria had shown good sensitivity towards the green synthesized ZnO NPs for the concentration $20\text{ }\mu\text{g mL}^{-1}$. It is quite interesting to note that all bacterial species tested in this study showed resistance to the synthetic antibiotic drug which in turn indicates the better antibacterial activity of the ZnO NPs than the commercially available synthetic drug.

Antifungal assay

Regarding the antifungal activity, all four fungal strains used in this study are found to be sensitive to the green synthesized ZnO NPs as well as to the commercially available antifungal drug Itraconazole. The antifungal activities of ZnO NPs are shown in Fig. 6 and the zone of inhibition values are presented in Table 2. The fungal species *Aspergillus flavus* had shown medium sensitivity to ZnO NPs with a concentration of $20\text{ }\mu\text{g mL}^{-1}$, whereas the remaining three fungal species showed good sensitivity to the ZnO NPs concentration of $20\text{ }\mu\text{g mL}^{-1}$. The encouraging aspect of this study is that the two fungal species *A. fumigatus* and *Penicillium sp.* are relatively more sensitive

to the ZnO NPs compared to the positive control. This may be due to the individual organisms response and their genotypic

characters which differs in their sensitivity pattern towards the single testing agent.

Table 1: Antibacterial activity of green synthesized ZnO NPs at different concentrations against pathogenic bacterial species of clinical sources.

Label	Bacteria	*Zone of inhibition (mm)			P
		Concentration of ZnO NPs ($\mu\text{g mL}^{-1}$)			
		5	10	20	
Gram-negative					
a	<i>K. pneumoniae</i>	-	-	10.3 \pm 0.57	-
b	<i>P. aeruginosa</i>	-	-	3.3 \pm 0.57	-
c	<i>E. coli</i>	-	-	-	-
Gram-positive					
d	<i>S. aureus</i>	-	2.3 \pm 0.57	5.3 \pm 0.57	-

Positive control : P, millimetre: mm, Microgram/millilitre: $\mu\text{g}/\text{mL}$

*zone of inhibition values are expressed as the mean of triplicate determination \pm standard deviation

Table 2: Antifungal activity of green synthesized ZnO NPs at different concentrations against pathogenic bacterial species of clinical sources.

Label	Fungi	*Zone of inhibition (mm)			P
		Concentration of ZnO NPs ($\mu\text{g mL}^{-1}$)			
		5	10	20	
a	<i>A. fumigatus</i>	-	-	5.3 \pm 0.57	3.3 \pm 0.57
b	<i>Penicillium sp.</i>	-	-	6.6 \pm 0.57	2.3 \pm 0.57
c	<i>A. flavus</i>	-	-	2.6 \pm 0.57	3.0 \pm 0.00
d	<i>A. niger</i>	-	-	3.0 \pm 1.00	2.3 \pm 0.57

Positive control: P, millimetre: mm, Microgram/millilitre: $\mu\text{g}/\text{mL}$

*zone of inhibition values are expressed as the mean of triplicate determination \pm standard deviation

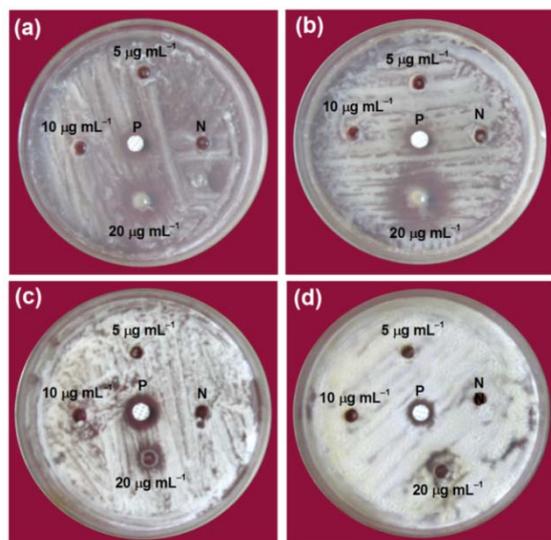


Fig. 6: Antifungal activity of ZnO NPs against a) *A. fumigatus*, b) *Penicillium sp.*, c) *A. flavus* and d) *A. niger* at different concentrations. Positive control: P, Negative control: N.

CONCLUSION

ZnO NPs were successfully produced by the green tea extract assisted synthesis. The blue-shifted UV-Vis absorption peak at 324 nm confirmed the nano-size of the synthesized ZnO particles. The average size of the NPs was 16 nm as obtained from XRD data. The FT-IR studies clearly indicated the reduction and capping biomolecules present in the green tea. The antibacterial assays revealed that the Gram-negative bacteria are sensitive to the ZnO NPs, while they showed resistance to the synthetic antibiotic.

Similarly the effective inhibitions of the fungi by the ZnO NPs are comparable to that of positive control.

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