Synthesis, characterization and antimicrobial activity of ZnO nanoparticles

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Abstract

Nowadays, methodologies of combining nanomaterials through ecological chemistry-based procedures have gained immense interest since nanomaterials have demonstrated to be of great use owing to their shape and size for numerous important applications. In the current examination, zinc oxide nanoparticles (ZnO NPs) were prepared using leaf extract of Catharanthus roseus (C.roseus). ZnO NPs were synthesized via both chemicals as well as biological methods which were subsequently confirmed by Dynamic Light Scattering (DLS). The effect of few physical parameters such as temperature (40 – 80 °C), reaction time (30 - 180 minutes), and stirring speed (200 – 400 rpm) was also investigated. The critical antibacterial action of biologically synthesized ZnO NPs has been observed against Bacillus pumilus MTCC 7092 and Saccharomyces cerevisiae MTCC 249T. The ZnO NPs have demonstrated antimicrobial adequacy against both the microbes. The study provides a rapid, cost-effective, and greener synthesis technique for ZnO NPs. According to DLS results, the average size of biologically synthesized ZnO NPs was 18 nm compared to the chemically synthesized ZnO NPs which had 1000 nm as their average size. NPs from both the methods were observed to be stable.

The biologically synthesized NPs were found to exhibit substantial antimicrobial activity as values of $1x10^{10}$ cfu/ml and $2.1x10^{10}$ cfu/mL were observed respectively for biologically synthesized ZnO NPs and the chemically synthesized ZnO NPs against B. pumilus. On the contrary, it was found to be $1.84x10^{10}$ cfu/mL in biologically synthesized ZnO NPs and $2.3x10^{10}$ cfu/mL in chemically synthesized ZnO NPs against S.cerevisiae. Since the lower cfu/ml as observed with biologically synthesized ZnO NPs implies inhibition of microbial growth, it can be concluded that biologically synthesized ZnO NPs exhibit better antimicrobial activity. Thus, ZnO NPs can be utilized as an antimicrobial agent against multidrug-resistant microorganisms. The associated research focused on the study of the concentration of ZnO NPs showing biological activity against pathogens.

Keywords: Nanobiotechnology, ZnO Nanoparticles, Green synthesis, *Catharanthus roseus*, Leaf extract.

Introduction

Nanoparticles (NPs) are referred to as the particles which range from 1-100 nm in size; because of this fluctuating size they diverge from there bulk resources in properties[1]. Their properties are exceptional, hence they have various applications like heavy industry, food science[2], information-technology[3] etc., but specifically have gained substantial notice in the area of medical sciences[4] such as nanodrugs and nano vaccines. Even though the information about the biocompatibility and threats of contact to nanoparticles is accurate, contact with these nanoparticles for humans can be spontaneous, for example, industrial contact or willingly comparable to the use of nano-enabled customer merchandises[5].

Exhaustive investigations in this field show the negative effects of nanoparticles *in-vitro* cellular systems. It is quite uncertain whether the available facts could accomplish the antagonistic properties of nanoparticles[5]. Therefore, an understanding of these molecular mechanisms of nanoparticles at a biological system interface is required.

To study the biological activities of the nanoparticles, different synthesis methods are being used so that an effective and efficient method can be developed against harmful microbes and environmental pollutants. Nanoparticles are synthesized having different structures playing a vibrant role in regulating their physical, chemical, optical and electronic properties. Imaging biomolecules, biological sheaths and tissues are the key research areas preferred by nanobiology scholars along with other issues which embrace the use of array devices and their application in nanophotonics for the elucidation of the molecular procedures in alive cells[6].

Nanoparticles can be synthesized by either top-down (disruption down of bulk material to nano-sized elements) or bottom-up (miniaturization of materials)[7]. Physical, chemical, or biological methods can be explored for NPs synthesis. The most frequently used is chemical synthesis involving multiple steps and is more noxious and harmful to the environment. The shortcomings of the chemical synthesis method can be decreased via green synthesis which embraces biological routes using microbes, floras, viruses, or their derivatives such as proteins and lipids[8]. In plant-based synthesis, diverse fragments can be used for the formation of nanoparticles like roots, leaves, shoot floret, etc.[9-11].

Several floras are having therapeutic properties like *Andrographis paniculata, Phyllanthus niruri*, and *Tinospora*

cordifolia[12], but their accessibility is rare. Hence, *C.roseus* becomes an imperative curative shrub because of its ease of availability, it can be probed for synthesizing nanoparticles via a biological method. Conventionally *Catharanthus roseus* has been used as a traditional treatment for diabetes, high blood pressure, and diarrhea[13]. Alkaloids and chemotherapeutic mediators from *C.roseus* are identified for their ache relieving activity in cancer treatment[10,11]. The bush is recognized to be effective for ailments like leukemia and diabetes[14] and is cultured mainly for its alkaloid content having anti-cancerous actions[15].

The techniques which are involved for characterization of nanoparticles and its interaction with protein are Ultraviolet-Visible spectroscopy (UV-Vis), Dynamic Light Scattering (DLS), Fourier Transform Infrared Spectroscopy (FTIR), Transmission Electron Microscopy (TEM), Fluorescence Spectroscopy, Scanning Electron Microscopy (SEM), Circular Dichroism (CD)[16,17].

Nanoparticles are effective against the toxic waste generated as a result of industrialization[7-11,15,17]. ZnO NPs show higher toxicity impacts than other metallic oxides NPs, for example, TiO_2[18-20] likely due to their particle shedding capacity. ZnO NPs show low dissolvability under unbiased conditions, however are promptly solvent under acidic conditions, for example, in lysosomes. As discussed earlier, numerous aspects are accountable for triggering of cyto-and-genotoxicity such as scattering, exterior functionalization, size, and concentration of NPs[21,22]. The focus of the current work is on the ZnO NPs for their biological activity against microorganisms and other environmental pollutants. In this work, different methods for organic synthesis of ZnO NPs using definite biomolecules existing in the plant extracts as pioneers exhibiting the biological activities of the synthesized nanoparticles have been explored.

Material and Methods

Materials: Fresh leaves of *Catharanthus roseus* were procured from the University Institute of Engineering and Technology, Chandigarh, India. Zinc Chloride ($ZnCl_2$), Sodium thiosulfate ($Na_2S_2O_3.5H_2O$), Yeast extract, dextrose, peptone and agar were used in analytical grade (Thermo Fischer Scientific, USA). *Saccharomyces cerevisiae* and *Bacillus pumilus* were procured from the Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh.

Methods

Preparation of Leaf extract: Fresh leaves of *C.roseus* were rinsed thoroughly using deionized water and 70% ethanol followed by air drying at room temperature. The washed leaves (8 g) were boiled in deionized water for about 15-20 minutes until the pigments got absorbed with subsequent extraction using the Whatmann filter paper. The leaf extract so obtained was stored at 37 ºC. This was supposed to be used in a weeks' time. The obtained leaf extract is speculated to act as the capping as well as the reducing agent during the nanoparticle synthesis.

Synthesis of Zinc Nanoparticles *via* green synthesis: 1 M $ZnCl_2$ was freshly prepared in DI water and then leaf extract was added to keeping it on constant stirring for incubation at room temperature. A brownish-yellow solution was formed signifying the formation of ZnO NPs. Aqueous zinc ions could be reduced by aqueous plant extract forming stable ZnO NPs as shown in fig. 1.

Synthesis of Zinc Nanoparticles via chemical synthesis: 8 mL freshly prepared $Na_2S_2O_3.5H_2O$ (1 M) and 10 mL $ZnCl_2$ (1 M) were used in the first phase of the synthesis followed by second addition of 2 mL $Na_2S_2O_3.5H_2O$ at 9 minutes as the stabilizing agent. The solution was observed for the settling of the precipitate which signified the completion of reaction followed by centrifugation to remove the precipitate that was formed. As a result, a slight milky colored solution indicated the formation of ZnO NPs and confirmed that $Na_2S_2O_3.5H_2O$ can be used as the reducing agent as shown in fig. 2.

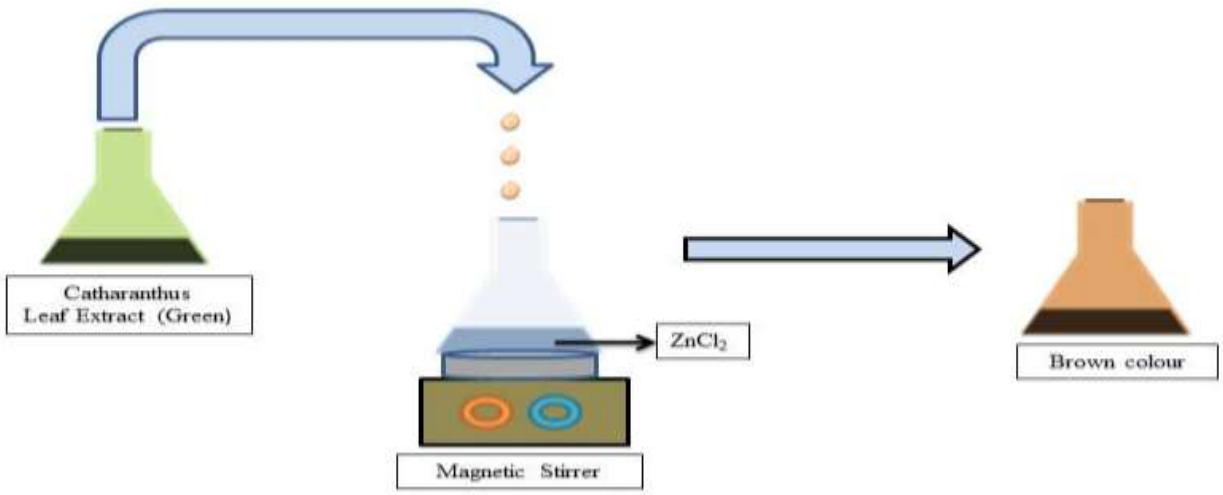

Fig. 1: Schematic reduction of $ZnCl_2$ by leaf extract

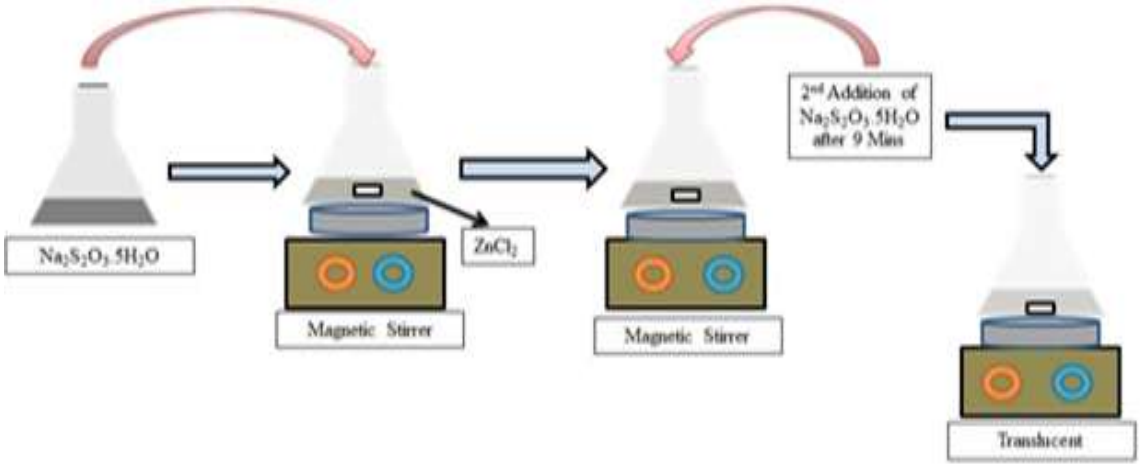

Fig. 2: Schematic reduction of $ZnCl_2$ by $Na_2S_2O_3.5H_2O$

Isolation and maintenance of the microbial cultures: The cultures were procured from MTCC IMTECH, Chandigarh in the lyophilized state. The lyophilized cultures were grown in their respective growth media and were kept at 37 °C (*Bacillus pumilus*) and 30 °C (*Saccharomyces cerevisiae*) for 24 hours. *Bacillus pumilus* was grown in nutrient agar (NA) at 37 °C for 24 hours *S.cerevisiae* was grown in yeast extract peptone dextrose agar (YPDA) medium at 30 °C for 24 hours. The cultures were subsequently sub-cultured to have viable microbes. Further, glycerol stocks were made to preserve the microbes for future use.

Characterization of Nanoparticles using DLS: DLS is based on the principle of "Brownian motion" in which a sample is illuminated by a laser beam and the fluctuations of the scattered light are detected at a known scattering angle θ by a fast photon detector. It determines the mean particle size in the limited size range. The parameters which are required to obtain the average hydrodynamic size of NPs are obtained from the instrument itself. It is calculated using the formula:

$D= kT/6\pi\eta R$

where D= Diffusion coefficient, R= Particle radius, k= Boltzmann constant, T= Temperature in Kelvin and Ŋ= Shear viscosity of the solvent.

Antimicrobial activity: The antimicrobial activity of ZnO NPs was determined against *B.pumilus* (MTCC 7092) and *S.cerevisiae* (MTCC 249T). The cultures were grown in the media plates for about 24-48 hours at 37 °C and 30 °C. The growth media were supplemented with $ZnCl_2$, $Na_2S_2O_3.5H_2O$ as well as chemically and biologically synthesized NPs. Further, these were serially diluted up to 10^{10} dilution factor. The dilutions were subject to control a (media with culture), control b (media + culture + $ZnCl_2$) and control c (media + culture + leaf extract/ $Na_2S_2O_3.5H_2O$).

The test sample comprised of biologically synthesized ZnO NPs to which the diluted culture was subjected whereas in other cases it comprised of chemically synthesized ZnO NPs to study the difference in the growth of the organisms. The controls have been referred to as (a), (b) and (c) respectively in the discussion, and the test sample has been labeled as (d). The antimicrobial activity of ZnO NPs was determined by observing the growth of the cultures after 24 hours of incubation at 37 °C and 30 °C.

Results and Discussion

The synthesis of ZnO NPs by chemical and green synthesis has been confirmed by the characterization technique which was performed using Zetasizer using Dynamic Light Scattering (DLS) method which determines zeta potential and polydispersity index. The size and stability of synthesized particles were confirmed by DLS measurement and zeta potential observed in fig. 3(a) and (b). The average hydrodynamic size was found to be 18 nm and +28mV for biologically synthesized nanoparticles whereas chemically synthesized NPs were found to be more than 1000 nm and -33.97 mV. In both the synthesis, the particles observed were stable.

Further, the activity of ZnO NPs was investigated against *B.pumilus* and *S.cerevisiae* in which it was detected that the biologically synthesized NPs were more efficient in arresting the growth of the microbes compared to their counterparts synthesized chemically by observing the growth patterns as shown in figures 4, 5, 6 and 7. The same results have been qualitatively depicted in table 1. The colony-forming unit (CFU) method was used to evaluate the bactericidal property of ZnO NPs. The cultures were spread on the culture plates and counted manually using the formula which is as follows:

CFU/mL= (No. of colonies x Dilution factor)/ Volume of culture used on plates

Figure 4 shows the effect of biologically synthesized NPs on *B.pumilus*. It was observed that the substantial growth could be achieved after 24 hrs of incubation in case of control experiment (a) as well as there was slight and overgrowth in control experiments (b), and (c) whereas reduced growth

was observed in the test sample (d). On the contrary, consistent growth could be observed in case of controls (a) and (c) establishing that the leaf extract was more capable in arresting the growth of *B. pumillus* compared to sodium thiosulphate used for the chemical preparation of ZnO NPs as shown in figure 5. However, the growth of *B. pumillus* could be controlled in the presence of biologically synthesized NPs. Similar investigations were also carried out for *S. cerevisiae*.

The plates containing *B.pumilus* culture were recorded to have about $3.1x10^{10}$ cfu/mL in control (a) $3x10^{10}$ in control (b) overgrowth in control (c) and treatment with biologically synthesized NPs gave $1x10^{10}$ cfu/mL whereas similar results were observed concerning control (a) and (b) in chemically synthesized but in control (c) in which $Na_2S_2O_3.5H_2O$ was added. It was observed that slight decrease in growth was obtained as the cfu/mL observed was $3.5x10^{10}$ and with treatment with chemically synthesized NPs, it was found to be $2.7x10^{10}$ cfu/mL.

In case of *S.cerevisiae* it was observed to have $3.4x10^{10}$ cfu/mL in control (a) $3.1x10^{10}$ in control (b) $2.52x10^{10}$ in control (c) and treatment with biologically synthesized NPs, it was observed to have $1.84x10^{10}$ cfu/mL whereas similar results were obtained concerning control (a) and (b) in chemically synthesized but in control (c) in which $Na_2S_2O_3.5H_2O$ was used, cfu/mL $2.6x10^{10}$ was obtained and on treatment with chemically synthesized NPs it was found to be $2.3x10^{10}$ cfu/mL. It is visible that biologically synthesized NPs have greater efficiency to kill microorganisms when compared with to chemically synthesized ones.

As the two synthesis methods produced different sized NPs, it can be speculated that this variation in size must have led to the different biological activity possessed by these synthesized NPs. The size of the chemically synthesized NPs was established to be 1000 nm as per DLS characterization whereas the smaller particle size of 10-100 nm was achieved from biological synthesis. Smaller particles are assumed to have enhanced interaction with microorganisms owing to their increased surface area, thus improving their biological activity against the microbes. The bigger size nanoparticles obtained via chemical synthesis could be explained based on the aggregation of the particles.

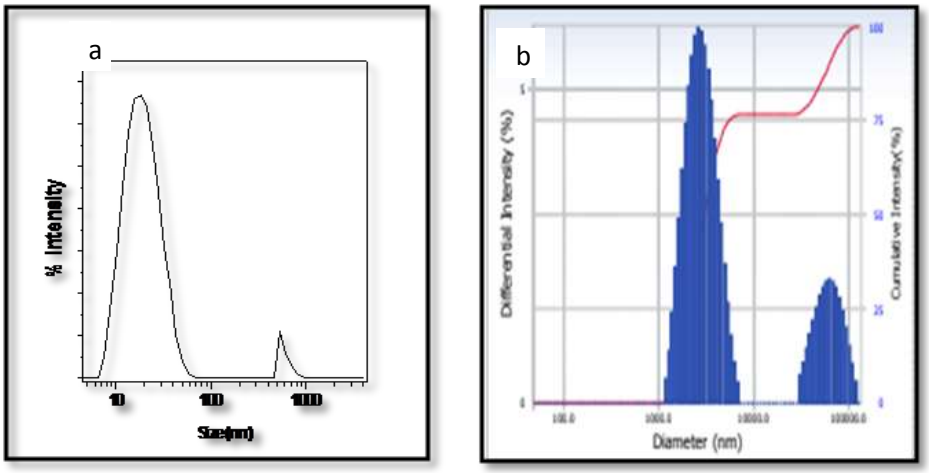

Fig. 3: DLS studies showing the size distribution of (a) green synthesized NPs and (b) chemically synthesized NPs.

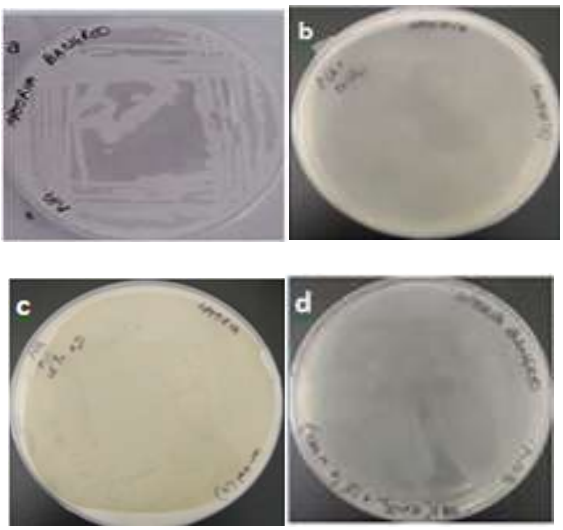

Fig. 4: Effect of biologically synthesized NPs on *Bacillus pumilus*

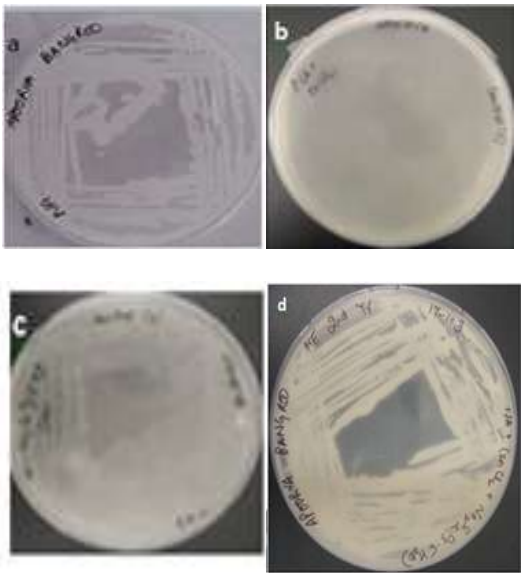

Fig. 5: Effect of chemically synthesized NPs on *Bacillus pumilus*

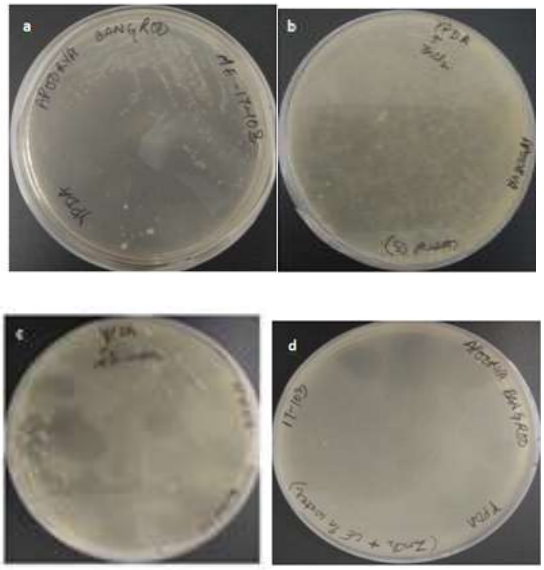

Fig. 6: Effect of biologically synthesized NPs on *Saccharomyces cerevisiae*

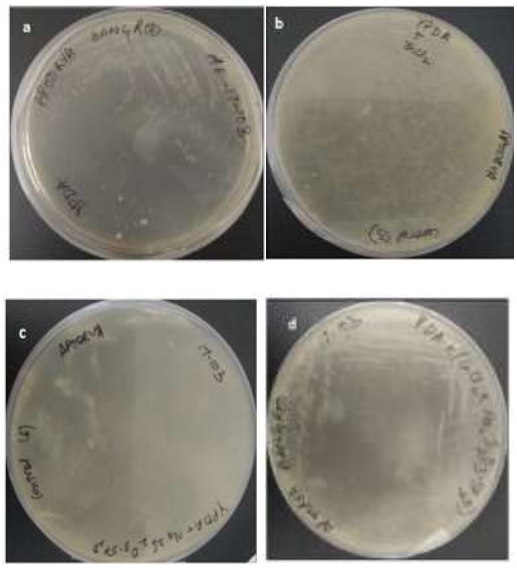

Fig. 7: Effect of chemically synthesized NPs on *Saccharomyces cerevisiae*

Table 1
Qualitative analysis of antimicrobial activity of ZnO NPs

Treatment	Test Samples	
	B.pumilus	***S.cerevisiae***
a	+	+
b	+	+
c	+	+
d	+++	+
e	++	++
f	+	-

(+) growth, (++) enhanced growth, (+++) over growth and (-) no growth

Conclusion

Study and developments in nanobiotechnology are renowned explanations for acknowledging this technology in routine life because it tends to deliver responses and alternatives to scientific, ecological, and health challenges. In the present manuscript, the use of different nanoparticles on different microorganisms has been investigated. With the growth pattern of the microorganisms, it was observed that plant synthesized NPs can effectively reduce the growth of microorganisms in comparison to the ones synthesized chemically.

Hence the mechanism of the nanoparticle can be understood inhibiting the growth of microorganisms which would give useful probe for designing the harmless and value-added nanoparticles for healing or treating several ailments which are correlated to pathogenic microorganisms and can be used as investigative kit or tool. Further, the investigation on the potential of nanoparticles against different pesticides and heavy metal water is proposed.

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