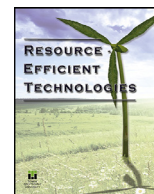




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Synthesis of zinc oxide nanoparticles using plant leaf extract against urinary tract infection pathogen

J. Santhoshkumar, S. Venkat Kumar, S. Rajeshkumar*

School of Biosciences and Technology, VIT University, Vellore 632014, TN, India

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ABSTRACT

In modern science, Nanotechnology is an ablaze field for the researchers. Zinc oxide nanoparticles (ZnO NPs) are known to be one of the most multifunctional inorganic nanoparticles with its application in treatment of urinary tract infection. Nanoparticles were synthesized using *Passiflora caerulea* fresh leaf extract and were characterized by UV-visible spectroscopy (UV-vis), X-ray diffractometer (XRD), Fourier transform infrared spectroscopy (FT-IR), Scanning electron microscopy (SEM), Energy dispersive analysis of x-ray (EDAX), Atomic force microscopy (AFM). Therefore, the study reveals an efficient, eco-friendly and simple method for the green synthesis of multifunctional ZnO NPs using *P. caerulea*. Urinary tract infection causing microbes were isolated from the disease affected patient urine sample. The synthesized nanoparticles have been tested against the pathogenic culture showed a very good zone of inhibition compared with plant extract. It indicates the biomedical capability of ZnO NPs.

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1. Introduction

Nanotechnology involves the use of materials having nanoscale dimensions in the range of 1–100 nm. Operating with nanomaterials has allowed researchers to have a much better understanding of biology. The green synthesis of nanoparticles has greatly reduced the use of physical and chemical methods. Various chemical methods have been proposed for the synthesis of zinc oxide nanoparticles (ZnO NPs), such as reaction of zinc with alcohol, vapor transport, hydrothermal synthesis, precipitation method etc [1–4]. The use of green synthesis method by the researchers is rapidly increasing due to usage of less toxic chemicals, eco-friendly nature and one step synthesis of nanoparticles [5]. The biological system involved in the green synthesis of nanoparticles are plants and their derivatives, microorganisms like bacteria, fungi, algae, yeast [6–9]. *P. caerulea* L. (Passifloraceae) is a medicinal plant native to South America, used against different pathogens causing various diseases such as diarrhea and their medicinal activity has been reported in many animal models [10]. Biosynthesis of ZnO NPs from plants such as *Aloe vera*, *Sargassum muticum*, *Eichhornia crassipes*, *Borassus flabellifer* fruit, and also in some bacterial and fungal species such as *Bacillus subtilis* and *Escherichia coli*, Ureolytic bac-

teria, *Lactobacillus plantarum* have been reported [11]. Nanoparticles synthesis within the size range of 10–100 nm have become an extensive research and concern due to their potential application in wide areas of science and technology. Metal oxide nanoparticles have been extensively used for medicinal purposes in the past decades. Metal oxide nanoparticles has environmental applications as it can act as catalyst which is helpful in reduction or elimination of the toxic hazardous chemicals from the environment [12]. Some of the metal oxide nanoparticles like, Fe₃O₄, TiO₂, CuO and ZnO are thoroughly been investigated for their various biological activity [13]. Among those, ZnO NPs are used in the elimination of toxic chemicals like arsenic, sulfur from water sources owing to their large surface area by volume ratio than the bulk materials [14]. Zinc oxide has remarkable application in micro- electronics, diagnostics, optoelectronic devices, biomolecular detection, surface acoustic wave devices like laser devices, electromagnetic coupled sensor [1]. They can act as an alternative source for degradation of atmospheric pollutants [14,15].

They also have potential application in the field of medicine like drug delivery, biological activities such as antimicrobial, antioxidant, etc., and diagnosis of diseases. Antimicrobial activity of ZnO NPs against various pathogens such as *B. subtilis*, *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus* and *E. coli* using disc diffusion method has been reported [16,17]. Also reported similar results by *Punica granatum* mediated synthesized ZnO NPs [19]. Some of the proposed mechanism responsible for antibacterial ac-

* Corresponding author.

E-mail addresses: ssrajeshkumar@hotmail.com, rajeshkumar.s@vit.ac.in (S. Rajeshkumar).

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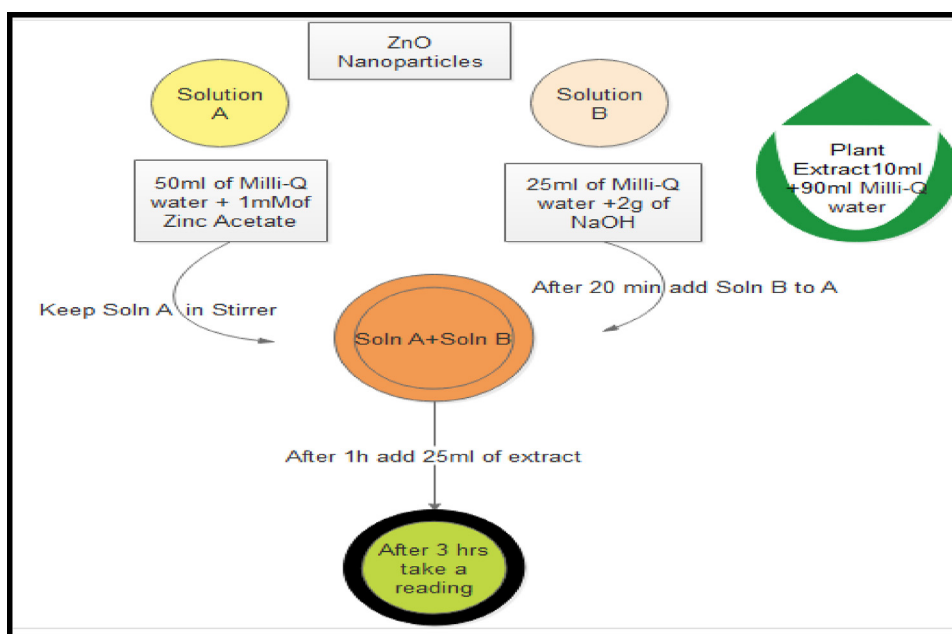


Fig. 1. Schematic representation of synthesis of ZnO NPs.

tivity of ZnO NPs includes disruption of the cell membrane, oxidative stress induction and generation of reactive oxygen species (ROS). It was reported that ZnO NP has showed high antibacterial activity against urinary infection disease [20]. Further the antibacterial activity of these biological synthesized ZnO NPs was evaluated against different pathogenic microorganisms. Our aim of the study is to synthesis ZnO NPs from the plant *P. caerulea* L. and their antibacterial activity against pathogens causing urinary tract infection.

2. Materials and methods

2.1. Plant collection

The leaves of *P. caerulea* L. (Passifloraceae) was collected in VIT. It is a medicinally important plant. Fresh green leaves, stem and flowers were harvested during the months of September to January.

2.2. Preparation of the plant extract

5 g of fresh leaves were washed with running tap water followed by Milli-Q water and then cut, and soaked in a 250 mL Erlenmeyer flask containing 100 mL Milli-Q. The solution was boiled at 70 °C for 8 min. The leaf extract was allowed to cool to room temperature, filtered through Whatman number-1 filter paper, and the filtrate was stored for further experimental use.

2.3. Synthesis of ZnO NPs

1 mM Zinc acetate [$\text{Zn}(\text{O}_2\text{CCH}_3)_2(\text{H}_2\text{O})_2$] was dissolved in 50 ml Milli-Q water and kept in stirrer for 1 h respectively [4]. Then 20 mL of NaOH solution was slowly added into the Zinc acetate solution and 25 mL of plant extract was added to the same. (Fig. 1). The color of the reaction mixture was changed after 1 h of incubation time. The solution was left in stirrer for 3 h Yellow color appeared after the incubation time confirmed the synthesis of ZnO NPs. The precipitate was separated from the reaction solution by centrifugation at 8000 rpm at 60 °C for 15 min and pellet was col-

lected. Pellet was dried using a hot air oven operating at 80 °C for 2 h and preserved in air-tight bottles for further studies.

2.4. Characterization of biosynthesized ZnO NPs

Optical properties of ZnO NPs were characterized based on UV absorption spectra with the wavelength range of 300–500 nm. X-ray diffraction (XRD) analysis was performed on X-ray diffractometer (PAN analytical X-Pert PRO) operating at 30 kV and 40 mA. The pattern was recorded by $\text{CuK}\alpha$ radiation with about 1.54060 Å X-ray crystallography is used for the determination of crystal density, purity and size of the nanoparticles. 2 mg of ZnO NPs was mixed with 200 mg of potassium bromide (FTIR grade) and pressed into a pellet for FTIR characterization. The sample pellet was placed into the sample holder and FTIR spectra were recorded in FTIR spectroscopy at a resolution of 4 cm^{-1} [18]. Shape and size were analyzed by using SEM. Topography of the nanoparticles was characterized using Atomic Force Microscopy (AFM). Elemental compositions of the synthesized nanoparticle were characterized using Elemental Dispersion Analysis of X-ray (EDAX).

2.5. Antibacterial activity of synthesized ZnO NPs

The antibacterial activity of synthesized ZnO NPs was performed by agar disc diffusion method against urinary tract infection pathogens *Klebsiella pneumonia*, *B. subtilis*, *E. coli*, *Serratia sp.*, and *Streptococcus sp.* Fresh overnight culture of each strain was swabbed uniformly onto the individual plates. The 25 μl , 50 μl and 75 μl of ZnO NPs solution impregnated disc were placed onto the plates and incubated for 24 h at 37 °C. Commercial antibiotic discs were placed as control. After incubation period got over, different levels of zonation formed around the disc was measured.

3. Results and discussion

3.1. Visual observation

ZnO NPs have attracted great attention because of their superior optical properties. Visual color change is the preliminary test for nanoparticle synthesis. (Fig. 2) represents the synthesis of ZnO

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