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Green synthesis and evaluation of antibacterial activity of zinc nanoparticles from *Calotropis procera* leaves

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Abstract
Zinc nanoparticles (ZnNPs) was experimentally synthesized by employing *Calotropis procera* leaves extract having particle size of 12.08 nm. The color of the ZnO / *Calotropis procera* extract solution changed from colorless dark orange brownish. Three infrared bands are observed at 3468 cm^{-1}, 1646 cm^{-1}, 1204 cm^{-1}, and 408 cm^{-1}, indicating the presence of proteins as capping agent for Zinc nanoparticles. The antimicrobial action of green synthesized ZnNPs was studied against *Escherichia coli* using agar plate method. As compared to control which showed 35 % zone of inhibition, the green synthesized nanoparticles significantly increased the zone of inhibition to 49 %. The research findings exhibited that ZnNPs revealed a strong antibacterial action and hence might be propagated as a new molecule having antimicrobial action for the curing of bacterial infection including multidrug resistant bacterial infection.

Keywords: *Calotropis procera*, *Escherichia coli*, nanoparticles, zinc

Introduction
Nanotechnology refers to that science which manipulates substance on an atomic, molecular and macromolecular at nano scale level [1]. The main principle of nanotechnology is to raise the surface area which manipulate particle in designing, characterize, manufacture, and practical application of structures at the atomic level around 0.20 nm up to around 100 nm. It presents improved, smarter, durable, hygienic and secure products for the home, communications, medicine, transportation, agriculture and for industry in general. Nano science is an innovator, revolutionary, transformative, most powerful, and highly potential, very dangerous as well as beneficial technology. In nanotechnology materials are reduced in size to the level of 10 [9] range exhibits diverse potentials compared to what, they showed on a macro scale level. Among the metallic nanoparticles, Zinc nanoparticles (ZnNPs) have gained increasingly attention due to its unique physical, biological and chemical properties [2]. ZnNPs are renowned to exhibit a high antimicrobial action against a variety of microorganisms such as bacteria, virus, and fungus due to their smaller size and higher surface area [3]. ZnNPs are also widely used for their anti - fungal, anti-inflammatory, and anti-viral properties.
The presence of multidrug resistance pathogens have increased the number of infectious disease and became the main cause of death in the world as per WHO. Broadly misuse and highly abuse of antibiotics are the prime reason of antibiotic resistance in the bacteria. Multidrug resistant strains of bacterial infection may lead to numerous impacts including increase of mortality and morbidity rates, prolong of hospitalization period, and economic loss detected multi-drug resistant non-typhoidal Salmonella among migrant food handlers, which may cause cross-contamination to the food products [4]. Thus, the development of a new and natural antimicrobial agent is needed as there is a growing concern in multidrug resistant food borne pathogens [5].
Calotropis procera is a species of flowering plant in the family Apocynaceae that is native to North Africa, Pakistan, tropical Africa, Western Asia, South Asia, and Indochina [6]. The green fruits contain a toxic milky sap that is extremely bitter and turns into a gluey coating which is resistant to soap. In the present study *Calotropis procera* was used to synthesize ZnNPs. The aim of the current research work study was to determine the antibacterial activity of green synthesized ZnNPs against *E. coli* by employing agar plate method.

Materials and Methods
Plant Material
Calotropis procera was obtained from botanical garden of OSGU, Hisar.

Chemicals and other materials: Zinc oxide (ZnO) was purchased from Sigma - Aldrich. All other chemicals and materials used for the present study wer of analytical grade.

Preparation of nutrient agar: Definite volumes of peptone (0.6%), yeast extract (0.15%) and di-potassium dihydrogen phosphate buffer (0.36%) was dissolved in double distilled H_2O and the pH was adjusted to 7.2. The prepared solution was sterilized by autoclaving at 15 psi pressure for 10 min [7].

Preparation of Zinc Nanoparticles

The *Calotropis procera* leaves extract was synthesized by following procedure. 20 g of *Calotropis procera* leaves were added to 1000 mL beaker along with 80 mL of distilled H_2O and maintained at 80 °C for 15 min previous to decanting it. The synthesized solution was filtered by 0.45 µm Milipore filter membrane and followed by 0.2 µm Millipore membrane filter. For synthesis of zinc nanoparticles, 150 mL of ZnO (1 mM) was reacted with 15 mL of the *Calotropis procera* extract in Erlenmeyer flask at nearly room temperature. Color changes of the solution were observed. (Figure 1) The synthesized green ZnNPs were characterized by UV-visible spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and transmission electron microscopy.

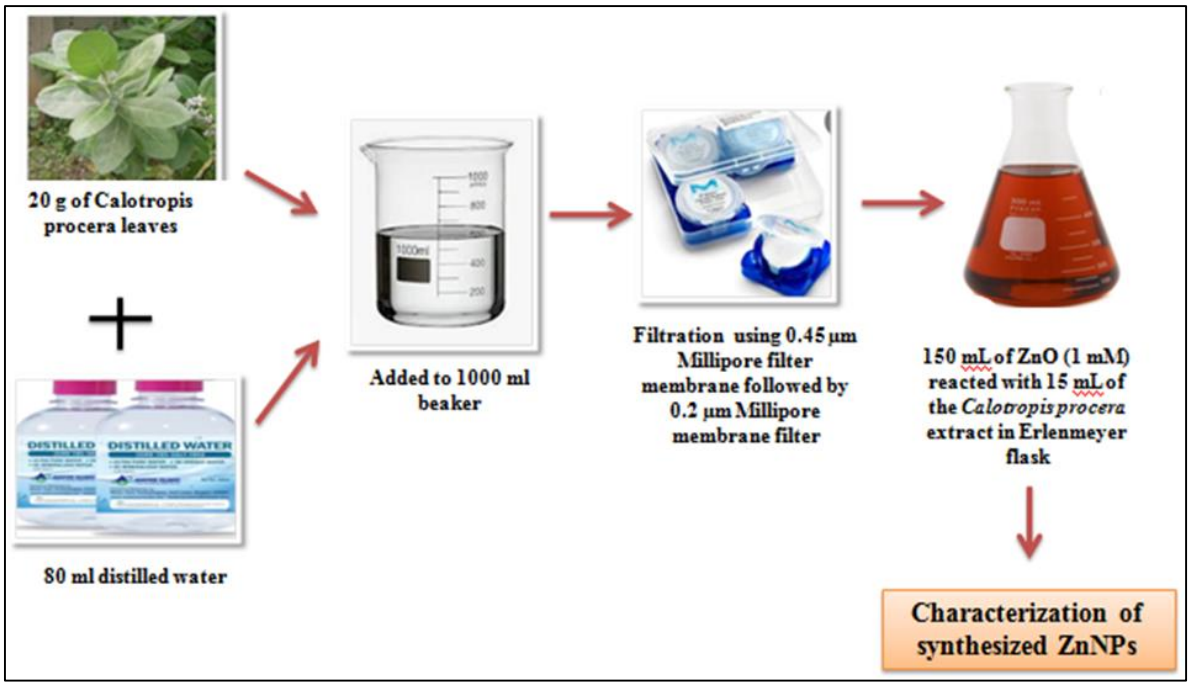

Fig 1: Preparation of Zinc Nanoparticles (ZnNPs) using *Calotropis procera leaves*

Characterization

The functional and composition of Zn nanoparticles were characterized by Fourier-Transform Infrared (FTIR, Perkin Elmer, Spectrum BX) spectroscopy in the range 4000- 280 cm^{-1}. [8] Additionally, the optical property of prepared Zn nanoparticles was analyzed via UV-visible (UV-Vis, Perkin Elmer, Lambda 35) absorption double beam spectrophotometer with a deuterium molecule and tungsten iodine lamp in the range from 300 - 600 nm at room temperature [9]. The morphology features of the prepared Zn nanoparticles were analyzed by instrument named as Transmission Electron Microscopy (TEM, Hitachi, H7100). Zn nanoparticles were sonicator for 15 min by a sonicator (50 Hz, Soniclean). Then, the dispersed solution was dipped to a copper grid at room temperature. After heat drying, sample was analyzed at voltage of 80 kV. The particle size distributions were determined using PSA.

Agar plate method

Agar plate method is one of the optimum methods where the test samples diffuse from the cup through an agar layer in a Petri dish or plate to such an extent that the growth of added microorganisms was restricted completely to a circular area or zone around the cavity containing the solution of a freshly prepared nanoparticles solution [10]. The antimicrobial activity was expressed as zone diameter in millimeters, which was measured by a scale. The zone of inhibition was measured by a scale and the measurements are tabulated. The samples were assessed by agar method, by comparing zone of inhibition between nanoparticles against control molecule [11].

Results and Discussion

Characterization of ZnNPs

The color change was noted by visual observation in the conical flask which contains ZnO solution with *Calotropis procera* extract. The color of the ZnO / *Calotropis procera* extract solution changed from colorless to light yellowish after 10 min and eventually to dark orange brownish. This color change indicates the synthesis of Zn nanoparticles in the solution. *Calotropis procera* extract without ZnO did not show any color changes.

The synthesis of Zn nanoparticles was further confirmed by using UV-visible spectroscopy (UV-vis), Fourier-Transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM). A broad absorption peak was observed at 355 nm, which is a characteristic absorption band peak for the Zn [12]. No other peak was observed in the spectrum which confirms that the synthesized products are Zn only.

FTIR measurement was carried out to recognize the possible biomolecules structure responsible for capping and reducing agent for the Zn nanoparticles prepared by *Calotropis procera* extract. Three infrared bands are observed at 3468 cm^{-1}, 1646

cm^{-1}, 1204 cm^{-1}, and 408 cm^{-1}. The intense broad band at 3468 cm^{-1} is due to N - H and O - H stretching mode in the linkage of the proteins. The medium intense band at 1646 cm^{-1} arises from the C = C stretching mode in amine I group which is commonly found in the protein, indicating the presence of proteins as capping agent for Zinc nanoparticles (Figure 2) which increases the stability of the nanoparticles synthesized [13]. On the other hand, the intense and broad peak at 408 cm^{-1} corresponded to the Zn metal.

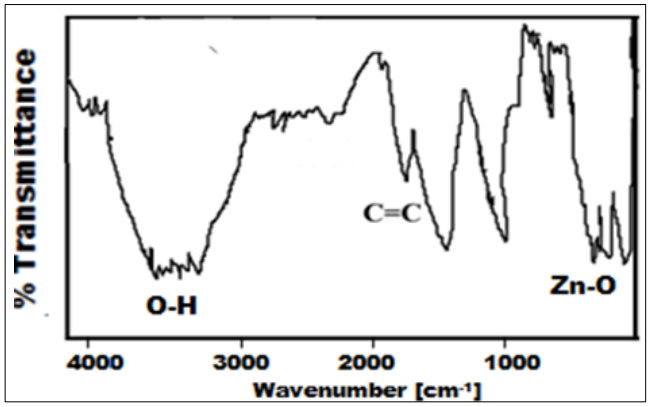

Fig 2: FTIR spectra of Synthesized Zinc Nanoparticles (ZnNPs) using *Calotropis procera leaves*

Transmission electron microscopy (TEM) has been employed to characterize the size, shape and morphology of synthesized Zinc nanoparticles [14]. The TEM image of zinc nanoparticles is shown in Figure 3. From the image, it is evident that the morphology of zinc nanoparticles is spherical which is in agreement with the shape of SPR band in the UV-vis spectrum. The average particles size measured from the TEM image is 12.08 nm (Figure 3).

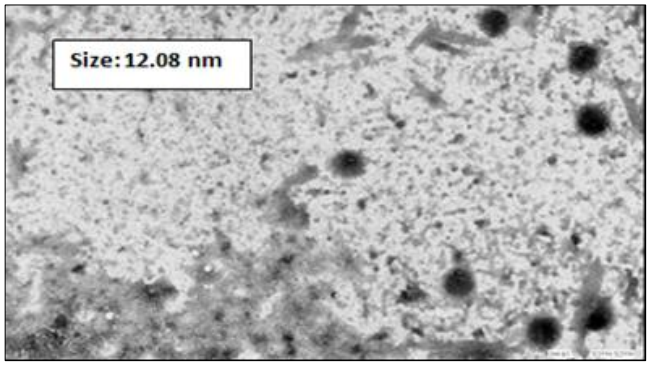

Fig 3: TEM image of nanoparticles

Antibacterial activity by agar plate method

Any color changes of the solution was observed. Zinc nanoparticles showed significant antibacterial activity against *E. coli*. The antimicrobial activity was expressed as zone diameter in millimeters, which was measured by a scale. The zone of inhibition was measured by a scale and the measurements were tabulated. As compared to control which showed 35% zone of inhibition, the green synthesized nanoparticles significantly increased the zone of inhibition to 49%. Thus, current findings suggested remarkable anti-microbial activity of green synthesized ZnNPs against *Escherichia coli* (Figure 4).

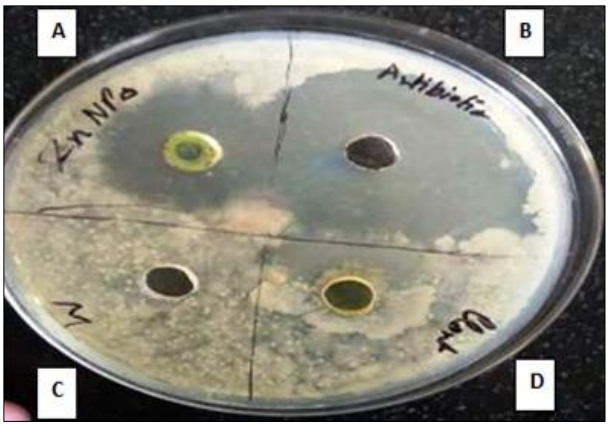

Fig 4: Visible clear zone produced by *Calotropis procera* extract mediated ZnNPs against E. coli. Using Agar plate. A: green synthesized ZnNPs; B: Only Antibiotic; C: Blank Water; D: Plant extract

The environmental friendly zinc nanoparticles synthesis processes have potential applications in various fields. Zinc nanoparticles can be functional in bio-labelling, food material packaging, antibacterial agent and drug delivery. Several studies on the usage of metal nanoparticles in the water filter have been carried out due to its antibacterial and as pesticide removal properties. Besides that, zinc nanoparticles play an important role in the medical area. Zinc nanoparticles act as biomarker in detection of early diagnosis and therapy monitoring such as the detection of tumor for cancer treatment and early diagnosis for Alzheimer's disease [15, 16].

Conclusion

During experiments, we reported a green approach for the synthesis of Zn nanoparticles using *Calotropis procera* leaves extract. It was concluded that the green synthesized zinc nanoparticles were composed of spherical particles which were highly crystalline. The particles sizes were controlled in the range from 5 to 15 nm. Furthermore, an increased zone of inhibition was also reported against *E.coli* by newly synthesized green ZnNPs. Hence, the present research work offers simple, green and efficient method to synthesize Zinc nanoparticles at normal temperature without using any harmful reducing agents such as sodium Borohydride and any capping or dispersing agent. Hence, the applications of ZnNPs might offer valuable services in diverse medical and non-medical fields.

Disclosure Statement

The author declares that there exist no relevant or material financial interests related to the research described in this paper.

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