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Biofabrication of Zinc Oxide Nanoparticles Using *Streptomyces cyaneofuscatus*

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Abstract. This article discusses a simple, cost-effective, and biocompatible method for biosynthesis of ZnONPs. For the first time, *Streptomyces cyaneofuscatus* cell free filterate, as a reducing and stabilizing agent, was used to prepare ZnONPs using zinc acetate as metal precursor. The prepared ZnONPs were characterized using multiple analytical techniques. The UV–Vis spectrum analysis of the biosynthesized ZnONPs exhibited an absorption peak at 375 nm. Fourier-transform infrared (FT-IR) spectroscopy suggested the presence of different functional groups of microbial biomolecules. XRD verified hexagonal wurtzite crystalline structure of ZnONPs. The zeta potential analysis showed good stability of ZnONPs (−26.6 mV). Furthermore, the EDX analysis explained the 73.11 % of zinc (Zn) and 26.89% of oxygen (O) elemental structure of fabricated ZnONPs which indicated the successful fabrication of ZnONPs by *S. cyaneofuscatus*.

1. Introduction

Nanotechnology is considered one of the most rapidly evolving fields, and it has great importance in different environemental, industrial and academic approaches, including biology, medicine, agriculture, engineering, chemistry and physics [1]. The widespread of nanotechnology applications emphasizes the need for sustainable ways to nanoparticle synthesis. The biological or green synthesis of nanoparticles has gained significant attention as a sustainable alternative to traditional physicochemical synthesis methods This approach utilizes biological entities such as microorganisms, enzymes and plant extracts to reduce metal ions precursors into nanoparticles, offering an environmentally friendly, cost-effective, and biocompatible routes for nanomaterial fabrication with many advantages, particularly in biomedical applications where purity and safety are very important factors [2]. Unlike conventional methods that often involve toxic chemical reagents and high energy consumption with health and environmental risks, green synthesis methods run under mild conditions with low energy and low environmental impact while maintaining high efficiency [3]. Microbial-derived nanoparticles are becoming a promising tool for nanoparticle synthesis. Microbes are fast growing, easy to cultivate and maintain and have ability to grow in different habitats. Also, microbial cells have ability to adapt in higher concentrations of metals and have specific potential to reduce metals into nanoparticles through their extracellular or intracellular mechanisms [4]. The fabrication of nanoparticles by microorganisms is achieved by microbial active biomolecules such as enzymes, proteins, amino acids and polysaccharides that act as reducing and stabilizing agents while controlling the size, shape, and dispersion of biosyntheized nanoparticles [5]. Particularly, zinc oxide is considered one of the most important inorganic oxides that has various applications like drug delivery, biosensors, photocatalytic degradation and personal care cosmetics due to its unique features such as low toxicity, high surface area [6]. Therefore, this research highlights the green approach used to fabricate zinc oxide nanoparticles (ZnONPs) using *Streptomyces cyaneofuscatus*. Standard protocol applied to determine the structure and morphological characterization of these biosynthesised ZnONPs.

2. Materials and methods

2.1. Microorganism and biosynthesis method

*Streptomyces cyaneofus*catus was previsouly collected from Marriott Lake, Alexandria, Egypt, and it was subjected to molecular identification and deposited in the GenBank database with accession number KY964508 by [7]. For extracellular fabrication of ZnONPs, cell-free filtrate of *S. cyaneofuscatus* fresh culture (100 mL) was mixed with the exact volume (1:1 (v/v)) of zinc acettate in the reaction vessels. After that, the reaction mixture was incubated under shaking condition at 150 rpm for 24 h at 30 °C. Two control flasks, medium containing *S. cyaneofuscatus* without zinc acetate and medium containing zinc acetate without *S. cyaneofuscatus*, were incubated under the same conditions of test flasks.

Changes in the reaction color and apperance of a white precipitate indicated the successful synthesis of ZnONPs. At the end of the experiment, the precipitate containing ZnONPs was harvested using centrifugation at 10,000 rpm for 20 min. Subsequently, the collected pellet was washed thrice with distilled water to eradicate any residues, and eventually dried in oven at 60°C for residual moisture removal. The dried white powder was further calcined for 2 hours at 300°C.

2.2. Characterization of biosyntheized ZnONPs

The ZnONPs were characterized by applying multiple analytical techniques.

2.2.1. Optical properties

In order to evaluate the optical features and charecteristic peaks of biosynthesized ZnONPs, UV-Visible spectrophotometer PerkinElmer was used in the range of 200–800 nm.

2.2.2. Functional properties

Fourier transform infrared (FTIR) spectral analysis was carried out using (FT/IR spectrophotometer, PerkinElmer, USA) at infra-red spectra range of 4000–400 cm^{-1} to define the functional groups in *S. cyaneofuscatus* -mediated ZnONPs.

2.2.3. Structural properties

The crystallinty of the biosyntheized ZnONPs were examined using Shimadzu XRD-6100 X-ray diffractometer in the scattering range (2θ) of 20–80°. Finally, data was analyzed by using JCPDS database.

2.2.4. Surface charge analysis

The charge and particle size of the biosynthesised ZnONPs, were detected using Dynamic light scattering (DLS) measurments on a ZetaSizer Nano Series Ver.7.02 (Malvern Ltd, UK).

2.2.5. Morphological features

The morphology and size of ZnONPs were investigated by transmission electron microscope (TEM; H7500, Hitachi, Japan).

2.2.6. Elemental characteristics

The elemental analysis of ZnONPs was conducted using EDX (JEOL JSM-6010LV, Japan).

3. Result and discussion

3.1. Biosynthesis process of ZnONPs

The formation of a white precipitate after adding zinc acetate to culture of *S. cyaneofuscatus* indicated the successful synthesis of ZnONPs as shown in Fig.1.

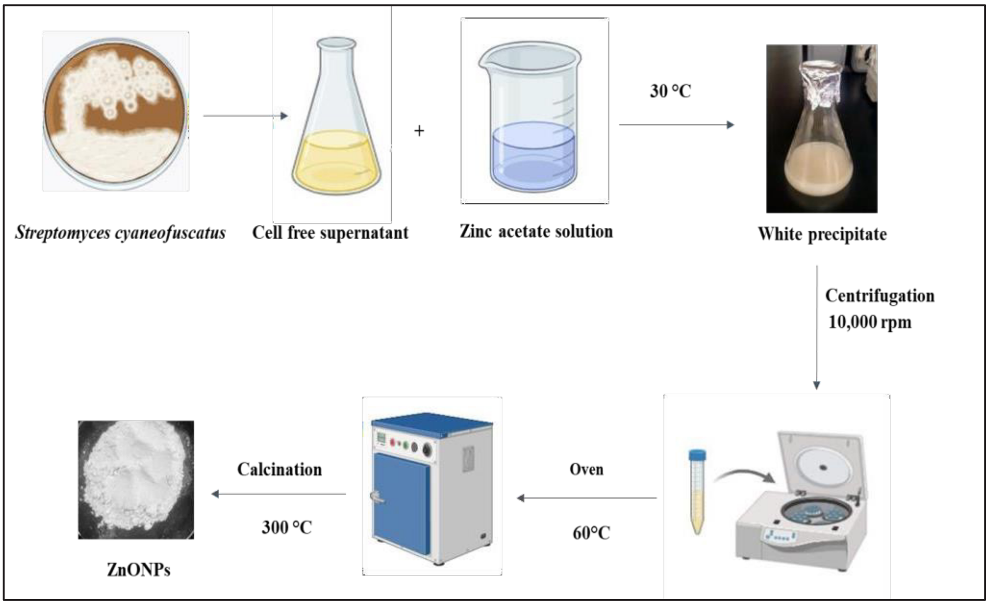


Fig. 1. Green synthesis of ZnONPs by *S. cyaneofuscatus***.**

3.2. Characterization of biosynthetized ZnONPs

3.2.1. Optical characteristics of ZnONPs

To confirm the successful formation of ZnONPs, UV–Vis spectrum analysis of ZnONPs biosyntheized by *S. cyaneofuscatus* was performed and presented in Fig.2. ZnONPs showed strong absorption peak at 375 nm. Furthermore, there are no other absorption peaks noted in UV–Vis spectrum which indicated the purity of the biofabricated ZnONPs. Similar results were obtained by [8], where biosyntheized ZnONPs exhibted the maximum absorbance peak at 374.23 nm. While in another study conducted by [9], ZnONPs synthesized from pomegranate flowers showed an absorption peak at 345 nm.

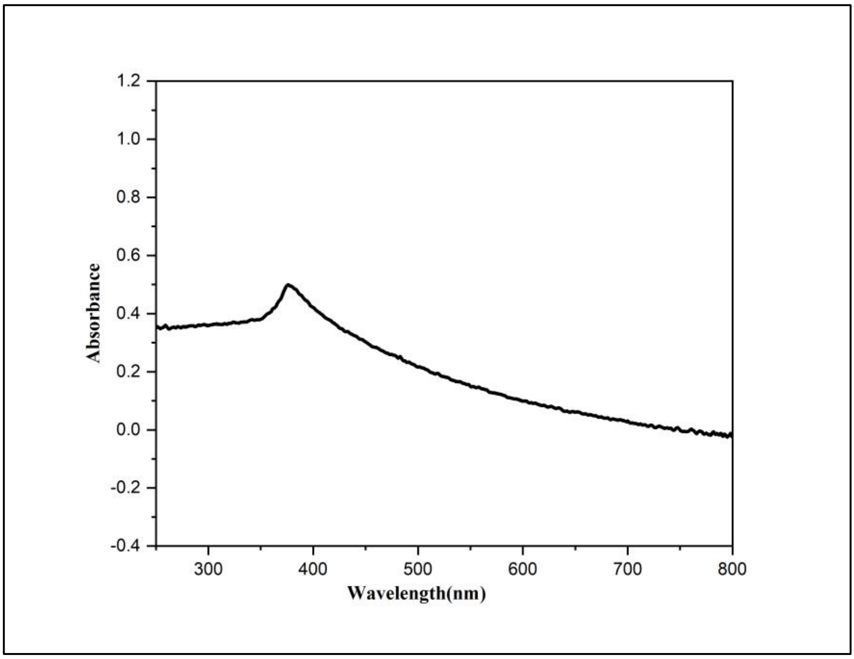


Fig. 2. UV–visible spectrum of biosyntheized ZnONPs.

3.2.2. FTIR analysis of ZnONPs

The FTIR spectrum of *S. cyaneofuscatus* -mediated ZnONPs using zinc acetate, as precursor salt, showed several absorption bands (Table 1 and Fig.3). Particularly, the peak at 447 cm^{-1} confirmed the successful fabrication of ZnONPs, with the 400–600 cm^{-1} regions demonstrating ZnO stretching vibrations. The presence of additional peaks in the FTIR spectrum beside the characteristic Zn-O stretching, may be assigned to several factors. One significant factor is surface functionalization. When ZnONPs are synthesized using *S. cyaneofuscatus,* organic biomolecules such as protein, carbohydrates and polysaccharides conjugated with nanoparticles surface. These organic biomolcules resulted in additional absorption peaks matching to different functional groups such as carbonyl (C=O), aromatic rings (C=C), hydroxyl (O-H) and amine(C-N). Similar results were obtained by [10] who used for *Syzygium aromaticum* for biosynthesis of ZnONPs.

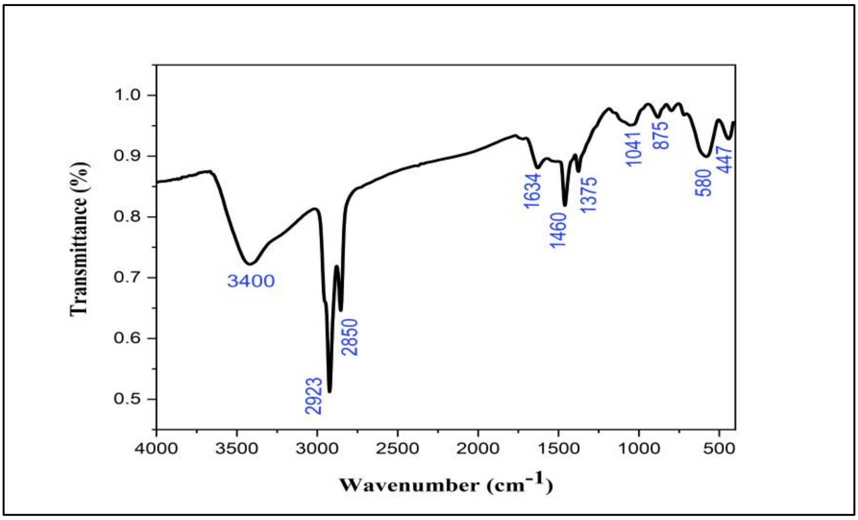


Fig. 3. FT-IR of green syntheized ZnONPs using *S. cyaneofuscatus.*

Table 1. FTIR Analysis of the biosyntheized ZnONPs

No	Absorption Peak (cm^{-1})	Functional Groups
1.	3400	–NH, O-H stretching vibrations
2.	2923	C-H asymmetric vibration
3.	2850	C-H symmetric vibration
4.	1634	C=C stretching vibration
5.	1460	C=O stretching vibration
6.	1375	C-H bending vibration
7.	1041	C–O stretching vibration
8.	875	C-N stretching vibration
9.	580	Hexagonal phase ZnO Zn-O
10.	447	stretching vibration

3.2.3. Structural properties

The crystallographic nature and identity of *S. cyaneofuscatus*- mediated biosynthesis ZnONPs were identified via XRD analysis. As observed, the XRD of ZnONPs showed well- defined and sharp peaks at 2θ values = 31.7 9 °, 34.56°, 36.27 °, 47.58 °, 56.55 °, 67.96 °,and 69.02 °, which correspond to (100), (002), (101), (102), (110), (103), (112) and (201) diffraction planes of hexagonal wurtzite of ZnO based on (JCPDS card number. 89-7102). These results results were in compatible with those obtained by [11,12] who fabricated ZnONPs using green synthesis methods. Notably, a small background hump was noted in the XRD pattern at 2θ before 30°, and this could be attributed to the association of microbial biomolecules with the surface NPs, which was commonly observed in green synthesis routes.

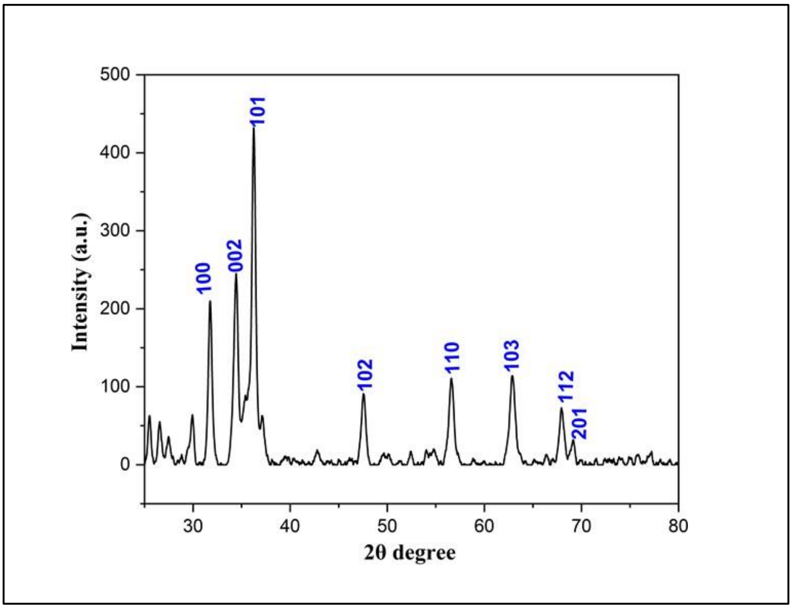
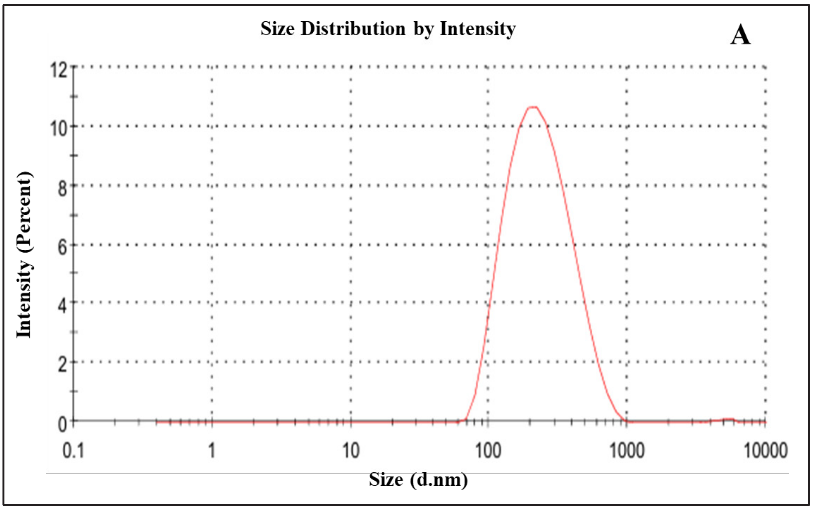


Fig.4. XRD pattern of biosynthesized ZnNPs via *S. cyaneofuscatus*.

3.2.4. DLS and zeta potential

The DLS test was applied to analyse the size distrubution, revealing the hydrodynamic size of the of the biosynthesized ZnONPs. As shown in Fig.5.A, the average size of ZnONPs was

205.9 nm, indicating the presence of large particles. The polydispersity index (PDI) of the ZnONPs was 0.206 which is comparable with values stated in several reports that consider PDI values below 0.5 to confirm a monodisperse system [13]. On the other hand, the stability of biosyntheized ZnONPs was assessed by zeta potential analysis which measures the surface charge of these particles. ZnONPs displayed zeta potential at –26.6 mV (Fig.5.B). This negative charge indicated the binding of negatively charged functional groups from *S. cyaneofuscatus* culture to the surface of ZnONPs which stabilize them and reduce their aggregateation, and it also confirmed the dispersive capacity of the biosynthesized ZnONPs [14]. The negatively charged biosyntheized ZnONPs were reported earlier in study by [15] who found that zeta potentials of green fabricated ZnONPs were - 17.49 mV.



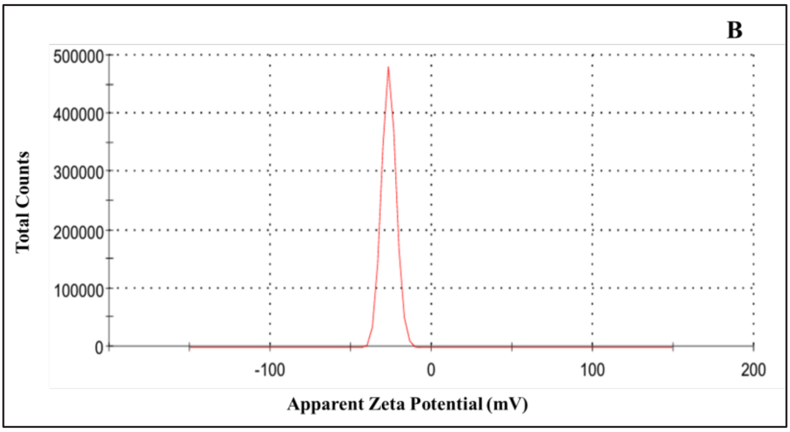


Fig. 5. particle size distribution (A) and zeta potential (B) of green syntheized ZnONPs.

3.2.5. TEM OF ZnONPs

The TEM image indicated that ZnONPs had rod shape to quasi-spherical with average particle size of 25.13 nm (Fig.6) using ImageJ software. Such morphology of ZnONPs was reported earlier in green synthesis methods by [16] who use *lilium ledebourii* for synthesis of rod-shaped ZnONPs, while some other studies reported the spherical shape of ZnONPs with green approaches [17, 18]. On the other hand, clustering of particles was observed in biosyntheized ZnNPs which is due to the presence of *S. cyaneofuscatus* organic biomolecules that provided stability to the ZnONPs by capping them. Notably, a slightly larger ZnONPs observed in DLS analysis, such difference in size between TEM and DLS measurements could be attributed to the presence of water and other biomaterials attached to the surface of ZnONPs.

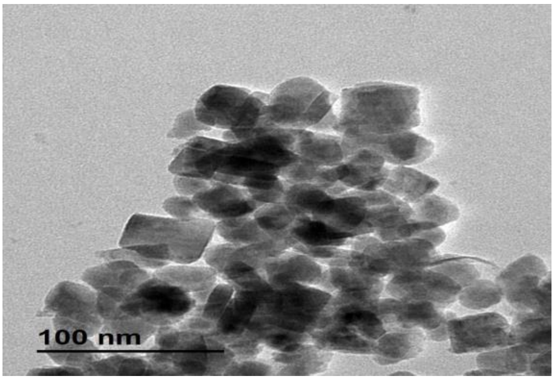


Fig. 6. TEM image of *S. cyaneofuscatus* – mediated ZnONPs.

3.2.6. EDX elemental composition

The composition of each element in the biosyntheized ZnONPs was obtained from EDX analysis. The EDX pattern of ZnONPs showed a strong peak of Zn at 1.0, 8.7 and 9.6 keV with weight percentage of 73.11 %. Also, a characteristic peak of oxygen at 0.5 keV with weight ratio of 26.89 % (Fig. 7). These findings proved that the presence of zinc in its oxide form and they were in agreement with earlier studies for green synthesized ZnONPs [19]. Also, the occurrence of Zn and O elements in EDX profile verfied the successful fabrication of ZnONPs using *S. cyaneofuscatus*.

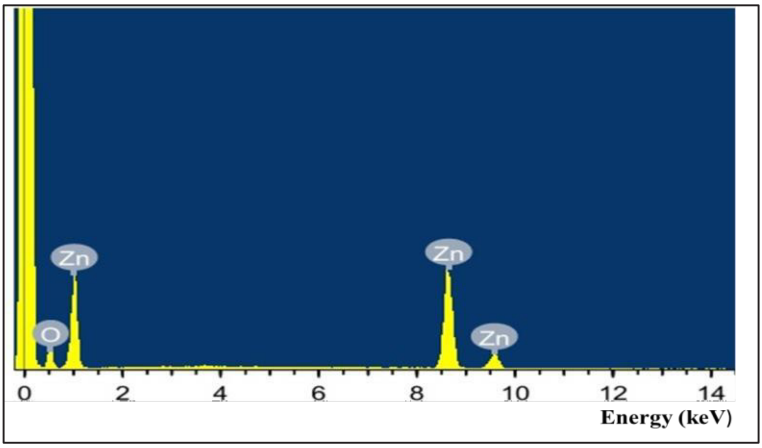


Fig. 7. EDX Spectrum of green syntheized ZnONPs.

3.3. Applications of biosynthesized ZnONPs

3.3.1. Biomedical applications of ZnONPs

Anticancer activity:

ZnONPs are vital metal oxide NPs for treatment of cancer cells due to their saftey and biocompatibility. ZnONPs display different types of surface charge behaviour owing to their electrostatic properties. Anticancer activity of ZnONPs owing to their ability for inducing ROS formation and also apoptosis. Electrostatic interactions between positively charged nanoparticles and target cells are believed to play akey role in cellular adhesion and uptake [20].

Antimicrobial activity:

The antimicrobial activity of ZnONPs relies on their capability to produce oxidative stress. They release Zn^{+} ions that interact with the respiratory enzymes, hindering their activity. Several studies reported that ZnONPs cause reactive oxygen species (ROS) formation as aresult of their effect the cell membrane of bacterial cells. Therefore, when bacterial cells interact with ZnONPs, cells take Zn^{+} ions, which inhibit activity of respiratory enzymes, and produce free radicals that lead to oxidative stress. ROS can disrbut bacterial membranes, genetic material, and mitochondria, causing the death of cells [21].

3.3.2. environmental applications of ZnONPs

ZnONPs can be used in several environmental approaches, such as remediation of wastewater and heavy metal, effectively removing contaminants and improving our ecosystem. These ZnONPs exhibit a large surface area and photocatalytic ability, can produce both OH° (hydroxide) and $O_2^{-\circ}$ (superoxide) radicals for the generation of ROS, resulting in oxidation and reduction of the pollutants eventually degradation of dyes, reduction and removal of heavy metal ions ($Cu^{2+,}$ $Pb^{2+,}$ Cr^{6+}, etc.) and degradation of pharmaceutical compounds [22].

Conclusions

The current study successfully establishes the biosynthesis of ZnO-NPs for the first time using *S. cyaneofuscatus*. This protocol for ZnONPs synthesis was cost-effective, biocompatible, eco-friendly and energy saving. The resulted ZnONPs were analyzed via different advanced techniques, such as UV-Vs spectroscopy, FT-IR, XRD, DLS, EDX and TEM, which indicated the successful fabrication of ZnONPs with average particle size of 25.13 nm from TEM image. Moreover, FTIR analysis showed the presence of bioactive molecules that congjuated with surface of ZnONPs, which act as capping, stabilizing and reducing agents, enhancing ZnONPs stability. Hence, these biosyntheized ZnONPs can be used in future work as stable substance that alternative to synthetics ones in different biomedical, pharmacuetical and environmental fiels.

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