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Full Length Article

Biosynthesis of Corn Silk Based Nanoparticles and their Efficacy Assessment against Uropathogenic *Escherichia coli*

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Abstract

This study aimed to find safe, bioactive and inhibitory substances against uropathogenic *Escherichia coli* (UPEC). Corn silk was used as a source to synthesize nanoparticles (NPs). Metal oxide nanoparticles were biosynthesized using plant extract as an alternative to conventional chemical synthesis. Zinc oxide nanoparticles (ZnO NPs) were generated from corn silk (*Zea mays* L.) and their anti–uropathogenic efficiency was tested against *E. coli.* Scanning electron microscopy (SEM) revealed a spherical shape of NPs within a diameter range of 73–121 nm. Furthermore, minimal bactericidal concentration (MBC) and minimal inhibitory concentration (MIC) methods were adopted to assess the antibacterial efficacy of synthesized ZnO NPs. Amoxicillin, a commercial antibiotic, was used as a standard to compare the experimental data. Biosynthesized ZnO NPs effectively inhibited the growth of all six UPEC samples at a concentration of 6.25 μg mL^{-1}. This study establishes a high efficacy of corn silk-based zinc oxide nanoparticles against UPEC bacteria. These nanoparticles could potentially serve as commercial antibiotics alternatives in the future. © 2022 Friends Science Publishers

Keywords: Alternative antibiotics; Corn silk of *Zea mays*; Green nanotechnology; Medicinal plants; Urinary tract infections; ZnO nanoparticles

Introduction

The occurrence of urinary tract infections (UTIs) has approximately reached 150 million per annum globally (Lo *et al*. 2017). UTIs represent a major cause of nosocomial and community-acquired infections among hospitalized patients in the United States (Stamm and Norrby 2001; Najar *et al*. 2009). Adult women face 30 times higher UTI risk than men. Generally, 50 to 60% of adult females develop at least one UTI during their life period (Foxman 2002; Alós 2005). The uropathogenic *E. coli* strain approximately causes 250,000 pyelonephritis cases and 70 to 90% of seven million acute cystitis cases in the United States per year (Foxman *et al*. 2000). World Health Organization (WHO) has documented *E. coli* based epidemic urinary tract infections, which are resistant to a broad range of medicines (Esteve-Palau *et al*. 2015; Hecke *et al*. 2017).

Nanotechnology has emerged as a foundation of several biological discoveries in the 21st century and it is considered the future industrial revolution (Um-e-Aiman *et al*. 2021). ZnO NPs are unique metal oxide nanoparticles possessing distinct chemical and optical characteristics. Their antimicrobial potential against various pathogens highlights their possible applications in the medical, biological and food industries (Sharma *et al*. 2020). Traditionally, ZnO NPs are synthesized through physical and chemical processes. However, the involvement of hazardous chemicals, high capital cost and high energy needs make these procedures undesirable (Hosseini and Sarvi 2015; Mohd *et al*. 2019). Green nanotechnology refers to nanomaterial synthesis using environment-friendly materials and it reduces the involvement of detrimental compounds in the manufacturing process (Abdel-Azeem *et al*. 2020). Metal nanoparticle manufacturing from medicinal plants, agricultural wastes and their byproducts is a rapidly emerging field (Sreelatha and Padma 2009; Solihah *et al*. 2012; Nezamdoost *et al*. 2014). The features like one-step nanoparticle synthesis, simplicity, eco-friendliness, cost-effectiveness, lower chemical toxicity, and reduced manufacturing time attract researchers toward green nanoparticle synthesis methods (Sundrarajan *et al*. 2015; Agarwal *et al*. 2017).

Bladder infection treating medicinal herbs has a successful long history of UTI treatment (Vijitha and Saranya 2017). Several studies have reported worldwide herbal treatment of UTIs and corn silk is a frequently used herbal medicine (Mithraja *et al*. 2012). The biological activity of corn silk components has been extensively studied for the treatment of gout, edema, nephritis, cystitis, kidney stones, and prostatitis (Hu and Deng 2011). Corn silk does not pose side effects and is considered completely

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safe for human consumption (Wang *et al*. 2011). During the current study, ZnO NPs were prepared using corn silk and their antibacterial efficacy was investigated against a uropathogenic *E. coli* strain.

Materials and Methods

Preparation of corn silk extract

Corn was obtained from a vegetable market in Jeddah. Distilled water was used to rinse the corn samples multiple times for removing contaminants and dust particles. Briefly, the corn silk was dried in a chamber and ground into a fine powder. 10 g of corn silk powder was added into a 250 mL flask containing 100 mL of distilled water. The mixture was continuously stirred and boiled at 85°C for 15 min. Finally, the corn silk was purified using filter paper and cooled to room temperature.

Synthesis of ZnO NPs

One hundred milliliters of the extract were mixed with 0.2 M zinc acetate dehydrate (2.6 g zinc acetate dehydrate diluted in 60 mL of distilled water) inside an Erlenmeyer flask. Then, 1 *M* NaOH (4 g NaOH dissolved in 100 mL of distilled water) was added to the solution for increasing the pH to 8. The solution was heated at 85–90°C for 8 h. Later on, the solution was centrifuged for 15 min at 4000 rpm and filtered. The resulting precipitate was heated at 500°C for 2 h to achieve a white powder of ZnO NPs (Hajinasiri *et al*. 2016).

Characterization of ZnO NPs

Surface functional groups of the nanoparticles were identified based on the vibrational frequency differences that were estimated through Fourier transformed infrared spectroscopy (FT-IR; Perkin Elmer, USA). Then, ZnO NPs samples were subjected to X-ray diffraction (XRD) analysis using a German XRD apparatus (Bruker as System, D8) within a scanning range of 10 to 90. Scanning Electron Microscopy (SEM) provided direct high-resolution images to assess the surface morphology of ZnO NPs. Furthermore, UV-Visible spectroscopy (UV-Vis) of biosynthesized ZnO NPs was carried out to examine their optical characteristics.

Antimicrobial activity of ZnO NPs

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the biosynthesized ZnO NPs were estimated against *E. coli* using diluted broth in 96-well plates (Wiegand *et al.* 2008; Yamani *et al.* 2016). A nanoparticle solution was prepared by dissolving 50 mg ZnO NPs powder in a solution of 4750 μL sterile distilled water and 250 μL of DMSO (5%). The antibiotic solution was prepared by dissolving 50 mg of Amoxicillin powder in 4750 μL sterile distilled water and mixed with 250 μL DMSO (5%) using a vortex mixer. MIC of the ZnO NPs and amoxicillin was estimated against *E. coli* (6 Samples) by following the diluted broth method. 50 μL of two-fold ZnO NPs dilution concentration (50 μg mL^{-1}) was prepared using MHB in a 96-well sterile flat-bottomed microtiter plate whereas antibiotic was diluted in a ratio of 1:2 from 50 μg mL^{-1} to 0.097 μg mL^{-1}. Then, 50 μL of bacterial suspension was added to each well to achieve a final concentration of 5×10^5 cfu per well. The plates were incubated for 24 h at 37°C. The presence of white turbidity on the well-bottom indicated the increase in concentration. MIC represented the lowest concentration without discernible growth. After overnight incubation at 37°C, 100 μL aliquots from each well were plated onto MHA to assess the MBC by calculating viable counts. The assay was performed in triplicate.

Results

Characterization of ZnO NPs

The reduction of zinc ions in the presence of plant extracts led to the formation of plant extract-capped ZnO NPs. The preparation of ZnO NPs was confirmed by their final yellow-brown color in the reaction mixture as compared to the initial dark brown color. The final yellow-brown color served as a positive indicator for the green synthesis of ZnO NPs.

UV-VIS analysis

Fig. 1 presents the UV-VIS spectra of reaction media after 24 h of treatment. The absorbance peak at 370 nm represents the synthesis of ZnO NPs in the reaction fluid.

XRD analysis

The crystalline nature of the corn silk-based green synthesized ZnO NPs were examined using XRD. Fig. 2 demonstrates the findings of the XRD analysis. Eleven significant peaks were observed at 27.19°, 29.02°, 31.91°, 34.64°, 36.44°, 47.68°, 56.69°, 63.07°, 66.54°, 67.98° and 69.16° corresponding to (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202). These results are in line with the JCPDS File card no. 01-075-0576, which confirmed the similarity of synthesized nanoparticles with zinc oxide's hexagonal phase.

FT-IR analysis

FTIR analysis of ZnO NP was conducted within a wavenumber range of 4000–400 cm^{-1} as shown in Fig. 3. Different bands were observed at 3401.55, 2925.24, 1647.22, 1437.83, 1032.43, 881.98, 694.66, 618.94 and 435.08 cm^{-1}. The presence of polyphenols, aldehydes, carboxylic acids, ketones, alcohol, amines, alkane, amides, and aromatic rings was believed to be originated from the plant extract that was used for the synthesis of zinc oxide nanoparticles. The results confirmed the presence of ZnO NPs.

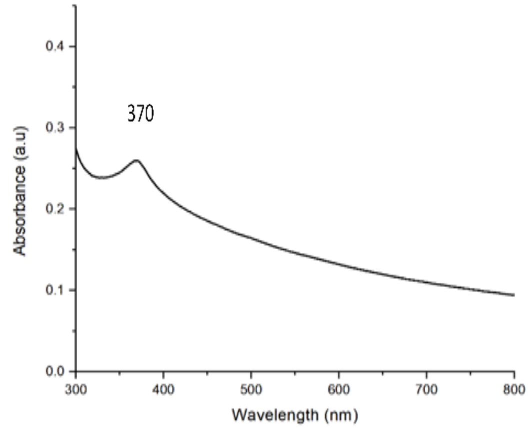

Fig. 1: UV–VIS spectrum of biosynthesized ZnO NPs

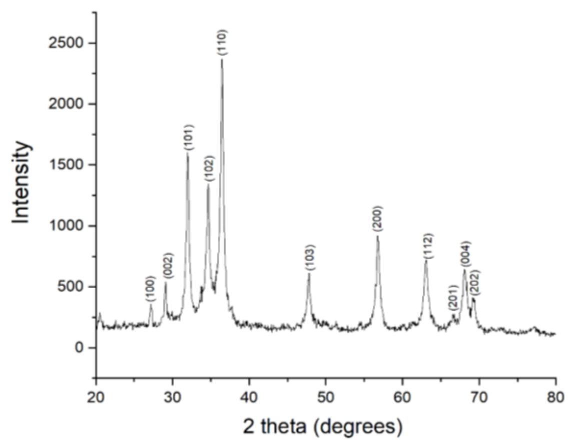

Fig. 2: XRD spectra of biosynthesized ZnO NPs

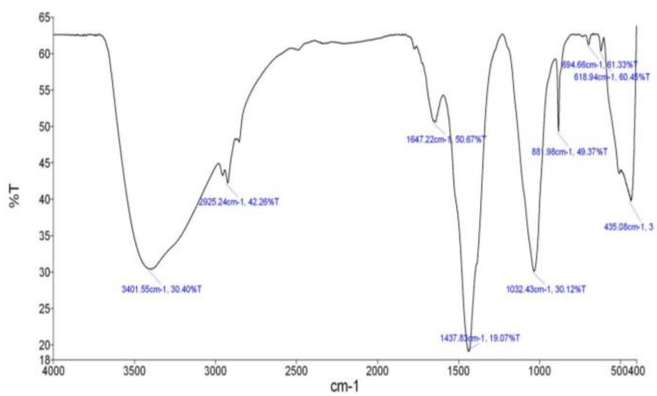

Fig. 3: FT-IR analysis of biosynthesized ZnO NPs

Scanning electron microscopy (SEM)

Scanning Electron Microscope (SEM) images revealed the surface morphology of synthesized ZnO NPs at various magnifications, as shown in Fig. 4. The majority of the nanoparticles were spherical and aggregated within a diameter range of 73–121 nm.

ZnO NPs minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

ZnO NPs inhibited the growth of all 6 samples at a concentration of 6.25 μg mL^{-1}. Amoxicillin was observed to be sensitive to three samples (1, 9 and 10). Amoxicillin inhibited the growth of sample no. 1 at a concentration of 50

Table 1: A Comparison of ZnO NPs and Amoxicillin MIC

Sample	Minimum Inhibitory concentration of ZnO NPs (μg mL^{-1})	Minimum Inhibitory concentration of Amoxicillin (μg mL^{-1})	VITEK Antibiotic sensitivity results of Amoxicillin
1	6.25	50	Sensitive
5	6.25	> 50	intermediate
6	6.25	> 50	intermediate
9	6.25	≤ 0.097	Sensitive
10	6.25	≤ 0.097	Sensitive
12	6.25	> 50	intermediate

Values are the mean of triplicate (n = 3) ± standard deviation (SD)

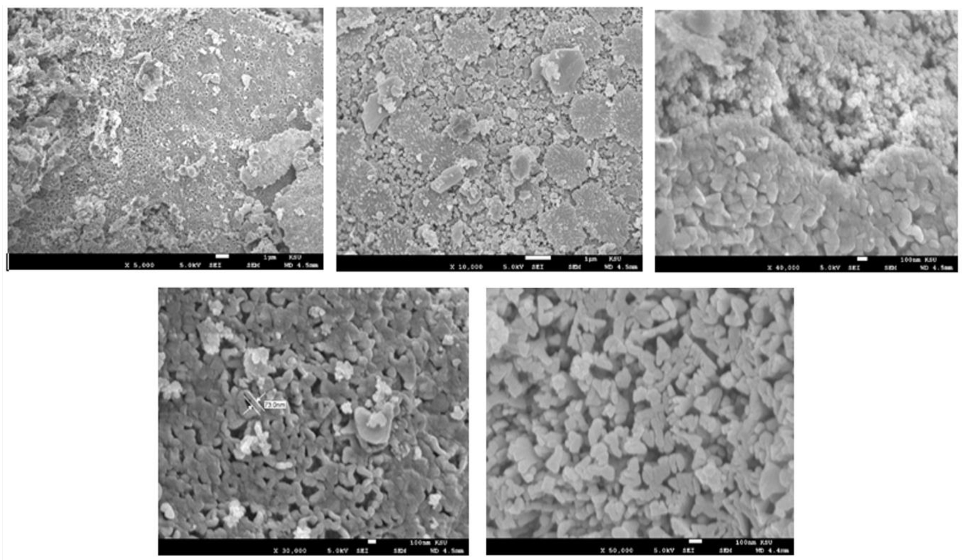

Fig. 4: SEM images of biosynthesized ZnO NPs using corn silk

μg mL^{-1} whereas no growth was observed in samples 9 and 10. Based on the results, the MIC was estimated to be ≤ 0.097 μg mL^{-1}. Moreover, Amoxicillin could not affect the growth of samples 5, 6, &12 leading to a MIC of > 50 μg mL^{-1}. The results revealed that samples 1, 9 and 10 were Amoxicillin sensitive whereas the Amoxicillin sensitivity of samples 5, 6, & 12 was intermediate. The MBC results demonstrated bacteriostatic effects of ZnO NPs on UPEC rather than bactericidal effects (Table 1).

Discussion

During this study, corn silk was used to biosynthesize ZnO nanoparticles, which effectively inhibited microbial growth (Hajinasiri *et al*. 2016). ZnO nanoparticles could play antifungal, anti-inflammatory, antibacterial and anti-cancer roles in the field of medicine (Sharmila *et al*. 2018). Therefore, the bio-production of ZnO NPs from plant extracts, bacteria, organic products, algae and fungi, has been established and it does not negatively impact the environment and human health (Lagopati *et al*. 2020). Plant extracts could act as reducing and stabilizing agents during nanoparticle synthesis (Sharma *et al.* 2019).

The change in solution color is a confirmatory sign of ZnO NPs formation. The green synthesis of ZnO NPs was further confirmed by treating Zn $(CH_3CO_2)_2{\cdot}2H_2O$ with corn silk extract. The color of the reaction mixture turned yellow-brown, which indicates the reduction of Zn $(CH_3CO_2)_2{\cdot}2H_2O$. UV-VIS spectroscopy is commonly used for the analysis of plasmon resonance excitation of ZnO nanoparticles. The only peak of the UV–VIS spectrum absorption was noted around 370 nm. The absence of any other peak in the spectrum indicated a high purity and crystallinity of ZnO NPs (Santhoshkumar *et al*. 2017). Multiple studies on plant extracts have reported an absorbance peak within a range of 300 to 500 nm (Ashwini *et al*. 2021). Saeed *et al*. (2021) have reported the synthesis of ZnO NPs from *Achyranthes aspera* leaf extract and their spectrum was also observed at a wavelength of 370 nm. During another study, ZnO NPs were chemically synthesized by following a solvothermal process. The spectrum of these ZnO NPs was also noted at a wavelength of 370 nm (Zak *et al*. 2011). Awwad *et al*. (2014) synthesized ZnO nanoparticles from the *Olea Europea* leaf extract, which exhibited a spectrum at a wavelength of 374 nm. Eleven major peaks were observed at 27.19°, 29.02°, 31.91°, 34.64°, 36.44°, 47.68°, 56.69°, 63.07°, 66.54°, 67.98° and 69.16° corresponding to 100, 002, 101, 102, 110, 103, 200, 112, 201, 004 and 202. These findings are in agreement with the JCPDS File card no. 01-075-0576, which confirmed the similarity of synthesized nanoparticles with the hexagonal phase of zinc oxide. Further analysis confirmed the absence of an extra peak (impurity) that indicates the high purity of the synthesized product. FTIR analysis of ZnO NPs was performed within a wavenumber range of 4000–400 cm^{-1}. Different bands were observed at 3401.55, 2925.24, 1647.22, 1437.83, 1032.43, 881.98, 694.66, 618.94 and 435.08 cm^{-1}. Similarly, Ebadi *et al*. (2019) have reported ZnO NPs absorption peaks within a wavenumber range of 400 to 700 cm^{-1}. These bands confirmed the successful synthesis of ZnO NPs. SEM analysis depicted a spherical shape of most of the nanoparticles having a shape agglomeration within a diameter range of 73–121 nm. ZnO nanoparticles synthesized from *Ixora coccinea* leaf extract were also noted to be spherical within a diameter range of 80-130 nm (Yedurkar *et al*. 2016). Santhoshkumar *et al*. (2017) have also reported the synthesis of spherical ZnO NPs from *P. caerulea* leaf extract having a diameter of 70 nm. The shape is a key feature that determines the NPs antimicrobial efficacy. Spherical NPs could easily penetrate the pathogenic cell wall and thus exhibit better antibacterial activity. In this regard, ZnO NPs synthesized from corn silk could be of great importance for the treatment of clinical pathogens (Naseer *et al*. 2020).

The results of the present study indicate strong antibacterial activity of zinc oxide nanoparticles against various strains of UPEC bacteria as compared to Amoxicillin. ZnO NPs successfully inhibited the growth of all six UPEC samples at a concentration of 6.25 μg mL^{-1}. These findings are similar to the biosynthesis of ZnO NPs from *Theobroma cacao L.* pod husks, which were tested against common foodborne pathogens such as *S. aureus*, and *E. coli* (Sarillana *et al*. 2021). They reported ZnO NPs minimum inhibitory concentrations against *E. coli* and *S. aureus* as 6.25 μg mL^{-1} and 12.5 μg mL^{-1}, respectively indicating a better ZnO NPs potential against *E. coli* than *S. aureus*.

Santhoshkumar *et al*. (2017) have also reported the efficacy of *P. caerulea* L. plant extract-based ZnO NPs against a urinary tract infection pathogen. These ZnO NPs caused a maximum zone inhibition against a gram-negative *E. coli* bacterium. During another study, ZnO NPs were prepared from *Berberis aristate* leaf extract, which acted as antibacterial agents against UTIs causing the *E. coli* pathogen (Chandra *et al*. 2019).

ZnO NPs biosynthesized from *Trifolium pratense* flower extract exhibited significant antibacterial activity against various pathogens including *P. aeruginosa* ATCC 6749, *S. aureus* ATCC 4163, and *E. coli* ATCC 25922 (Dobrucka and Dlugaszewska 2016). Sharmila *et al*. (2018) have demonstrated better antibacterial properties of *Bauhinia tomentose* leaf extract-based Zinc oxide nanoparticles against gram-negative bacteria as compared to gram-positive bacteria. Similarly, ZnO nanoparticles biosynthesized from *Lippia adenosis* leaf extract were found to be effective against both gram-positive (*E. feacalis* and *S. aureus*) and gram-negative (*K. pneumonia* and *E. coli*) bacteria (Demissie *et al*. 2020).

MBC results revealed bacteriostatic effects of ZnO NPs rather than bactericidal effects at the tested concentrations. Higher ZnO NPs concentrations might be required to achieve bactericidal efficacy. These results are in line with Dogan and Kocabas (2020) who have also reported strong bacteriostatic effects of ZnO NPs instead of bactericidal efficacy against gram-negative and gram-positive bacteria.

Conclusion

During this study, ZnO NPs were successfully biosynthesized using corn silk extract as a reducing and stabilizing agent. The technique followed during this study was novel, eco-friendly, cost-effective, and simple with minimum use of chemicals as compared to traditional physical and chemical methods. ZnO NPs synthesized from corn silk were stable, effective and safe with inhibitory potential against uropathogenic *E. coli*. MIC (6.25 μg mL^{-1}) revealed ZnO NPs efficacy on all six selected samples. The results confirmed the antimicrobial potential of ZnO NPs against UPEC. Therefore, ZnO NPs could be potentially used in pharmaceutical industries to prepare nanomedicines.

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Author Contributions

HAY: conceptualized the study, wrote the manuscript and is responsible for the content and similarity index of the manuscript. YFZ: collected the samples, performed the practical study, and reported the results.

Conflict of Interest

The authors declare that they have no competing interests.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

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