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Biofabrication of zinc oxide nanoparticles using fruit extract of *Rosa canina* and their toxic potential against bacteria: A mechanistic approach

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ABSTRACT

The use of plant extract in the biosynthesis of nanoparticles (NPs) can be an eco-friendly approach and have been suggested as a possible alternative to classic methods namely physical and chemical procedures. In this study, the biosynthesis of zinc oxide (ZnO) NPs by both "conventional heating" (CH) and "microwave irradiation" (MI) methods has been reported. Stable and spherical ZnONPs were produced using zinc nitrate and flesh extract of *Rosa canina* fruit (rosehip) which was used as a precursor. The flesh extract acts as a reducing and capping agent for generation of ZnONPs. The structural, morphological and colloidal properties of the as-synthesized NPs have been confirmed by X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDX), Fourier transform Infrared (FT-IR) and Dynamic Light Scattering (DLS). In comparison with the CH method, the MI method has some advantages such as significantly short reaction time (within 8 min) owing to the high heating rate and thus the accelerated reaction rate. Both methods led to the synthesis of nearly identical NPs with respect to shape and size according to the results of DLS, XRD and SEM techniques. The possible mechanism for synthesis pathway has been proposed based on FT IR results, XRD patterns, potentiometric data and antioxidant activity. In addition, the antibacterial activity of as-prepared ZnONPs was investigated against several bacteria such as *Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhimurium*. Moreover, the efficacy of ZnONPs to treat cancer cell lines were measured by means of cell viability test via MTT assay in which concentrations of 0.05 and 0.1 mg/mL of ZnONPs induced a very low toxicity. Thus, the present investigation reveals that ZnONPs have the potential for various medical and industrial applications.

1. Introduction

Nowadays nanoscience and nanotechnology have become one of the most important research areas in physics, chemistry, engineering and biology [1]. Particles with size less than 100 nm are considered due to their special properties, such as low melting point, unique optical properties, high catalytic activity, and unusual mechanical properties [2]. Based on scientific reports, ZnO is a unique material with a wide band gap of 3.37 eV possessing numerous attractive properties [3]. In addition ZnO is considered as a potential material in the field of optoelectronics, solar cells, gas sensors, catalysts, pharmaceuticals and cosmetics [4–9]. Moreover, ZnONPs have also been used as an anti-microbial preservative for wood and food products [10,11].

In general, three kinds of methods have been applied for the generation of metal oxide NPs in solution including: (i) the "normal" synthesis under reflux conditions in the oil bath using CH, (ii) the autoclave synthesis, and (iii) the most recent synthesis using MI [12]. As it is known, the walls of the reaction flask in CH method are heated by convection or conduction. In this process the core of metal oxide NPs needs longer time to achieve the desired temperature and accordingly, this leads to inhomogeneous temperature profiles within the flask. One possibility to overcome this drawback is the use of MI method, which allows the rapid and homogeneous heating of the reaction mixture to the target temperature without heating the entire oil bath. MI not only helped in employing green chemistry approach, but also led to the revolution in production of both organic and inorganic NPs [12].

As it is known, in one hand, by using classical methods for preparing NPs such as physical and chemical procedure some toxic chemical remains in products that may have adverse effects in medical application. Although, on the other hand, a lot of researches have been prepared for metal and metal oxide NPs by using green synthesis methods; however, the problem has not been solved properly. Indeed, such methods are not really green in their principle whereas extracellular biosynthesis is one of the most promised pathways of green synthesis. Recently, a variety of studies have been carried out to survey the biosynthesis of various metal ions such as Ce, Ag and Au [13–18].

Rosa canina or *dog rose* is a medicinal plant belonging to the family *Rosaceae*. Rosehip or the small fruit of *Rosa canina* is known for their high content of vitamin C. In addition, rosehip has a high level of antioxidants such as flavonoids and phenolic compounds. Based on reports,

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rosehip possesses preventive and curative activity against a wide range of diseases such as renal, inflammatory, gout and gastric disorders [19].

In the present research, for the first time, we describe the extracellular biosynthesis of ZnONPs, as a green technique, using rosehip extract with two methods (CH and MI). Moreover, the structural, morphological and colloidal properties and mechanistic aspects of the as-synthesized NPs have been investigated. In addition, the antibacterial activities as well as MTT assay for cell viability of as-prepared ZnONPs have been studied.

2. Materials and methods

2.1. Materials

All the chemicals in this research were of analytical grade. The rosehip was collected from around Mahabad city, a county with an area of 2591 km^2, approximately located at 36°46′N, 45°43′E of West Azerbaijan Province (the west northern of Iran). The fruit was washed with deionized water and dried at room temperature and then were used for further tests.

2.2. Preparation of extract

The harvested fruits were washed with distillated water and kept in shadow to dry. Then seed and flesh of the fruit were separated. The flesh was powdered and weighted carefully. About 10 g of fruit powder was shaken with 100 ml deionized water for 24 h and heated in oven at 50 °C for 15 min. Light red color of mixture implied that the extraction has occurred. In order to achieve clear extraction, the mixture filtered, centrifuged and finally stored in refrigerator for further experiments.

2.3. 1-Diphenyl-1-2-picrylhydrazyl (DPPH) scavenging assay

The rosehip extract and ZnONPs were screened for free radical scavenging activity by DPPH method [20]. The scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm using a UV-spectrophotometer.

Radical scavenging activity was calculated using the formula (Eq. (1)):

$$\%I = (A_{control} - A_{sample})/A_{control} \times 100 \quad (1)$$

Where, %I is % of radical scavenging activity, $A_{control}$ is the absorbance of the control sample (DPPH solution without test sample) and A_{test} is the absorbance of the test sample (DPPH solution with test compound). All tests were performed in triplicate and the results were averaged.

2.4. Extracellularly synthesis of ZnONPs using CH method

In order to synthesize ZnONPs, 5 ml of 5×10^{-2} M zinc nitrate solution was mixed with 10 mL of the extract. The zinc nitrate was dissolved in the extract solution under constant stirring using magnetic stirrer. After complete dissolution of the mixture, the final pH of the solution was fixed at 6.0. Then the solution was kept under vigorous stirring at 150 °C for about 5 h until color change occurs. Finally, it was allowed to cool at room temperature and the supernatant was separated. This mixture was centrifuged at 5000 rpm for 10 min and a solid precipitate was obtained. The solid product was centrifuged twice at 5000 rpm for 15 min after thorough washing and dried at 80 °C for 4 h. This precipitate was collected and dried and then heated in air heated furnace at 400 °C for 4 h. The pale white colored solid was obtained. This solid was completely powdered using a mortar that got a fine powder for further characterizations.

2.5. Extracellular synthesis of ZnONPs using MI method

A typical route was applied according to CH method except that the mixture 4 times and each time for 2 min was placed under microwave irradiation at 320 W. A deep red colored suspension was created that was centrifuged at 5000 rpm. The precipitate was obtained which was centrifuged twice at 5000 rpm after washing. The obtained pale white colored precipitate was heated according to the same condition mentioned above (CH method) in air-heated furnace.

2.6. Characterization of ZnONPs

Crystalline structure and grain size were identified by XRD (D500, Siemens Diffractometer-Germany) Cu-K_a radiations ($\lambda = 1.54$ Å) in 2θ range from 10° to 80°. FTIR spectra were measured with a TENSOR 27-Bruker Spectrometer in the 400–4000 cm^{-1} region. The size and shape of NPs were characterized by SEM and EDX techniques using MIRA3 FEG-SEM. In addition, the size and size distribution, polydispersity index (PDI) and zeta potential were identified using DLS Nanotrac Wave. TEM studies were carried out on a Zeiss LEO 912 Omega instrument, operating at 120 kV. TEM specimen was made by evaporating one drop of solution of the sample in ethanol onto carbon coated copper grids. Grids were blotted dry on filter paper and investigated without further treatment.

2.7. Anti-bacterial assay of ZnONPs

The ZnONPs that were synthesized using *Rosa canina* extract were tested for antimicrobial activity by agar disk diffusion method against *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia coli*. The pure cultures of bacteria were subcultured on nutrient agar medium. Each strain was put uniformly onto the individual plates using sterile cotton swabs. Filter paper disks (Whatman no. 3, 6 mm diameter) were sterilized by autoclaving. Ten milliliters of the nanoparticle's solution was loaded onto each paper disk and allowed to air dry. The dry disks were placed on the previously inoculated agar. After incubation at 37 °C for 24 h, the different levels of zone of inhibition of bacteria were measured.

2.8. MTT assay for cell viability

A549 alveolar adenocarcinoma cells (9×10^3 cells/well) were incubated in 96-well plates; each containing 200 μL of supplemented cell culture media for 24 h at 37 °C and 5% CO_2. The cells were divided in 5 groups in triplicates: blank ZnONPs (different concentrations: 0.05, 0.1, 0.25, 0.5 mg/mL) were treated. After an incubation period of 24 h, the spent media were removed and the plate wells were washed with phosphate-buffered solution. Briefly, 50 μL of 2 mg/mL MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-trazolium bromide) and 150 μL of culture medium was added to each well. The cells were incubated at 37 °C and 5% CO_2 for 4 h and then the media was discarded and dimethyl sulfoxide and Sorenson buffer were added to each well as solubilizer buffer. Finally, absorbance was read using an ELISA plate reader (BioTeck, Bad Friedrichshall, Germany) at 570 nm wavelength.

3. Results and discussion

3.1. Structural characterization of ZnONPs

3.1.1. XRD pattern

XRD pattern of the prepared NPs have been shown in Fig. 1a.

The peak positions with 2θ values of 31.76°, 34.42°, 36.25°, 47.53°, 56.59°, 62.85°, 67.94° and 69.08° are indexed as to the (100), (002), (101), (102), (110), (103), (112) and (202) planes, respectively, according to ICDD data (card No. 01-079-0206) for extracellularly biosynthesis