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Simple biosynthesis of zinc oxide nanoparticles using nature's source, and it's *in vitro* bio-activity

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ABSTRACT

Nanoparticles with antimicrobial activity, especially as a new class of biomedical materials for use in increasing the level of public health in daily life have emerged. In this study, green synthesis of zinc oxide) ZnO(nanoparticles was studied by *Cuminum cyminum* (cumin) as novel natural source and zinc nitrate [$Zn(NO_3)_2$] as Zn^{2+} source. The results showed that parameters such as concentration, time, temperature and pH have a direct impact on the synthesis of zinc nanoparticles and change in any of the factors causing the change in the process of synthesis. The properties of synthesized nanoparticles were examined by UV–visible Spectrophotometer, X-ray diffraction spectroscopy and transmission electron microscopy (TEM). The UV–visible spectroscopy presented the absorption peak in the range of 370 nm. Transmission electron microscopy images of synthesized nanoparticles are mainly spherical or oval with an average size of about 7 nm. The effect of antimicrobial nanoparticles calculated using disk diffusion method and broth MIC and MBC in different strains of bacteria, which showed that gram positive and negative were sensitive to zinc oxide nanoparticles. The sensitivity of gram-negative bacteria was more.

1. Introduction

Nano means one billionth of meter. Nano (word) taken from nanus that in classical Greek means dwarf or anything smaller than usual. Nano world consist of atoms, and molecular machines such as ribosomes. Nanotechnology is applicable in various fields such as medical science [1–7], pharmaceutical, food, biotechnology [8], environment [9], electronics [10], computers, materials, aerospace, defense, energy, transportation and promising a wide revolution in science and technology [11]. Nano materials are materials said to have at least one dimension sized from 1 to 100 nm [12]. In the past decade, scientific development in the fields of biosciences, chemistry, physics, engineering have led to the deep attention of researchers and scientists to different properties of nanomaterial such as nanoparticles, dendrimers, quantum dots, Nano shells, nanotubes, Nano machines and their application in a wide range of science.

Antimicrobial agents considerably helped to prevent and treat infectious diseases in humans and animals and many of them plays an important role in promoting growth in humans and animals and increasing feed function [13]. But improper use of common antibiotics, such as the indiscriminate use of powerful antibiotics with broad-spectrum or its widespread use in the food industry, animal husbandry, toiletries and household cleaners led to the increase of resistance bacteria such as multi-drug resistance (MDR) microbial strains [14]. The misuse and overuse of antibiotics has led to the

Abbreviations: Fig., Figure; g, Gram; HCL, Hydrochloric acid; M, Molar; ml, Milliliter; mM, Milli molar; NaOH, Sodium hydroxide; nm, Nanometer; NPs, Nanoparticles; SPR, Surface plasmon resonance; TEM, Transmission electron microscopy; Vis, Visble; XRD, X-ray diffraction; ZnO, Zinc oxide; Tab., Table; PDA, Potato dextrose agar.

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emergence of *Escherichia coli* and *Salmonella* which are antibiotic-resistant [15]. Bacterial strains from a range of species such as *Salmonella*, *Staphylococcus aureus*, *Enterococcus* species, Enterobacteriaceae, *Pseudomonas* and specifically *Acinetobacter* are very resistant to antibiotics, so that treatment of diseases and infections caused by these strains, become intractable problem in medicine [16,17]. This problem led to a need for research to discover and develop new and urgent approaches to deal with the bacteria [18].

Nano science has shown that if you reduce the size to nanometers, very different and unique properties such as optical, electrical conductivity, hardness, active area and chemical reactivity will be achieved [19,20]. The effect of antimicrobial metal nanoparticles such as silver, zinc, copper and titanium, is one of the different and important properties of nanoparticles [21,22].

Antimicrobial nanoparticles resulted in a plan for strategic planning and new applications of nanoparticles in medical and biotechnology. Nanoparticles with high antimicrobial activity as biomedical materials are a new class of biomedical materials with special effects to enhance the level of public health. The nanoparticles can be active compounds or molecules and react with bacterial cells and inactivate the bacteria. Highly efficient and low-cost Chemical methods are presented for the synthesis of nanoparticles. But pollution from chemicals and high energy used in these methods could be a problem for human health and the environment [23].

Recently, the biological process of producing nanoparticles that is based on the use of natural resources has attracted scientific interest [24]. The synthesis of nanostructures that deals with the use of natural resources is called biosynthesis or green synthesis. Green synthesis of nanoparticles have created a common point between nanotechnology and biotechnology and led to the development of new materials that are used in many fields.

Silver nanoparticles [25,26], the magnetic iron oxide [27,28], titanium dioxide [29], zinc oxide [30,31] and copper oxide [22,32] are one of the important nano-materials that have been studied, and can be synthesized by using physical, chemical and biological methods. Some bacteria, fungi, yeasts and plants have shown their ability in regenerating and accumulation of metal ions and nanoparticles [33,34]. Biosynthesis can be intracellular [35] or extracellular [36,37]. Extracellular methods are more advantageous due industrial-scale production ability and ease of control on the environment. For this reason; researchers have chosen the production of nanoparticles in biological systems, with minimal environmental risks and the simple and eco-friendly (biocompatible) production methods. The properties of nanoparticles (size, optical properties, electrical, etc.) can be synthesized by manipulating the biosynthesis of controlling parameters such as pH, temperature, substrate concentration and changing the exposure time of substrate [38–40].

Park and et al. [41] studied cytotoxicity and genotoxicity effects of nanoparticles with different sizes (20, 80, 113 nm) and reported that in all toxicity endpoints studied, smaller nanoparticles (20 nm) were more toxic than the larger nanoparticles. The cytotoxicity of nanoparticles strongly depends on the concentration and increases with increasing the concentration of nanoparticles. Identification and use of natural resources as a biological source is very attractive in biosynthesis of nanoparticles. Nanostructures produced by natural sources with different shapes and sizes are more stable and have a rapid synthesis process.

Among the many types of nanoparticles with applications in medicine, such as zinc oxide nanoparticles show semiconductor and piezoelectric properties. Zinc oxide nanoparticles are used in various fields, including visible light, ultraviolet, catalytic activities, optical, deodorizing, the treatment of diabetes [31], cosmetics, gas sensors, ultraviolet light absorbing materials, piezoelectric devices, photodiode, solar cells, glass sunscreen, light-emitting diodes, lasers, paints, varnishes and rubber industry, chemical absorbents, catalysts for light demolition instead of titanium nanoparticles, semiconductor manufacturing, ultra-violet filter feeders and especially in the fields of Medical and pharmaceutical sciences [30,42–44]. There are a variety of physicochemical methods for the production of zinc oxide nanoparticles [30,45], which has some disadvantages, including lack of stability of the solution, not uniform particle size, nanoparticles gross, low efficiency and require advanced equipment for production [46]. Green synthesis of zinc oxide nanoparticles have been done by extracellular cumin extract aims to develop the methods and environmentally friendly synthesis was investigated. Ever study of the synthesis of zinc oxide nanoparticles using cumin (*Cuminum cyminum*) is not released. This biological source is aromatic and have medicinal properties [47] and frequency found in the East Mediterranean region, especially the mountains of Kerman, Iran. Medicinal and natural products are used in many natural products.

This study was carried out for identify and develop the new generation of antimicrobial agents to control infections, the antimicrobial properties of the green synthesized ZnO nanoparticles are examined [48].

In the following, methods and results from the synthesis of zinc oxide nanoparticles and UV–Vis spectroscopy analysis made UV–visible spectroscopy, X-ray diffraction)XRD(, TEM, and antimicrobial effects of zinc oxide nanoparticles synthesis will be explained.

2. Materials and methods

2.1. Green synthesis of zinc oxide nanoparticle

A total of 20 g of cumin seeds clean and free of dust were disinfected by 3% sodium hypochlorite for 3 min and then washed with distilled water 3 times and each time was 2 min. The more seeds disinfected with 70% alcohol for 2 min and then washed with distilled water three times and each time was 2 min. Then sterile water was added with the ratio of 1: 10 (per gram of seed, 10 ml of water), and were kept in the dark for two days at 25 °C. After two days, the liquid phase was filtered with Whatman No. 1 filter paper and the obtained filtered extract was used for the synthesis of nanoparticles. In order to synthesis, the zinc nitrate stock was prepared by adding 0.0297 gr zinc nitrate to 50 cc distilled water and then the final volume reached to 100 ml through adding distilled water. To biosynthesis zinc oxide nanoparticles, zinc nitrate were used at concentrations of 0.1, 0.25, 0.5, 0.75 and 1 mM. The 10 ml of the obtained extract was added to 90 ml zinc nitrate with mentioned concentrations separately. The zinc nitrate was not added to the control. The mixture (extract + zinc nitrate) was incubated at 70 °C for 2 h and calcined at 300° (2 h) to obtained white powder.

2.2. Specification

The process of light absorption of organic molecules is usually attributed to the excitation of electrons in molecular orbitals. Some of the metal nanoparticles show a similar absorption behavior. Due to the lack of molecular orbital's in the structure of metallic nanoparticles, the absorption of light in this case is related to the phenomenon of surface Plasmon resonance (SPR). Put simply, we can say that a sea of electrons is floating on the surface of a metallic nanoparticle. In certain metals (specifically with the ability to show SPR), surface absorption of the light leads to the volatility of electrons. In this study the samples were studied with the wavelength range of 400–700 nm. Previous studies have shown that an