Antioxidant Properties of *Dracocephalum kotschyi* and Synthesis of Zinc Oxide Nanoparticles using an Herbal Extract to assess the Antibacterial Properties of the Biologically Synthesized Nanostructure

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Abstract
Synthesis of zinc oxide (ZnO) nanoparticles is considered to be highly beneficial since these agents have numerous applications in pathogen detection and cancer therapy, as well as unique thermal, optical and electronic properties. The present study aimed to perform the green synthesis of ZnO nanoparticles using the extract of Dracocephalum kotschyi. Green methods of nanoparticle synthesis have several advantages such as simplicity, low cost, eco-friendly nature and providing high-level production. Contrary to chemical methods, green approaches do not require high temperatures and pressure or toxic chemical compounds to be carried out. In this experimental study, synthesis of ZnO nanoparticles was performed using the herbal extract of Dracocephalum kotschyi and zinc acetate solution (0.02 M). In addition, various concentrations of the herbal extract were added to 50 milliliters of the zinc acetate solution and the mixture was stirred at different periods in order to evaluate the effects of the extract concentrations and reaction time. Change of color from dark yellow to pale yellow indicated the production of ZnO nanoparticles. With the assumption that the antioxidant compounds in the plant could restore metal ions into nanoparticles as a reducing agent, Dracocephalum kotschyiwas assessed in terms of antioxidant properties using the α, α-diphenyl-β-picrylhydrazyl (DPPH) technique.

Moreover, IC_{50} was calculated and the formation of ZnO nanoparticles with the absorbing peak at approximately 210 nanometers was determined using UV-visible spectroscopy.The size and morphology of the synthesized nanoparticles were also determined using Scanning electron microscopy (SEM), which indicated that the ZnO nanoparticles were in the form of nanoshells with the approximate size of 33 nanometers. In addition, X-ray diffraction spectroscopy was carried out to evaluate the purity of the ZnO nanoparticles, which confirmed the presence of zinc and oxygen. The type of the chemical bonds in the ZnO nanoparticles was determined using infrared spectroscopy, which confirmed the presence of a bond between oxygen and zinc. Finally, the antibacterial properties of the synthesized nanostructures were investigated against Escherichia coli and Staphylococcus aureus and the results confirmed the antibacterial properties of the synthesized ZnO nanostructures.

Keywords: ZnO Nanoparticles, Antibacterial Properties, *Dracocephalum kotschyi*, Antioxidant, Green Synthesis

Introduction

Today, the design and manufacturing of materials with completely new features and properties and size range of 1-100 nanometers have become possible with the emergence of nanotechnology[1]. The key difference between nanotechnology and other technologies is the scale of the utilized materials and structures in this technology[2]. Nano-scale structures (e.g. nanoparticles and nano-layers) have an extremely high surface-to-volume ratio[3]. Owing to their unique properties (e.g. high surface-to-volume ratio), nanostructures have several applications including the construction of lithium-ion batteries, fuel cells and solar cells; therefore, they could be regarded as commercial nanostructures.

Metal oxide nanostructures have beneficial applications in biological and medical sciences; such examples are drug delivery, cancer therapy and fluorescence imaging[4]. In scientific and research areas, metal oxide nanostructures are of utmost importance due to their broad applications in energy storage and photonic devices and sensors, as well as their medical and biological applications[3]. Zinc oxide (ZnO) nanoparticles have exhibited notable properties such as semi-conductivity and piezoelectric features. As a result, ZnO nanoparticles have been reported to be abundant nanostructured materials.

ZnO nanoparticles are utilized in numerous fields such as ultraviolet light detectors, chemical adsorbents, optical degradation catalysts (as a substitute of titanium nanoparticles) and the construction of semi-conductors and ultraviolet filters. Furthermore, these nanoparticles are commonly used in the medicine for the treatment of hospital-acquired infections with gram-positive and gram-negative bacteria[5].

Dracocephalum kotschyi is a semi-woody plant with the length of 20 centimeters, multiple wooden stems, petiolate and egg-formed leaves and yellow-white flowers which blossoms at the end of April and lasts until the middle of June. This plant is used to relieve rheumatic and joint pains. In addition, *Dracocephalum kotschyi* has been reported to have anticancer properties due to the presence of xanthomicrol. Among the other major components of *Dracocephalum kotschyi* are limonene, alpha-terpineol, verbenone, perillyl alcohol and caryophyllene.

According to the literature, the therapeutic effects of the extract of *Dracocephalum kotschyi* include the reduction of fever, joint pain and rheumatism, memory reinforcement, treatment of multiple sclerosis, acceleration of the blood flow and treatment of dizziness, tinnitus and headache, which are comparable to hyoscine and indomethacin[6,7].

In a research in this regard, Jamdagni et al[8] performed the green synthesis of ZnO nanoparticles using the extract of *Nyctanthes arbor-tristis* (olea or olive family). In the mentioned study, the herbal extract was applied as a biological reducing agent in the synthesis of ZnO nanoparticles and obtained from zinc acetate dihydrate[8]. In another research, Gnanasangeetha et al[9] successfully synthesized ZnO nanoparticles using the aqueous extract of *Acalypha indica*. Furthermore, several studies have confirmed the antimicrobial activity of ZnO nanostructures against the pathogens associated with food (e.g. pathogenic bacteria). *S. aureus* and *E. coli*are considered to be the most important food pathogens[9].

In another study, Karimi et al[10] used the extract of *Allium jesdianum* in the synthesis of ZnO nanoparticles. In the mentioned research, medium-sized nanoparticles (30 nm) with multidimensional, round particles were synthesized. Furthermore, Rafiei et al[11] utilized the extract of *Eucalyptus melliodora* and zinc sulfate solution (0.1 M) in order to synthesize round nanoparticles with the size of 30-50 nanometers.

Accordingly, to the findings, the synthesized nanoparticles exerted antibacterial effects against *S. aureus*, *Bacillus cereus*, *E. coli* and *Pseudomonas aeruginosa*[11]. In the mentioned research, the biosynthesis of ZnO nanoparticles was performed using natural extracts and various salts based on different instructions, resulting in the synthesis of nanoparticles in various shapes and sizes[12].

With this background in mind, the present study aimed to evaluate the green synthesis of ZnO nanostructures using the extract obtained from the aerial parts of *Dracocephalum kotschyi*, while assessing the antioxidant properties of this plant and antibacterial properties of the synthesized nanoparticles.

Material and Methods

All the materials used in this study were purchased from Merck Company, Germany. *Dracocephalum kotschyi* was collected from the heights of Bojnurd in North Khorasan province, Iran. The plant is depicted in figure 1 and the plant species was confirmed by a botanical specialist.

Figure 1: *Dracocephalum kotschyi*

Double-distilled water was applied at all the experimental stages and 2,2-diphenyl-1-picrylhydrazyl free radical and standard industrial antioxidant of Butylated hydroxyl toluene (BHT) was utilized as well. The bacteria required to evaluate the antibacterial effects of the synthesized nanoparticles (*E. coli and S. aureus*) were purchased from the Pasteur Institute of Iran.

Extract Preparation: Initially, the aerial parts of the plant were collected and dried indirect sunlight. Afterwards, the dried plants were powdered, 10 grams of which was weighed and boiled in100 milliliters of double-distilled water at the temperature of 70°C for 15 minutes. The obtained mixture was filtered using a filter paper and the filtered solution was preserved at the temperature of 4°C for further use.

Antioxidant Properties of *Dracocephalum kotschyi*: Initially, the prepared extract was concentrated using a rotary device and dried in plates for several days, powdered and preserved in special containers. Methanol solutions were obtained from the powder of *Dracocephalum kotschyi* and BHT at various concentrations in order to assess the antioxidant properties of the herb. In total, 3.9 milliliters of α, α-diphenyl-β-picrylhydrazyl (DPPH) methanol solution were added to each sample and the samples were preserved in the dark at room temperature for 50 minutes. Sample adsorption was determined at 517 nanometers (Table 1).

Data on the evaluation of the antioxidant activity of *Dracocephalum kotschyi* and BHT extract are presented in table 1.

Synthesis of ZnO Nanostructures: At this stage, zinc acetate solution (0.02 M) was used to synthesize the nanoparticles. In order to evaluate the effect of the concentration, 50 milliliters of the zinc acetate solution were poured into three 100-milliliterglass Erlenmeyer flasks. Following that, various concentrations of the *Dracocephalum kotschyi* extract (0.5, 1.5 and 2.5 ml) were added to the flasks. Prior to the tests, the pH of the solution was set at 12 using the NaOH solution. Afterwards, the solution was placed in a magnetic stirrer for 21 and two hours. However, the flasks were covered with aluminum foil so as to prevent possible oxidation with air oxygen. After the tests, color change from dark yellow to pale yellow (colloid solution) indicated the production of the ZnO nanoparticles as in figure 2.

The UV-visible spectrum was obtained from the final solution after the expected time followed by the centrifugation of the solution in triplicate for SEM and XRD analyses. In the first two times, the precipitate was collected with distilled water and washed with 99.1% ethanol at the third stage and the resulting material was centrifuged. Finally, the solution in the tube was discarded and the remaining precipitate was preserved in an oven at the temperature of 80˚C for 12 hours in order for the synthesized precipitate (ZnO nanoparticles) to be completely dried. Afterwards, the powder of the ZnO nanoparticles turned white and was preserved foil-covered in closed containers for spectroscopy.

Figure 2: Colloid Solution Containing ZnO Nanostructure Synthesized by *Dracocephalum kotschyi* Extract

Results and Discussion

Antioxidant Activity of the *Dracocephalum kotschyi* Extract: According to the obtained results, the IC_{50} values of BHT and *Dracocephalum kotschyi* extract were obtained as follows:

IC_{50} (BHT)=0.01923 g/ml
IC_5 (extract)=0.03771 g/ml

The insignificant difference between the IC_{50} of the aqueous extract of *Dracocephalum kotschyi* and BHT (industrial antioxidant) indicated the significant antioxidant properties of the plant. As such, the *Dracocephalum kotschyi* extract could substitute BHT. Furthermore, the elevated concentration of the material enhanced the antioxidant properties (Table 1).

Effects of Extract Concentration and Reaction Time on the Synthesis of the ZnO Nanoparticles: The direct effects of the extract concentration and reaction time on the synthesis of the ZnO nanoparticles using the *Dracocephalum kotschyi* extract became evident after the assessment of these variables (Table 2). According to the UV-Vis spectrum (Figure 3), the increased concentration of the *Dracocephalum kotschyi* extract and higher incubation duration were associated with the increased maximum adsorption (λ_{max}).

X-ray Diffraction Pattern of the Synthesized ZnO Nanoparticles: At this stage, the crystalline structure of the synthesized ZnO nanostructures was assessed via X-ray diffraction spectroscopy. Comparison of the XRD spectra of the synthesized samples is depicted in figure 4 with the standard spectra indicating the presence of the ZnO nanoparticles.

Table 1
Antioxidant Activity of *Dracocephalum kotschyi* Extract and BHT

BHT and Aqueous Extract Amount(g)	BHT Standard Adsorption	BHT Inhibition Rate	Extract Adsorption	Inhibition Rate of Extract
0.00025	0.291	76.437	1.320	-6.88
0.0005	0.190	84.61	1.102	10.769
0.001	0.101	91.821	0.590	52.226
0.002	0.064	94.81	0.321	74.008
0.004	0.057	95.384	0.161	86.963
0.008	0.055	95.546	0.116	90.60
0.016	0.048	95.870	0.113	90.85

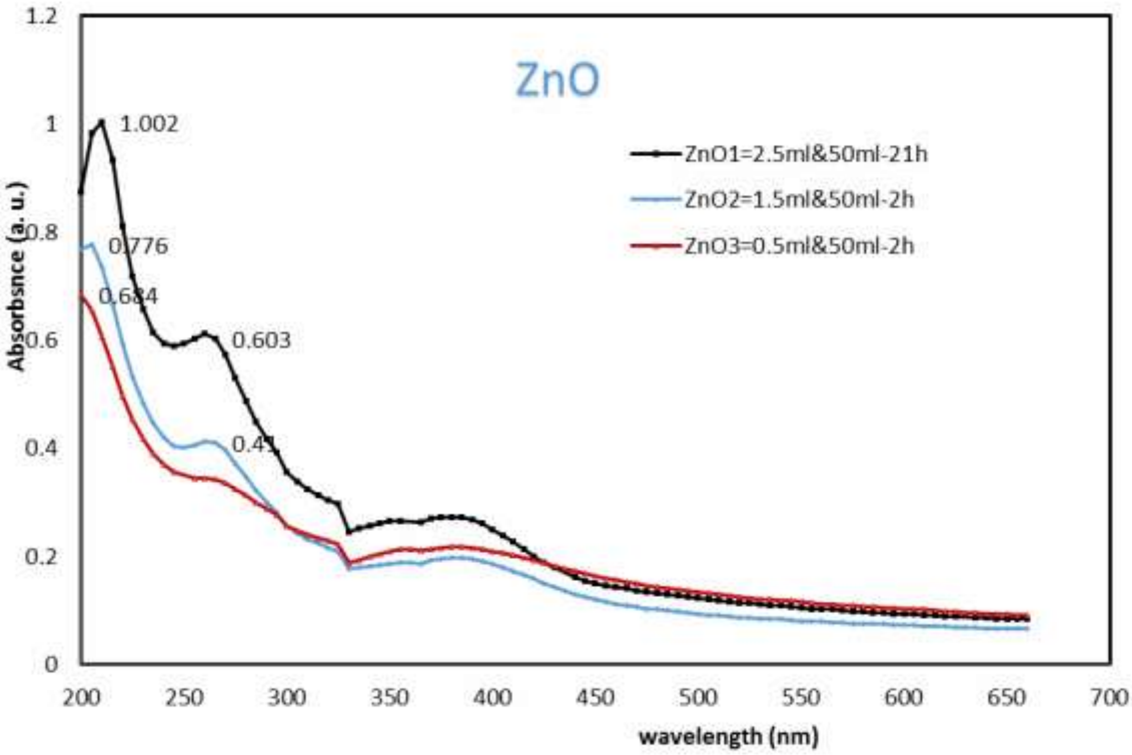

Figure 3: UV-Visible Spectrum of Colloid Solution Containing ZnO Nanoparticles at Various Reactions Times and Extract Concentrations

Table 2
Effectso f Extract Concentration and Reaction Time on Synthesis of ZnO Nanoparticles

Sample	Wavelength	λ_{max} (nm)	Extract Concentration(ml)	Reaction Time (h)
ZnO_1	210	1.002	2.5	21
ZnO_2	205	0.776	1.5	2
ZnO_3	200	0.684	0.5	2

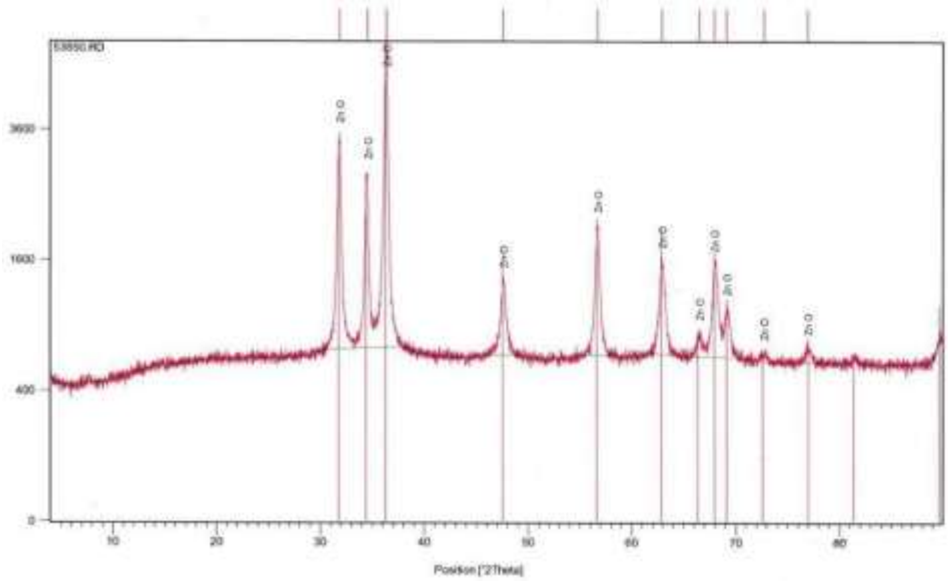

Figure 4: XRD Spectrum of ZnO Nanoparticles Synthesized by *Dracocephalum kotschyi* Extract

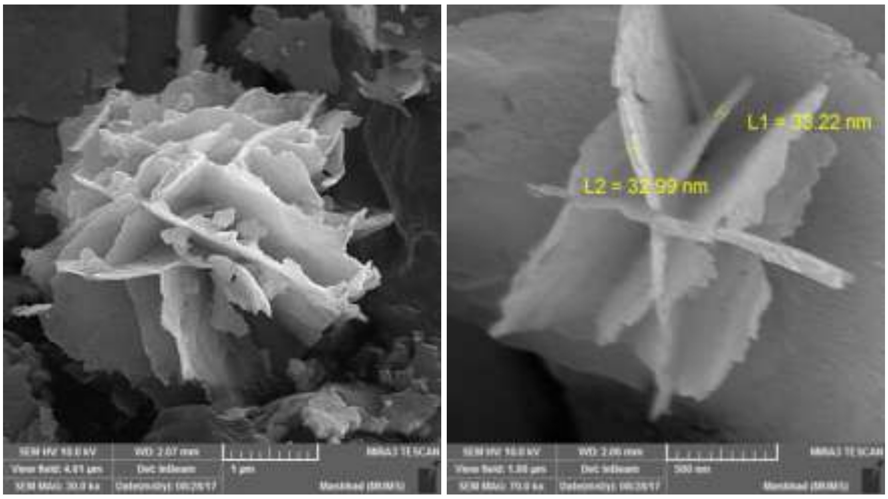

Figure 5: SEM Images of ZnO Nanoshells

Scanning Electron Microscopy (SEM): The morphology and size of the ZnO nanoparticles synthesized by SEM were evaluated (Figure 5). According to the findings, the ZnO nanostructures had the morphology of nanoshells and an approximate size of 33 nanometers. In addition, the clear, uniform distribution of the synthesized nanoparticles was observed which is considered to be an advantage of the applied synthesis method.

Energy-dispersive X-ray Spectroscopy (EDS): Energy-dispersive X-ray spectroscopy (EDS) is a method used for the evaluation of the structure and elements of various compounds. In the current research, the EDS spectrum of the synthesized ZnO samples was prepared to recognize the elements in the ZnO nanostructure (Figure 6). The presence of zinc and oxygen elements in the samples was confirmed based on the EDS spectrum and the purity of the synthesized nanomaterials was presented.

Fourier-transform Infrared Spectroscopy (FT-IR): Evaluation of the chemical structure and type of the chemical bonds of the ZnO nanoparticles was performed using infrared spectroscopy (Figure 7). Accordingly, the absorption spectrum observed at the regions of 3,400 and

3,600 $cm^{-1}$was associated with the stretching and bending vibrations of the H-O bond. This group could be associated with the organic compounds of the plant, which were absorbed by the surface of the ZnO nanoparticles. Nanoparticles have layers of compounds in the extract which could help stabilize nanoparticles[13]. In this regard, the severe absorption at560 cm^{-1} was associated with the stretching vibrations of the Zn-O bond.

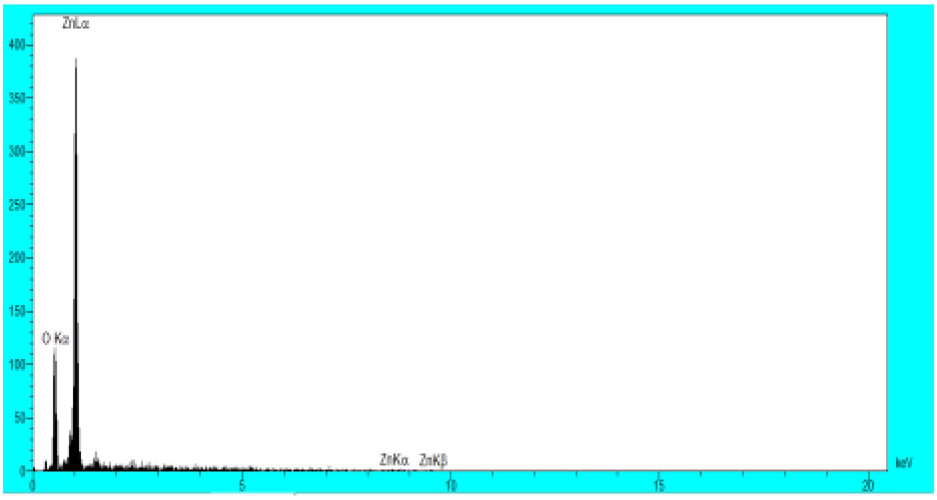

Figure 6: EDS Spectrum of Synthesized ZnO Nanoparticles

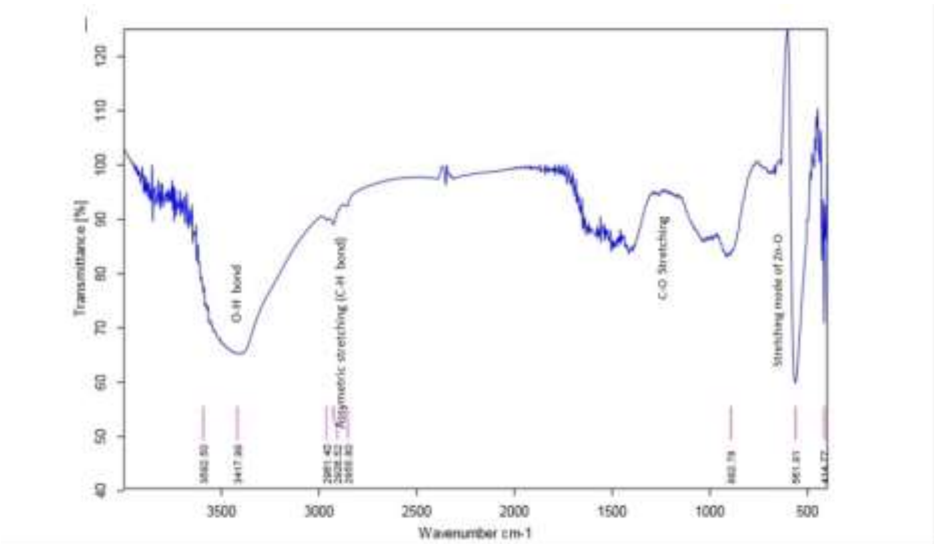

Figure 7: FT-IR Spectrum of Synthesized ZnO Nanoparticles

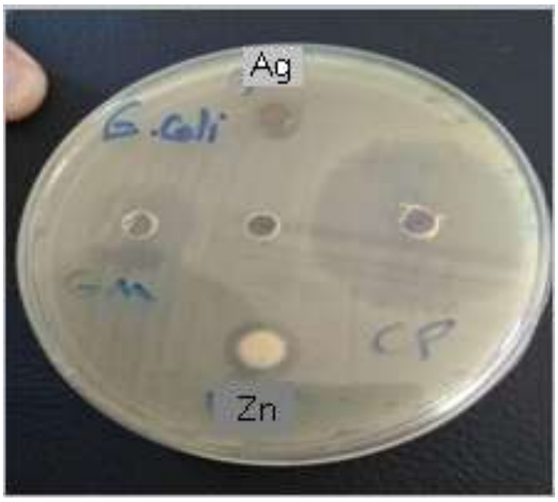

Figure 8: Evaluation of Antibacterial Properties based on Growth Inhibition Zone in Synthesized Nanoparticles and Control Antibiotics against *E. coli* and *S. aureus*

Table 3
Antibacterial Effects of Synthesized ZnO Nanoparticles and Standard Antibiotics against *E. coli* and *S. aureus*

Bacterium	Diameter of Growth Inhibition Zone (mm)		
	ZnO Nanoparticles	Gentamicin	Cefalotin
E. coli	12	20	35
S. aureus	20	23	30

Antibacterial Properties of the Synthesized ZnO Nanostructures: At this stage, the growth inhibition zone in the presence or absence of the ZnO nanostructures was measured in order to evaluate the sensitivity of *E.coli* and *S. aureus*, which were produced in the research. Initially, 20 micro liters of the standard strains of *E. coli* and *S. aureus* were added to a tube containing three milliliters of liquid culture medium and incubation was carried out in a microwave oven at 250 rpm and temperature of 37°C for 24 hours. After 10 minutes, the sample in the tubes was centrifuged, washed with phosphate buffer saline three times and cultured on Agar medium in four directions.

Following that, sterilized paper round sheets were placed on the culture medium. At the next stage, 20 micrometers of the samples (ZnO nanostructure, gentamicin and cefalotin) were poured on the sheets in four directions and the sheets were placed in the oven at the temperature of 37°C. After 24 hours, the diameters of the growth inhibition zone around each round plate were measured on a millimeter scale (Table 3).

In this experiment, the obtained results regarding the antibacterial activity of the nanostructures were compared with the antibiotic species used as control. Accordingly, the ZnO nanostructures synthesized by the *Dracocephalum kotschyi* extract indicated effective antibacterial activity against the mentioned bacterial pathogens.

Discussion

Within the past few decades, the preparation and evaluation of nanoparticles have attracted the attention of researchers in the fields of applied sciences. To date, various physicochemical methods (e.g. chemical reactions, reverse micelles, thermal decomposition and laser radiation) have been applied for the synthesis of nanostructures. Some of the main limitations of these methods include low production rate, application of hazardous materials and high loss of energy. Biological synthesis of nanostructures is a method that has attracted the attention of most of scientists owing to its advantages such as low costs, non-toxicity and eco-friendly nature.

Furthermore, high levels of secondary metabolites and significant antioxidant properties in plants are involved in the restoring and stabilizing of nanoparticles. In the present study, we used the herbal extract of *Dracocephalum kotschyi*, which is a native species in Iran used in traditional medicine to reduce fever, joint pain and rheumatism. Initially, DPPH free radical and BHT antioxidants were used to estimate the antioxidant activity of *Dracocephalum kotschyi* and the IC_{50} and BHT values were obtained (Table 1). With regard to the insignificant difference between the IC_{50} values of *Dracocephalum kotschyi* and BHT, it could be concluded that the herbal extract has high antioxidant activity and could be used to synthesize ZnO. In order to synthesize ZnO nanoparticles, we applied the combination of the *Dracocephalum kotschyi*extract and zinc acetate solution (0.02 M) at the optimal conditions of pH=12 (NaOH).

As is depicted in figure 2, the clear yellow solution turned into a very pale yellow colloid mixture, which indicated the production of white precipitate on the ZnO nanoparticles. After preparing the UV-visible spectrum using the samples, the synthesized nanoparticle was absorbed at 200-700 nanometers (Figure 3). According to the information in table 2, the maximum adsorption of the nanoparticle was within the range of 200-210 nanometers.

With respect to the impact of reaction time on the preparation of the ZnO nanoparticles, the duration of 21 hours was selected as the optimum time for the synthesis of the ZnO nanoparticles. As can be seen in figure 3, the evaluation of the effect of concentration showed the increased concentration of *Dracocephalum kotschyi*was associated with the higher absorption rate of ZnO. In the current research, the concentration of the herbal extract was determined to be 2.5 milliliters and SEM was applied to measure the size, morphology and uniform distribution of the produced nanoparticles.

As shown in figure 5, the ZnO nanoparticles had the morphology of nanoshells with the mean size of 33 nanometers. In addition, the nanoparticles had the same size distribution. It is notable that changes in the temperature, pH and duration and concentration of the interactions between the salt solution and herbal extract may affect the range variations in the nanoparticle size[14].

Conclusion

In the present study, the dried white powder of the ZnO nanoparticles was used to analyze the X-ray energy. According to the findings, the presence of zinc and oxygen in the samples was confirmed (Figure 6). Moreover, the synthesized nanoparticles were observed to have high purity.

Considering the formation of distinct, sharp peaks in the assessment of the XRD spectrum, it could be concluded that the ZnO nanoparticles were successfully synthesized in all the test conditions (Figure 4).According to the information in table 3 and observations regarding the diameters of the growth inhibition zone (Figure 7), it seems that ZnO nanoparticles have antibacterial properties and are comparable to standard antibiotics. Therefore, the biological synthesis of nanoparticles could have medical applications due to these antibacterial properties.

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