

Research Article

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Effectiveness of different accelerated green synthesis methods in zinc oxide nanoparticles using red pepper extract: Synthesis and characterization

<https://doi.org/10.1515/gps-2022-0053>

received February 01, 2022; accepted April 20, 2022

Abstract: Potential ability of red pepper extract in zinc oxide nanoparticles (ZnO NPs) fabrication via three accelerated heating techniques, namely, conventional heating with stirring (at 100°C and 700 rpm, for 30 min), autoclave (at 15 psi and 121°C, for 15 min), and microwave irradiation (power of 800 W for 3 min) was assessed. Fourier transform infrared spectroscopy and gas chromatography mass spectroscopy indicated that the prepared extract contained 20 active compounds including alcohols, aldehydes, ketones, esters, and organic acids with several functional groups such as hydroxyls. Results indicated that ZnO NPs with spherical and hexagonal structures have been formed using three different heating methods, and the minimum crystal size for the fabricated ZnO NPs was 88.44 nm, which was attained using heating by autoclave. Antioxidant activity of the synthesized ZnO NPs was determined using the red pepper extract, and accuracy of this method was 80.21%. Finally, results indicated that the formed ZnO NPs had high antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus faecalis* and high antifungal activity against *Aspergillus niger* and *Aspergillus flavus*.

Keywords: accelerated heating method, antimicrobial activity, green synthesis, red pepper extract, zinc oxide nanoparticles

1 Introduction

Among novel technologies, nanotechnology is a concept that has been quickly developed in recent years for applications in various areas. Materials in nanoscale have gained much attention due to their unique physico-chemical properties, and those have been widely utilized in the production of different new systems, structures, devices, and nanoparticles in various fields with imminent suggestions [1,2].

Green synthesis of inorganic materials by plants and their derived extracts has been considered due to the presence of numerous natural bioactive substances, such as carbohydrates, phenols, terpenoids, ketones, aldehydes, proteins, enzymes, and amides in these natural sources [3,4]. Zinc oxide nanoparticles (ZnO NPs), as a metal oxide NPs, have been fabricated and used in various areas due to their unique stability, low poisonousness, higher resistance to heat, higher antimicrobial, and photocatalytic activities [5]. In fact, ZnO NPs have been widely used in plastics, ceramics, cement, lubricant, dye, adhesion, pigment, packing, cosmetics, and textile industries [6,7]. In addition, ZnO NPs have been recognized as strong antibacterial, antifungal, and antiviral agent toward wide ranges of the microorganisms [7,8]. There are three approaches to the synthesis ZnO NPs, namely, physical, chemical, and biological [9]. Physical methods are accomplished in high-temperature conditions using microwave oven or autoclave, which need high energy [10]. Chemical fabrication methods are based on employing chemical agents for both reducing of the metal ions and stabilizing of the formed NPs, and the chemical residuals in final products can limit the utilization of these NPs in some areas such as food, medicine, and cosmetics. To overcome the mentioned limitations, researchers did their best to find alternative fast, clean, cost-effective, and environment-friendly methods for the metal NP synthesis [11]. Biological synthesis techniques are known as green methods and have several

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advantageous due to their cheapness, resource availability, fast synthesizing process, single-stage nature, and easy scalability [12]. Compared to the conventional synthesizing methods, the synthesis of metal and metal oxide NPs by plant extracts is time consuming due to low concentration of bioactive components of the natural extracts for reduction of ions and convert those into NPs. Incorporation of the heat accelerated techniques such as autoclave, ultrasonication, ultraviolet (UV) irradiation, and microwave radiation to the biological synthetic methods can considerably increase the rate of NP formation [13–16].

Red pepper is obtained from the bell pepper genus and the Solanaceae (nightshades) family. It is used as a spice in different foods. Several studies indicated that the red pepper extract contains valuable bioactive compounds such as flavone, flavonol, xanthine, free steroids, cellulose, and reducing sugars, as well as small amounts of copper, zinc, magnesium, and iron. Most of these bioactive compounds have reducing and stabilizing properties, which make those more suitable in the synthesis of organic NPs [15]. Different studies have showed the antimicrobial activity of ZnO NPs [17–19]. In an era of eco-friendly expansion, methods of the green synthesis of ZnO NPs from plant extracts have become a focus of research attention because of the benefits of environmental sustainability, simplicity, and low price. It is important to prepare ZnO NPs with a morphology suitable for the purpose intended. Compared with physical and chemical methods, the preparation of ZnO NPs from plant extracts can be controlled, so that NPs obtained have a specific size and morphology.

Therefore, the main objectives of the present study were as follows: (i) preparation of the red pepper extract and determining its main compounds, (ii) synthesis and characterization of the ZnO NPs using red pepper extract and three different heating methods based on microwave, autoclave, and conventional heater with stirring, and (iii) studying the physico-chemical properties, antimicrobial, anti-fungal, and antioxidant activities of the formed ZnO NPs.

2 Materials and methods

2.1 Materials

Red peppers were purchased from a greenhouse in Tabriz, an east north city of Iran. Ethanol and double distilled water (DDW) were provided by Dr. Mojalali Company (Tehran, Iran) and $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, bought from Merck (Merck GmbH & Co. KG, Darmstadt, Germany). *Staphylococcus aureus* (PTCC 1764), *Escherichia coli* (PTCC 1330), *Pseudomonas*

aeruginosa (PTCC 1310), *Enterococcus faecalis* (PTCC 1237), *Aspergillus niger* (5010 PTCC), *Aspergillus flavus* (5006 PTCC), and *Penicillium aculeatum* (PTCC-5167) were provided from Persian type culture collection (PTCC, Tehran, Iran). Nutrient agar and potato dextrose agar were purchased from Biolife Company (Milan, Italy).

2.2 Preparation and characterization of the red pepper extract

The provided red peppers were eluted by DDW for the removal of their dust and dried at room conditions (20°C and relative humidity of 45%), for 7 days. Afterward, red pepper dried powder was prepared utilizing a domestic grinder (MX-GX1521, Panasonic, Tokyo, Japan), and 1 g of the powder was added into 100 mL of DDW, boiled for 5 min, and filtered by filter paper (Whatman No. 1). Finally, the prepared extract was stored at 4°C for further analyses and the synthesis of ZnO NPs.

Fourier transform infrared spectroscopy (FT-IR), using a Bruker Tensor 27 spectrometer (Karlsruhe, Germany) and KBr salt, in the wavenumber ranging 400–4,000 cm^{-1} , was utilized to determine the main functional groups existed in the prepared red pepper extract. The main bioactive compounds of the red pepper extract were determined by a gas chromatography technique (gas chromatography mass spectroscopy (GC-MS), Agilent 6890, Santa Clara, CA, USA). A capillary column (HP-5 30 \times 0.25 mm) in combination with mass spectrometry (HP5989A) ionizing electrons at 70 eV were used in the GC-MS system. Helium was also used as a carrier gas.

2.3 Synthesis of ZnO NPs

To fabricate ZnO NPs, 2 g of zinc nitrate was dissolved in 20 mL of the prepared red pepper extract, and mixture solution was then exposed into three different heating methods based on autoclave, microwave oven, and heater with stirring equipment. According to the literature, fabrication conditions using each technique have been selected [13–16].

In conventional heating method, the mixture solution was heated using a magnetic heater with stirring, set at rotation speed of 700 rounds per minute and temperature of 100°C, for 30 min. In the hydrothermal method, solution containing zinc salt and red pepper extract was placed in an autoclave, at 15 psi and 121°C for 15 min.

In microwave heating technique, the prepared solution was placed in a home microwave oven (MG-2312W, LG Co., Seoul, South Korea) with a power of 800 W and a maximum temperature of 250°C for 3 min. Then, the heated mixture solutions with three mentioned methods were poured into ceramic cups and placed in the furnace, adjusted at 350°C for 2 h. ZnO NPs were obtained as resultant yellow powder and stored in dark and closed bottles for further analyses.

2.4 Characteristics of the resulted ZnO NPs

Structural characteristics and morphology of the synthesized ZnO NPs were evaluated using X-ray diffractometry (XRD; D5000, Siemens Co., Karlsruhe, Germany) using Cu-K α irradiation and 2 theta ranging 10–80 and a scanning electron microscope (SEM; CamScan MV 2300, Teskan, the Czech Republic) with magnification of 200 nm [16].

The average crystal size of the synthesized ZnO NPs was calculated by the Debye-Scherrer equation (Eq. 1):

$$D = 0.89\lambda/\beta \cos \theta \quad (1)$$

where D , λ , β , and θ were related to the crystal size, the wavelength of X-ray, full width at half maximum, and diffraction angle, respectively [20].

Antioxidant activity of the fabricated ZnO NPs was assessed using described method by Anzabi, based on the inhibitory capacity of 2,2-diphenyl-2-picrylhydrazyl (DPPH) and Equation 2 [16]:

$$I\% = (A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100 \quad (2)$$

where $I\%$ represented the inhibition percentage of the free radical, and A_{control} and A_{sample} are the absorbance of the solutions without and with the ZnO NPs, respectively. The absorbance was measured by a UV-spectrometer (250–800 nm, Perkin Elmer, Überlingen, Germany), adjusted at wavelength of 570 nm.

2.5 Antibacterial and antifungal activities of the synthesized ZnO NPs

To assess bactericidal effects of the fabricated ZnO NPs, based on agar diffusion method, which was explained by Torabfam and Jafarzadeh-Malmiri [21], bacterial suspensions having 1.5×10^8 colony forming units of *S. aureus*, *E. coli*, *P. aeruginosa*, and *Enterococcus faecalis* were provided. Diameter of the formed inhibition zones around the holes containing ZnO NPs, in the incubated plates at 37°C and after 24 h, was measured and reported as antibacterial activity of the synthesized ZnO NPs.

Antifungal activity of the fabricated ZnO NPs against *A. niger*, *A. flavus*, and *P. aculeatum* was investigated based on the mycelia growth inhibition method, described by Mohammadlou et al. [22]. The antifungal assay was accomplished in 7 days of incubation at 26°C.

2.6 Statistical analysis

All physico-chemical properties and antimicrobial activities of the synthesized ZnO NPs were done in three replications. The data statistical analyses were interpreted by analysis of variance using Minitab software (V.16, Minitab Inc., PA, USA). Tukey's test was used for the comparison of the means at the significance level of 5%.

3 Results and discussion

3.1 Specifications of the prepared extract

FT-IR spectrum of the prepared red pepper extracts is shown in Figure 1. As can be clearly observed in this figure, six absorption peaks, centered at wavenumber of 690.71, 1,074.39, 1,411.08, 1,638.77, 2,078, and 3,445.92 cm⁻¹, were detected. The most expansive absorption peak (3,445.92 cm⁻¹) was related to the OH stretching vibrations of the hydroxyl groups, which were mainly observed in alcohols and phenolic compounds and are capable to reduce zinc ions and convert those into stable ZnO NPs [23]. The observed peak at 2,078.20 cm⁻¹ was related to the C=O bond vibrations and N–H bond, which were found in octadecadienoic acid and formamid [24]. The centered peak at 1,638.77 cm⁻¹ was related to the OH bending vibration and shows high water absorption over the sample with regard to the sharpness of this peak. The detected peak at 1,411.08 cm⁻¹ was related to the C–C aromatic stretching materials, which belonged to polyphenols and ethanol. Furthermore, the asymmetrical stretching vibrations of the carboxylic groups were related to the centered peak at 1,412 cm⁻¹ and related to the organic acids. The peak centered at 1,074.39 cm⁻¹ was related to the C–O stretching, which could be observed in alcohols and vanillin [25,26]. According to the GC-MS analysis, main bioactive compounds of the prepared extract are presented in Table 1. It was found that almost 20 active compounds were recorded during 36 min of the retention time (Figure 2). Alcohols, aldehydes, ketones, esters, amides, amines, terpenoids,

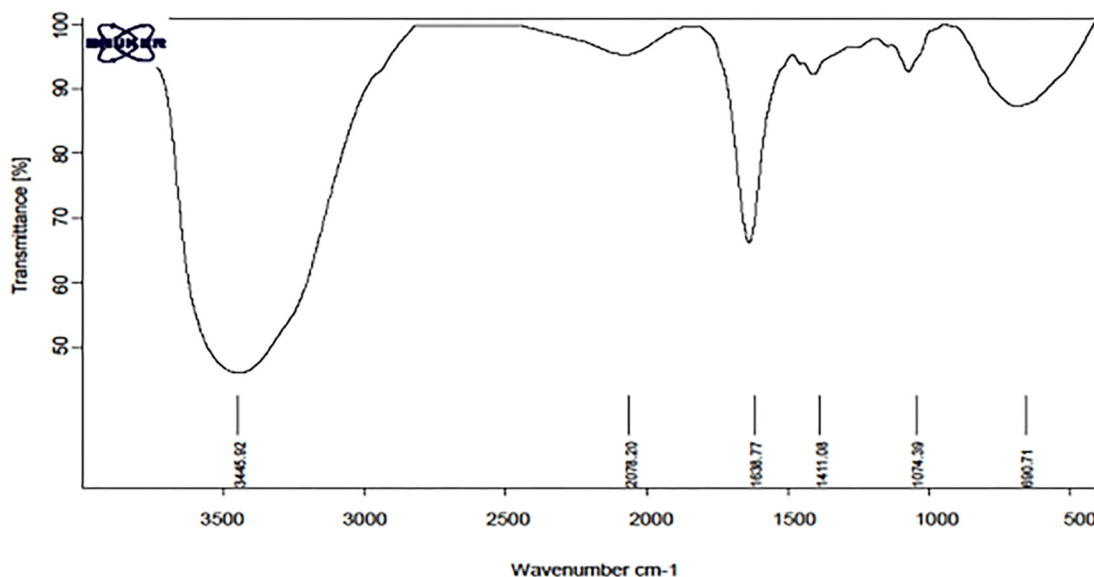


Figure 1: FT-IR spectrum of the prepared red pepper extract.

and organic acids were the main components that have key roles in the synthesis of ZnO NPs.

3.2 Characteristics of the fabricated ZnO NPs

In the green synthesis of ZnO NPs using red pepper extract, the mechanism of the formation of NPs can be explained by the fact that $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ dissociated into Zn^{2+} in solution and hydroxyl functional groups in the prepared red pepper extract could be combined with these ions to form $\text{Zn}(\text{OH})_2$. ZnO NPs were then prepared by drying $\text{Zn}(\text{OH})_2$ during heating using three different methods, prior to calcination in a furnace. In fact, formation of ZnO NPs using red pepper extract was completed through the precipitation method [27]. XRD patterns of the fabricated ZnO NPs using red pepper extract through three different heating methods are shown in Figure 3a–c. As can be clearly observed in Figure 3a, using the conventional heating method, numerous centered peaks at 10.34° , 11.20° , 27.24° , 31.67° , 34.34° , 36.14° , 39.25° , 43.16° , 47.42° , 56.49° , 62.76° , 66.40° , 67.83° , 68.90° , and 76.82° , 2θ positions were observed. These were linked to the (001), (020), (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (202), and (104) planes. The most intense peak, 31.67° , was related to the diffraction plane of 100 and was fully coincided with the JCPDS-36-1451 standard for ZnO NPs. The intensity of peaks and their relative thinness indicated the high crystallinity of the formed NPs [28,29]. The average

crystal size of the synthesized ZnO NPs using red pepper extract and conventional heating method was 98.75 nm.

The XRD pattern of the fabricated ZnO NPs by autoclave heating method and red pepper extract is shown in

Table 1: Main bioactive compounds of the red pepper extract

No.	Volatile compounds	Retention time (min)	Relative content (%)
1	Ethanol	1.54	2.80
2	Formamide	10.77	0.68
3	Benzyl alcohol	11.56	0.82
4	Benzenemethanol	12.47	0.4
5	2-Propenal, 3-phenyl	15.71	8.35
6	Cinnamaldehyde	17.68	0.15
7	Vanillin	18.78	1.1
8	Ethyl vanillin	19.22	0.91
9	Alpha-muurolene	19.39	0.28
10	Delta-cadinene	19.74	0.21
11	2-Butanone	20.52	0.34
12	5-Butylpentan-5-olide	22.42	0.51
13	Hexadecanoic acid	25.61	0.35
14	9-12-Octadecadienoic acid	27.17	0.15
15	9-Octadecenoic acid (z)	27.98	1.3
16	9-Octadecenoic acid (e)	28.16	0.05
17	3-Heptanone,6-(dimethylamino)	28.25	0.78
18	9-Oxa-bicyclo[3.3.1]nona-3,6-dien-2-one	31.10	0.07
19	Di-(2-ethylhexyl) phthalate	31.97	0.16
20	Cholesta-3,5-diene	36.17	0.12

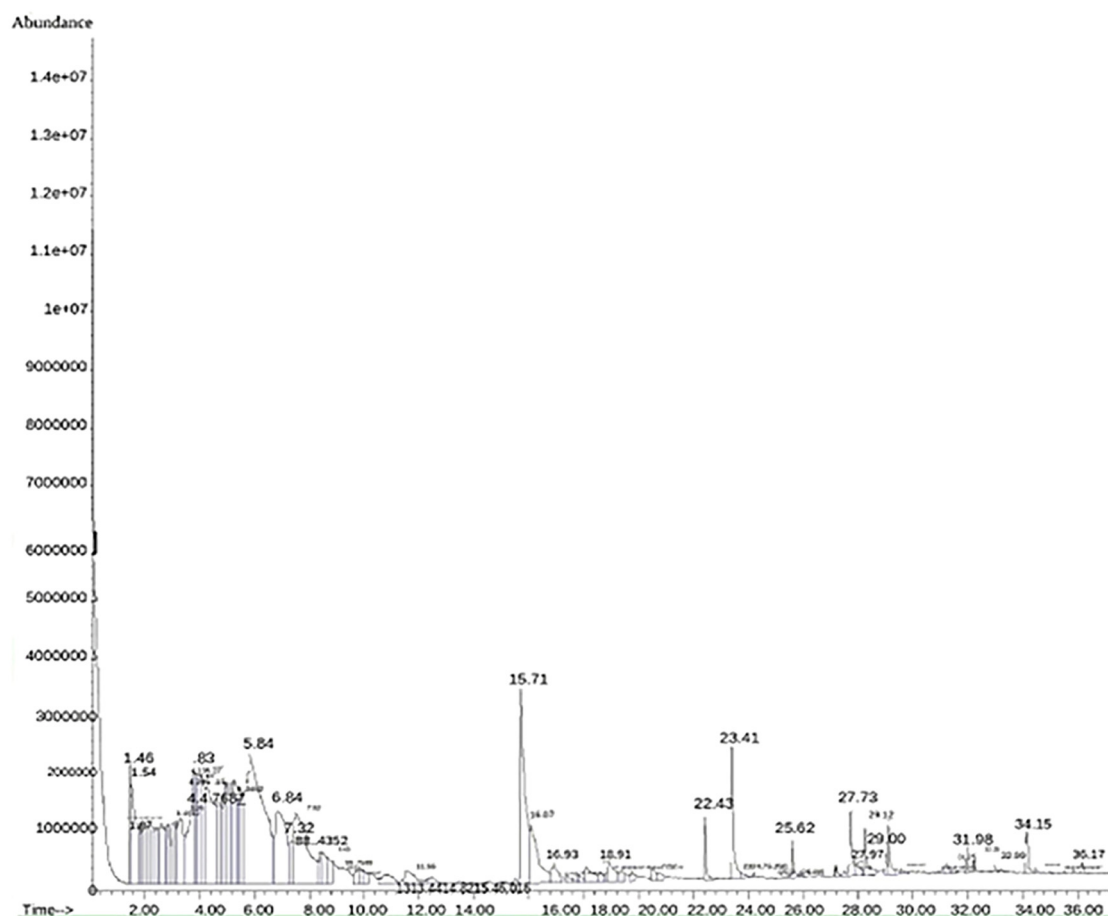


Figure 2: GC-MS chromatogram of the prepared red pepper extract.

Figure 3b. Diffractive peaks are observed at various 2θ values in this figure, which were associated with the (100), (002), (101), (102), (110), (103), (200), (112), (201), (202), (104), and (203) planes. The highly intense peak at 31.71° , connected to the (100) diffraction plane, which was fully corresponded with the standard XRD pattern of ZnO NPs. The average crystal size of the formed ZnO NPs by this method was 88.44 nm.

The XRD pattern of the fabricated ZnO NPs by microwave heating method (Figure 3c) presents several peaks at various 2θ values, which were correlated with the (100), (002), (101), (102), (110), (103), (200), (112), and (201) planes. The high-intense peak was centered at 36.07° of 2θ , related to the standard diffraction plane of (100) and completely coincided with (JCPDS-36-1451) standard. The average crystal size of the synthesized ZnO NPs was 91.16 nm.

The peak shift in XRD spectra of the synthesized ZnO NPs using three different methods was related to the structural changes on the formed ZnO NPs. In fact, by changes in heating time and temperature, which were observed in three fabricated techniques, there may be an increase or a

decrease in the crystallite size, depending on the nature of the peak shifting, which is closely related to broadening or shrinking of the XRD peaks [20,30,31]. However, peak shifting was small in the presented XRD spectra of the synthesized ZnO NPs (Figure 3). Obtained results showed good agreement with the findings of Çolak and Karaköse [30]. They synthesized ZnO NPs using the aqueous extract of *Thyme vulgaris* using conventional heating, with average crystallite sizes ranging from 42 and 85 nm. Furthermore, Vahidi et al. indicated that the fabricated ZnO NPs using *Pelargonium zonale* leaf extract under microwave irradiation, autoclave, and conventional heating methods had average crystalline size of 51, 60, and 61 nm, respectively [31].

SEM images of the fabricated ZnO NPs by the red pepper extract and three heating methods are shown in Figure 4a–c. Obtained results indicated that ZnO NPs with spherical and hexagonal structures have been formed using three different heating methods.

Obtained results, based on XRD analysis, indicated that the minimum crystal size for the fabricated ZnO NPs was 88.44 nm, which was attained using heating by autoclave.

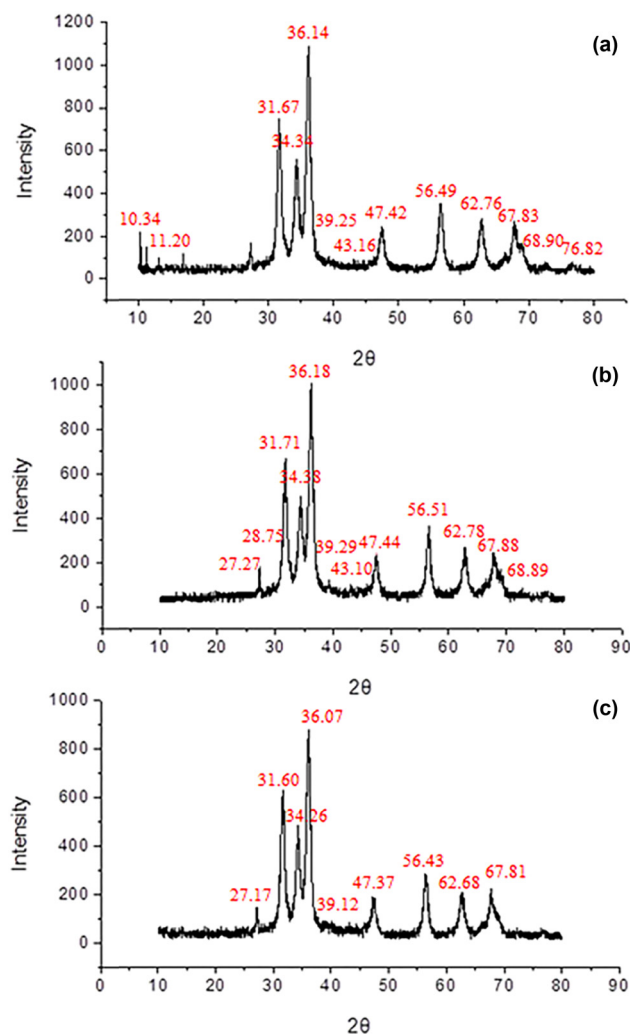


Figure 3: XRD patterns of the fabricated ZnO NPs using red pepper extract through conventional heating (a), autoclave (b), and microwave irradiation (c).

Therefore, the autoclave heating method was selected as more suitable technique in the fabrication of ZnO NPs using red pepper extract. Further analyses were done on the fabricated ZnO NPs by this heating method. It seems that higher temperatures could completely accomplish the reducing reaction and form ZnO seeds with the uniform size [16].

3.3 Antibacterial activity of the formed ZnO NPs using red pepper extract and autoclave

Table 2 presents the antibacterial activity of the formed ZnO NPs using red pepper extract, as manifested in the created clear zones around the holes around four different

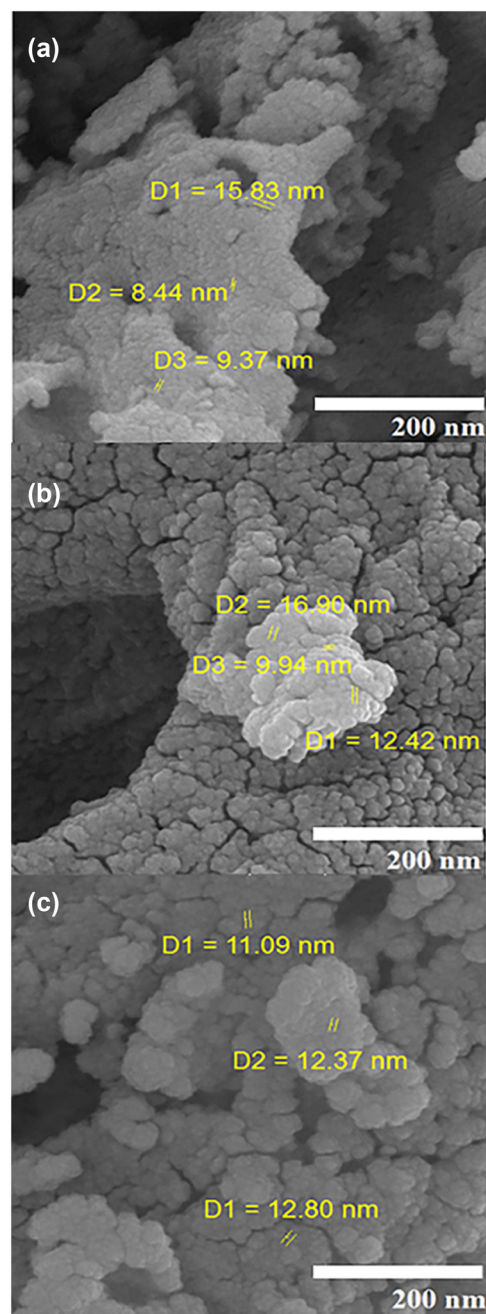


Figure 4: SEM images of the fabricated ZnO NPs by the red pepper extract and conventional heating (a), autoclave (b), and microwave irradiation (c).

Gram-positive and Gram-negative bacterial strains. Obtained result indicated that the synthesized ZnO NPs had inhibitory effects on all bacteria strains, except *P. aeruginosa*. In fact, results revealed that the Gram-positive bacteria strains were more sensitive than Gram-negatives toward ZnO NPs. This could be related to differences in their cell wall structures [16,30]. According to the findings of Behbahani et al., all Gram-negative bacteria possess lipopolysaccharide external

Table 2: Diameter of clear zones (mm) around the holes in the plates, containing synthesized ZnO NPs using autoclave heating methods and four different bacteria strains

Bacteria strains	<i>E. coli</i>	<i>Enterococcus faecalis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
Diameter of clear zone (mm)	14	12	14	0

walls operating as barriers against hydrophobic compounds. Since many extract compounds are hydrophobic, they cannot pass through the walls easily [32]. The primary mechanism of the antibacterial activities of the ZnO NPs can be attributed to their photochemical properties. It seems that the oxygen and H₂O absorbed in the ZnO NPs surfaces establish connections with load carriers derived from light refraction and reactive oxygen forms, such as singlet oxygen and hydroxyl radicals, leading to lipid membrane peroxidation and antibacterial effects [32]. Recent studies report promising insights for

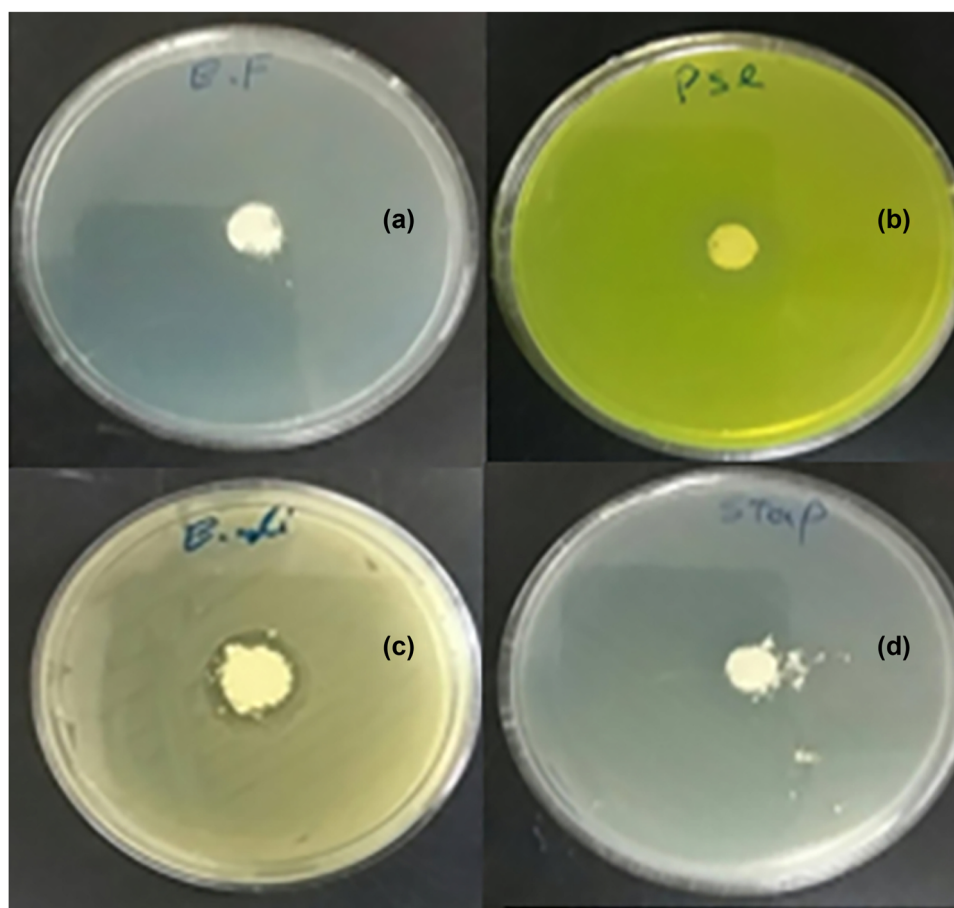


Figure 5: Created clear zones around the holes pored with the fabricated ZnO NPs in the plates that were inoculated with *Enterococcus faecalis* (a), *P. aeruginosa* (b), *E. coli* (c), and *S. aureus* (d).

Table 3: Mycelia growth (mm) of three different fungi strains, in the plates containing ZnO NPs and control plates

Fungi strains	<i>A. niger</i>	<i>A. flavus</i>	<i>P. aculeatum</i>
Mycelia growth (mm) in the sample plate after 2 days of incubation	10	10	0
Mycelia growth (mm) in the control plate after 2 days	23	22	7
Mycelia growth (mm) in the sample plate after 4 days	22	22	0
Mycelia growth (mm) in the sample plate after 4 days	40	50	8

developing ZnO NPs with considerable adaptability and negligible poisonousness that can be applied for medical uses [32,33]. Figure 5 shows the created clear zones around the

holes pored with the fabricated ZnO NPs in the plates. Obtained results were in line with findings of Vahidi et al. [31]. They also synthesized ZnO NPs using *Pelargonium*

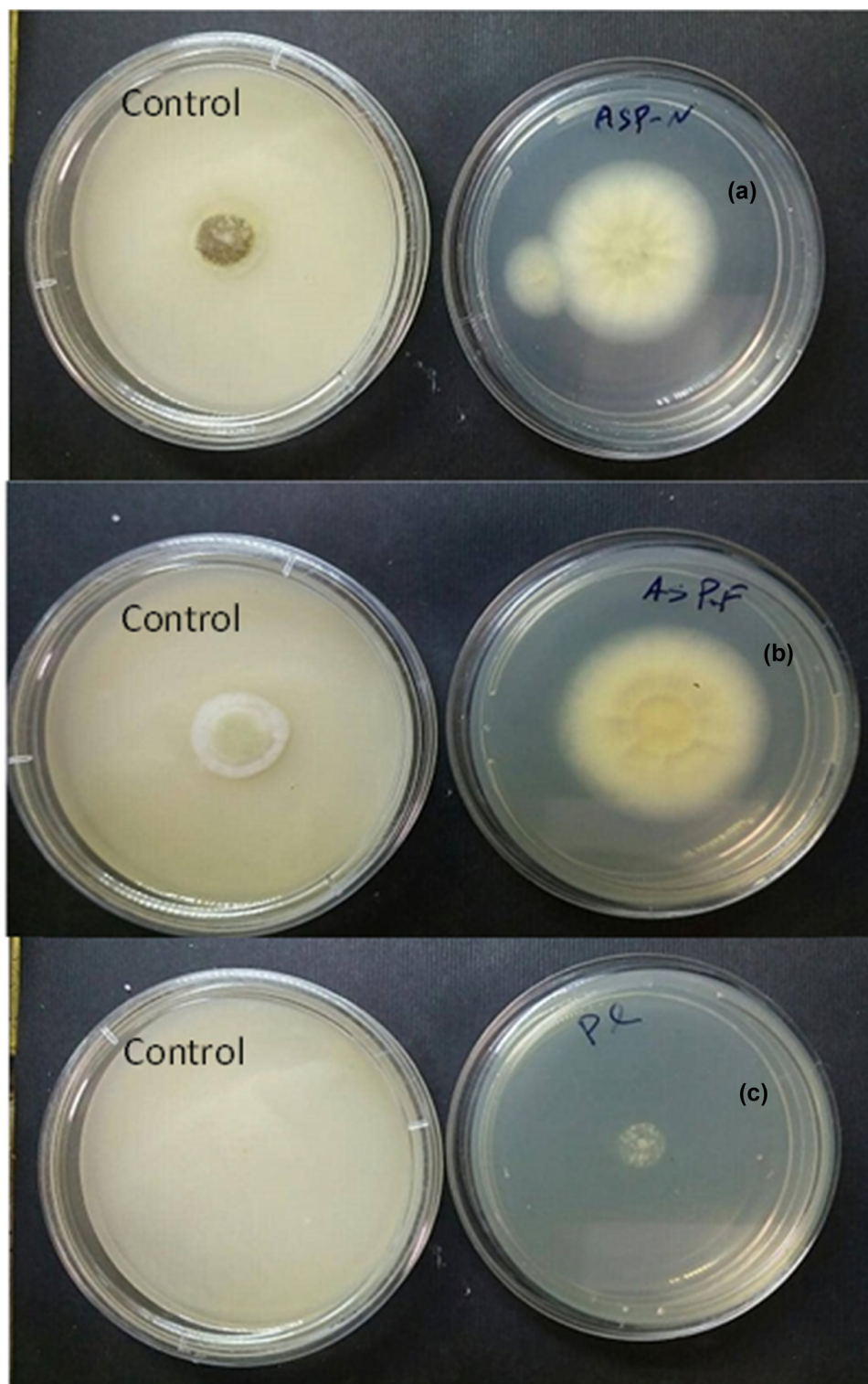


Figure 6: Mycelia growth of *A. niger* (a), *A. flavus* (b), and *Penicillium aculeatum* (c) in the plates containing fabricated ZnO NPs as compared to the control plates (without ZnO NPs) after 4 days of incubation at 26°C.

leaf extract and hydrothermal method, and assessment of bactericidal effects against *E. coli* and *S. aureus* indicated that the fabricated ZnO NPs had antibacterial activity toward these two bacterial strains with clear zone diameters of 10 and 13 mm, respectively.

3.4 Antifungal activity of the fabricated ZnO NPs using red pepper extract and autoclave heating methods

Table 3 presents antifungal activities of the formed ZnO NPs using prepared red pepper extract, against *A. niger*, *A. flavus*, and *P. aculeatum*. Obtained results indicated that the growth of *A. niger* and *A. flavus* has been limited nearby to 50% compared to those growth in the control plates after 4 days of incubation (Figure 6a and b). While, in the plate that was amended with the fabricated ZnO NPs, growth of *P. aculeatum* was significantly ($p < 0.05$) limited after 4 days of incubation (Figure 6c). Sharma et al. indicated that the antifungal activity of ZnO NPs could be related to the rupture of the fungal cell membrane due to attachment of the NPs and reduction of the activity of the fungus enzymes [34]. Gunalan et al. concluded that the ZnO NPs formed an effective antimicrobial and antifungal factor against pathogenic microorganisms, and the antimicrobial activity of NPs depended on the nanoparticle dosage, contact time, particle size, and synthesizing method [35]. Naval et al. showed that the ZnO NPs were effective fungicides not only in agricultural programs and nutrient packing but also in controlling the growth of pathogenic bacteria and fungi strains, such as *A. flavus* and *A. fumigatus* [36].

3.5 Antioxidant activity of the produced ZnO NPs using red pepper extract and autoclave heating methods

Antioxidant activity of the synthesized ZnO NPs using red pepper extract and the selected heating method was 80.21%. The DPPH radical was violet initially and changed into yellow when that was reacted with hydrogen. According to the findings of Anvarinezhad et al., the synthesized ZnO NPs using clove extract and hydrothermal technique had high antioxidant of 85.23% [37]. It seems that the nature of plant extract and their main bioactive compounds had strong effect on antioxidant activity of the formed ZnO NPs [16,31].

4 Conclusions

Over recent years, green methods in synthesis of ZnO NPs based on plant extracts have attracted considerable attention due to the benefits to environmental protection, lack of toxicity, and low cost. The present study showed that the red pepper extract contained main active biological compounds that can be used as reducing and capping agents in fabrication of ZnO NPs with crystal form and different shapes. Furthermore, among three different heating methods, autoclave technique was established based on under pressurized liquid system adjusted at high temperature that could accelerate the formation of ZnO NPs with small crystal size and high antioxidant and antimicrobial activities. Plant extract have been known as natural antioxidant and antimicrobial agents because of the existence of valuable bioactive molecules, such as flavonoids, phenols, and organic acids in various parts of the plants. It seems that by optimization of the synthesized parameters, it will be possible to fabricate ZnO NPs with higher antioxidant, antibacterial, and antifungal activities for more applications in food, cosmetics, drug, and medicinal formulations.

Acknowledgments: The authors appreciate the supports of Islamic Azad University – North Tehran Branch to accomplish this research.

Funding information: The authors state no funding was involved.

Author contributions: Hadi Eslami: methodology, validation, investigation, resources, data curation, and analysis; Hoda Jafarizadeh-Malmiri: writing – final manuscript, writing – review and editing, supervision, and project administration; Hosein Ali Khonakdar: formal analysis, data analysis, and design of experiments.

Conflict of interest: The authors state no conflict of interest.

Data availability statement: All data generated or analyzed during this study are included in this published article.

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