Afsaneh Vahidi, Hamideh Vaghari, Yahya Najian, Mohammad Javad Najian and Hoda Jafarizadeh-Malmiri*

Evaluation of three different green fabrication methods for the synthesis of crystalline ZnO nanoparticles using *Pelargonium zonale* leaf extract

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Abstract: Zinc oxide nanoparticles (ZnO NPs) were green synthesized using *Pelargonum zonale* leaf extract under three different heating methods, and their characteristics were evaluated using X-ray diffractometry (XRD), Fourier-transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay and antibacterial well diffusion method. The FT-IR analysis indicated that the *Pelargonium* leaf extract contained hydroxyl and amide I groups which were related to the proteins, carbohydrate, tannins and phenolic compounds of the extract and had an essential role in the reduction of the zinc ions and synthesis of the ZnO NPs. The obtained results revealed that the synthesized spherical individual ZnO NPs as well as the number of aggregates using microwave irradiation, autoclave and conventional heating (heater-stirrer) methods had average crystalline size of 51, 60 and 61 nm. Furthermore, antioxidant activities of the fabricated ZnO NPs were 7.8, 4.1 and 5.5% by using conventional heating, autoclave and microwave irradiation, respectively. The obtained results indicated that all the formed ZnO NPs had bactericidal effects against to the both Gram negative and Gram positive bacteria strains. However, the synthesized ZnO NPs using conventional heating method had the highest antibacterial activities toward both studied bacteria strains.

Keywords: autoclave heating; green synthesis; microwave irradiation; *Pelargonium zonale*; zinc oxide nanoparticles.

***Corresponding author: Hoda Jafarizadeh-Malmiri,** Faculty of Chemical Engineering, Sahand University of Technology, East Azarbaijan, Tabriz, Iran, e-mail: h_jafarizadeh@sut.ac.ir; h_jafarizadeh@yahoo.com
Afsaneh Vahidi: Faculty of Chemical Engineering, Sahand University of Technology, East Azarbaijan, Tabriz, Iran
Hamideh Vaghari: Faculty of Chemical Engineering, Sahand University of Technology, East Azarbaijan, Tabriz, Iran; and Research and Development Department, Najian Herbal Group, East Azarbaijan, Tabriz, Iran
Yahya Najian and Mohammad Javad Najian: Research and Development Department, Najian Herbal Group, East Azarbaijan, Tabriz, Iran

1 Introduction

Metal oxide nanoparticles (NPs) have been extensively synthesized and utilized in different areas because of their distinctive optical, physico-chemical, thermal and biological attributes [1]. In fact, as compared to the organic NPs, metal oxide NPs indicate unique stability, lower toxicity and higher heat resistance and selectivity [2]. Among the metal NPs, zinc oxide nanoparticles (ZnO NPs) are highlighted due to their numerous applications in the plastic, ceramics, cement, lubricants, paints, adhesives, pigments, packaging, cosmetics and textile industries [3, 4]. Furthermore, ZnO NPs have been known as strong antibacterial and antifungal agents [4–6]. In fact, ZnO NPs, by the generation of the reactive oxygen on their surfaces, can effectively influence the permeability of the microorganisms' cytoplasmic membrane and inhibit their normal activities [1, 2].

There are three different ZnO NPs synthesis methods, namely, physical (i.e. thermal evaporation and pulsed laser deposition), chemical (i.e. sol-gel, sonochemical and spray pyrolysis) and biological techniques based on using the microorganisms, polysaccharides, plants and enzymes [7]. The physical synthesis methods require high vacuum and are energy consuming [8]. The chemical fabrication approaches are based on consuming chemical solvents and reagents in which their residuals in the final products can limit the application of the formed NPs and is not environmentally friendly. To overcome the mentioned physical and chemical NP synthesis methods, the development and achievement of alternative NP synthesis methods which are fast, clean, cost-effective and eco-friendly and also require mild reaction conditions are necessary these days [9]. Recently, the biological synthesis processes for the metal oxide NPs based on utilizing the natural compounds as reducing and stabilizing agents have attracted scientific interest. Numerous researches have been completed on the green synthesis of the ZnO NPs using plants and their derivatives such as leaf, root, flower and fruit [10, 11]. As compared to the biosynthesis of the NPs using the microorganisms, NP green synthesis based on plant extract has several advantages including the availability of the resources, not needing the culture

and downstream processes, rapid and one-step synthesis process, and easy to scale-up fabricated process [12]. *Pelargonium* belongs to the family *Geraniaceae* and has high potential applications in cosmetics, medicines and food products. Several studies indicated that *Pelargonium* leaves extract contains flavonoids, phenolic compounds, organic acids, proteins, enzymes and polysaccharides which have key roles in the reduction and stabilization of the metal NPs such as silver NPs [13].

Synthesis of the metal oxide NPs using plant extract is time-consuming as compared to the traditional synthesis techniques due to the lower concentration of the existing bioactive components. It decreases the yield and production rate of the synthesized NPs [14]. To increase the rate of the NP production rate, green synthesis of the NPs using plant extract can be coupled with three different heating methods, namely, reflux conditions, autoclave heating and microwave irradiation [15]. These methods can drastically accelerate the rate of the metal oxide NP formation in the aqueous solutions [16].

As a result, the main aims of this study were to (i) evaluate the synthesis potential of the *Pelargonium* leaves extract to fabricate ZnO NPs, (ii) synthesis of the ZnO NPs using *Pelargonium* leaves extract utilizing three heating methods, namely, microwave (at 250°C for 3 min), autoclave (at 121°C and 1.5 bar for 15 min) and conventional heating (at 150°C for 2 h), and (iii) study physico-chemical properties and antimicrobial activities of the fabricated ZnO NPs.

2 Materials and methods

2.1 Materials

Pelargonium zonale was provided from a greenhouse (Tabriz, Iran). $Zn(NO_3)_3 \cdot 6H_2O$ was used as ZnO salt that was purchased from Sigma-Aldrich. Deionized double distilled water (Mojalali Co., Tehran, Iran) was used for plant extraction and preparation of all aqueous solutions. *Staphylococcus aureus* (PTCC 1112) and *Escherichia coli* (PTCC 1270) were obtained from microbial Persian type culture collection (PTCC, Tehran, Iran). Nutrient agar (NA) was provided from Biolife (Biolife Co., Milan, Italy).

2.2 *Pelargonium* leaf extract preparation

Pelargonium leaves were cut and washed to remove their dusts using double-distilled water and were dried at ambient conditions (at 20°C and 45% relative humidity) during 7 days. After that 1 g of the dried powder which was obtained using a domestic miller (MX-GX1521; Panasonic, Tokyo, Japan) was added into the boiling 100 ml deionized water for 5 min and then filtered using filter paper (Whatman no. 1). The provided extract was kept at a cold temperature (4°C) throughout the experiments.

2.3 Synthesis of ZnO NPs

For preparation of the ZnO NPs, 2 g of the provided zinc nitrate salt was added into 20 ml of provided *Pelargonium* leaf extract and the mixture solutions were exposed to the three different heating methods namely autoclave, microwave irradiation and conventional heating (heater-stirrer) which were described below.

2.3.1 Conventional heating synthesis: In this method, the mixture solution was heated at 150°C for 2 h using a magnetic heater-stirrer adjusted at 700 rpm. Then, the prepared solution was placed into a ceramic crucible cup and kept in furnace (400°C for 2 h). The resulted yellow coloured powder, as fabricated ZnO NPs, was then studied.

2.3.2 Autoclave synthesis: In this method, the mixture solution was put in autoclave (15 psi and 121°C) for 15 min. Then, the provided solution was put into a ceramic crucible cup and transferred to the furnace set at mentioned conditions to form ZnO NPs. The resulted yellow coloured powder was then studied.

2.3.3 Microwave synthesis: In this method, the provided mixture solution was put into a domestic microwave oven (MG-2312W, LG Co., Seoul, South Korea) with the power of 800 W and a maximum oven temperature of 250°C for 3 min. As the same is mentioned in the previous methods, the solution was put into the furnace and the produced yellow coloured ZnO NPs powder was used to more physico-chemical and antimicrobial analysis.

2.4 Physico-chemical characteristics of the fabricated ZnO NPs

In order to find the main existing functional groups in the prepared *Pelargonium* leaf extract, Fourier-transform infrared spectroscopy (FT-IR) was utilized by using a Bruker Tensor 27 spectrometer (Bruker, Karlsruhe, Germany) and KBr pellets ranging from 4000 to 400 cm^{-1}. By X-ray diffractometry (XRD: D5000, Siemens Co., Karlsruhe, Germany) using Cu Kα radiation and scanning electron microscopy (SEM, CamScan MV 2300, Tescan, Czech Republic), the structural properties and morphology of the fabricated ZnO NPs were evaluated. The average crystalline size of the synthesized ZnO NPs was obtained using Debye-Scherrer formula (Equation 1).

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

where, D, λ, β and θ are the crystalline size, X-ray wavelength, full line width at the half-maximum elevation of the main intensity peak, and Bragg angle, respectively [17].

For antioxidant activity evaluation of the formed ZnO NPs, the described method by Anzabi, based on the scavenging ability on 2,2-diphenyl-2-picrylhydrazyl (DPPH) was used. The scavenging ability was obtained by Equation 2 [14]:

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

where, I% is the inhibition percent and $A_{control}$ and A_{sample} are the absorbance of the control solution (without the main component)

and the solution containing main component, respectively, which were measured using a UV-visible spectrophotometer (250–800 nm, Perkin Elmer, Überlingen, Germany).

2.5 Antibacterial activities of the fabricated ZnO NPs

For monitoring of the bactericidal effects of the fabricated ZnO NPs to the *Staphylococcus aureus* and *Escherichia coli*, well diffusion method was used according to the described procedures by Torabfam and Jafarizadeh-Malmiri [18]. The antibacterial activities of the fabricated ZnO NPs with three different methods could be related to the diameter of the created clear zone.

2.6 Statistical analysis

Physico-chemical analysis of the prepared extracts was carried out in three replications. Data were interpreted by analysis of variance (ANOVA) using Minitab v.16 statistical package (Minitab Inc., PA, USA). Tukey's comparison test was used to compare the mean values. All comparison was made at 5% level of significance.

3 Results and discussion

3.1 FT-IR analysis of the *Pelargonium* leaf extract

To identify and study the existing functional groups in the *Pelargonium* leaf extract, FT-IR spectra of *Pelargonium* leaf extract was obtained (Figure 1). This figure indicates that four absorption peaks were centered at 669.44, 1636.59, 2068.15 and 3451.04 cm^{-1}. The widest spectrum absorption peak (3451.04 cm^{-1}) was related to the stretching vibrations of OH (hydroxyl groups) which was mainly obtained in the carbohydrate, tannins and phenolic compound structures, and the highlighted peak centered at 1636.59 cm^{-1} was attributed to the amide I group, which was related to the proteins [13]. The presented OH groups in the leaf extract had a key role in the green fabrication of the ZnO NPs. In fact, hydroxyl groups of glucuronic acids present in hydrolysable tannins, reacted with zinc ions and resulted intermediate complexes which were oxidized and converted into the quinine forms. Quinine acted as reducing and capping agents and finally reduced zinc to the ZnO NPs [5]. It seems that the phenolic compounds of the leaf extract had the main potential of the reduction of zinc ions to form ZnO NPs. On the other hand, the opportunities of the nucleation and growth of the formed NPs increased by increasing the heating temperature due to the increasing collision rate of the fast reduced zinc ions and formation of the zinc elements in the synthesis solution [18].

3.2 XRD analysis of fabricated ZnO NPs

XRD patterns of the fabricated ZnO NPs using *Pelargonium* leaf extract and three different methods, namely, conventional heating (heater-stirrer), autoclave and microwave irradiation are indicated in Figure 2A–C, respectively. As clearly observed in Figure 2A, the peak position with 2θ values of 32.0069°, 34.7027°, 36.4775°, 47.7821°, 56.7634°, 63.0365°, 66.5725°, 68.0871°, 69.2823°, 72.7665°, 77.1089° and 81.6504° were indexed as (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (202) and (104) planes, which were in line with the International Center of Diffraction Data card (JCPDS-36-1451) and verified the

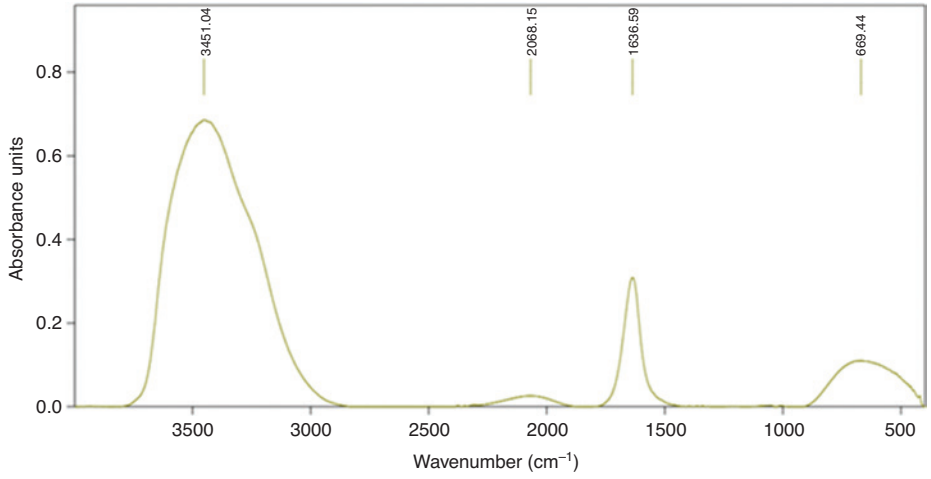

Figure 1: FT-IR spectrums of *Pelargonium zonae* leaf extract.

formation of a crystalline hexagonal structure for the formed ZnO NPs [19]. The average crystalline size of the fabricated ZnO NPs using *Pelargonium* leaf extract and conventional heating method was 61 nm.

The XRD pattern of the synthesis ZnO NPs using autoclave heating and *Pelargonium* leaf extract, which is indicated in Figure 2B, showed the diffraction peaks centered at 2θ values of the 31.9974°, 34.6735°, 36.5438°, 47.7855°, 54.3528°, 56.7967°, 63.0736°, 66.5680°, 68.1375°, 69.2402°, 72.7907°, 77.2468°, and 81.5953° which were indexed as (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (202), (104) and (203) planes. The average crystalline size of the formed ZnO NPs during this procedure was calculated to be 60 nm.

The XRD pattern of the synthesis ZnO NPs using microwave irradiation (Figure 2C) indicated various peaks placed at 2θ values of 32.0101°, 34.5839°, 36.4560°, 47.7213°, 56.7126°, 56.8791°, 63.0107°, 66.5589°, 68.0753°, 69.1841°, 72.7673°, 77.0398° and 81.6445°, which were indexed as (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), (202), (104) and (203) planes. The average crystalline size of the synthesized ZnO NPs was calculated to be 51 nm. The same XRD patterns obtained in the present study using three different heating methods verified the formation of ZnO NPs using *Pelargonium* leaf extract. The minimum crystaline size of the synthesized ZnO NPs under microwave irradiation (51 nm), as compared to that of ZnO NPs obtained using another two

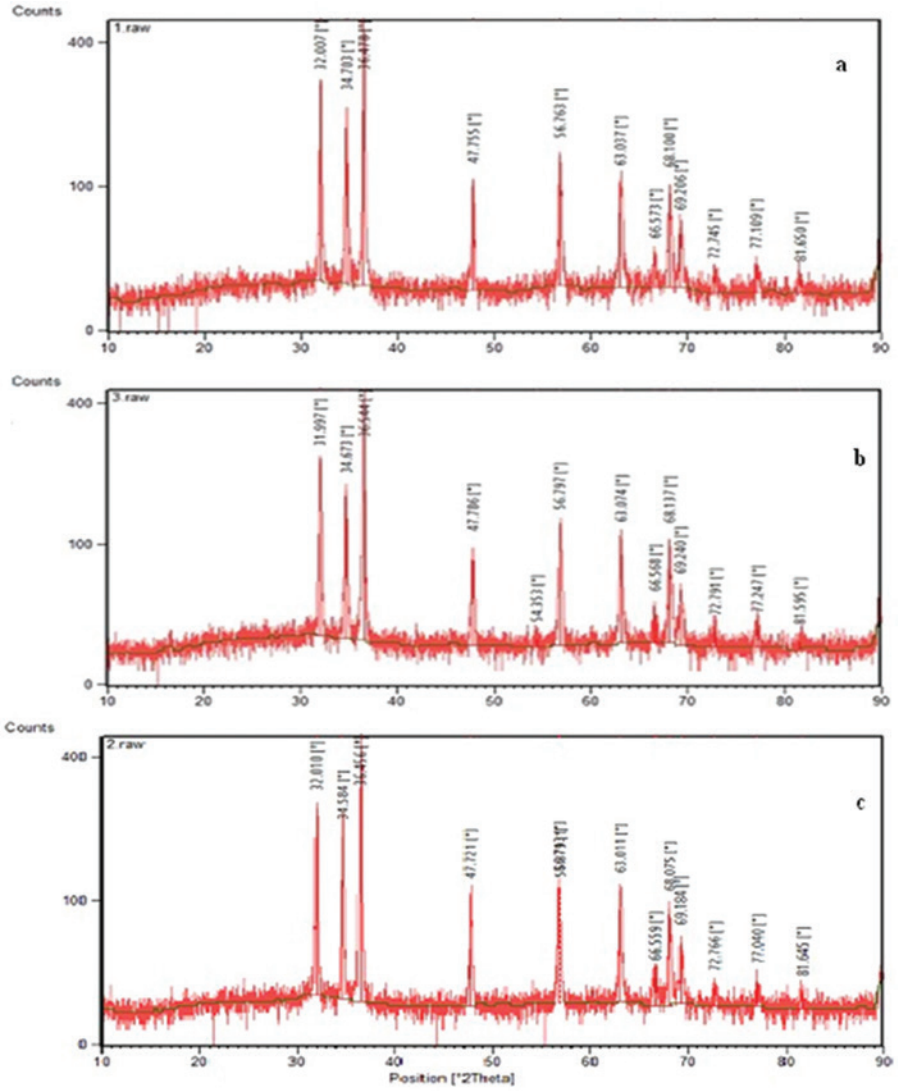

Figure 2: XRD pattern of the fabricated ZnO NPs using *Pelargonium* leaf extract and three different methods namely conventional heating (heater-stirrer) (A), autoclave (B), and microwave irradiation (C).

heating methods, can be explained by the fact that in the microwave heating fabrication technique for formation of ZnO NPs to achieve high temperatures within a limited time, uniform heat distribution was formed through the mixture solution containing zinc salt and *Pelargonium* leaf extract and increased movement of the formed ZnO NPs, which in turn, inhibited growth of particles by coagulation.

The obtained results were close to the findings of Çolak and Karaköse [20]. They synthesized ZnO NPs using aqueous extract of *Thyme* and deionized water with average crystallite sizes of 42 and 85 nm, respectively.

3.3 Morphology

The typical SEM images for the fabricated ZnO NPs using *Pelargonium* leaf extract and three heating methods, namely, conventional heating (heater-stirrer), autoclave and microwave irradiation are shown in the Figure 3A–C, respectively. Figure 3 indicates that the individual and aggregate ZnO NPs were formed and the formed particles had spherical shape.

3.4 Antibacterial

The antibacterial activities of the fabricated ZnO NPs using *Pelargonium* leaf extract and three heating methods, namely, conventional heating (heater-stirrer), autoclave and microwave irradiation is indicated in Table 1. The obtained results revealed that all the formed ZnO NPs had bactericidal effects against both the Gram negative and Gram positive bacteria strains. However, this effect was higher on *Staphylococcus aureus* (Figure 4A) as compared to the *Escherichia coli* (Figure 4B). Furthermore, the synthesized ZnO NPs using conventional heating method had highest antibacterial activities toward both the studied bacteria strains as compared to those fabricated using another two different synthesis methods. The main mechanism of the bactericidal activities of ZnO NPs can be related to their photochemical property. It seems that the adsorbed oxygen and H_2O on the surface of ZnO NPs interact with photoinduced charge carriers and reactive oxygen forms such as singlet oxygen and hydroxyl radical which these compounds cause membrane lipid peroxidation and show bactericidal effect [21–23]. The obtained results were in line with the findings of Shakeel et al. [24]. They also indicated that the synthesized ZnO NPs using *Abutilon*

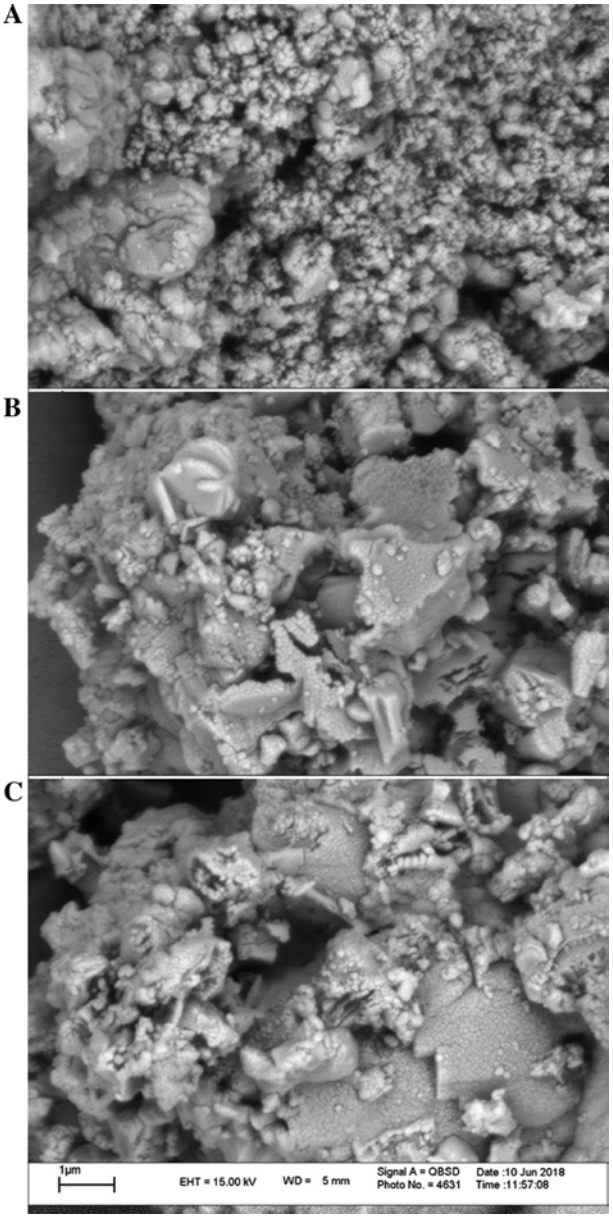

Figure 3: SEM images for the fabricated ZnO NPs using *Pelargonium* leaf extract and three heating methods, namely, conventional heating (heater-stirrer) (A), autoclave (B), and microwave irradiation (C).

Table 1: Diameter of clear zones (mm) around the synthesized ZnO NPs using *Pelargonium* leaf extract and three heating methods, namely, conventional heating (heater-stirrer), autoclave, and microwave irradiation, is indicated.

Synthesized methods	*Staphylococcus aureus*	*Escherichia coli*
Conventional heating	20 ml	14 ml
Autoclave heating	13 ml	10 ml
Microwave irradiation	15 ml	12 ml

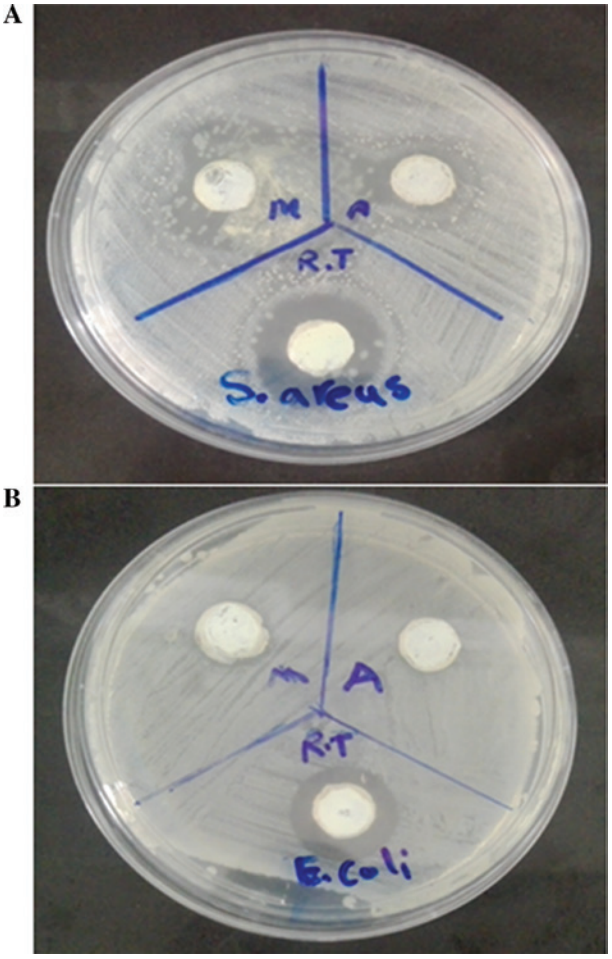

Figure 4: Antibacterial activities of the fabricated ZnO NPs using *Pelargonium* leaf extract and three heating methods to *Staphylococcus aureus* (A) and *Escherichia coli* (B).

indicum, *Clerodendrum infortunatum* and *Clerodendrum inerme* had high antibacterial activity against both Gram negative and Gram positive bacteria strains.

3.5 Antioxidant

The obtained results indicated that antioxidant activities of the synthesized ZnO NPs using *Pelargonium* leaf extract and three different synthesis methods, namely, conventional heating, autoclave and microwave irradiation were 7.8, 4.1 and 5.5%, respectively. In fact, the highest antioxidant activity was achieved using conventional heating at 150°C and 2 h. The DPPH radical is purple in colour and upon reaction with hydrogen donors, changes to yellow in colour [25]. It seems that at the highest temperature and reaction time, the reaction between DPPH radical and hydrogen donors existing in the leaf extract was completed and the antioxidant value increased.

4 Conclusions

Clean, rapid and one-step fabrication method was developed using *Pelargonium zonale* leaf extract to the synthesis of the ZnO NPs. For this reason, three different heating methods, namely, conventional heating, autoclave and microwave irradiation, were utilized to accelerate the rate of the NP formation. The obtained results demonstrated the high potential application of the *Pelargonium zonale* in the fabrication of the ZnO NPs. Furthermore, the ZnO NPs with the highest antioxidant and antibacterial activities were fabricated using conventional heating method and the ZnO NPs with the lowest particle size was synthesized using microwave irradiation. Such developed green synthesis method can be utilized to make other metal and metal oxide NPs.

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Conflict of interest statement: The authors declare that they have no conflict of interest.

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