Anticorrosion, antimicrobial and antioxidant study of ZnO nanoparticles synthesized from *Punica granatum* (Pomegranate) extract

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Abstract

Metal and metal oxide NPs have shown to be perfectly synthesized by using plant extracts with high efficiency, low cost and low toxicity. Our goal was to synthesize ZnO NPs by using an extract of pomegranate seeds and investigate the anticorrosion, antimicrobial and antioxidant properties of the synthesized ZnO NPs. The results have shown that the use of pomegranate in the green synthesis of ZnO NPs gave a good yield, with a low cost and non-toxic approach. The electrophoretic deposition (EPD) was used to coat stainless steel (S.S) by synthesized ZnO NPs in an alcoholic solution at room temperature producing a good coating against corrosion. The corrosion properties were investigated in a saline solution and a temperature range of (293–323) K. The effect of ZnO NPs against the growth of two Gram-negative bacteria *Escherichia coli* and *Klebsiella sp*., as well as the two Gram-positive bacterial strains *Staphylococcus epidermidis* and *Staphylococcus aureus*, as well as *Candida albicans* was at a very good scale. ZnO NPs have shown good properties as antioxidants to scavenge DPPH radicals. These therapeutic properties of ZnO NPs make them valuable in the medical field.

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1. Introduction

Pomegranate (*Punica granatum L.*) is a Mediterranean fruit that has long been used in traditional medicine in many countries. It is consumed as juice, concentrate, canned beverage, wine, jam, and jelly in India [1]. Pectin, ascorbic acid, and flavonoids are all present in modest amounts in fresh juice [2].

Pomegranate is a shrub native to Asia that belongs to the *Punicaceae* family [3]. The herb has been used for astringents, hemostatics, antidiabetics, antihelmintic, and diarrhea and dysentery [4, 5]. Organic acids, polyphenols, flavonoids, anthocyanins, alkaloids, fatty acids, and vitamins are among the macromolecules and metabolites found in the pomegranate plant [6]. Pomegranate's antibacterial, anthelmintic, anti-inflammatory, and antioxidant activities have been attributed to its high phenolic content [7, 8]. It's also utilized as an antiseptic/antiviral agent, as well as in the treatment of oral mucosa inflammation and genital herpes [9]. The juice appears to have anti-leprosy properties [10].

Infections caused by bacterial pathogens are routinely treated with antibiotics [11]. Antibiotics are antibacterial medicines that function by decreasing enzyme activity as well as interfering with DNA, RNA, and protein creation to stop bacteria from growing. These processes eventually cause the bacterial cell membrane structure to be disrupted, resulting in cell death. Recent research has shown that bacterial infections are developing resistance to several antibiotics, decreasing the efficiency of these drugs [12].

Nanoparticles (NPs) are small objects with a diameter of 1–100 nm that can be used in a variety of applications. Metal and metal oxide NPs are possible to make and have applications in medicine, biotechnology, and a variety of other industries [5]. ZnO NPs are useful as surface coatings of alloys such as stainless steel (S.S). The enhanced surface area of alloys by ZnO NPs due to protection of the surface from corrosion [13]. Formulated metal and metal oxide nanoparticles, particularly those made from plant extracts, have been investigated as potential bio-control agents in recent years [14]. Furthermore, when used as therapeutic agents against illnesses caused by multidrug-resistant Gram-negative as well as Gram-positive bacteria, these biosynthesized metal and metal oxide nanoparticles have shown to be very effective. However, the consistency, dimension, size distribution, surface functionality, morphology, form, and kind of material used in the synthesis all have a role in the efficacy of the metal and metal oxide nanoparticles generated [15]. In this regard, we have attempted to synthesize zinc oxide nanoparticles (ZnO NPs) by using water extract of pomegranate seeds and investigating the antibacterial, antifungal and antioxidant properties of the prepared nanoparticles.

2. Experimental part

2.1. Materials

The pomegranate fruits were purchased from the local market in Wasit, Iraq. Ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, and zinc nitrate hexahydrate were obtained from Merck (Germany).

2.2. Preparation of the pomegranate extract

The pomegranate fruits were washed to remove the dust and other wastes and sterilized with 70% ethanol, then washed again with deionized water. The fruit peel was removed and the

seeds of the pomegranate were extracted. The seeds then were placed in the fruit juicer (Silvercrest, Germany) and a concentrated juice of pomegranate was obtained.

2.3. Preparation of ZnO NPs

For the synthesis ZnO NPs, The approximation method was used by Ifeanyichukwu *et al.* [16]. 5.85 g of $Zn(NO_3)_2 \cdot 6H_2O$ were added to a beaker containing 300 mL of deionized water to prepare 0.1 M of the zinc salt solution. 100 mL of the zinc solution was added dropwise (by using a burette) to 100 mL of the concentrated pomegranate extract, under continuous stirring with a magnetic bar for 2 hours at 60°C. The red solution of the pomegranate turned into a light-yellow foggy solution. The solution was centrifuged at 5000 rpm for 15 minutes, and the precipitate was dried at 80°C for 12 hours. The obtained powder of ZnO NPs was characterized by using UV-Vis spectrophotometry and field emission scanning electron microscopy (FESEM) technologies.

2.4. Electrophoretic deposition of ZnO NPs solution

A deposition cell device was utilized to deposit ZnO NPs solution on a piece of stainless steel (S.S) surfaces. A 16 V D.C power supply was used to power the electrodes. An inert (Auxiliary) electrode made of a large piece of stainless steel or Platinum is employed in the deposition process cell. The substrate of S.S fixed with a 1 cm distance between it and the inert electrode. By mixing 1% ZnO NPs powder with ethanol as the solvent, ZnO NPs solution was produced (adding 1.5 g NPs to 150 mL ethanol). An ultrasonic (50 W) stirrer was used to mix the solution for 30 minutes to homogenize it. Utilizing the EPD technique, the solutions were used for coating S.S. components [13, 17].

2.5. Corrosion study

The electrochemical measurements were carried out using Mlab (Germany, 2000) potentiostat and controlled by a computer and MLabSci software which were used for data acquisition and analysis under static conditions. The corrosion cell that was employed contained three electrodes: a carbon steel working electrode, an auxiliary platinum electrode with a 1 cm^2 surface area, and a silver-silver chloride reference electrode. This reference electrode served as the reference for all potentials presented in this study [18, 19]. To create a steady state open circuit potential (E_{ocp}), the working electrode was submerged in the test solution for 15 minutes. Then, electrochemical measurements were carried out in the potential range 200 mV. Aerated solutions have been used for all electrochemical tests performed at 208–328 K.

2.6. Antibacterial study

The concentrated extract of the pomegranate seeds and the prepared ZnO NPs were examined against two Gram-negative bacterial strains *Escherichia coli* as well as *Klebsiella sp*., and also two Gram-positive bacterial strains *Staphylococcus epidermidis* as

well as *Staphylococcus aureus*, as well as one fungi (*Candida albicans*). In Petri dishes, the well diffusion method was used. Three wells in the agar medium were made in a radius of 6mm, and 50 µL of the pomegranate extract, 50 µg/mL and 100 µg/mL of ZnO NPs were added to the corresponding wells. The plates were incubated at 37°C for one day, and the inhibition zones were determined.

2.7. Antiradical study

The activity of pomegranate extract and ZnO NPs to scavenge DPPH was determined in a spectrophotometric method [20]. The pomegranate concentrate extract was evaporated and the precipitate was used to prepare a series of concentrations in methanol (10, 20, 40, 80, and 100 µg/mL). ZnO NPs were used to prepare similar series of concentrations in methanol. Then, a weight of 0.36 g of DPPH was dissolved in 4 mL methanol. 0.15 mL of the DPPH solution was mixed with 3 mL of each of the prepared concentrations, and with deionized water as control. The tubes were allowed to stand in dark for 30 minutes, and then the absorbance of each tube was determined at 517 nm. The activity of each material was calculated from the following Equation (1):

$$\%Activity = \frac{\left(A_{\text{DPPH}} - A_{\text{test}}\right)}{A_{\text{DPPH}}} \quad (1)$$

3. Results and Discussion

3.1. Characterization of ZnO NPs

Figure 1 shows the UV-Vis chart of the pomegranate extract and the prepared ZnO NPs.

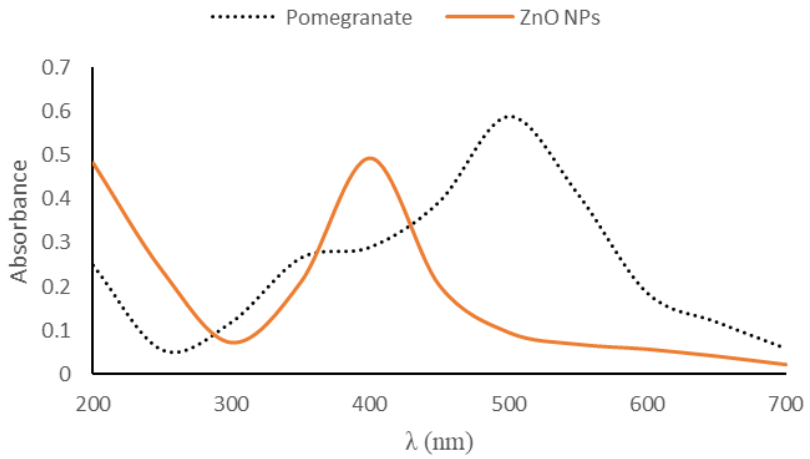

Figure 1. The UV-Vis chart of pomegranate and ZnO NPs.

The pomegranate extract has shown a maximum peak of around 500 nm, and a great shift was seen in the ZnO NPs which appeared around 400 nm. This shift of peaks and the

better arrangement of the absorbance data indicate the formation of ZnO NPs. A previous study indicated a peak of ZnO NPs prepared by pomegranate extract around 412 nm [21].

The FESEM image of ZnO NPs (Figure 2) indicates the presence of nanoparticles with agglomeration and rough spherical-like surfaces.

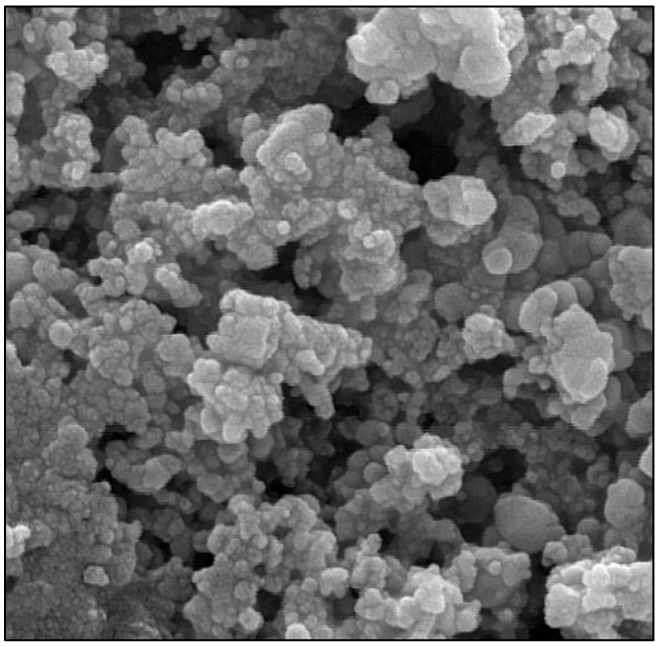

Figure 2. FESEM image of ZnO NPs.

3.2. Corrosion study

3.2.1. Potentiodynamic polarization

The potentiodynamic polarization curves of S.S coating by ZnO NPs are demonstrated in Figure 3. For more clarity, the potentiodynamic polarization curves of the S.S substrate are also presented. It can be shown that the corrosion potential of S.S. coated with ZnO NPs is more negative than that of uncoated S.S., meaning that all of the latter will electrochemically protect the substrate by dissolving in the medium and producing corrosion products with low solubility.

The corrosion potential values of the S.S substrate ranged from 478.5–672.5 mV, where the negative values of E_{corr} were increased with increasing temperature from 298–328 K. The corrosion current density (i_{corr}) of S.S coating by ZnO NPs is lower (0.017–0.0592 $\mu A \cdot cm^{-2}$) compared to uncoated S.S (20.64–91.9 $\mu A \cdot cm^{-2}$), at a temperature range 298–328 K. One cause for this finding under external anodic polarization parameters may be derived from the distribution and existence of ZnO nanoparticles with inhibitors impregnated in the metal matrix.

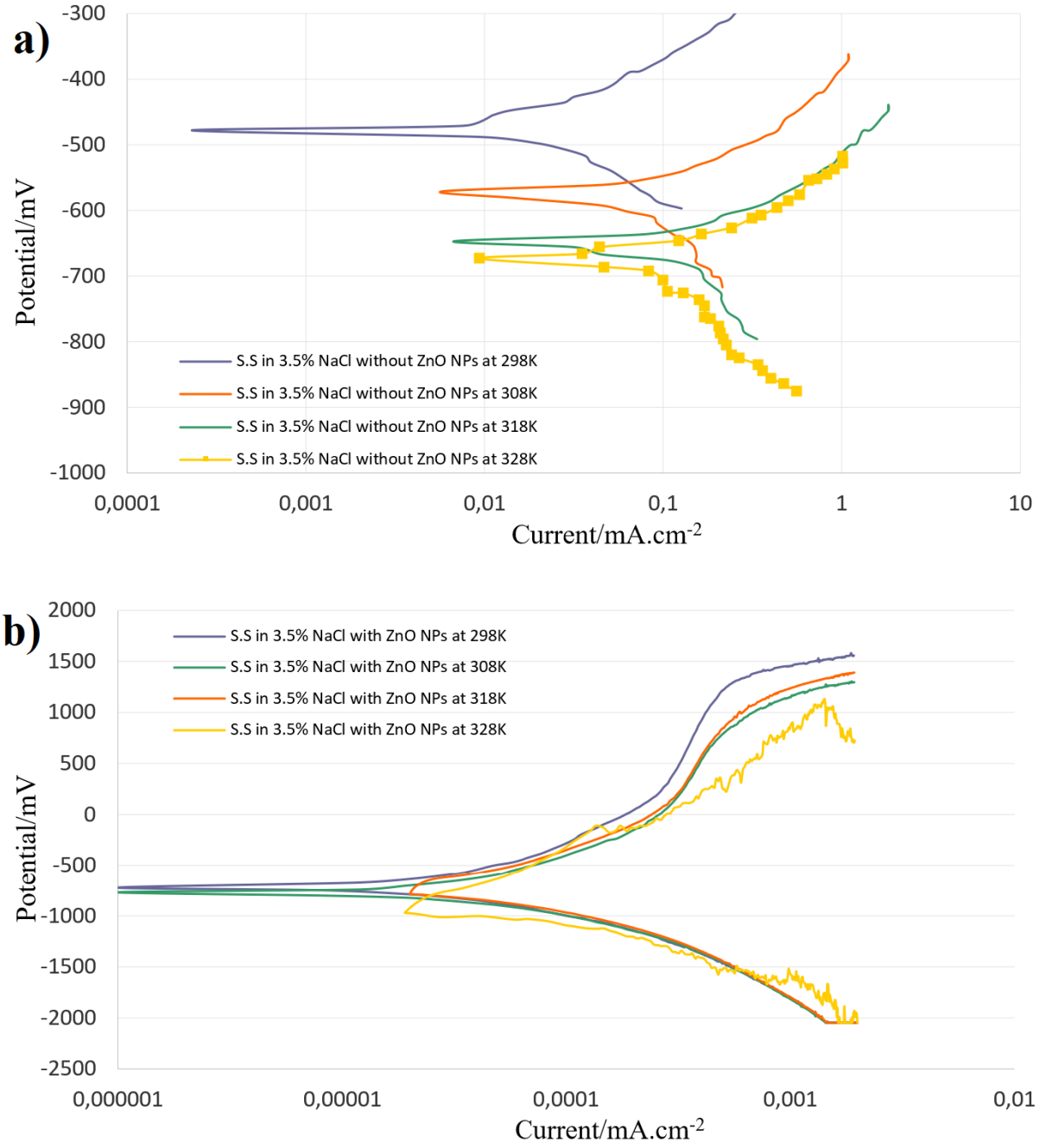

Figure 3. S.S alloy polarization plots in a saline medium at 298–328 K range; a) without coating and b) after coated by ZnO NPs.

This protection provided by ZnO NPs, gave the *PE*% reach 99.9% for all temperatures and increase the resistance polarization from 1688.735 to $539.8 \cdot 10^4$ k$\Omega \cdot$cm^2 at 298 K. Where the polarization resistance is based on the Stern–Geary Equation (2) [22]:

$$R_p = \frac{b_a \cdot b_c}{2.303\left(b_a + b_c\right)} \cdot \frac{1}{i_{corr}} \tag{2}$$

Table 1. Corrosion kinetic parameters for S.S alloys in saline at different temperatures.

Material	Temp. K	$-E_{corr}$ mV	I_{corr} $\mu A \cdot cm^{-2}$	b_a $mV \cdot dec^{-1}$	$-b_c$ $mV \cdot dec^{-1}$	$\%PE_T$	R_p $k\Omega \cdot cm^2$	$\%PE_R$
Uncoated S.S	298	478.5	20.64	159.6	161.5	–	1688.735	–
	308	571.7	61.16	106.5	231.2	–	517.660	–
	318	647.8	85.33	94.4	91.2	–	236.044	–
	328	672.5	91.9	123.3	322.1	–	421.303	–
S.S coated by ZnO NPs	298	717.1	0.017	517.5	357.3	99.92	$539.8 \cdot 10^4$	99.97
	308	766.4	0.03	708.6	459.6	99.95	$403.5 \cdot 10^4$	99.99
	318	733.7	0.0323	790.7	493.5	99.96	$408.5 \cdot 10^4$	99.99
	328	912	0.0592	405.4	626.6	99.94	$180.5 \cdot 10^4$	99.98

3.2.2. Thermodynamic and kinetic study

The effect of temperature on the rate of corrosion of uncoated and coated S.S. by ZnO NPs was investigated at temperatures between 298 and 328 K. The Arrhenius Equation (3, 4) was used to calculate the apparent activation energies as shown in Figure 4 [23].

$$i_{corr} = A\exp\left(\frac{-E_a}{RT}\right)\cdot 100 \tag{3}$$

$$\log i_{corr} = \log A - \frac{-E_a}{2.303RT} \tag{4}$$

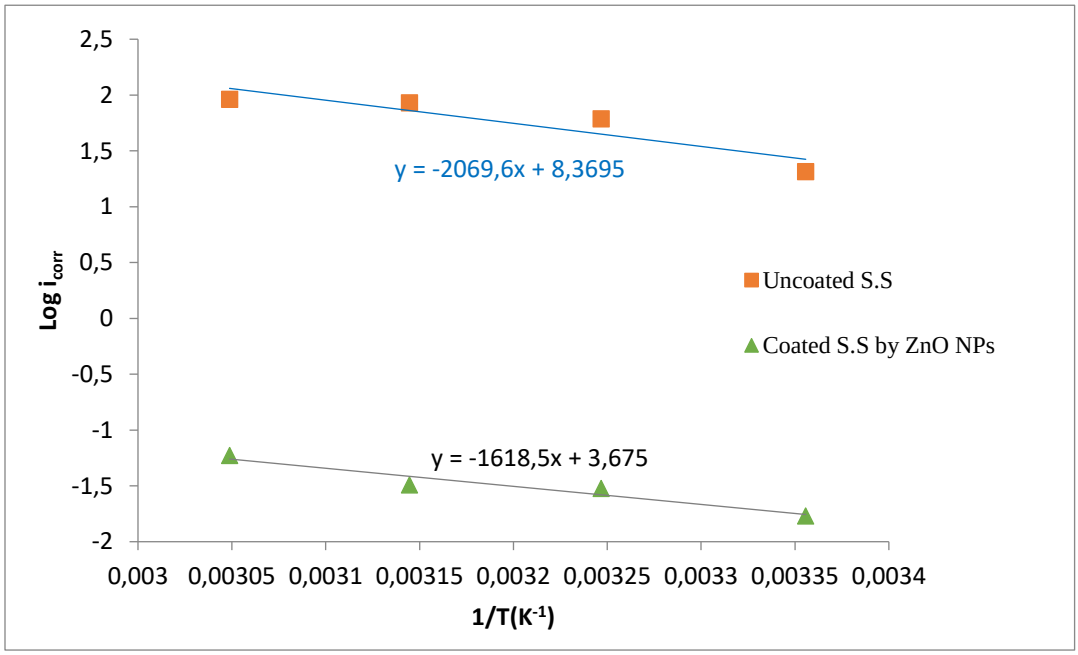

Figure 4. Arrhenius Plot of $\log i_{corr}$ *versus* $1/T$ for the corrosion of S.S in saline solution.

The transition state equation was used to determine the values of the activation entropy (ΔS^*) and enthalpy (ΔH^*) for the corrosion of uncoated and coated S.S (Figure 5).

$$i_{corr} = \frac{RT}{Nh}\exp\left(\frac{\Delta S^*}{R}\right)\exp\left(\frac{-\Delta H^*}{RT}\right) \quad (5)$$

$$\left(\log\frac{i_{corr}}{T}\right) = \log\frac{R}{Nh} + \frac{\Delta S^*}{2.303R} - \frac{\Delta H^*}{2.303RT} \quad (6)$$

While the values of the activation free energy ΔG^* were calculated by using Gibbs Equation (7):

$$\Delta G^* = \Delta H^* - T\Delta S^* \quad (7)$$

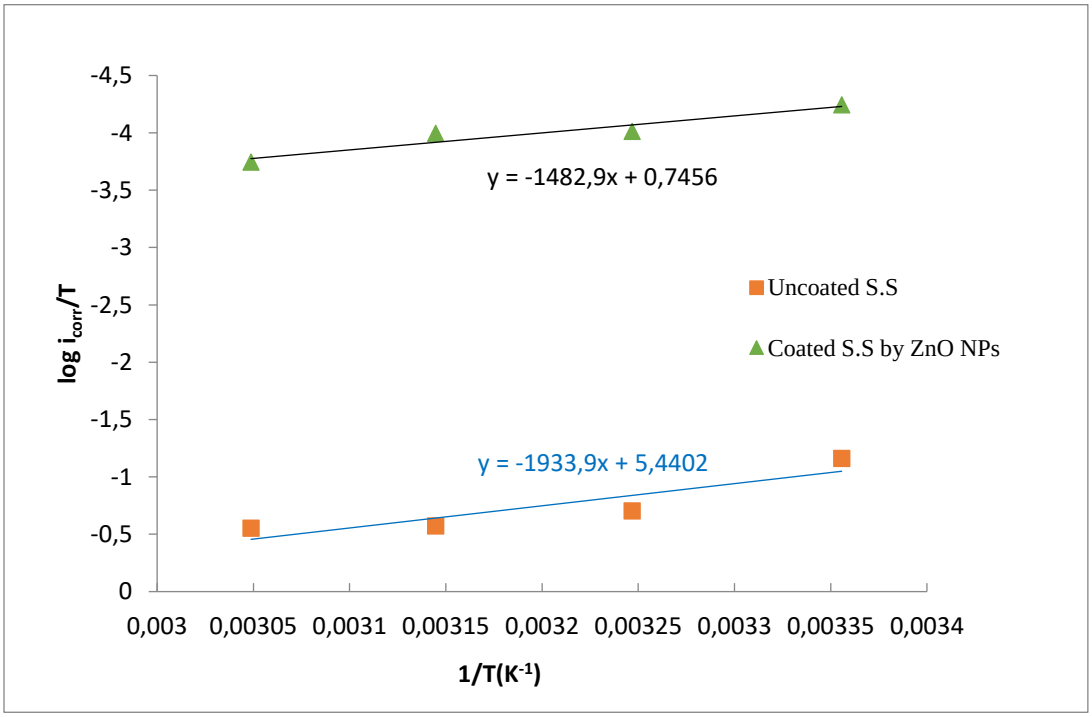

Figure 5. A plot of logi_{corr}/T *vs.* 1/*T* for the corrosion of S.S in saline solution.

Table 2. Kinetic parameters of in presence inhibitor and absence at different media.

Solution	ΔG_a^* (kJ·mol^{-1})				ΔH_a^* kJ·mol^{-1}	ΔS_a^* kJ·mol^{-1}·K^{-1}	E_a kJ.mol^{-1}	A mol·cm^{-2}·s^{-1}
	298 K	308 K	318 K	328 K				
Uncoated S.S	64.86	65.79	66.73	67.66	37.03	–0.0934	39.626	$1.41 \cdot 10^{26}$
S.S coated by ZnO NPs	83.01	84.84	86.68	88.51	28.39	–0.1833	30.991	$2.85 \cdot 10^{27}$

3.3. Antimicrobial effects

Table 3 contains the inhibition zones created through the presence of pomegranate extract and ZnO nanoparticles [35]. The extracted juice of pomegranate has shown to have an antimicrobial effect against all of the strains that have been used in this study, where the most significant inhibitory effect was observed against *Candida albicans*. ZnO NPs have exhibited a greater inhibitory effect compared to the pomegranate extract.

Table 3. Inhibition zones of water and methanol extracts of peppermints.

Type of microbe	**Pomegranate extract**	**ZnO NPs**	
		50 µg/mL	**100 µg/mL**
E. coli	7.5	10.5	13.4
Klebsiella sp.	9	11.1	15.8
S. epidermidis	8.1	10.2	12.5
S. aureus	9	13.5	16
Candida albicans	10.5	12.5	15.5

The extract of pomegranate contains many phytochemicals with therapeutic behaviours. Polyphenols, in pomegranate extract, have been confirmed to display a tough radical scavenging influence, and also antibacterial activity against Gram positive and Gram negative strains [24]. Pomegranate extract was reported to inhibit the growth of a wide spectrum of bacterial and mould strains [25–27]. Metal oxide NPs, including ZnO NPs, have been shown to exhibit antimicrobial properties. The mechanism by which these NPs have inhibited the growth of microorganisms is by inducing reactive oxygen species (ROS) which leads to apoptosis [16, 28].

3.4. Antioxidant effects

Figure 6 shows the antioxidant activity of ascorbic acid as standard material, the IC50 of ascorbic acid was obtained as 26.94 µg/mL to scavenge DPPH radicals, this was in agreement with a previous study [29]. Pomegranate extract has shown good antioxidant behaviour in the scavenging DPPH radicals, where the IC50 was 37.77 µg/mL. Several studies have reported an antioxidant property exhibited by the pomegranate extract solutions in different solvents, which was attributed to the presence of certain phytochemicals such as polyphenols [30–32]. On the other hand, ZnO NPs were shown to have the minimum antioxidant properties among the three tested materials, where the IC50 was 50.53 µg/mL. The antioxidant behaviour exhibited by green synthesized ZnO NPs was reported [33, 34].

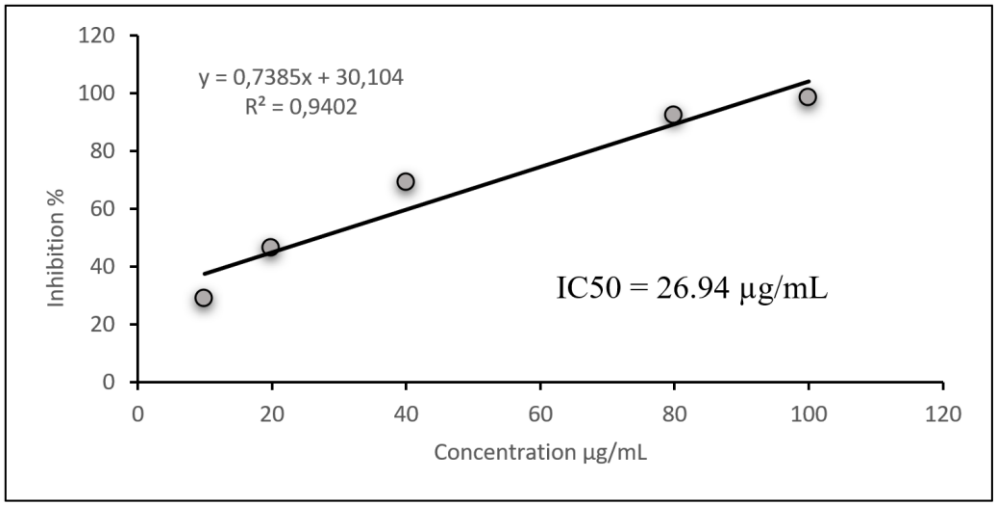

Figure 6. The antioxidant activity of ascorbic acid to scavenge DPPH.

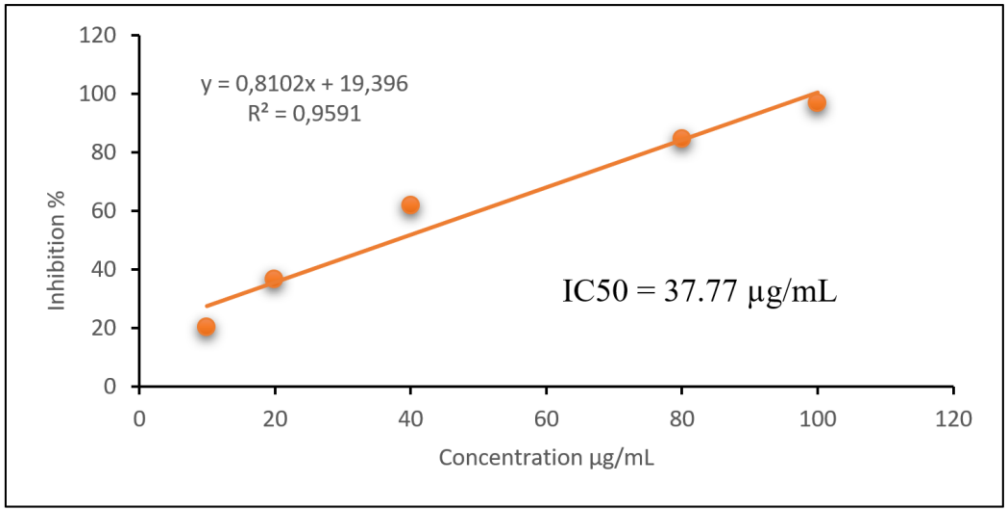

Figure 7. The antioxidant activity of pomegranate extract to scavenge DPPH.

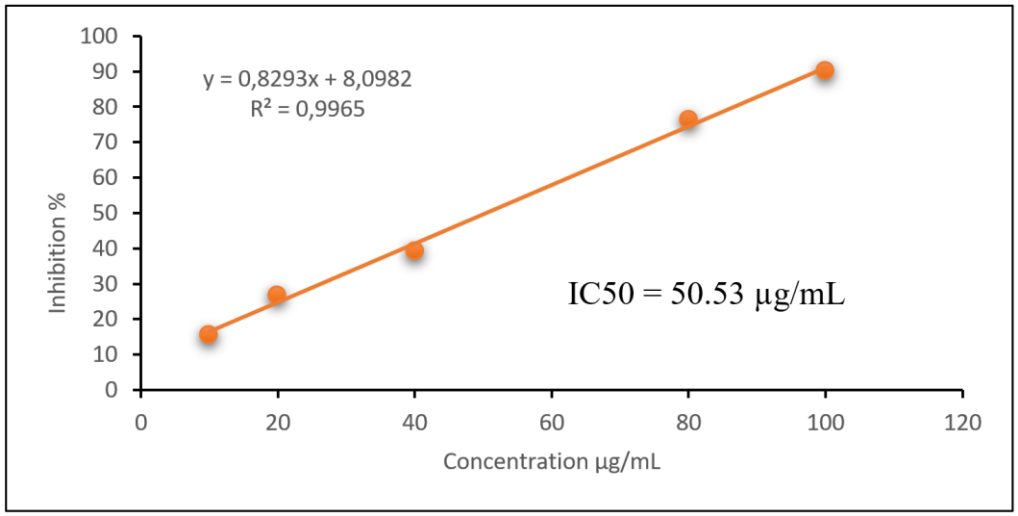

Figure 8. The antioxidant activity of ZnO NPs to scavenge DPPH.

Conclusion

The use of pomegranate in the green synthesis of ZnO NPs has shown to give good yield, with a low cost and non-toxic approach. Pomegranate content of phytochemicals has shown multiple acting roles, including anticorrosion, antimicrobial, and antioxidant agents. Coating S.S by synthesized ZnO NPs by using the EPD technique shows good results for the protection of S.S against corrosion, where the *PE*% reaches 99.9% in the temperature range of (298–328 K).

The effect of ZnO NPs against the growth of two Gram-negative bacteria *Escherichia coli* and *Klebsiella sp*., and also two Gram-positive bacterial strains *Staphylococcus epidermidis* and *Staphylococcus aureus*, as well as *Candida albicans* was at a very good scale. ZnO NPs showed a good property as antioxidants to scavenge DPPH radicals. These therapeutic behaviors of ZnO NPs make them valuable in the medical field.

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