

## Research Article

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# Green approach in fabrication of photocatalytic, antimicrobial, and antioxidant zinc oxide nanoparticles – hydrothermal synthesis using clove hydroalcoholic extract and optimization of the process

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**Abstract:** Zinc oxide nanoparticles (ZnO NPs) were hydrothermally fabricated, using hydroalcoholic clove extract. GC-MS analysis demonstrated that Eugenol is the main bioactive compound of the prepared extract. Experiments were designed, based on the central composite design. The effects of different amounts of zinc nitrate (2–6 g) and clove extract (10–30 mL) were evaluated for antioxidant and bactericidal properties of the formed ZnO NPs using the response surface methodology. The attained results demonstrated that more desirable NPs with maximum antioxidant activity (85.23%) and bactericidal effect, against *Escherichia coli* and *Staphylococcus aureus*, as manifested in the diameter of formed clear zones of 11.12 and 12.11 mm, respectively, were resulted using 3.98 g of the zinc salt and 20.30 mL of the clove extract. Furthermore, XRD and SEM analysis results revealed that the fabricated ZnO NPs had a hexagonal shape with a particle size of 50 nm and could degrade 70% of methylene blue during UV radiation.

**Keywords:** antioxidant activity, clove extract, hydrothermal synthesis, photocatalytic activity, zinc oxide nanoparticles

## 1 Introduction

With the development of nanotechnology, enormous revolution has been created by the researchers to fabricate new materials on nanoscale, such as metal nanoparticles (NPs), carbon nanotubes, and metal oxide NPs, improve properties of the materials as compared to their in bulk scale, and develop nanomaterial applications in wastewater treatment, food and agriculture, cosmetics, medicine and pharmaceuticals, packaging, tissue engineering, biotechnology, plastic and ceramics, petroleum and textile industries [1,2]. Bio-synthesis of metal and metal oxide NPs with plant extract is a novel branch of nanobiotechnology, which has been established based on green chemistry [3]. In fact, inorganic NPs' green synthesis with plants and their derivative extracts, due to the presence of carbohydrates, proteins, enzymes, and phytochemicals, such as phenols, terpenoids, ketones, aldehydes, and amides in the plant's structure, has roused considerable interest among scientists due to its cost-effective and environment-friendly nature [4,5].

Zinc oxide nanoparticles (ZnO NPs) are of more applicable metal oxide NPs due to their photocatalytic and photo-oxidizing capacity, and antimicrobial activity [2]. ZnO NPs have been recognized as robust antimicrobial compounds against various pathogenic and spoiling bacterial and fungal strains due to generation of reactive oxygen, which can alter the permeability of cytoplasmic membrane of the live cells and inhibit their normal activities [6]. ZnO NPs, due to their biocompatibility, biosafety, and non-toxicity, have also been known as safe and elite nanomaterials, as same as carbon nanotube, grapheme, and gold, and have been widely utilized in numerous disciplines [1]. For example, ZnO NPs have gained cutting-edge applications in electronics, communication, biosensor, cosmetics, environmental protection, food packaging, biology, and medicinal industry, these days [7].

Clove (*Syzygium aromaticum* Linn) belongs to the family *Myrtaceae* with aromatic dried flower buds. Clove contains 10% fixed oil, 15–20% essential oil, 6–7%

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non-essential ether extract, and 13% tannin, besides glycosides and flavonols [8]. Eugenol(4-allyl-2-methoxyphenol) is the chief component that exists in clove essential oil with high antimicrobial, antioxidant, and insecticidal activities. According to the food and drug administration, it is classified as a natural food additive that is generally regarded as safe [9]. Clove has been added to foods not only as a flavoring agent, but also as a preservative due to its antioxidant and antibacterial properties. In fact, clove extract, due to its Eugenol content, acts as a preservative in food to prevent foodborne pathogens and spoilages [10].

For that reason, the key objects of the present work were to (i) prepare a clove hydroalcoholic extract and determine its main bioactive compounds, (ii) evaluate the potential application of the clove extract to synthesize ZnO NPs, (iii) optimize hydrothermal synthesis conditions to fabricate ZnO NPs with more desirable physicochemical and biological properties, and (iv) assess the removal, antioxidant and antibacterial attributes of the synthesized ZnO NPs using achieved optimal fabrication settings.

## 2 Materials and methods

### 2.1 Materials

Clove dried buds were provided from a local traditional market (Tehran, Iran). Zinc nitrate hexahydrate ( $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ) was purchased from Merck (Merck GmbH & Co. KG, Darmstadt, Germany). 2,2-Diphenyl-2-picrylhydrazyl (DPPH) was provided by Sigma Company (St. Louis, Missouri, USA). Dimethyl sulfoxide (DMSO) was bought from Merck (Merck GmbH & Co. KG, Darmstadt, Germany). Distilled water, as solvent, was purchased from Dr Mojallali Industrial Chemical Complex Co. (Tehran, Iran). Methylene blue dye (C.1.52015) was provided by Merck (Merck GmbH & Co. KG, Darmstadt, Germany). *E. coli* (PTCC 1395) and *Staphylococcus aureus* (PTCC 1189) were provided by the microbial Persian-type culture collection (PTCC, Tehran, Iran). Plate count agar (PCA) was bought from Biolife (Biolife Co., Milan, Italy).

### 2.2 Preparation of the clove extract

Provided clove buds were ground using a domestic miller (MX-GX1521, Panasonic, Tokyo, Japan) and 60 g of the ready powder was added into 300 mL of mixture

solution containing distilled water and ethanol with a ratio of 70:30 (V/V). The mixture solution was stored at 30°C for 24 h and afterward, it was filtered by No. 1 Whatman filter paper. Finally, the ethanol was removed using a rotary evaporator (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) attuned at 60°C, 0.4 atm, and 200 rpm (rotation speed). Provided clove extract was deposited in a dark vial and retained in a refrigerator (4°C) for further processes and analysis.

### 2.3 Synthesis of ZnO NPs

In order to synthesize ZnO NPs, different amounts of zinc nitrate (2–6 g) were added into different amounts of the provided clove extract (10–20 mL) and placed into a laboratory autoclave set at a temperature of 121°C and a pressure of 1.5 atm for 15 min. After this, the mixture solutions containing ZnO NPs were decanted into ceramic crucible cups and placed into the laboratory furnace adjusted at 350°C for 2 h to result in pale yellow powder of ZnO NPs.

### 2.4 Physicochemical analysis of the clove extract

Brix value (°Bx) of the provided aqueous clove extract was assessed using a refractometer (Index Instruments Ltd, Kissimmee, FL, USA) and its pH was evaluated by a laboratory pH meter. The main existing functional groups of the clove extract were assessed by a Fourier transform infrared (FT-IR) spectrometer on a Bruker Tensor 27 spectrometer (Bruker, Karlsruhe, Germany) using KBr pellets in the 4,000–400  $\text{cm}^{-1}$  region. To recognize the main bioactive components that existed in the clove extract, a gas chromatography instrument (Agilent 6890, Santa Clara, CA, USA) with a 30 m  $\times$  0.25 mm HP-5 capillary column which was coupled with a HP 5989A mass spectrometer (operated in electron ionization mode at 70 eV) and Helium, as carrier gas, was employed.

### 2.5 ZnO NPs' characteristics

X-ray diffractometry (XRD: D5000, Siemens Co., Karlsruhe, Germany) using Cu  $K\alpha$  radiation and scanning electron microscopy (SEM, CamScan MV 2300, Tescan, Czech Republic) were used to evaluate structural and morphological attributes of the resulted NPs [11]. Mean crystalline size of the produced ZnO NPs (nm) was calculated by the Debye–Scherrer formula [12]. Antioxidant activity of the

resulted ZnO NPs was measured, based on scavenging ability of the formed NPs on DPPH (% inhibition percent), as described by Anzabi [6]. For this analysis, a UV-visible spectrophotometer (250–800 nm, Perkin Elmer's Co., Rodgau, Germany) set at a wavelength of 517 nm was used.

In order to evaluate the photocatalytic activity of the hydrothermally resulted ZnO NPs using clove extract, according to the described method by Sayyar and Jafarizadeh-Malmiri [13], a 5 ppm methylene blue aqueous solution was provided as an organic contaminant medium. A coated thin cubic glass ( $2 \times 2 \text{ cm}^2$ ) with synthesized ZnO NPs was placed in a 100 mL glass jar having 50 mL of the provided methylene blue solution and the container was exposed to UV-visible irradiation for 2 h, while the solution was magnetically stirred. For this reason, a 150 Watt lamp with a wavelength of 360 nm was placed on top of the container, with a distance of 3 cm. The maximum absorption band of the solution at the beginning and at the end of UV radiation was measured using a UV-visible spectrophotometer at 663 nm. Reduction in that value, at the end of radiation, indicates decomposition of methylene blue by synthesized ZnO NPs.

## 2.6 Bactericidal effect of the resulted ZnO NPs

Antibacterial activity of the synthesized ZnO NPs was estimated via well diffusion method, as termed by Sayyar and Jafarizadeh-Malmiri [14]. After the preparation of bacterial suspensions containing  $1.5 \times 10^8$  colony-forming units of bacteria in 1 mL of those (based on using 0.5 McFarland Standard), 1 mL of each providing suspension was spread on the surface of the set PCA in the plates and a few holes, with a diameter of 8 mm, were made in the inoculated media. Ten mg of the resulted ZnO NPs was dissolved in 10 mL of mixture solution containing DMSO and distilled water with a ratio of 1:10 (V/V) and 100  $\mu\text{L}$  of the solutions containing fabricated ZnO NPs was added into the holes and the plates were located in a laboratory incubator adjusted at 37°C for 24 h. The diameter of the formed clear zone nearby the holes could be directly correlated to the bactericidal effect of the resulted ZnO NPs.

## 2.7 Experimental design and data statistical analysis

Central composite design (CCD) and response surface methodology (RSM) were utilized in experimental design and assessment of influences of two synthesis factors,

including amounts of zinc salt powder ( $X_1$ , g) and clove extract ( $X_2$ , mL), on antioxidant ( $Y_1$ , %) and bactericidal activities of the fabricated ZnO NPs toward *E. coli* ( $Y_2$ , mm) and *S. aureus* ( $Y_3$ , mm). Due to advantages of the RSM over classical one-variable-at-a-time optimization, such as the generation of large amounts of information from a small number of experiments and the possibility of evaluating the interaction effect between the variables on the responses, it is a useful technique to assess the relationships between the synthetic variables and response variables [15,16]. According to the CCD, 13 experimental runs were provided with five replications for the center point (Table 1) via Minitab software (v.16 statistical package, Minitab Inc., Pennsylvania State, PA, USA). A second-order polynomial equation (equation (1)) was applied to interrelated dependent factors into the two independent factors. Where  $\beta_0$  is a constant,  $\beta_1$ ,  $\beta_{11}$ , and  $\beta_{12}$  are related to the linear, quadratic, and interactive terms, correspondingly [16,17].

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (1)$$

Suitability of the models was studied based on the coefficient of determination ( $R^2$ ), adjusted coefficient of determination ( $R^2\text{-adj}$ ), and lack of fit ( $P$ -value). ANOVA was also used to provide the significance determinations of the resulted models in terms of the  $P$ -value. Small  $P$ -values ( $P < 0.05$ ) were considered as statistically significant [18]. Surface and contour plots were also utilized to better visualize the influences of the synthesized factors on the response factors [19,20]. Graphical and numerical optimizations were applied to predict optimal area and exact values of independent variables to fabricate ZnO NPs with maximum antioxidant and antibacterial activities.

# 3 Results and discussions

## 3.1 Specification of the prepared clove extract

Obtained results indicated that pH and brix values of the prepared hydroalcoholic clove extract were 4.13°Bx and 14°Bx, respectively. Typical FT-IR absorption spectra of the prepared clove extract are displayed in Figure 1a. As this figure shows, numerous absorption peaks were observed, with some of them due to the structure of chief bioactive materials that existed in the clove extract. The main peak centered at  $3520.39 \text{ cm}^{-1}$  was due to stretching vibration of the  $-\text{OH}$ , with this group originating from Eugenol having a key effect on the reduction of zinc ions. Other studies have

**Table 1:** CCD, independent variables' levels and experimental values for the response variables

Sample no.	Amount of zinc salt (g)	Amount of clove extract (mL)	Antioxidant activity (%)	Antibacterial activity against <i>E. coli</i> (mm)	Antibacterial activity against <i>S. aureus</i> (mm)
1	4.0	20	85.5	11	12
2	4.0	20	85.5	11	12
3	4.0	30	77.7	14	15
4	2.0	20	73.9	15	14
5	4.0	20	85.0	11	12
6	2.6	27	74.8	14	15
7	5.4	27	85.1	10	12
8	6.0	20	89.8	9	10
9	4.0	10	90.5	8	8
10	4.0	20	85.8	11	12
11	5.4	13	90.6	8	8
12	4.0	20	85.8	11	12
13	2.6	13	86.5	10	12

also demonstrated that the formation of ZnO NPs was due to the reduction capacity of the phenolic compounds present in the plant extract [21–23]. In fact, hydroxyl group of the phenolic compounds has the ability to reduce metal ions and convert them into stable metal and metal oxide NPs.

The centered peak in the range of 2844.52–2936.63  $\text{cm}^{-1}$  was related to  $\text{CH}_2$  and  $\text{CH}_3$ , which could be found in the alcoholic compounds. The stretching band centered at 1638.29  $\text{cm}^{-1}$  was interconnected to the carbonyl ( $\text{C}=\text{O}$ ) group that originated from Carvone. The stretching band placed at 1,513  $\text{cm}^{-1}$  was related to the aromatic  $\text{C}=\text{C}$  group, which could be found in the Eugenol or Eugenol acetate. Bending peaks centered in the range of 818.30 to 913.97 and 995.18  $\text{cm}^{-1}$  were correlated to the disubstituted C–H and trisubstituted C–H which were found in the Limonene [24,25].

GC-MS chromatogram of the clove extract is shown in Figure 1b. As shown in this chromatogram, approximately 23 detected components were indicated in the extract, within 22 min of retention time. As can be seen in this figure, Eugenol was the main known bioactive compound of the clove extract, which had a maximum peak height at a retention time of 12.71 min, respectively.

### 3.2 Models' generation

Based on the obtained experimental values for the antioxidant and bactericidal activities of the resulted ZnO NPs (Table 1), second-order polynomial models were made to predict response factors of the produced ZnO NPs as function of the amounts of zinc salt and prepared clove

extract. Regression coefficients for the terms of models, with their  $R^2$  and  $P$ -value of the lack of fit, are shown in Table 2. Results revealed that the higher values of the  $R^2$  and  $R^2$ -adj ( $>0.9779$  and  $>0.9621$ ) for all three studied response variables, and  $P$ -values of the lack of fit higher than 0.05 for them, verified high acceptability of the created models according to the obtained experimental data [18,19]. As can be observed in this table, linear term of amount of the prepared clove extract had significant ( $P < 0.05$ ) effect on all three selected response factors, while only linear term of amount of the zinc salt had significant ( $P < 0.05$ ) effect on the antioxidant activity of the synthesized ZnO NPs. Results also demonstrated that quadratic term of amount of zinc salt had significant ( $P < 0.05$ ) effect on all three selected dependent variables, while, only quadratic term of amount of the extract had significant ( $P < 0.05$ ) effect on the antibacterial activity of the resulted ZnO NPs against *S. aureus*. Furthermore, the results demonstrated that the interactive term of the synthesis factors had an insignificant effect on the bactericidal property of the formed NPs, against *S. aureus*.

### 3.3 Effectiveness of synthesis variables on the antioxidant activity of the fabricated ZnO NPs

According to Table 1, antioxidant activity of the synthesized ZnO NPs using clove extract varied from 73.9% to 90.6%. Effects of the studied fabrication variables on response variables of the resulted ZnO NPs are shown in Figures 2 and 3. As evidently presented in Figure 2a, at fixed and low amounts of the clove extract, by rising amount of zinc salt,

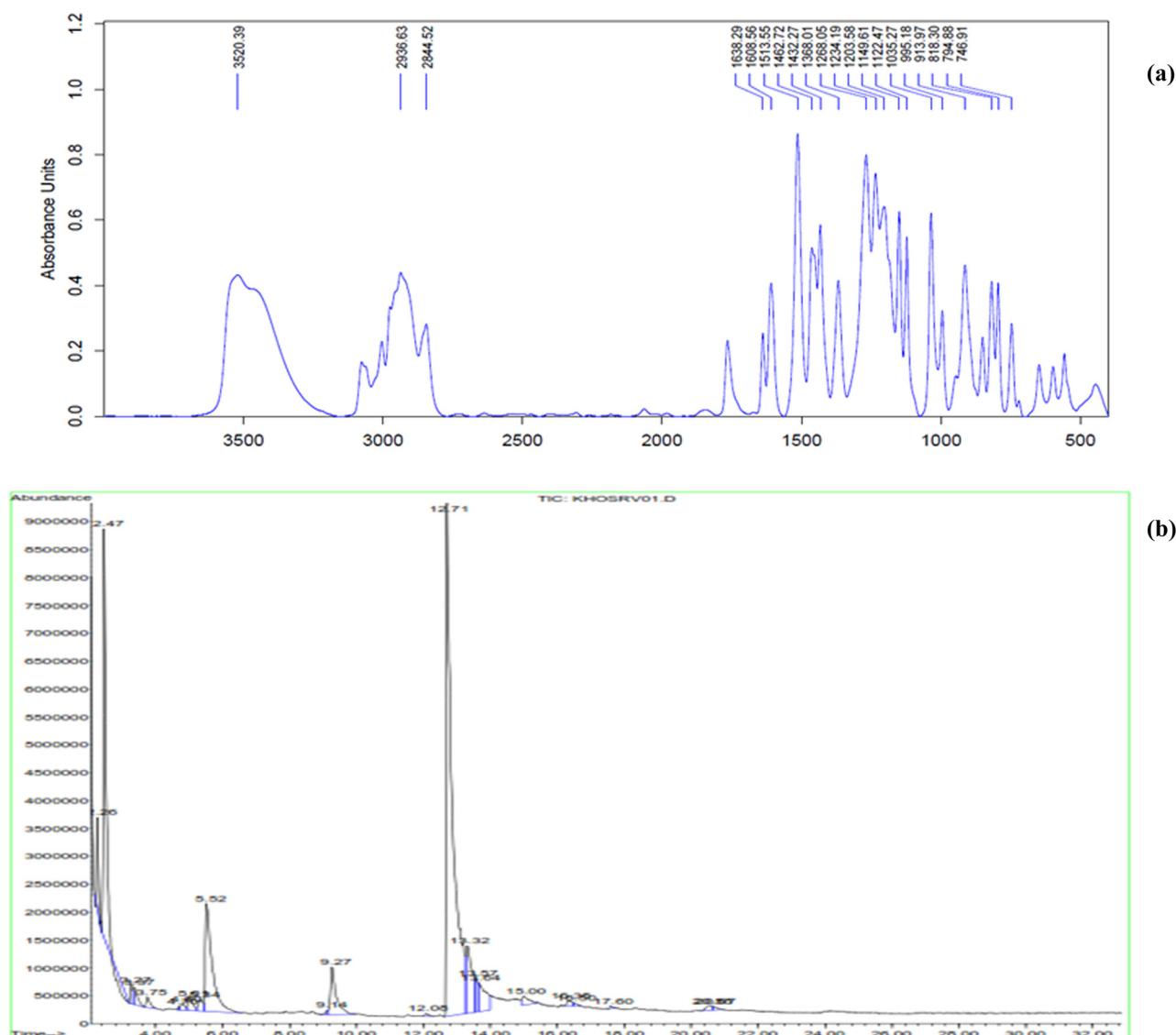


Figure 1: FT-IR spectra (a) and GC-MS chromatogram (b) of the clove (*Syzygium aromaticum* Linn) extract.

the antioxidant activity of the synthesized NPs did not change significantly ( $p < 0.05$ ). However, at fixed and high amounts of the clove extract, by rising amount of zinc salt, the antioxidant activity of the resulted ZnO NPs significantly ( $p < 0.05$ ) increased. Obtained results can be designated by the point that, at high values of the clove extract, the concentration of the main bioactive compounds such as Caryone and Eugenol, which played a key role in zinc ions' reduction and converting them into NPs, is high [7,26]. Therefore, higher amounts of the clove extract could result in ZnO NPs with a higher concentration, as compared to those fabricated using lower amounts of the prepared extract. Attained results were in line with the finding of Suresh et al.

[27]. They reported that by rising amount of *Artocarpus gomezianus* extract, antioxidant activity of the fabricated ZnO NPs increased. Existence of curvature in Figure 2a indicated that the interactive term of both the selected independent variables had significant ( $p < 0.05$ ) effect on the antioxidant activity of the resulted ZnO NPs and was reconfirmed by a lower  $P$ -value of their interactive term, as can be observed in Table 2.

As presented in Figure 3a, the maximum antioxidant activity was achieved in the formed ZnO NPs that were made using higher amounts of zinc salt. In fact, due to the antioxidant activity of ZnO NPs, their antioxidant activity has a direct relationship with their concentration [6,8,9].

**Table 2:** Coefficients of determination  $R^2$ ,  $R^2$ -adj, lack of fit ( $P$ -value of the regression) and ANOVA for the terms of the final model based on the  $P$ -value

Coefficients of determination	Antioxidant activity (%)	Antibacterial activity against <i>E. coli</i> (mm)	Antibacterial activity against <i>S. aureus</i> (mm)
$\beta_0$ (constant)	86.795	4.009	10.5
$\beta_1$ (main effect)	5.464	-0.797	-1.62
$P$ -Value	0.009	0.053	0.078
$\beta_2$ (main effect)	-1.248	0.858	0.4
$P$ -Value	0.006	0.001	0.038
$\beta_{11}$ (quadratic effect)	-0.905	0.218	0
$P$ -Value	0.001	0.001	0.048
$\beta_{22}$ (quadratic effect)	-0.013	-0.001	-0.005
$P$ -Value	0.063	0.403	0.016
$\beta_{12}$ (interaction effect)	0.272	-0.124	0.025
$P$ -Value	0.002	0.025	0.077
$R^2$	0.9861	0.996	0.9779
$R^2$ -adj	0.9745	0.9927	0.9621
$P$ -Value (lack of fit)	0.133	0.089	0.089

### 3.4 Effectiveness of synthesized variables on the bactericidal property of the formed ZnO NPs

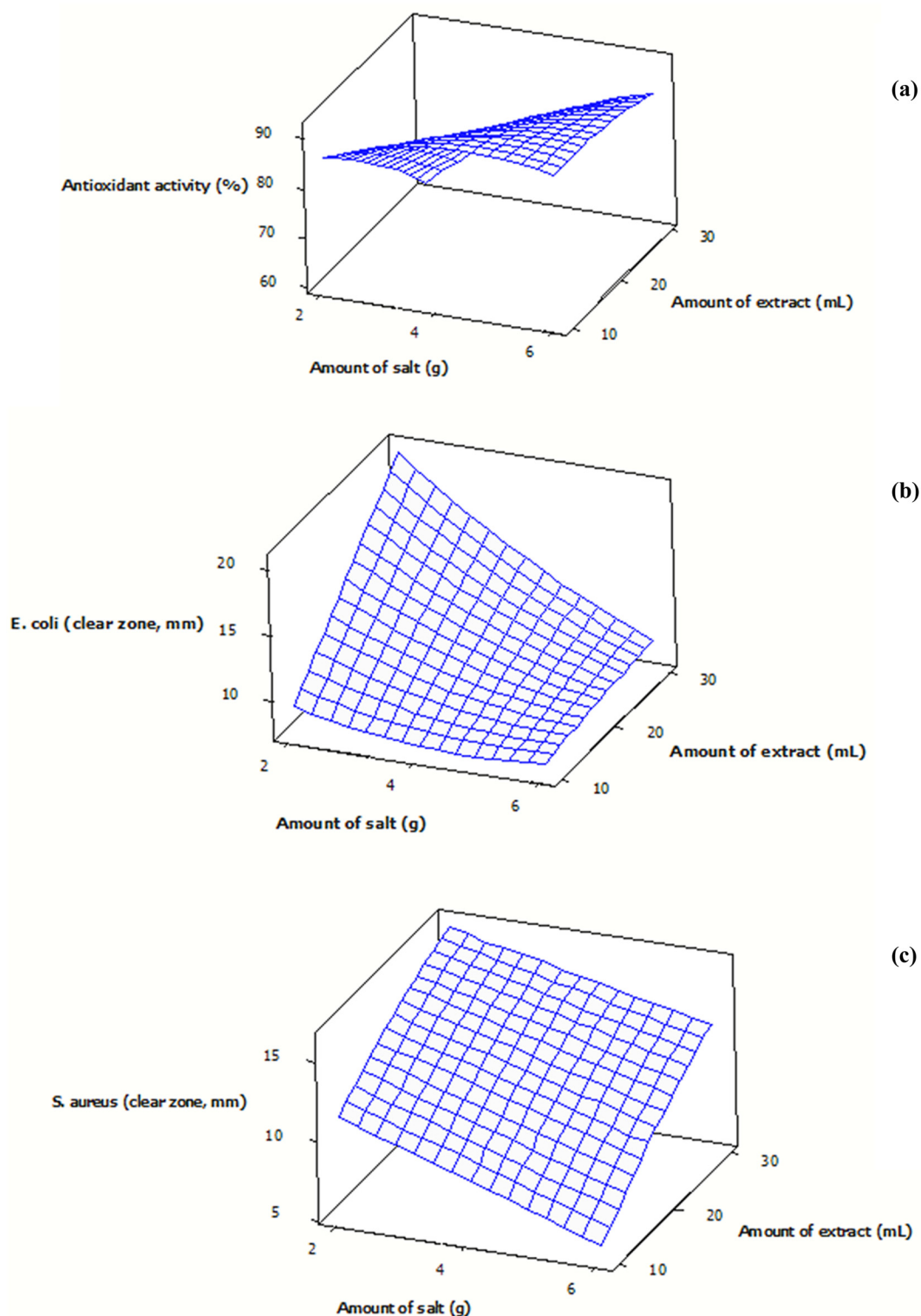
According to Table 1, antibacterial property of the resulted ZnO NPs, as manifested in the diameter of the created clear zone, changed from 8 to 15 mm, toward both selected Gram-positive and Gram-negative bacterial strains. Figure 2b shows the influences of the selected independent variables on the bactericidal property of the resulted ZnO NPs against *E. coli*. According to Figure 2b, at a fixed and low amount of zinc salt, by rising the amount of the prepared extract, the bactericidal effect of the resulted ZnO NPs, toward *E. coli*, significantly ( $p < 0.05$ ) increased, while, at constant and high amount of zinc salt, by rising amount of extract, the bactericidal property of the resulted NPs did not change. It seems that at high amounts of zinc salt and clove extract, the number of formed NPs is much more, which causes agglomeration of the NPs and increases their particle size. However, at lower amounts of the zinc salt and higher amounts of the clove extract, agglomeration of the formed ZnO NPs was limited due to a low concentration of the resulted NPs and a high concentration of natural stabilizers such as proteins and carbohydrates that existed in the clove extract. Therefore, synthesized NPs, using this mentioned condition, had a small particle size and maximum surface area to volume ratio, which, in turn, increased the attachment of the formed NPs to the cell membrane, changed that permeability, and caused cell death [28]. Existence of curvature in Figure 2b indicated that the

interactive term of both the selected factors had significant ( $p < 0.05$ ) effect on the bactericidal property of the resulted ZnO NPs, against *E. coli*, as manifested in a lower  $P$ -value of their interaction term in Table 2. According to Figure 3b, ZnO NPs with the highest antibacterial activity against *E. coli* were synthesized, using minimum and maximum values of the zinc salt and clove extract, respectively.

Effects of synthesized parameters on the bactericidal property of the formed ZnO NPs toward *S. aureus* are presented in Figures 2c and 3c. Same results, as achieved for bactericidal properties of the formed ZnO NPs against *E. coli*, were obtained for those of the fabricated ZnO NPs against *E. coli*. However, the interaction of both independent variables did not show significant effect on the bactericidal property of the formed NPs toward *S. aureus*, as there is no curvature in Figure 2c. Based on Figure 3c, minimum bactericidal properties of the NPs are obtained by the synthesis of ZnO NPs using the highest and lowest amounts of the zinc salt and clove extract, respectively. Obtained results were in agreement with the finding of Jones *et al.* [29]. They found that the produced ZnO NPs using plant extracts had bactericidal effect toward both Gram-positive and Gram-negative bacterial strains.

### 3.5 Optimization of the synthesis process

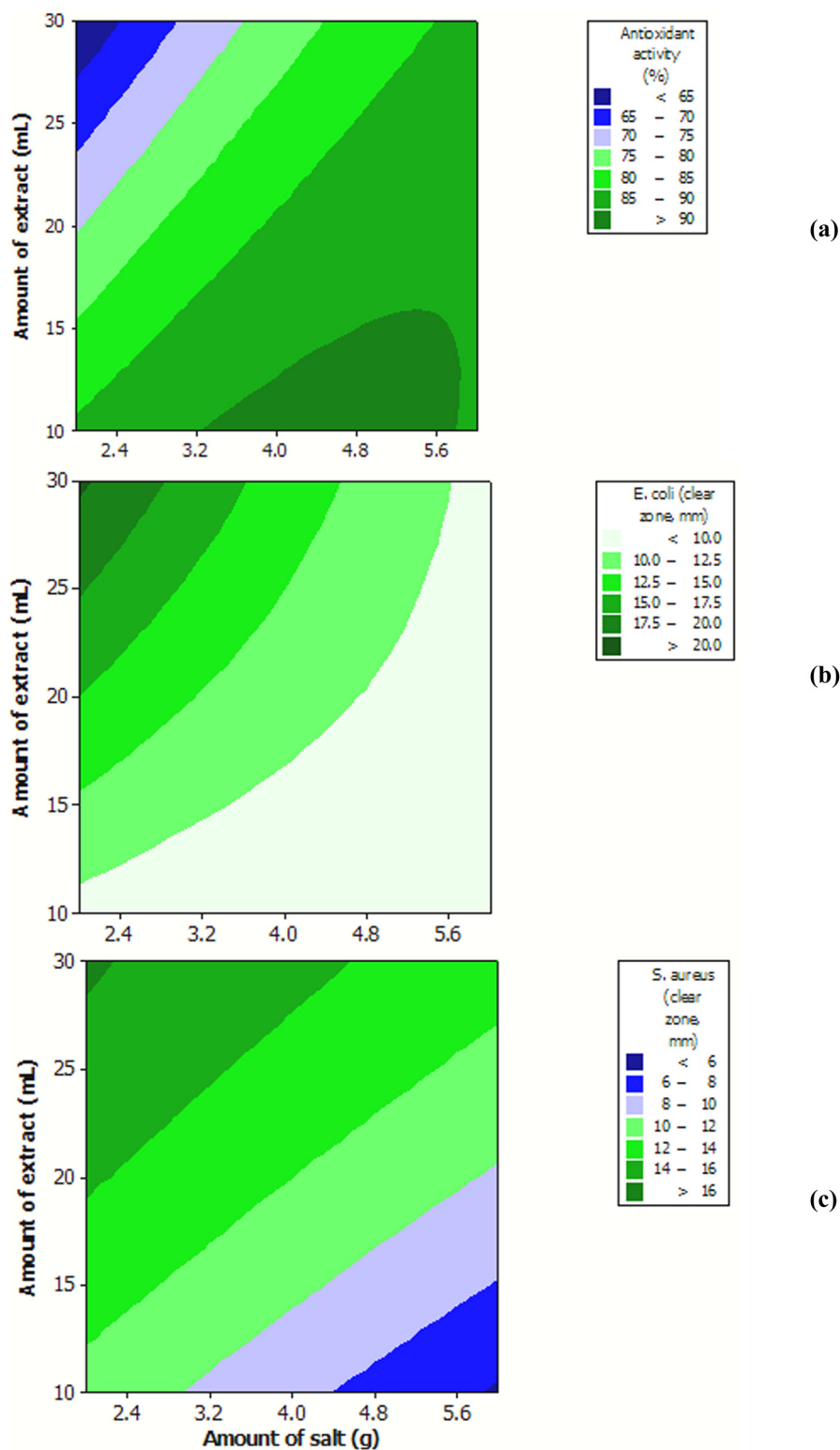
Optimum synthesis conditions in ZnO NPs fabrication are considered as the conditions that result in NPs with



**Figure 2:** Surface plots for antioxidant activity (a), antibacterial activities against *E. coli* (b) and *S. aureus* (c) of the synthesized ZnO NPs, as function of amounts of zinc salt (g) and clove extract (mL).

maximum antioxidant and antibacterial activities. Obtained numerical optimization result revealed that the hydrothermally green synthesis of ZnO NPs using 3.98 g zinc salt and

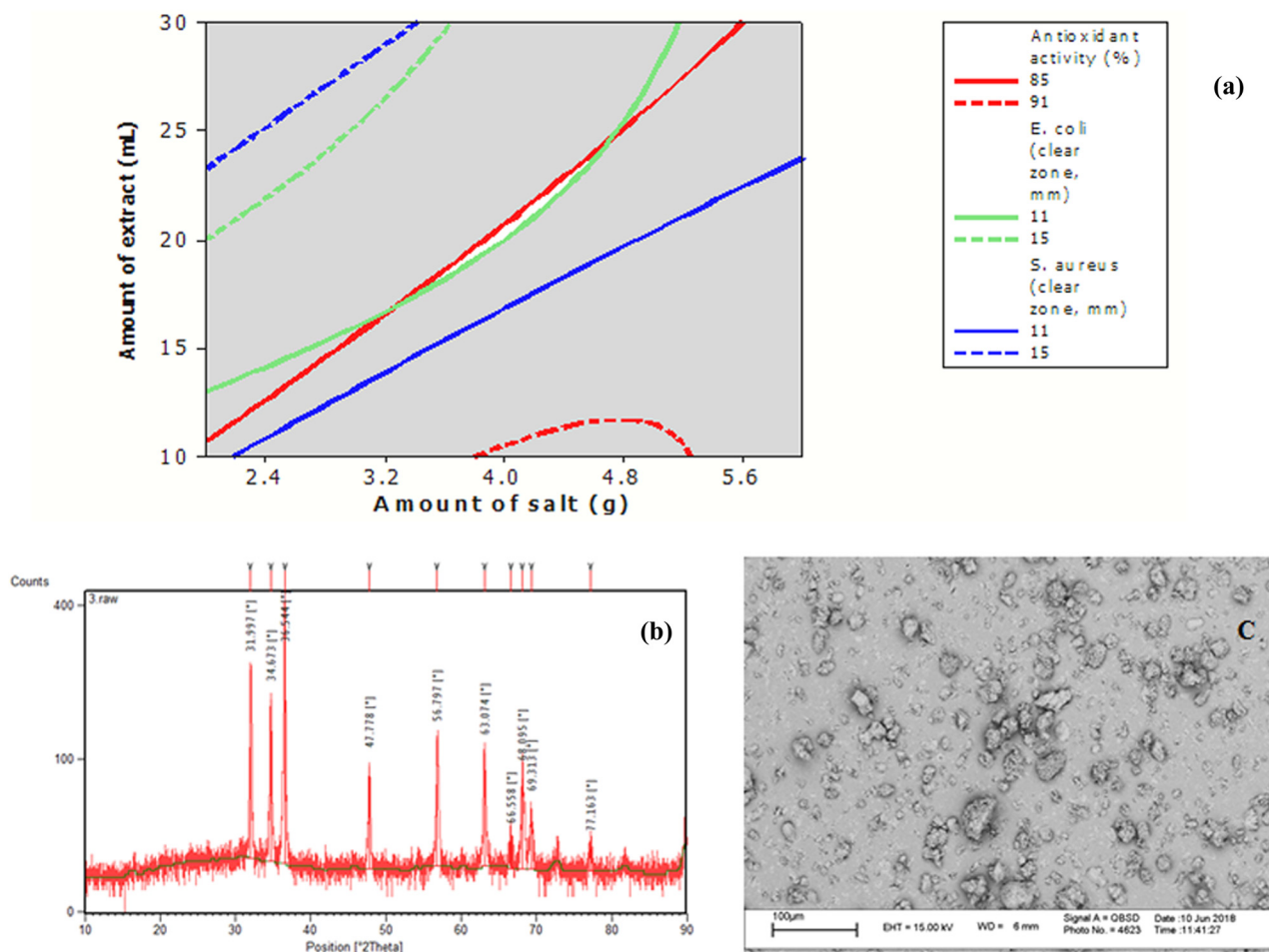
20.30 mL clove extract could result in forming ZnO NPs with an antioxidant activity of 85.23% and a bactericidal effect toward *E. coli* and *S. aureus*, as manifested in the diameter



**Figure 3:** Contour plots for antioxidant activity (a), antibacterial activities against *E. coli* (b) and *S. aureus* (c) of the synthesized ZnO NPs, as function of amounts of zinc salt (g) and clove extract (mL).

of made clear zones of 11.12 and 12.11 mm, respectively. In fact, *E. coli* and *S. aureus* are known as the indexes of Gram-negative and Gram-positive bacterial strains [15].

Graphical optimization is shown in Figure 4a, in which the white color zone is related to the optimum area for both independently synthesized parameters. Furthermore, three



**Figure 4:** Overlaid contour plot of ZnO NPs' antioxidant and antibacterial activities with acceptable levels as function of amounts of zinc salt (g) and clove extract (mL) (a), XRD pattern (b), and SEM image (c) of the fabricated ZnO NPs using clove (*Syzygium aromaticum* Linn) extract and obtained optimum synthesis conditions.

extra approval tests by attained optimal synthesis parameters were conducted and the obtained results indicated insignificant differences between the experimental and predicted values of antioxidant and antibacterial activities of the resulted ZnO NPs, which verified the validity and accuracy of the generated models using RSM [30].

### 3.6 Structural, morphological, and photocatalytic attributes of the formed ZnO NPs using optimal conditions

The XRD pattern of hydrothermally produced ZnO NPs by the clove extract is shown in Figure 4b, which was in line with the XRD pattern of the produced ZnO NPs with *Berberis vulgaris* extract by Anzabi (2018) and certified the production of a crystalline hexagonal structure for

the resulted ZnO NPs. Mean crystalline size of the NPs made by the clove extract was 50 nm.

A typical SEM image of the resulted ZnO NPs using clove extract is shown in Figure 4c. As can be clearly observed in this figure, hexagonal ZnO NPs in individual and aggregated states, which were in line with the finding of Vahidi et al. [2], were formed. They produced ZnO NPs with the same morphological attributes using *Pelargonium* leaf extract.

Furthermore, obtained results demonstrated that the formed ZnO NPs using clove extract had a high degradation potential of methylene blue during 2 h, and the resulted ZnO NPs could degrade 70% of the methylene blue. During UV irradiation of the formed ZnO NPs in the solution containing dye, and based on the theory of generation of “electron-hole” pairs, the energetic electrons traversed up from the valence to the conduction band, which had the potential to generate reactive oxygen species and due to their high reactivity, the degradation of the dyes [22,23].

Obtained result was in line with the finding of Sayyar and Jafarizadeh-Malmiri [13]. They removed 86% of methylene blue using fabricated ZnO NPs by curcumin.

## 4 Conclusions

The bottom-up green approach based on using subcritical water, as a safe and non-toxic solvent, and clove hydroalcoholic extract, as a natural reducing and stabilizing agent, was utilized to fabricate ZnO NPs. Obtained results indicated that clove extract, due to the presence of strong reducing bioactive compounds such as Eugenol and stabilizing biomolecules such as carbohydrates, had rapid and one-step synthesis potential to form ZnO NPs, which makes this synthesis method, as a simple, environment-friendly, low-energy consuming, and cost-effective technique, as compared to the conventional physical and chemical metal oxide NP fabrication methods. Furthermore, results also revealed that the RSM could be excellently utilized to provide models, optimize the production process parameters, and calculate antioxidant and bactericidal activities of the resulted ZnO NPs. Due to more desirable antioxidant, antibacterial, and photocatalytic activities of the resulted ZnO NPs using obtained optimal conditions, the synthesized ZnO NPs can be widely utilized in different food, pharmaceutical, and cosmetic formulations.

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

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