



Antioxidant, Protoscolicidal, Hemocompatibility, and Antibacterial Activity of Nickel Oxide Nanoparticles Synthesized by *Ziziphus spina-christi*

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Abstract

Over the past several years, the greener fabrication of metal oxide nanoparticles has attracted significant attention due to their simplicity, eco-friendliness, availability, and nontoxicity. This paper focused on the fabrication of nickel oxide nanoparticles (NiO-NPs) using the leaf extract of *Ziziphus spina-christi* L. and evaluating its potential biological activities. The characterization of synthesized NiO-NPs was confirmed using ultraviolet–visible spectroscopy, field emission-scanning electron microscope, energy-dispersive X-ray spectroscopy, and X-ray diffraction. Furthermore, protoscolicidal, antibacterial, and antioxidant activities and hemocompatibility of NiO-NPs were investigated. The findings revealed that the NiO-NPs were crystalline on nanoscale between 50- and 90-nm particle sizes. The NiO-NPs showed high scolicidal activity against *Echinococcus granulosus*. The viability of the treated protoscoleces exponentially decreased with an increase in the concentration of NiO-NPs. The NiO-NPs exhibited effective antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. NiO-NPs also possess a H₂O₂ scavenging activity in a dose-dependent manner. This study revealed that the *Z. spina-christi* L. leaf extract is an effective reducing and capping agent for the production of NiO-NPs; it showed critical biological properties. Moreover, NiO-NPs have a potent antioxidant activity and low toxicity on the erythrocytes and appear hemocompatible.

Keywords Green synthesis · *Ziziphus spina-christi* · Nickel oxide nanoparticles · Antioxidant · Protoscolicidal · Hemocompatibility · Antibacterial

1 Introduction

Nowadays, NPs of some metals or metal oxides are widely used as a therapy to treat different diseases and progress human health due to their antimicrobial action; these NPs exhibit antibacterial, antiviral, and antiparasitic efficacies [1, 2]. Uddin et al. [3] concluded that the green synthesis of NiO-NPs by leaf extracts of *Berberis balochistanica* is inexpensive, easy, and safe. This is recommended to use as nanomedicine and nanofertilizer.

Nickel and nickel oxide nanoparticles (NiO-NPs) possess unique physical and chemical properties, due to their specific magnetic, catalytic, and electronic characteristics in magnetism, and biomedicines, besides electronics [4].

NiO-NPs are the first-known P-type semiconductors with a wide bandgap of 3.6 to 4.0 eV and found that they exhibit significant electrochemical stability, high reactivity, excellent biocompatibility, and bacterial resistivity [5]. In a recent study, the cytotoxic efficiency of NiO-NPs against human hepatic cancer cells, breast cancer cells, and colon cancer cell lines by the MTT assay was investigated. They concluded that fabricated NiO-NPs showed acceptable cytotoxic properties against the tested cancer cell lines, therefore, they could be considered a good option for cancer treatment [6].

Nickel oxide nanoparticles (NiO-NPs) are usually synthesized by several physical and chemical techniques like sol–gel, hydrothermal, precipitation, and solvothermal [7]. Most of these methods are expensive, need highly sophisticated instruments, are toxic, and are non-eco-friendly for the production of NPs [8]. To overcome these problems,

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a greener and eco-friendly approach to NP synthesis has been recommended [9]. Nevertheless, the biogenic synthesis method has drawn the attention of investigators due to its biocompatibility and eco-friendly process, which involves a green synthetic way that is less toxic. It is significant because the current therapeutic approaches have toxicity effects and microbial multidrug resistance properties.

Plant extracts were used as capping and reducing agents to fabricate different NPs, which improved the discipline of nanoscience application [10, 11]. Therefore, this work focuses on the biosynthesis of NiO-NPs by the green method using *Z. spina-christi*. The biomolecules, secondary metabolites, and coenzymes present in the plants reduce metal ions to nanoparticles. Such nanoparticles are considered potential antioxidants and promising candidates for cancer treatment. Thus, the synthesis of eco-friendly nanoparticles from combustion solutions is one of the simplest and easiest synthetic approaches toward uniform mixing plant extract with precursor/oxidizing agents [7]. Their superior physico-chemical characteristic considers them a promising candidate for advanced material applications.

The current protoscolicidal agents used to avoid secondary hydatidosis and hypersensitivity reactions during hydatid cyst surgery are unsafe and have several adverse side effects [12]. Therefore, finding an effective non-surgical method to treat the hydatid cyst disease becomes one goal of the study.

Several publications have pointed out that NiO-NPs have numerous chemical and biological applications, for instance, adsorption of pollutants and dyes, anti-inflammation, antibacterial, antifungal, anticancer, and cytotoxic abilities [13].

The *Z. spina-christi* was called sidr (as Quran Lote trees). It is an important cultivated tree and one of the few genuinely native tree species of Iraq that is still growing mainly in the south of Iraq. It is a typical edible and medicinal plant native to Iraq. The genus of this plant is known for its medicinal characteristics as a hypoglycemic, hypotensive, anti-inflammatory, antimicrobial, antinociceptive, antioxidant, antifungal, antitumor, liver-protective agent, and immune responses stimulant. Flavonoids, alkaloids, and saponins are the significant phytochemicals described in this plant [14].

Moreover, *Z. spina-christi* extract was also proved to protect against aflatoxicosis [15]. This study aimed to photosynthesize NiO-NPs using leaf extract of *Z. spina-christi* for the first time. This would essentially aid in evaluating NiO-NPs as protoscolicidal, antimicrobial, and antioxidant agents and assess their hemocompatibility by blood hemolysis test.

The novelty of the present work is the utilization of *Z. spina-christi* leaf extract as a substantial reducing and capping agent for NiO-NPs fabrication, in addition to investigating their antioxidant, antiparasitic, antibacterial activities, and hemocompatibility.

2 Materials and Methods

2.1 Chemicals and Materials

Double-distilled water has been used for preparation for all experimental processes. All the used chemical materials such as nickel chloride $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (99%) from Thomas Baker CAS number 7791–20-0, sodium hydroxide, and eosin stain are purchased from Sigma-Aldrich, USA. Mueller–Hinton agar (Rashmi Diagnostics Private Limited), amikacin, and amoxicillin antibiotic disks were supplied by Oxoid Ltd., Basingstoke, X3296 Hampshire, England.

Solvents and chemicals were obtained from Merck and utilized without additional purifications.

2.2 Preparation of the Plant Extract

The preparation of *Z. spina-christi* L. (Sider) leaf extract was achieved as the fresh plant leaves were collected from a naturally grown plant in Erbil city, Kurdistan, Iraq. The leaves were washed, air-dried, and cut into small pieces. Twenty-five grams of fresh leaves was added to 200 mL of double-distilled water and stirred at 80 °C for 40 min. Then the leaf extract was allowed to cool down to room temperature and filtered through Whatman No. 1 filter paper. Later, the pure filtrate extract was stored for further experiments.

2.3 Green Fabrication of Nickel Oxide Nanoparticles

The green preparation of NiO-NPs was performed using *Z. spina-christi* leaf extract based on a previous study with slight modifications [16]. One gram of nickel chloride hexahydrate $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ as Nickel precursor salt was dissolved in 50 mL of deionized water and stirred for 20 min. Then, 100 mL plant leaf extract was added and stirred at 70 °C for 30 min. A color change was observed initially, indicating the likely formation of NiO-NPs. Then, 1 M of NaOH solution was gradually added, and the pH of the mixture was adjusted to 12.

The solution was left stirring for 2 h, and NiO-NP powder was collected by water evaporation at 100 °C, then calcinated in a furnace at 400 °C for 2 h [17]. After that, the NiO-NP particles were carefully washed three times with double-distilled water to remove the contaminant materials, washed with ethanol, and then dried.

Fig. 1 Schematic depiction of green synthesized nickel oxide NiO-NPs from *Z. spina-christi* leaf extract

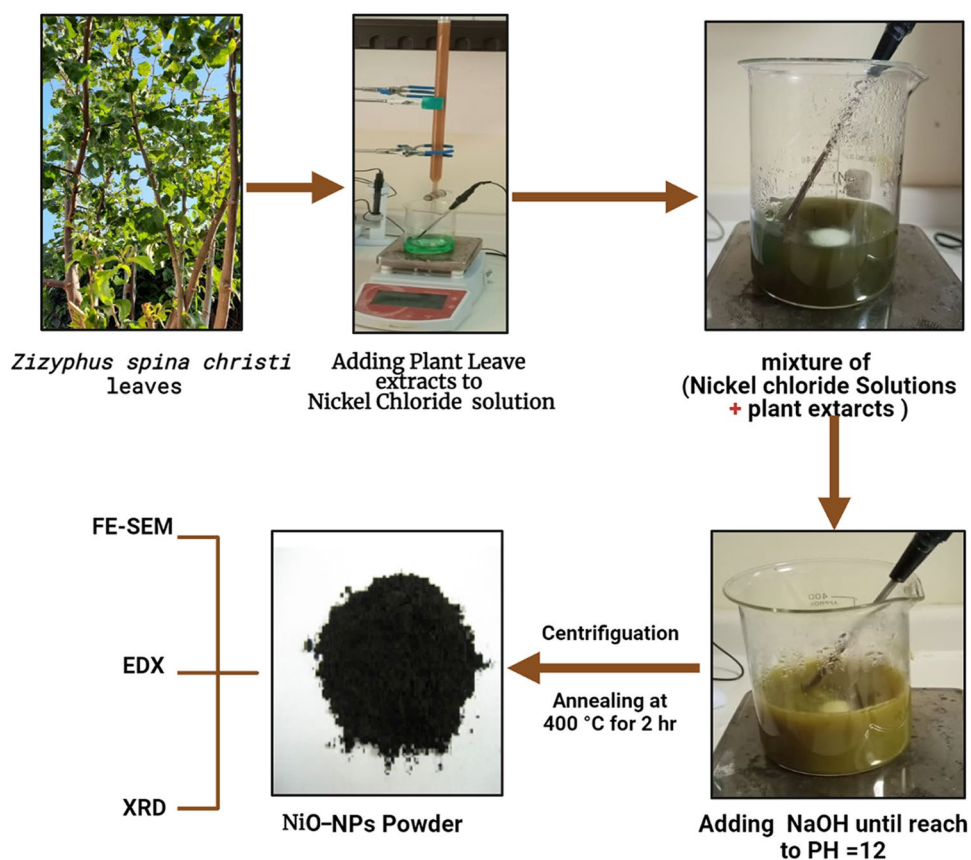


Fig. 2 A proposed mechanism for green synthesis of NiO-NPs

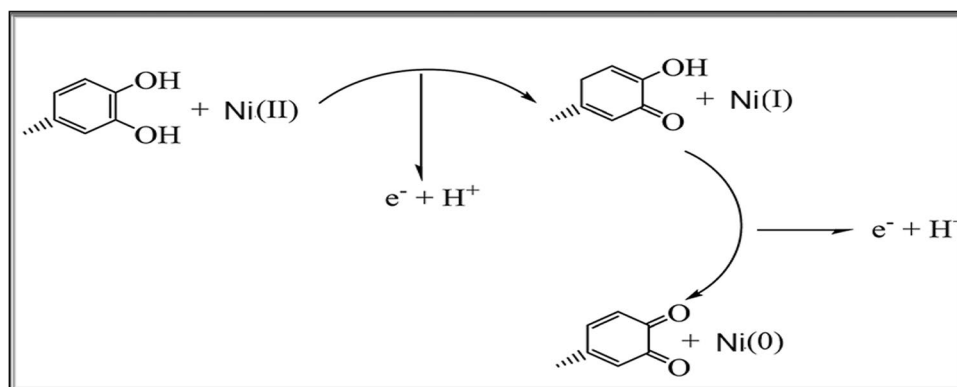


Figure 1 shows the schematic diagram of the biosynthesis and characterization of NiO-NPs. The expected mechanism for the biosynthesized NiO-NPs is shown in Fig. 2.

2.4 Characterization of Nickel Oxide Nanoparticles

The physicochemical properties of the green prepared NiO-NPs were investigated using various analytical techniques. UV–Vis absorption spectra were performed on a double-beam UV–visible spectrum of the reaction solution (Super Aquarius

spectrophotometer-2000) to confirm NiO-NPs' formation. It was scanned from 200- to 850-nm wavelengths. The morphology, shape, size, and elemental composition of NiO-NPs were analyzed by field emission scanning electron microscopy (FE-SEM, Quanta 450) and energy-dispersive X-ray spectroscopy (EDX), respectively. Furthermore, the crystal structure of nanoparticles was investigated by X-ray diffraction (XRD) using PAN analytical X'Pert PRO (Cu K α = 1.5406 Å) [18], with a scanning rate of 1°/min in the 2 θ range from 20° to 80°. The crystallite size of NiO-NPs was calculated by using the Debye Scherrer equation:

$$D = k \lambda / \beta \cos \theta$$

$k = 0.9$ Scherrer constant and θ is the angle determined using 2θ values that are the maximum intensity peak in the XRD series, which correspond to the maximum intensity peak of the Cu-K α radiation at half maximum symbolized by λ and β .

2.5 Biological Applications

2.5.1 Hydrogen Peroxide (H_2O_2) Scavenging Activity

Hydrogen peroxide scavenging activity was assessed by method as described by Keshari et al. [19] and Keshari et al. [20].

Two millimolars of hydrogen peroxide (H_2O_2) was prepared in phosphate-buffered saline (PBS: 50 mM, pH 7.4). 0.1 mL of different concentration (6.25–100 μ g/mL in 50 mM PBS, pH 7.4) of NiO-NP solution was added to 0.3 mL PBS (50 mM, pH 7.4). 0.6 mL H_2O_2 solution was added to it, then the mixture was vortexed. After 10 min, the absorbance was determined at 230 nm using UV–Vis spectrophotometer. Ascorbic acid (vitamin C) was used as standard, and PBS (50 mM, pH 7.4) was used as blank. The following formula estimated the percentage of H_2O_2 scavenging activity:

$$\text{Scavenging\%} = \frac{(OC - OS)}{OC} \times 100$$

The OC represents the absorbance of the control (all the reagents excluding the test sample) and the OS absorbance of NiO-NPs/vitamin C.

2.5.2 Protoscolicidal Activity

Hydatid cysts of *E. granulosus* were collected from the naturally infected liver of sheep from the slaughterhouse in Soran city, Kurdistan, Iraq. The hydatid fluid was aseptically aspirated from the cyst. The collected fluid with protoscoleces was left for 15 min to settle down the protoscolices. Then, the supernatant was discarded, and the protoscolices were washed with normal saline. The viability of protoscoleces was detected using a 0.1% aqueous eosin dye. After incubation for 5 min with the stain, the stained protoscoleces were considered dead and the colorless protoscoleces were considered viable. If the viability of the protoscoleces in the hydatid fluid is as high as 97% or more, it was suitable for this experimental work [21].

The activities of NiO-NPs were evaluated against *E. granulosus* parasite as previously described by Jalil et al. [10]. Protoscolicidal procedures were done using different concentrations of ultra-sonicated NiO-NPs including 0.1, 0.2, 0.3, and 0.4 mg/mL. Briefly, 100 μ L of protoscoleces was added to 2.5 mL of the prepared concentrations. The test tubes were incubated at 37 °C for various periods. The supernatant was gently removed at the end of each incubation period. The mortality percentage was calculated by gently mixing 10 μ L of 0.1% eosin with 20 μ L of the protoscoleces pellet. The protoscoleces were smeared on a microscopic slide coated with a coverslip and inspected under an optical microscope after 10 min. The percentage of viability was evaluated by counting 200 protoscoleces from each treatment. Normal saline (0.9% NaCl) was considered a negative control, and 5% NaCl was positive. The experiments were repeated three times.

The following formula determined the viability percentage of the protoscoleces:

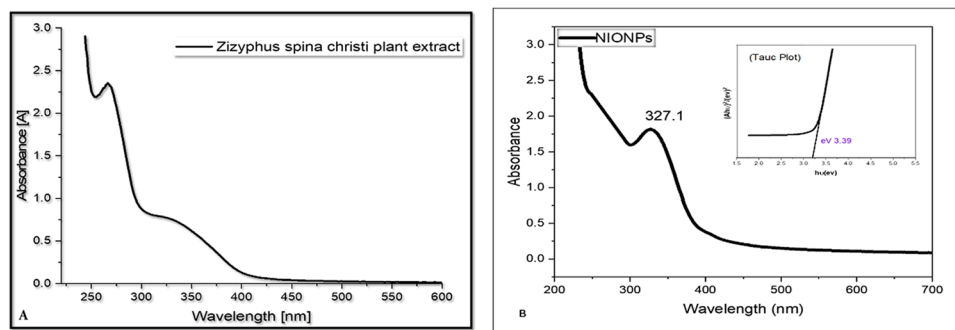
$$\text{Viability of protoscoleces} = \frac{\text{Number of viable protoscoleces}}{\text{Total number of protoscoleces}} \times 100$$

2.5.3 Blood Compatibility of NiO-NPs

Hemolysis assays using erythrocytes (RBCs) were achieved to test the hemolytic ability of NiO-NPs. Four milliliters of fresh human blood from healthy volunteers and used with formal bioethics approval were obtained in an ethylenediaminetetraacetic acid anticoagulant tube. The blood was centrifuged at 1500 rpm for 10 min. After centrifugation, the buffy coat and plasma were removed from the supernatant, and the pellet was washed three times with PBS. The RBCs were diluted to 8 mL with PBS buffer. Then, 200 μ L of the diluted RBC suspension was added to 800 μ L of the various concentrations of NiO-NP suspension in PBS (1100, 550, 275, 137.75, 68.75, 34.38, and 17.1) μ g/mL. The reaction mixture was incubated for 1 h at 37 °C. Then the solutions were centrifuged at 1500 rpm for 10 min, and the supernatant was collected. The released amount of hemoglobin from the supernatant was measured at a wavelength (570 nm), using a UV–vis spectrophotometer. Deionized water and sterilized PBS were utilized as positive control and negative control, respectively [22]. The data were calculated as % hemolysis affected by different concentrations of NiO-NPs using the following formula [23, 24].

$$\text{Hemolysis \%} = \frac{[(\text{Specimens absorbance}) - (\text{Negative control absorbance})]}{(\text{Positive control absorbance} - \text{Negative control absorbance})} \times 100$$

Fig. 3 The UV–Vis absorption spectrum of the plant extract of *Z. spina-christi* (A) represents the UV–Vis spectrum of the biosynthesized NiO-NPs (B)



2.5.4 Antibacterial Activity of NiO-NPs

The antibacterial activity of the biosynthesized NiO-NPs from *Z. spina-christi* leaves was investigated against clinical pathogenic isolates of Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*) by agar well diffusion assay to test the bacterial susceptibility. Muller Hinton agar medium was prepared in Petri dishes, and the bacterial cultures were swabbed on test media with a sterile cotton swab. Wells (6 mm) loaded with 10 μ l of different concentrations of NiO-NPs were carefully prepared. The standard antibiotic, amoxicillin, 30 μ g/disk for *E. coli* and amikacin 10 μ g/disk for *S. aureus* were used as a positive control. The bacterial pathogens were obtained from the Biology Department, Faculty of Science, Soran University. The plates were then incubated at 37 °C for 24 h. After incubation, the antibacterial activity of the biosynthesized NiO-NPs was evaluated according to the diameters of the inhibition zones. The inhibition zone diameter was measured by millimeter (mm) in triplicate [25].

2.5.5 Statistical Analysis

The inhibition concentration (IC₅₀) values was calculated using Microsoft Office Excel 2016. The data was analyzed by GraphPad Prism software version 8. The one-way ANOVA and Tukey's method was used for multiple comparisons. $p < 0.05$ was considered statistically significant. All experiments were performed in triplicate, and the results are presented as mean $\pm$ SE.

3 Results and Discussion

3.1 Characterization of Green Synthesized NiO-NPs

3.1.1 UV–Vis Spectrophotometer of Plant Extract and NiO-NPs

UV–Vis images of the plant extract absorption peaks appeared around 270 and 320 nm (Figure 3A). These two

peaks are related to the leaves' phytochemicals associated with cinnamoyl and benzoyl phenolic systems. They would be a source for the green synthesis of metallic and metal oxide nanoparticles.

In the present work, the plant extract expressed two peaks at 270 and 320 nm related to the cinnamoyl and benzoyl phenolic systems. They would be a source for the green synthesis of metallic and metal oxide nanoparticles. The peak at 270 nm represents the benzoyl systems of antioxidant phenolics; Moustafa et al. [26] mentioned that flavonol compounds displayed dual absorption peaks, one at 273 nm with a shoulder at 329 nm, indicating the existence of 3,49-hydroxy groups. The *Z. spina-christi* extract contains several phytochemical compounds such as phenolic content, tannins, polyphenols, saponins, and cardiac glycosides that supposed to act as a reducing, capping, and stabilizing agent for NiO-NP biosynthesis [27].

(Figure 3B) displays the optical absorption peaks within the limit of 200–850 nm to be at 327 nm, confirming the green-based synthesis of NiO-NPs. These findings agree with the previous study conducted by El-Kemary et al. [18]. Also, UV–vis spectroscopy of NiO-NPs was reported in another study to possess an absorption peak at 330 nm [28]

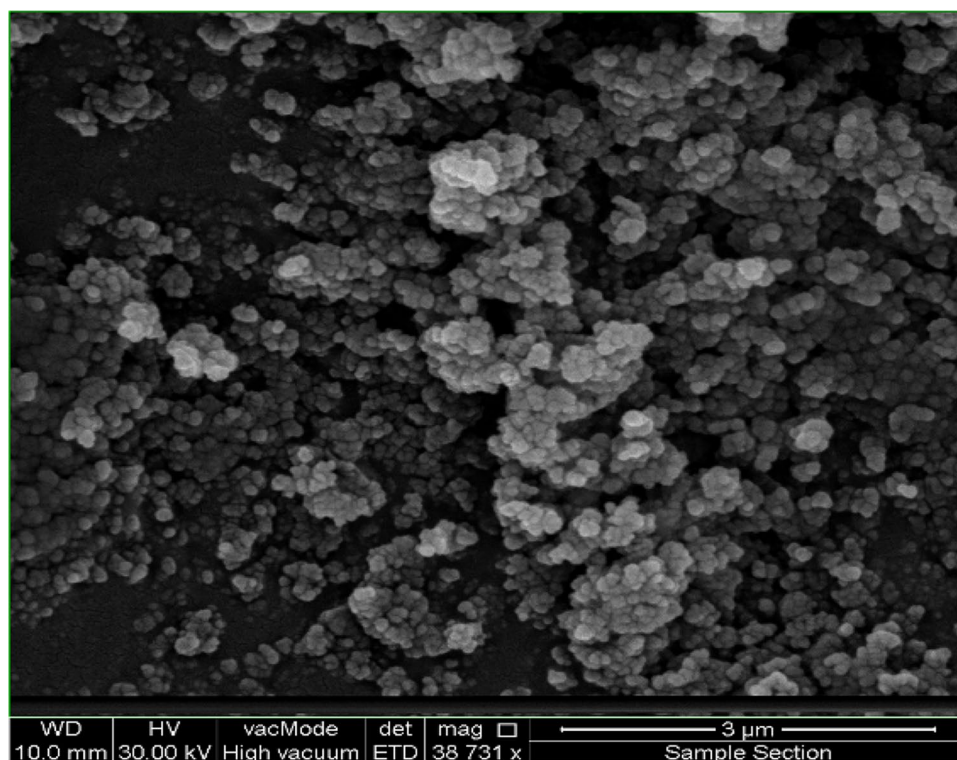
Meanwhile, the color change in reaction mixtures was seen from light green to dark green after adding plant extract. This result confirms the formation of NiO-NPs. The figure shows no additional peaks, which is good evidence that there are no impurities in the crystalline of the biosynthesized NiO nanoparticles.

The energy bandgap of the prepared NiO-NPs is calculated by Tauc equation,

$$(\alpha h\nu)^{1/n} = A(h\nu - E_g)$$

where α is the absorption coefficient, h is Planck's constant, ν is the photon's frequency, A is absorbance, and E_g is the bandgap, for direct allowed transitions: $n = 1/2$ [29].

Fig. 4 FE-SEM image of the purified NiO-NPs



A plot of $(\alpha h\nu)^2$ versus $h\nu$ is shown in the inset of Fig. 3, and the linear portion of the curve is extrapolated to the $h\nu$ axis to determine the bandgap.

The optical bandgap of the biosynthesized NiO-NPs of the present study was assessed as 3.39 eV, which is in agreement with the value (3.42 eV) documented by Davar et al. [30] and (3.5 eV) mentioned by Barzinjy et al. [28].

This difference in the band gaps of NiO resulted from the absorption edges, which shifted slightly. This shift points to the increased bandgap, ascribed to particle size reduction [31]. No linear correlation was found for $n = 1/2$, proposing that the biosynthesized NiO-NPs are semiconducting with a direct transition at this energy [32].

3.1.2 Morphological Study

Figure 4 depicts the morphology of the biogenic NiO-NPs as almost all spherical shapes, with some forming an agglomerated cluster, appearing on the scale of nanoscale in the SEM image. The synthesized NiO-NPs possess uniformly spherical NPs, with grain sizes ranging from 50 to 90 nm. On the other hand, agglomeration formation was also detected. The agglomerated NiO-NPs revealed an amorphous shape. Landi et al. [33] reported that the agglomeration of NiO-NPs is due to van der Waals forces that pull the particles together. This condition occurs mainly in nanoparticles of smaller sizes. Also, an investigation showed that agglomeration could be attributed to

NiO-NPs having high surface energy and high surface tension of the ultrafine nanoparticles [34]. Also, this aggregation is expected due to the magnetic interaction besides the polymeric adherence nature of NPs [35].

3.1.3 EDX and Elemental Mapping

Figure 5 explains the pureness of NiO-NPs that was examined using energy-dispersive X-ray spectroscopy (EDX) analysis. The EDX data confirms the presence of nickel and oxygen atoms in the sample while exhibiting the Ni and O peaks free of any other impurities. Also, the chemical elements of NiO-NPs consisted of 17.7% mass of oxygen and 82.2% nickel elements. The ratio of atomic percentages of Ni to O is 1.26; additionally, the existence of Au in the EDX spectrum is due to the coating of NiO-NPs with a tiny layer of a gold coat to enhance the quality of the image. Figure 6 shows the elemental mapping of NiO-NPs, which contains the nickel and oxygen distribution with almost no impurity.

3.1.4 XRD

The X-ray diffraction pattern of NiO-NPs is presented in Fig. 7 as it was scanned between 20 and 80 at an angle of 2θ (2 theta) degrees. The diffraction peaks observed at the theta angles 37.24° , 43.26° , 62.84° , 75.34° , and 79.30° can be allocated to the (111), (200), (220), (311), and (222) planes, respectively.

Fig. 5 EDX analysis of the biosynthesized NiO-NPs

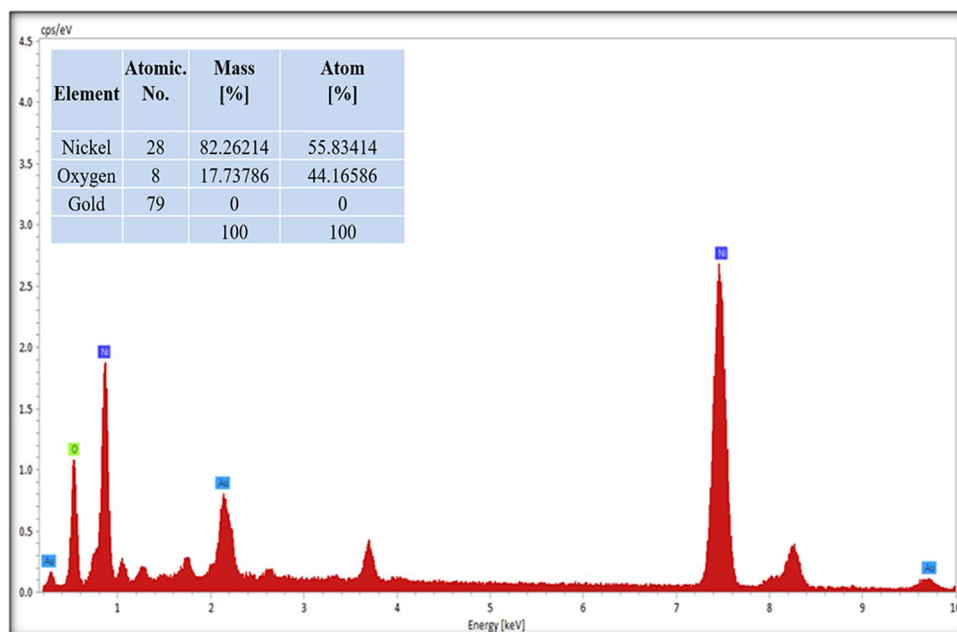


Fig. 6 Elemental composition distribution of the biosynthesized NiO-NPs

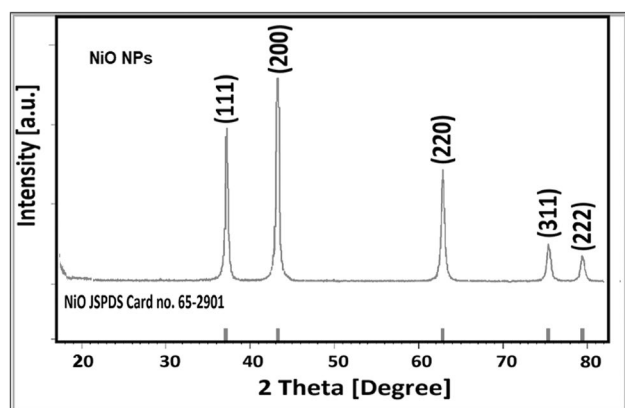
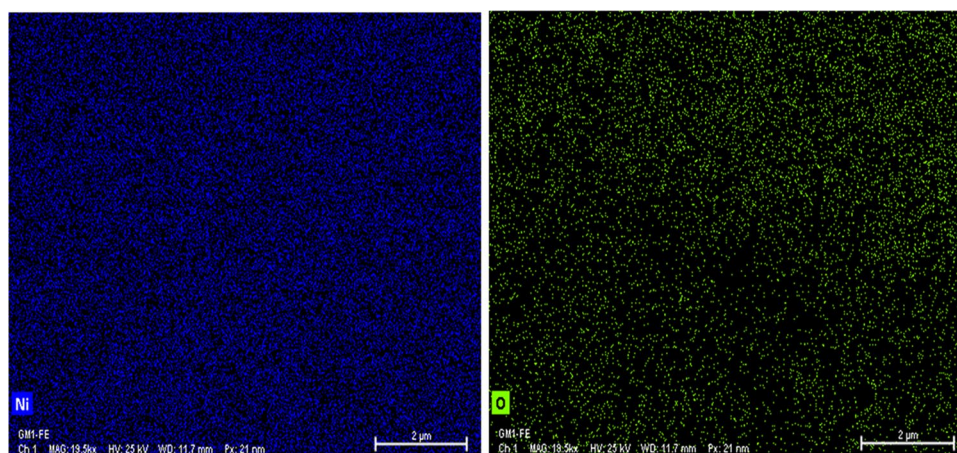
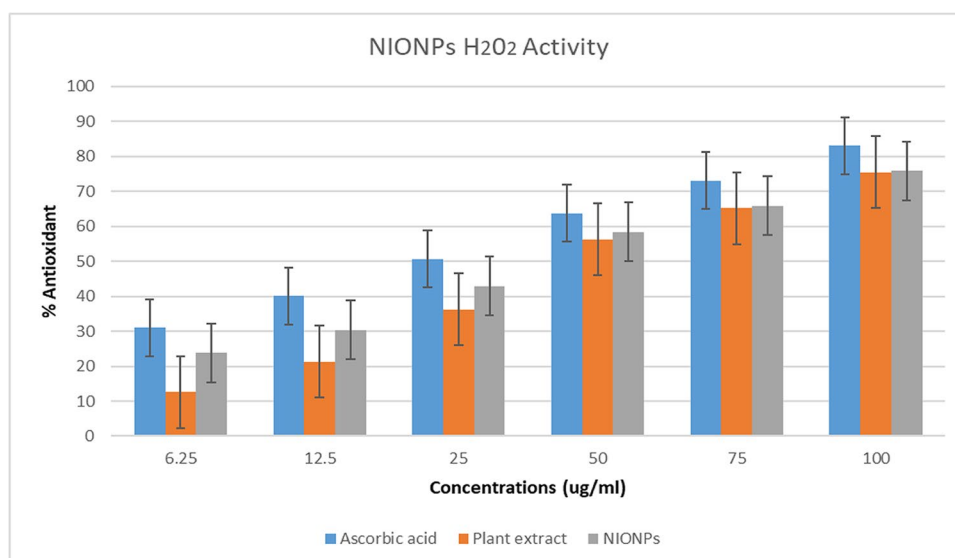


Fig. 7 X-ray diffraction patterns of the biosynthesized NiO-NPs

This pattern confirmed the crystallinity of green synthesized NiO nanostructures. A cubic structure phase of NiO (JSPDS card no. 65–2901) with $a=b=c=4.201 \text{ \AA}$ was indexed in all the diffraction patterns (Fig. 7). Furthermore, the XRD patterns of the NiO nanoparticles peaks and high intensity exhibited sharpened peaks, which indicated the high quality of NiO has been synthesized by the green method. In addition, the XRD patterns show no extra peak, indicating that all precursors and impurities have been destroyed and no impurity peaks have been left, as proven previously from SEM and EDX analysis. The full width at half maximum (FWHM) value of the XRD spectra can be used to study the crystal quality of the produced NiO crystal structure. Therefore, Debye–Scherrer equation can be used to calculate the average crystallite size from the XRD data

Fig. 8 H₂O₂ scavenging activity of NiO-NPs at different concentrations compared to ascorbic acid as standard and plant extract



$$D = \frac{0.95\lambda}{\beta_D \cos \theta}$$

where D is the average crystallite size; λ is the wavelength of the incident X-ray (0.154 nm), i.e., Bragg's diffraction angle; and β_D is the FWHM. The average crystallite dimension computed from the Debye–Scherrer equation is ~20 nm. Nevertheless, Debye–Scherrer's equation is suitable for estimating the size of spherical and semispherical particles besides nanocrystalline material [36]. The documented crystallography structure of the NiO-NPs is agreed with the results of a previous study [37]. The findings gained from EDX and XRD studies confirmed the purity of the green prepared NiO-NPs.

It is supposed that a higher annealing temperature can increase the intensity of diffraction peaks. It was suggested that the crystallite size of NiO-NPs parallelly increases with the increase of the annealing temperature [38].

3.2 Biological Applications

3.2.1 Hydrogen Peroxide (H₂O₂) Scavenging Activity

The present findings confirmed that NiO-NPs have IC₅₀ (the concentration that gave 50% of inhibition) as 45.7 hydrogen peroxide scavenging activity while the IC of standard vitamin is 31.6. The plant extract has IC as 53.1 hydrogen peroxide scavenging activity. This result proved that the NiO-NPs have good hydrogen peroxide scavenging activity compared with the standard ascorbic acid and *Z. spina-christi* plant extract, as depicted in Fig. 8. Saikia et al. [39] investigated the inorganic NiO-NP antioxidant activity using modified DPPH. Their

results showed significant antioxidant potency compared to iron oxides. This study described NiO-NPs as novel antioxidants that might result in a new approach to NiO activity in the biological system.

The plant extracts contain bioactive compounds like phenolic compounds and flavonoids, which are significant in the antioxidation process [40].

Oxygen radicals can be produced by mitochondria during the reduction of oxygen to generate energy, which induces DNA damage. These changes may cause diseases, e.g., heart problems, muscle failure, diabetes, and cancer [41].

Antioxidants acted against free radicals and were recognized as powerful therapies to destroy free radicals and regenerate damaged cells. More importantly, laboratory findings have shown that antioxidants can significantly prevent cancer [42].

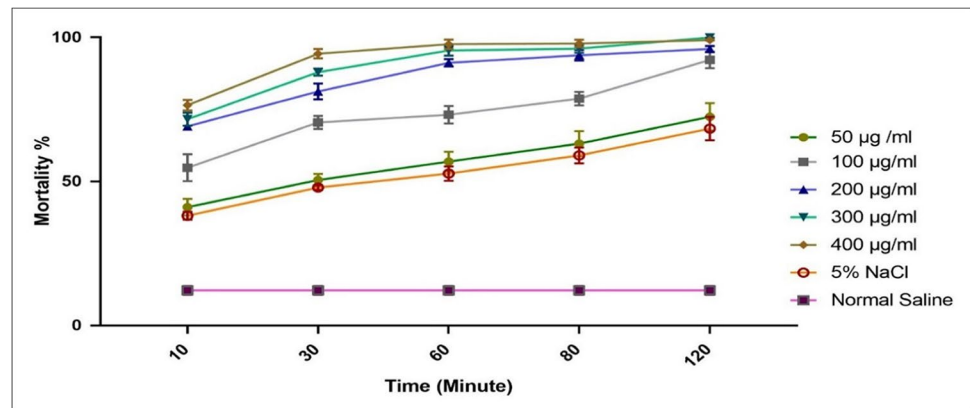
The green synthesized NiO-NPs possess antioxidant hydrogen peroxide scavenging activity.

H₂O₂ is produced in vivo by some oxidase enzymes. There is increasing evidence that directly or indirectly, its reduction produces hydroxyl radical (OH·). H₂O₂ leads to severe damage in the biological systems. When a scavenger material is incubated with H₂O₂, the decay of H₂O₂ can be spectrophotometrically measured at 230 nm [43].

Hydrogen peroxide can react with iron to form hydroxyl radicals (HO·); they recorded two iron chelators, deferoxamine, and hydroxy benzyl ethylenediamine, which accelerated apoptosis, suggesting that hydroxyl radicals act as an intracellular mediator of neutrophil apoptosis [44].

Recent studies recommended applying callus extract of *Ziziphus spina-christi*–prepared ZnO-NPs and SeO-NPs as beneficial natural antioxidants for health preservation against diverse oxidative stress related to degenerative diseases [45].

Fig. 9 Scolicidal activity of NiO-NPs against protoscoleces of *E. granulosus*



3.2.2 Protoscolicidal Effect of NiO-NPs

Surgery remains the superior therapeutic option for *Echinococcus granulosus* infection, but other methods also play an essential role in disease management [46]. Many scolical agents are used to inactivate the protoscoleces during surgery because leakage of hydatid fluid is a reason for recurrence and leads to secondary hydatidosis [47]. All scolical materials possess adverse impacts. It can induce hypernatremia, convulsions, intracranial bleeding, necrosis, and myelinolysis [48]. Treatment of cystic echinococcosis still depends on benzimidazole. This drug exhibited severe adverse impacts like leucopenia, alopecia, and hepatotoxicity, besides thrombocytopenia [49]. Therefore, due to the unavailability of effective treatment for cystic echinococcosis, there is a necessity for novel compounds [2].

The in vitro effects of NiO-NPs against *E. granulosus* protoscoleces were investigated utilizing light microscopy. The biosynthesized NiO-NPs has had potent protoscolical activity that increased proportionally with the incubation time. All concentrations of 400, 300, and 200 µg/mL of NiO nanoparticles exhibited more rapid effects with non-significant differences, which reached their noticeable effect after 1 h of exposure. Then the activity increased after 80 and 120 min to reach their peaks by killing all the treated parasites (100% mortality). It caused contraction and damage of all the protoscoleces with a clear attachment of the nanoparticles to the parasite. The effects were followed by loss of hooks, bleb formation, and irreversible tissue vacuolation, leading to death compared to the untreated control protoscoleces incubated in normal saline, as shown in Fig. 9 and Fig. 10. Similar nanoparticle-induced damage was observed on protoscoleces treated with biosynthesized Ag NPs and ZnO NPs by other researchers within in vitro and in vivo models [10, 50, 51].

The antiparasitic activity of the biosynthesized nanoparticles was reasoned by their high surface area, which results in additional mechanical, chemical, electrical, optical,

magnetic, electro-optical, and magneto-optical characteristics that differed from their original ones [52].

3.2.3 Blood Compatibility

The hemolytic value reflects the strength of the interaction between the nanoparticles and the erythrocytes, which characterizes the damage to the integrity of the erythrocyte membrane, leading to hemoglobin release [53]. A blood hemolysis experiment was performed to assess the effect of NiO-NPs on erythrocytes and their toxicological properties, which depends on the spectrophotometric measurement of hemoglobin after exposure of the erythrocytes to different concentrations of NiO-NPs. The hemolysis assay was tested and compared with negative (phosphate-buffered saline, pH 7.4) and positive (double-distilled water) controls. The hemolytic activity of NiO-NPs exhibited dose-dependent activity.

In regard to this, NiO-NPs were non-hemolytic at a lower concentration. At the 1100 µg/mL concentration, NiO-NPs caused 11.18% hemolysis. The lowest concentration of NiO-NPs just led to 1.85% hemolysis. Lysis of RBCs at different concentrations of NiO-NPs was significantly ($P \leq 0.05$) less than the positive control value, as shown in Fig. 11. The present finding proposed that the bio-fabricated NiO-NPs from *Z. spina-christi* leaves were biocompatible at all concentrations below 1100 µg/mL, as in Fig. 11.

Lingaraju et al. [7] documented that the green synthesized NiO-NPs from *Euphorbia heterophylla* (L.) did not express any hemolytic effects against the erythrocytes within the concentration (40, 60, 80, and 100 µg) and were considered biocompatible for biological applications. Luna-Vázquez-Gómez et al. [54] pointed out that according to the American Society for Testing and Materials, less than 5% of hemolysis is considered null, more than this limit, and up to 10% is supposed as low. In contrast, $HR > 10\%$ indicated incompatibility between blood and the persistent luminescence nanoparticles [55]. Moreover,

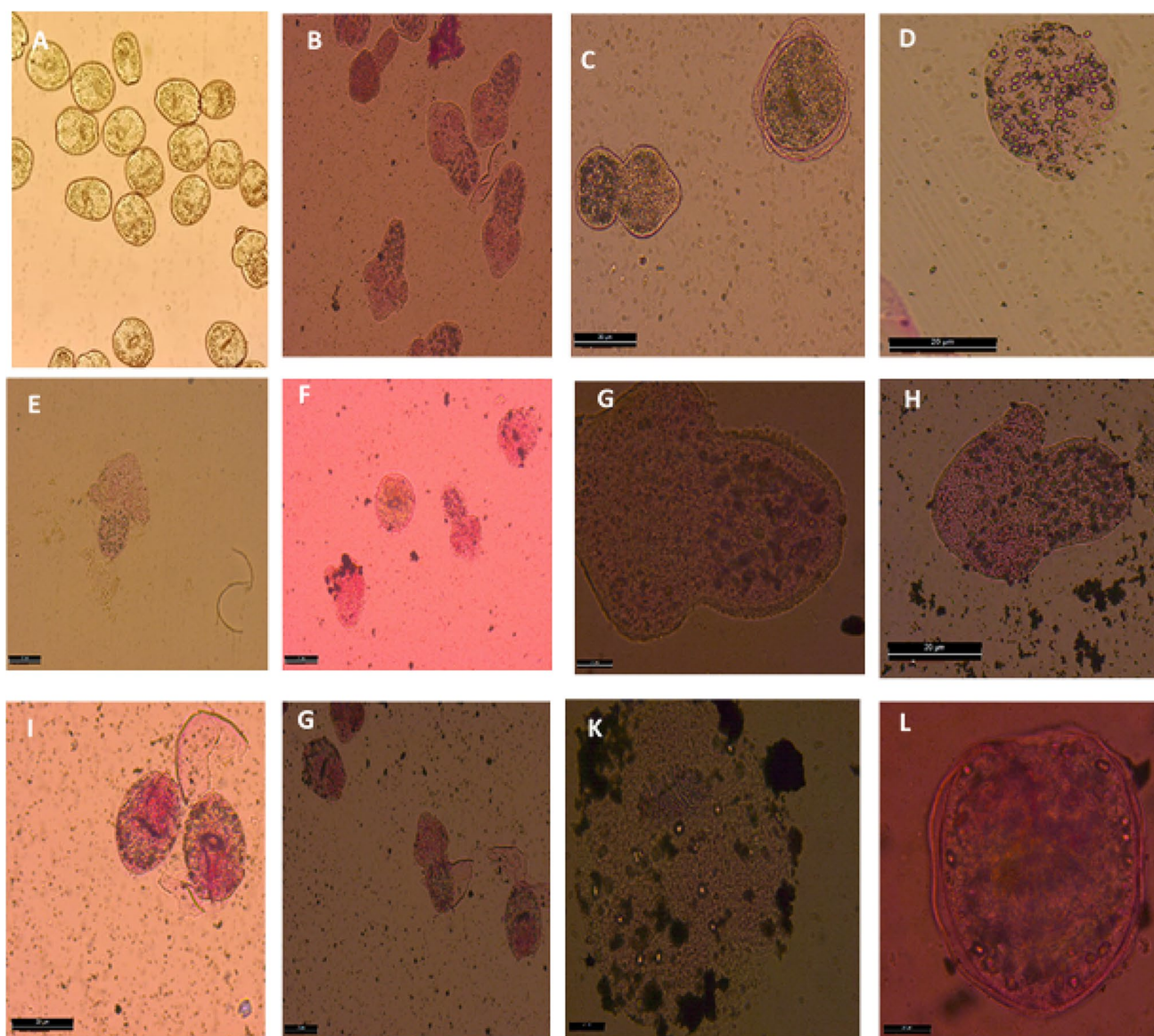


Fig. 10 Images by light microscopy. **A** Viable green protoscoleces after staining with 0.1% eosin. **B** Dead protoscoleces after 2 h of exposure to NiO-NPs. **C**, **D**, **E** Partial absorption of the dye, indicat-

ing their tegumental alteration. **F**, **I**, **J** Illustrate dead invaginated and evaginated protoscoleces. **G**, **H**, **K**, **L** High magnification of dead protoscoleces with NiO-NP attachment

hemolysis analysis was performed in another study and concluded that NiO-NPs possess good hemocompatibility [56].

3.2.4 Antimicrobial Activity

The antibacterial activity of the biosynthesized NiO-NPs was investigated against both *S. aureus* Gram-positive and *E. coli* Gram-negative bacteria by zone of inhibition technique. The NiO-NPs exhibited significant antibacterial effects against the tested bacteria with different susceptibility as a dose-dependent effect. The largest zone of inhibition for *E. coli* (10 mm) was observed compared to *S. aureus*

(8 mm) by the same concentration of NiO-NPs. Figure 12 and Table 1 illustrate these results.

During the last few decades, the overuse and misuse of broad-spectrum antibiotics have been proposed as responsible for the emergence of antibiotic-resistant bacteria, for instance, methicillin-resistant *Staphylococcus aureus* [57, 58]. Therefore, monitoring the antibiotic susceptibility of pathogens is crucial for treating antibiotic-resistant bacterial diseases in case of continuous antibiotic exposure. Proper combination medication is required to treat pathogens with different degrees of antibiotic resistance [59].

Fig. 11 Hemolytic activity of NiO-NPs compared to negative and positive controls as phosphate-buffered saline and double-distilled water, respectively

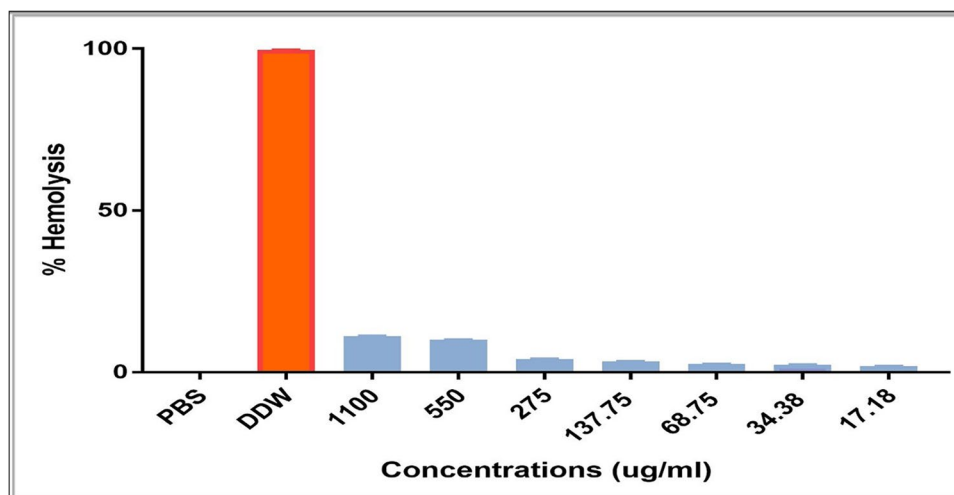


Fig. 12 Antibacterial activity of NiO-NPs against Gram-positive and Gram-negative bacteria



Table 1. Diameters of inhibition zones (mm) of different concentrations of NiO-NPs against bacteria compared to standard antibiotics

NiO-NPs µg/ ml Antibiotic	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
100 µg NiO-NPs	–Ve	–Ve
200 µg NiO-NPs	–Ve	–Ve
300 µg NiO-NPs	–Ve	6 mm ± 3
400 µg NiO-NPs	10 ± 2	8 mm ± 2
Amoxicillin 30 µg/disk	11 mm	–

The precise mechanisms of heavy metals' bactericidal action are still not fully understood. Previous publications described the possible pathways of the bactericidal action of NiO-NPs. Generally, it is mentioned that the metal oxides possess a positive charge, whereas the microorganisms have negative charges; this leads to the electromagnetic attraction between the

microorganisms and the metal oxides, resulting in oxidation and death of microorganisms [60]. The NiO-NP surfaces have a positive charge, whereas the bacterial cell wall possesses a negative charge, therefore, it generates an electrostatic interaction destroying the bacterial wall and cytoplasm [61]. Moreover, NiO-NPs produce reactive oxygen species that result in protein oxidation and DNA damage, finally killing the bacteria [62].

The antibacterial activity mechanisms of NiO-NPs were clarified by evaluating the amount of ROS generation at the NiO-NP interface. The effect of ROS production on the bacterial membrane was assessed by BacLight assay and morphological characterization of the bacterial membrane by FE-SEM. The proposed generation of oxidative stress at the NiO-NP interface leads to membrane damage and bacterial death [63]. Several previous studies also proved more similar antibacterial activities of NiO-NPs against Gram-positive and Gram-negative bacteria [3, 62, 64]. Vijaya Kumar et al. [61] reported the antibacterial activity of the green synthesized NiO-NPs using *Calotropis gigantea* leaves against *S. aureus* and *E. coli*. This activity is attributed to the reactive oxygen species, large surface area, and nanoparticle size. NiO-NPs yield (hydroxyl,

superoxide radical, singlet oxygen, besides alpha-oxygen) by the Fenton reaction, which results in lipid peroxidation, DNA destruction, and protein oxidation which can eradicate the bacteria. The same findings were recently revealed by Prabhu et al. [64] that the biosynthesized NiO-NPs treated with *Clitoria ternatea* flower extract showed potent antibacterial activity. They reasoned it by the adsorption of phytochemical materials of the *C. ternatea* flower extract on the surface of NiO-NPs, which worked together with NPs to inhibit and kill the bacteria.

Electrostatic interaction between bacteria and nanoparticles generates reactive oxygen species, which is suggested to be responsible for bacterial cell death [65]. The probable bactericidal mechanisms for NP reactions with bacteria include strong interaction of cations Ni^{2+} with the negatively charged bacterial cells that lead to collapse. The second reaction results in electronic excitation from valance to conduction band upon light irradiating NiO surface. Further electronic reaction with O_2 generates O_2^- radicals resulting in H_2O_2 production. The $\cdot\text{OH}$ production happened by the interaction of h^+ with water. This reaction resulted in the formation of reactive oxygen species $\cdot\text{O}_2^-$ and $\cdot\text{OH}$ species, which play an essential role in breaking down lipid or protein molecules present in the outer cell surface of bacteria [66].

Also, nickel ions (Ni^{2+}) produced from the NiO-NPs penetrate the cell wall, causing damage to the DNA, protein, and mitochondria and disturbing the electron transport, resulting in cell death. Diffusion and gathering of NiO-NPs on the cell membrane lead to membrane permeability alteration resulting in protein leakage (Baek and An, 67).

Other studies revealed that the NiO-NPs have more potent antibacterial action against Gram-positive bacteria than Gram-negative bacteria, particularly against multiple antibiotic-resistant *S. aureus* [63, 68].

Moreover, the particle size of nanomaterials affects the antibacterial activity; most studies have documented that their activity increases with minimizing NP size [69].

Nickel is a crucial nutrient for several microorganisms because it participates in various cellular processes. Numerous microbes can sense cellular nickel ion concentrations and uptake this nutrient by ATP-binding cassette-type transport systems or nickel-specific permeases. Also, this metal ion is incorporated explicitly into nickel-dependent enzymes [70].

Electrostatic forces, the creation of Ni^{2+} ions, and the release of reactive oxygen species were defined as possible ways of the bactericidal action of NiO-NPs. Hence, understanding the bactericidal mechanisms of NiO-NPs can help construct a predictive alternative to overcome bacterial resistance.

4 Conclusion

In the current study, green eco-friendly biosynthesis of NiO-NPs was performed using leaf extract of *Ziziphus spina-christi* as a reducing, capping, and stabilizing agent for the first time. Characterization of NiO-NPs was achieved using UV-vis, XRD, SEM, and EDX; the results revealed their crystallinity and purity. Antibacterial and antioxidant activities of NiO-NPs were evaluated, and the obtained results showed that NiO-NPs have potential antioxidant and antibacterial activity. The broad peak of the XRD pattern indicates the nanocrystalline behavior of the particles. The SEM images confirmed that NiO nanoparticles had a spherical shape on the nanoscale. The purity and elemental percentages of NiO-NPs were confirmed by EDX analysis. Our results conclude that biologically synthesized NiO-NPs exhibited multifunctional properties and could be used against cystic echinococcosis and other pathogens.

Data Availability Not applicable.

Code Availability Not applicable.

Declarations

Ethical Approval Not applicable.

Consent for Publication We, the author of this manuscript, give our consent for the publication of identifiable details of the above-titled manuscript to be published in this journal.

Conflict of Interest The authors declare no competing interests.

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