

Phytochemical-Driven Green Synthesis of NiO Nanoparticles Using *Ocimum basilicum* and *Urtica dioica*: Correlating Phytochemical Surface Characteristics With Antimicrobial Activity

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Abstract

Green synthesis provides a sustainable route for producing metal oxide nanoparticles whose physicochemical and biological characteristics may be influenced by plant-derived surface components. In this study, nickel oxide nanoparticles were synthesized using aqueous extracts of *Ocimum basilicum* leaves and flowers (OB-NiONPs) and *Urtica dioica* seeds (UDS-NiONPs), and their structural, optical, morphological and antimicrobial properties were comparatively investigated. The nanoparticles were characterized using UV–Vis spectroscopy, FTIR, XRD, SEM–EDX and TEM analyses, and their antimicrobial activities were evaluated against Gram-positive and Gram-negative bacteria and three yeast strains. OB-NiONPs and UDS-NiONPs exhibited absorption maxima at 282 and 276 nm, respectively, while their optical band-gap energies, estimated using the direct-allowed-transition representation of the Tauc relation, were 3.47 and 3.50 eV. XRD patterns supported cubic NiO as the major crystalline phase, with average coherent crystallite sizes of 12.33 nm for OB-NiONPs and 13.38 nm for UDS-NiONPs. TEM-derived mean particle sizes were 5.14 and 11.90 nm, respectively; however, the particle and crystallite dimensions were not considered directly equivalent because of their different measurement principles. FTIR analysis indicated the association of plant-derived functional groups with the nanoparticles, whereas SEM and TEM revealed differences in morphology, aggregation and particle-size distribution. EDX detected Ni and O together with weak Cl signals, indicating that minor residual chloride-containing species could not be excluded. Antimicrobial activity was concentration- and strain-dependent. UDS-NiONPs produced inhibition zones of 23.33 mm against *Staphylococcus aureus* and 24.00 mm against *Enterobacter aerogenes* at 1 mg, whereas OB-NiONPs showed comparatively greater activity against *Pseudomonas aeruginosa* and similar antifungal activity against *Candida albicans* and *Yarrowia lipolytica*. These findings suggest that the observed antimicrobial differences cannot be explained by particle size alone and may also involve variations in phytochemical surface functionalization, morphology and nanoparticle–microorganism interactions. Direct measurements of surface composition, ROS generation and Ni²⁺ release are required to clarify the underlying mechanisms.

1. Introduction

In our rapidly changing world, resistance to diseases is increasing day by day [1] [2]. Many pathogenic bacteria have become more resistant to antibiotics [3]. This situation has led to antibiotic resistance becoming a global health crisis. There is a constant need to develop new and effective antimicrobial agents. When the risk of bacterial spread is also taken into account, the significance of this issue is abundantly clear [4].

Developments in nanotechnology over the past 20 years have facilitated the preparation of materials at the nanoscale. Nanotechnology produces and develops materials for new treatment methods against resistant diseases [5]. The most distinctive feature of this technology lies in the fact that the material, which has a controllable shape, possesses very small and unique physical, chemical and biological properties [6]. These nanotechnology-based materials, whose applicability has been proven in many fields, continue to attract the interest of scientists. Among these nanomaterials, metal/metal oxide

nanoparticles, with their small volumes and large surface areas, can be used effectively in healthcare applications [7, 8].

In recent years, the synthesis of nanomaterials has increasingly favoured the green chemistry methodology—an environmentally friendly approach that requires less energy and avoids complex synthesis processes [9–12]. This is because the high toxicity of the chemicals used in traditional synthesis methods, the significant energy wastage in physical methods, the difficulties in preparing materials of the desired size, and the generation of hazardous waste in quantities that could harm the environment are all disadvantages that limit the use of physical and chemical methods [13]. The green chemistry approach, which aims to transform industrial and chemical applications, holds significant potential for addressing these disadvantages. In this method, parts of plants such as roots, leaves and flowers, as well as biological materials such as fungi and bacteria, are used as reducing and stabilising agents [14–16]. Thanks to their easy accessibility and safe handling properties, phytochemically rich plants play a notable role as reducing agents in synthesis processes. Among these nanomaterials, NiO nanoparticles—which are extremely small in size, possess catalytic properties due to their semiconducting structure, and are known for their chemical stability—are attracting significant interest. As p-type semiconductor materials, NiO NPs possess a large surface area and a broad bandgap energy ranging from 3.00 to 4.00 eV, which makes them a preferred choice for applications [17–19]. This means that the optical, electrical and catalytic properties of NiO NPs can be finely tuned according to particle size. Thus, nickel oxide nanoparticles (NiO NPs) have been shown to possess promising potential not only for various industrial applications such as battery electrodes, gas sensors, electronics, catalysts and photocatalysis, but also for antimicrobial applications [20–31].

In addition to traditional synthesis methods for synthesising NiO-NPs, environmentally friendly synthesis methods with low impact on human health and the environment are attracting interest [32]. In this regard, the green synthesis approach is being adopted as a non-toxic, cost-effective and environmentally sensitive method for the production of nanomaterials. The green synthesis method involves the synthesis of metals or metal oxides formed as a result of the reaction between metal salts and the extract of any plant composed of bio-components or a microorganism [9, 33]. In particular, plant extracts reduce metal salts to produce effective metal nanoparticles with significant antioxidant, antimicrobial, anti-inflammatory, anti-cancer and cytotoxic properties [34, 35]. The abundance of bioactive compounds such as polyphenols, flavonoids and terpenoids in plants, combined with the ease of accessing plant materials, ensures that plants are widely preferred for this synthesis method [36, 37]

NiO nanoparticles synthesised using the green method exhibit potent antimicrobial properties, having been shown to be effective against Gram-positive and Gram-negative bacteria and even certain fungal strains [38–40]. This may be attributed to this antimicrobial activity is associated with mechanisms such as the nanoparticles disrupting cell membrane integrity, triggering the formation of reactive oxygen species (ROS) and thereby causing oxidative stress, and/or the released nickel ions interacting with intracellular components[41].

U. dioica is a perennial flowering plant that grows wild in nitrogen-rich, moist riverbeds, with seeds that begin to ripen in August [42]. *U. dioica* (stinging nettle) seeds can exhibit potent biological activity due to their antioxidant and anti-inflammatory effects [43]. *Ocimum basilicum* L. is of interest in the medical, pharmaceutical and cosmetic industries due to its valuable fragrance and medicinal properties [44]. A common feature of *Ocimum basilicum* L. and *U. dioica* is that they contain numerous phytochemicals, such as flavonoids, phenols, alkaloids and terpenoids, which can convert and stabilise metal salts into other forms [45, 46].

In this study, NiO NPs were successfully synthesised using extracts from the leaves and flowers of *Ocimum basilicum* L. and the seeds of *U. dioica*, prepared separately and inspired by green chemistry methodology. The morphological, structural and chemical properties of the synthesised NiO NPs were elucidated through a series of detailed characterisation procedures. Most importantly, the antimicrobial potential of the particles was tested against various pathogenic microorganism strains, exploring potential solutions for combating infections resistant to conventional antibiotics. In addition to comparing the physicochemical properties of the synthesized nanoparticles, this study investigates how different phytochemical environments provided by *O. basilicum* and *U. dioica* extracts influence the surface chemistry and consequently govern the antimicrobial performance of NiO nanoparticles.

1. Materials and instrumentation methods

1.1. Chemical Substances and Application Reagents

Nickel(II) chloride hexahydrate ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$) was purchased from Sigma-Aldrich. The leaves and flowers of the *Ocimum basilicum* L. plant were collected together in June in the province of Bitlis, Turkey (38.472565, 42.309400). Stinging nettle (*U. dioica*) seeds were also collected from a riverbank in the city of Bitlis, Turkey (38.482687, 42.159946).

The leaves, flowers and seeds were washed separately first with tap water and then with deionised water, before being dried in a shaded environment for 15 days. The dried plant parts were ground into a powder and stored in a dry environment for extraction. For the XRD analysis of NiONPs obtained via green synthesis, a Rigaku instrument was used; for FTIR analysis, a Shimadzu FTIR spectrometer; for the surface morphology of the nanoparticles, a HITACHI HT 7700 transmission electron microscope; and to confirm the formation of the nanoparticles, the maximum absorbance value was measured using a Photolab 7600 UV -Vis spectrophotometer. The extraction and synthesis work was carried out under standard ambient conditions.

1.2. Preparation of *U. dioica* seeds and *O. basilicum* L. extract

After the stinging nettle (*U. dioica*) seeds and the leaves and flowers of *O. basilicum* L. were collected, they were thoroughly washed with deionised water to remove any potential contamination. The *U. dioica* seeds and the leaves and flowers of *O. basilicum* L. were first broken down into smaller pieces using a

grinder. Subsequently, 25 g samples were placed into 500 mL beakers. 300 mL of deionised water was added, and the mixture was heated and stirred at 80°C for 1 hour at 150 rpm using a magnetic stirrer. After allowing the mixtures to cool to room temperature, they were filtered twice. The extracts obtained as a result of the extraction were stored at 4°C in a sealed container, with the sides covered in aluminium foil to protect them from light [47]. Figure 1(a) illustrates the steps involved in the extraction process.

1.3. Green Synthesis of NiO Nanoparticles Coated with Organic Molecules

A 0.1 M solution of nickel chloride (II) hexahydrate ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$) was prepared for use as the precursor salt in the synthesis of NiONPs coated with organic molecules. 80 mL of the $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ solution was placed in a beaker, and 20 mL of *O. basilicum* L. extract was added drop by drop. The same procedure was carried out for *U. dioica* seed extracts. The mixtures were continuously stirred at 150 rpm on a magnetic stirrer at 40°C for 50 minutes, and the resulting colour change was observed.

During the reaction, the mixture gradually developed a dark coloration, indicating the formation of a solid Ni-containing product. Because colour change alone cannot confirm the formation of NiO, the crystalline phase of the recovered material was subsequently evaluated by XRD analysis. The recovered solid was washed three times with deionized water and collected by filtration using Whatman No. 1 filter paper after each washing step. The washed product was subsequently dried at 80°C for 12 h and gently ground to obtain a homogeneous powder. The nickel oxide nanoparticles prepared with *U. dioica* seed extracts were designated as UDS-NiONPs, whilst those prepared with *O. basilicum* L. extract were designated as OB-NiONPs. The samples were stored for characterisation and subsequent applications. Figure 1(b) illustrates the steps involved in preparing the nanoparticles using the green synthesis approach.

Under the applied low-temperature conditions, phytochemicals present in the plant extracts may have facilitated Ni^{2+} complexation, hydrolysis, nucleation and stabilization of the resulting nanoscale material. A possible pathway involves the initial formation of nickel hydroxide or hydroxy-complex intermediates, followed by partial dehydration to NiO during heating and drying. However, these intermediate species were not directly monitored in the present study; therefore, this pathway should be regarded as a proposed formation mechanism. The presence of the cubic NiO phase in the final products was supported primarily by XRD analysis.

1.4. Characterisation of Phytocapsule-coated UDS-NiONPs and OB-NiONPs

Absorption spectrum analysis, a preliminary characterisation method used to confirm the formation of NiONPs, was carried out using a UV-Vis spectrophotometer (Photolab 7600) covering the wavelength range of 200–800 nm. The Tauc method was applied to determine the optical bandgap of the NiONPs. The bandgap energy was calculated by extrapolating the linear region in the graph plotted according to the following Eq. (1).

$$(\alpha h\nu)^n = B(h\nu - E_g) \quad (1)$$

Here, h is the Planck constant, α is the absorption coefficient, ν is the photon frequency, $h\nu$ is the energy of the incident photon, and E_g is the energy of the optical bandgap. Both A and n are constant values determined by the probability and modes of the transition. When $n = 0.5$, this indicates a direct transition, whereas when $n = 2$, it indicates an indirect transition.

An X-ray diffractometer was used to determine the crystal structure and average size of the nanoparticles obtained after synthesis, as well as to characterise the peak structure. XRD measurements were carried out at 2θ angles ranging from 10° to 80° . The Scherrer equation [48] was used to calculate the average crystallite size (2)

$$D = \frac{0.94 \lambda}{\beta \cos \theta} \quad (2)$$

In the above equation, D corresponds to the crystal particle size, k to the shape factor (generally taken as 0.9), λ to the wavelength of Cu-K α X-rays (taken as $\lambda = 0.154060$ nm), FWHM to the full width at half maximum, and θ to the Bragg diffraction angle.

SEM (Scanning Electron Microscopy) and the accompanying EDX (Energy-Dispersive X-ray) spectroscopy, which form the basis for the characterisation of NiO NPs from both structural and chemical perspectives, were used to analyse the elemental composition and structure of the nanoparticles.

FTIR analysis was performed to identify the functional groups of the plant extract immobilised on the surface of NiO NPs synthesised under the influence of organic compounds present in extracts of *Ocimum basilicum* L. and stinging nettle seeds. FTIR spectrum measurements were carried out using a Shimadzu instrument in the wavelength range of 4000 to 400 cm^{-1} . To examine the size, shape, crystal structure and morphology of the NiO NPs at the nanometre scale, the nanoparticles were analysed using TEM (Transmission Electron Microscopy), and the structural properties of the particles were determined.

1.5. Antimicrobial Activity

A total of ten microorganisms were employed in this investigation, including *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Bacillus megaterium* DSM 32, *Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 9027, *Enterobacter aerogenes* ATCC 13048, *Klebsiella pneumoniae* ATCC 13883, *Yarrowia lipolytica*, *Candida albicans* ATCC 10231, and *Saccharomyces cerevisiae*. Bacterial strains were cultivated and activated in Nutrient Broth, whereas fungal strains were grown in Potato Dextrose Broth. For antimicrobial assays, Nutrient Agar and Potato Dextrose Agar were utilized.

To evaluate the antimicrobial efficacy of the synthesized nanoparticles, standard antibiotic discs Erythromycin (E-15), Ampicillin/sulbactam (SAM-20), Rifampicin (RD-5), Amikacin (AK-30), and Fluconazole (FCA-25) were included as reference controls. The antimicrobial activity of the stock solution (10 mg/mL), prepared in dimethyl sulfoxide (DMSO), was assessed using the agar well diffusion

technique [49]. Sterile wells of 10 mm diameter were created in the inoculated agar, and 100 μ L aliquots containing 1, 0.5, and 0.25 mg of the test substance were dispensed into each well.

Bacterial cultures were incubated in Nutrient Broth at 37°C for 18 hours to achieve activation, while yeast cultures were incubated in Potato Dextrose Broth at 25°C for 24 hours. Subsequently, sterilized Nutrient Agar and Potato Dextrose Agar, cooled to 45–50°C, were inoculated with the respective 24-hour microbial cultures. After thorough mixing, approximately 15 mL of the inoculated medium was aseptically poured into sterile Petri dishes (9 cm diameter) to ensure uniform distribution. Following solidification, wells were aseptically formed in the agar and filled with the prepared test solutions. The plates were then held at 4°C for 1 hour to facilitate diffusion of the substances. Thereafter, bacterial plates were incubated at 37°C for 18–24 hours, while yeast plates were incubated at 25°C for 24–37 hours [50].

2. Results and Discussion

In this study, NiO nanoparticles were synthesised from $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ salt using an extract derived from stinging nettle seeds, employing an environmentally friendly approach. The spectroscopic properties of the synthesised NiO nanoparticles were determined using methods such as SEM, TEM, UV-Vis, FT-IR and XRD. Subsequently, the efficacy of both nanoparticles against Gram-positive and Gram-negative bacterial strains and certain fungi was investigated and compared with that of some antibiotics.

2.1. UV–Visible spectral analysis

The optical properties of the UDS-NiONPs and OB-NiONPs synthesised from *U. dioica* seeds and *basilicum* L. extracts were investigated using a spectrophotometer, with a wavelength range of 200–800 nm selected. The maximum absorption values of the NiONPs were determined, their optical properties were examined, and the bandgap energies of the nanoparticles were calculated using the Tauc plot generated accordingly.

The absorption peak (282 nm) observed in the UV -Vis spectrum of the OB-NiONPs, observed between 270–322 nm (282 nm), and the maximum absorption peak observed in the spectrum of the UDS-NiONPs shown in Fig. 2(b) within the 270–300 nm wavelength range (276 nm) reflect the characteristic properties of nickel oxide nanoparticles. Similar values to these absorption peaks have also been recorded in previous studies [14, 51]. In one study, absorption peaks ranging from 280 to 322 nm were observed in NiO nanoparticles synthesised at different temperatures, affecting the efficacy of Arabic gum [52].

During the formation of nanoparticles, an electronic transition occurred through the transfer of electrons from the phytochemicals absorbed to the unpaired electrons in the empty d orbitals of nickel. Consequently, these peaks represent the characteristic optical transition for NiO nanoparticles. In other words, the observed absorbance value reflects the transition of electrons from the valence band to the conduction band. It can be concluded that the peaks observed in both nanoparticles result from the

accumulation of reducing and stabilising compounds—such as phenolic compounds and flavonoids present in the structure of stinging nettle seeds and *Ocimum basilicum* L. extracts—on the nanoparticle surface, which stabilise the nanoparticles, influence their optical properties, and contribute to the formation of their size, crystal structure and surface morphology [27, 53].

The bandgap values of OB-NiONPs and UDS-NiONPs were determined using the extrapolation curve plotted on the Tauc plot. The band-gap energies were estimated as 3.47 and 3.50 eV using the direct-allowed-transition representation of the Tauc relation. These values confirm that the NiO nanoparticles, synthesised using a methodology inspired by green chemistry, possess a semiconducting structure. The fact that the E_g values are smaller than those of bulk NiO NPs (4 eV) reflects the quantum confinement effect associated with the expansion of the surface area as the nanoparticle size decreases [54, 55]. These values in the band gap may originate from defects on the nanoparticle surface. In particular, the wrapping of phytomolecules around the nanoparticle may have caused this result. However, this situation may positively influence adhesion to the cell wall and penetration into the cell by breaching the Wall [56].

2.2. FT-IR study

Figure 3(a) and (b) show the Fourier Transform Infrared (FT-IR) spectra of the OB-NiONPs and UDS-NiONPs synthesised using *basilicum* L. and *U. dioica* seed extracts. Analysis conducted using a Thermo Scientific FT-IR spectrophotometer provided detailed information on the chemical bonds and functional groups associated with the structure of the nanomaterials. It confirms whether the nanoparticle has formed and verifies the extent of the plant extract's presence on the surface.

The broad and intense peaks at 3523.95 cm^{-1} and 3502.73 cm^{-1} , and the broad and intense peak at 3446.79 cm^{-1} in the UDS-NiONPs are attributed to the -OH groups of polyphenols, flavonoids and alcohols, which are thought to be associated with the extract bound to the nanomaterial. It is thought that the water molecules observed here are adsorbed onto the surface of the nanoparticle [57, 58]. OB-NiONPs at 2974.23 cm^{-1} and 2918.30 cm^{-1} and those of UDS-NiONPs at 2924.09 cm^{-1} and 2852.22 cm^{-1} indicate the presence of aliphatic C-H bonds in fatty acids, terpenoids or other organic molecules found in plant extracts [59, 60]. The low-frequency absorption peaks observed at 2312.65 cm^{-1} and 2314.48 cm^{-1} in the spectra are attributed to the $\text{C}=\text{O}$ bonds in CO_2 molecules originating from the ambient atmosphere during the measurement [61, 62]. The peaks observed in the OB-NiONP spectra at 1749.44 cm^{-1} , 1689.64 cm^{-1} and 1635.64 cm^{-1} , and those detected in the UDS-NiONP spectra in the 1680.00 cm^{-1} – 1629.84 cm^{-1} are $\text{C}=\text{O}$ (carbonyl) stretching vibrations found in carboxylic acids, esters or ketones, which have the ability to stabilise and perform a reducing function. Here, carboxylic acids or polyphenols are involved in both the reduction and the binding to the metal surface, thereby enveloping the nanoparticle [63–66]. This is attributed to the N-H bending and C-N stretching vibrations of Amide II, which constitutes the structure of the common peak observed at 1535 cm^{-1} [67]. The peaks observed at 1415.75 cm^{-1} and 1338.60 cm^{-1} in the OB-NiONP spectrum and at 1373.32 cm^{-1} and 1338.60 cm^{-1} in the UDS-NiONP spectrum correspond to C-O stretching (alcohols, ethers) and C-H bending vibrations

[68, 69]. The sharp peak at 1049 cm^{-1} is attributed to the C–O stretching vibrations of the polysaccharides or alcohols responsible for the formation and stabilisation of the nanoparticle [70, 71]. Peaks detected below 800 cm^{-1} (peaks in UDS-NiONPs: $790, 677, 570, 518, 479\text{ cm}^{-1}$; peaks in OB-NiONPs: $690, 646$ and 524 cm^{-1}) are the most significant evidence confirming the formation of NiO nanoparticles [72, 73].

The graphs demonstrate that UDS-NiONPs and OB-NiONPs have been successfully synthesised, and that bioactive molecules constituting the structure of stinging nettle seeds have been detected on the surface of the nanoparticles.

2.3. XRD Study

An X-ray diffraction instrument is the most important analytical tool used to determine the crystal structure of solid materials. As X-rays pass through a material, they produce diffraction patterns. These patterns are unique to each material, reflecting its spatial arrangement.

The XRD pattern of the OB-NiONPs synthesised using *basilicum* L. plant extracts is shown in Fig. 4(a). As can be seen in the figure, sharp and distinct peaks characterise the crystal structure of the OB-NiONPs. In the graph, at 2θ angles of $37.25^\circ, 43.27^\circ, 62.86^\circ, 75.40^\circ$ and 79.39° , the (111), (200), (220), (311) and (222) light-diffracting hkl planes. The crystal structure, with reference code 01-073-1523, is in exact agreement with the data from the International Centre for Diffraction Data (ICDD). The synthesised NiO nanoparticles belong to the cubic crystal system with lattice constants: $a = b = c = 4.1800\text{ \AA}$, and the space group: Fm3m. The average crystal size, calculated using the Scherrer Eq. (2), is 12.33 nm . The average crystal sizes of NiONPs previously prepared using the green synthesis method were almost identical to this value [74].

The XRD pattern prepared using nettle seed extract is shown in Fig. 4(b). The sharp peaks visible in Fig. 4(b) indicate that the material has a high degree of crystallinity. In the graph, the peaks corresponding to the two-theta angles of $37.26^\circ, 43.27^\circ, 62.86^\circ, 75.36^\circ$ and 79.32° have been assigned to the (111), (200), (220), (311) and (222) planes, respectively. These hkl planes are consistent with the characteristic diffraction planes of NiO and are seen to match exactly with the ICDD reference code 01-071-1179. The synthesised NiO nanoparticles belong to the Fm3m space group with lattice constants $a = b = c = 4.1780\text{ \AA}$ in the cubic crystal system. The average crystallite size was calculated to be 13.38 nm using the Scherrer equation. It is known that previously synthesised NiO nanoparticles also possess crystals of this size [75].

Peaks are also observed that indicate the presence of planes not detectable in the graphs (marked with an *). Studies have reported that these peaks are caused by bioactive compounds found in stinging nettle seeds and *O. basilicum* L. extracts, and that they contribute significantly to the crystal structure[58].

2.4. Study of Scanning Electron Microscope with EDX (SEM + EDX)

The morphological structure of the OB-NiONPs, as observed by scanning electron microscopy, and their associated elemental composition (EDX data) are shown in Fig. 5. The images reveal that functional groups from the *Ocimum basilicum* L. plant extract occupy a significant proportion of the surface structure. These groups, which play a role in the formation of the structure, have led to the formation of agglomerated structures and irregular shapes on the material's surface. During the synthesis of the nanoparticles, the removal of water molecules was achieved through a drying process. Consequently, the functional groups in the plant extract, which act as reducing and stabilising agents, were not removed. Currently, the effects of NiO nanoparticles prepared using the green synthesis method are being investigated in various fields, including medical applications, sensors and photocatalysts, following calcination at temperatures of 400°C and above, after the activity of the functional groups in the structure has been virtually eliminated [76, 77]. Our aim here is to demonstrate how significant the effect of functional groups on the surface of NiO nanoparticles is in terms of their applications.

An examination of the elemental analysis reveals the co-occurrence of Ni and O in the EDX, whilst the presence of a granular morphology characteristic of metal oxides in the SEM indicates that the synthesis of NiO nanoparticles was successful [78]. The weak Cl signal detected by EDX may originate from residual chloride-containing precursor species that were not completely removed during washing. Therefore, although XRD supports the presence of crystalline NiO as the major phase, the EDX result suggests that minor residual chloride-containing species cannot be excluded.

Scanning electron microscope images were taken and EDX analysis was performed to determine the morphological structure and elemental composition of NiO nanoparticles synthesised using nettle seed extract. The surface morphology and particle distribution of UDS-NiONPs are shown in Fig. 6, with microscopic images taken at both low (1.0×) and high (10.0×) magnifications. Based on the SEM images, it is estimated that the particles generally concentrate and aggregate on the surface. Furthermore, the particles observed in the surface topography shed light on the agglomerated structures formed following the green synthesis. UDS-NiONPs possess a more porous structure compared to OB-NiONPs. The high Ni and O ratios in the EDX data indicate that the synthesis has taken place, whilst the amount of C in the environment suggests that phytochemicals have stabilised the nanoparticles by enveloping them. In this context, UDS-NiONPs, with their increased surface activity and enhanced biocompatibility, demonstrate potential for advantageous use not only in biomedical applications such as antibacterial and anticancer treatments but also in fields such as photocatalysis, sensors and energy storage.

Weak Cl signals were detected in the EDX spectra of both nanoparticle systems. These signals may originate from residual chloride-containing precursor species that were not completely removed during washing. Although XRD supports crystalline NiO as the major phase in both samples, the presence of minor residual chloride-containing species cannot be excluded. Further surface-sensitive analyses would be necessary to determine their chemical state and relative abundance.

2.5. Study of Transmission Electron Microscope (TEM)

The transmission electron microscope (TEM) is one of the microscopy techniques that provides the highest resolution for determining the size, morphology and distribution of nanomaterials.

Figure 7 shows TEM images of OB-NiONPs and a particle-size distribution histogram. The particles exhibit a size distribution ranging from 2 to 11 nm. The average particle size corresponds to 5.144 nm. It has been reported that some previously synthesised NiONPs also exhibit sizes close to these values [79, 80]. It should also be noted that the particles appear to be spherical or semi-spherical in shape, and that agglomeration is minimal. These results demonstrate that OB-NiONPs possess an exceptionally high surface area and reactivity, suggesting their potential for use in antimicrobial applications.

The mean particle size of the OB-NiONPs determined by TEM was 5.144 nm, the average coherent crystallite size estimated using the Scherrer equation was 12.33 nm. As the estimated crystallite size exceeded the particle size obtained from TEM, these values should not be interpreted as directly equivalent. This discrepancy may stem from the limited number of particles analysed by TEM, the narrow field of view, uncertainty in defining particle boundaries, and assumptions related to peak broadening analysis. Consequently, the TEM histogram and Scherrer calculations were re-evaluated independently, and the number of particles included in the TEM analysis was specified in the histogram graph.

Figure 8 shows the TEM image of UDS-NiONPs and the histogram of the nanoparticles. In the TEM image, the dark spots are NiO nanoparticles. Although these particles generally have a spherical appearance, they also exhibit a homogeneous distribution. The reason for this homogeneous distribution is that the bioactive compounds in the stinging nettle seed extract have coated the surface of the NiO NPs, facilitating their dispersion and stabilisation [81].

The histogram of the nanoparticles shows a Gaussian distribution with an average size of 11.90 nm and a standard deviation of 7.075 nm. Helan and colleagues calculated the average size of the NiO NPs they synthesised using neem leaf extract to be approximately 10 nm [82].

The fact that the XRD and TEM results for UDS-NiONPs are very close to one another (13.38 nm vs 11.90 nm) indicates that the synthesis carried out using stinging nettle seed extract resulted in high crystallinity and the production of particles of similar size. EDX results show that the NiO NPs synthesised using nettle seeds are richer in nickel content compared to those synthesised using basil extract. This is consistent with the peak intensities observed in the XRD analysis. The nettle seeds have effectively acted as a stabilising agent, producing a nickel-rich product.

2.6. Antimicrobial activity

The antimicrobial activities of OB-NiONPs and UDS-NiONPs were investigated at 3 different concentrations against 3 Gr+, 4 Gr-, and 3 fungi. The results are given in the Table 1. As can be seen, when the effects on bacteria were examined, the UDS-NiONPs exhibited the highest antibacterial activity against *E. aerogenes* and *S. aureus*. The UDS-NiONPs showed no antimicrobial activity against *B. megaterium* and *S. cerevisiae*. Similarly, it was observed that the OB-NiONPs also showed no

antimicrobial activity against *B. subtilis*, *B. megaterium*, *S. aureus*, *E. aerogenes*, and *S. cerevisiae*. In contrast to the activity of the OB-NiONPs, the UDS-NiONPs exhibited the best activity against *S. aureus* and *E. aerogenes*. When their antifungal effects were examined, the OB-NiONPs and UDS-NiONPs were found to have similar levels of antifungal activity against *C. albicans* and *Y. lipolytica*. Furthermore, no antifungal activity was observed against *S. cerevisiae*.

It was determined that the antimicrobial and antifungal activities of the substances partially increased with increasing concentration. Compared to the antibiotics in the Table 2, the synthesized UDS-NiONPs, in particular, was found to have equal or higher activity levels against some bacteria and fungi. Against *S. aureus* and *E. aerogenes*, the UDS-NiONPs exhibited better antibacterial activity than all antibiotics except erythromycin.

Table 1
Antimicrobial activities of substances on microorganisms

Microorganisms		OB-NiO			UDS-NiO		
		1 mg	0.5 mg	0.25 mg	1 mg	0.5 mg	0.25 mg
Gr+	S. aureus	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	23.33 ± 0.58	20.00 ± 1.00	18.00 ± 0.00
	B. subtilis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	16.00 ± 0.00	14.33 ± 0.58	0.00 ± 0.00
	B. megaterium	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Gr-	E. coli	16.33*±0.58	13.00 ± 0.00	0.00 ± 0.00	18.00 ± 1.00	16.33 ± 0.58	14.00 ± 0.00
	K. pneumoniae	15.00 ± 0.00	13.00 ± 0.00	0.00 ± 0.00	20.33 ± 0.58	17.00 ± 1.00	15.00 ± 0.00
	P. aeruginosa	19.00 ± 1.00	17.00 ± 0.00	15.00 ± 0.00	15.00 ± 0.00	13.00 ± 0.00	0.00 ± 0.00
	E. aerogenes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	24.00 ± 1.00	22.33 ± 0.58	17.00 ± 0.00
Fungus	C. albicans	20.00 ± 1.00	16.33 ± 0.58	14.00 ± 0.00	18.00 ± 0.00	16.33 ± 0.58	14.00 ± 0.00
	Y. lipolytica	18.00 ± 1.00	15.00 ± 0.00	13.00 ± 0.00	16.00 ± 0.00	15.00 ± 1.00	13.00 ± 0.00
	S. cerevisiae	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
* Inhibition zone (mm)							

Table 2
Antimicrobial activities of antibiotics on microorganisms

Microorganisms		Antibiotics				
		E-15	SAM-20	AK-30	RD-5	FCA-25
Gr+	S. aureus	21.00*±1.00	10.00 ± 0.00	9.33 ± 0.58	17.67 ± 0.58	0.00 ± 0.00
	B. subtilis	20.33 ± 0.58	14.00 ± 0.00	10.67 ± 0.58	21.00 ± 0.00	0.00 ± 0.00
	B. megaterium	25.00 ± 0.00	0.00 ± 0.00	10.00 ± 1.00	16.00 ± 0.00	0.00 ± 0.00
Gr-	E. coli	19.00 ± 0.00	12.67 ± 0.58	13.00 ± 1.00	18.00 ± 0.00	0.00 ± 0.00
	K. pneumoniae	19.00 ± 1.00	15.67 ± 0.58	10.67 ± 0.58	19.00 ± 1.00	0.00 ± 0.00
	P. aeruginosa	19.33 ± 0.58	0.00 ± 0.00	14.00 ± 0.00	8.00 ± 0.00	0.00 ± 0.00
	E. aerogenes	27.00 ± 1.00	10.00 ± 1.00	9.00 ± 0.00	16.67 ± 0.58	0.00 ± 0.00
Fungus	C. albicans	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	23.00 ± 1.00
	Y. lipolytica	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	22.00 ± 1.00
	S. cerevisiae	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
* Inhibition zone (mm)						

The TEM results indicate that, although UDS-NiONPs have a larger volume than OB-NiONPs, their effect against *S. aureus* and *E. aerogenes* bacteria was significantly more lethal. This result demonstrates that nanoparticles cannot be evaluated solely on the basis of their volume. It also demonstrates the effectiveness of the phytochemicals derived from stinging nettle seed extract, rather than merely the size effect of the UDS-NiONPs. The phytochemicals and fatty acids present in the structure of the stinging nettle have effectively transformed the surface of the nickel oxide nanoparticles into a protective shield, thereby playing an active role in disrupting the bacterial cell wall. Studies have reported that phytomolecule-coated NiONPs exhibit antibacterial activity against *S. aureus*, *B. subtilis* and *E. coli* [36, 83, 84]. OB-NiONPs, which are smaller in size, have demonstrated similar performance to UDS-NiONPs in penetrating fungal cells. The mechanism by which nickel oxide nanoparticles prepared via green synthesis eliminate bacteria involves their dissolution into ions upon exposure to the environment and the subsequent penetration of Ni²⁺ ions. As can be seen from the EDX elemental mapping of the UDS-NiONP structure, the Ni content is very high (roughly five times that of the control), and

phytochemicals are present in this proportion (in terms of C and O content). In this context, the release of Ni^{2+} into the bacterial cell occurs intensively.

The proposed mechanism by which the NiONPs we synthesised damage a bacterial cell membrane and penetrate into the cell is illustrated in three stages in Fig. 9.

In the first stage, electrostatic attraction occurs between the lipopolysaccharides and other negatively charged components on the bacterial surface. As a result of this attraction, the NiO nanoparticles adhere to the cell surface. In many bacteria, movement begins towards the peptidoglycan—the main component of the cell wall that maintains and protects the cell's integrity.

In the second stage, NiONPs that bind to the cell wall begin to trigger the production of reactive oxygen species (ROS). Numerous studies have reported that reactive oxygen species ($\text{OH}^\bullet$, $\text{O}_2^{\bullet-}$, H_2O_2) are responsible for bacterial death [85]. These ROS can lead to lipid peroxidation in the cell membrane and disruption of membrane integrity; as a result, the damaged cell membrane may become permeable. It has been reported that membrane integrity is disrupted when peptidoglycan is damaged, particularly when exposed to external factors such as nanoparticles and antibiotics [86].

In the third stage, Ni^{2+} ions begin to move freely within the cell, whose membrane integrity has been compromised, entering the cytoplasm where they interfere with metabolic processes, leading to cell death. Further research is required to elucidate the mechanism of interaction between NiONPs and bacteria and fungi. However, many studies suggest that nanoparticles enter the cytoplasm and halt vital cellular processes via redox reactions. The antibacterial activity of the NiONPs we synthesised can largely be attributed to the Ni^{2+} ions they contain [12, 39, 87]. However, it is important to bear in mind that each effect depends on the material's physicochemical properties, synthesis conditions and the organic structures it contains. EDX analysis also indicates that the Ni content of UDS-NiONPs is significantly higher than that of OB-NiONPs. The superior antibacterial performance of UDS-NiONPs against *S. aureus* and *E. Aerogenes* can be attributed to their high Ni concentration. The higher presence of Ni^{2+} ions indicates a greater concentration of toxic agents, leading to faster ROS formation within the cell. This results in irreversible damage to the bacterial membrane and exposes the bacterium to lethal oxidative stress.

4. Conclusion

This study successfully demonstrated the green synthesis of NiO nanoparticles using *U. dioica* seed and *O. basilicum* extracts and established a clear relationship between surface chemistry mediated by phytochemicals and antimicrobial performance. Comprehensive structural characterization confirmed the successful formation of highly crystalline cubic NiO nanoparticles, while optical analyses verified the semiconducting nature of these nanoparticles with bandgap energies of 3.47 and 3.50 eV. FTIR and SEM–EDX analyses also revealed that phytochemical compounds derived from plant extracts are bound to the nanoparticle surface, thereby contributing to the nanoparticles' physicochemical properties.

Although they exhibit similar crystal structures, the two nanoparticle systems demonstrated distinctly different antimicrobial activities. While UDS-NiONPs exhibited superior antibacterial activity, particularly against *Staphylococcus aureus* and *Enterobacter aerogenes*, OB-NiONPs demonstrated a similar level of antifungal activity despite their smaller particle sizes. These findings indicate that antimicrobial performance cannot be explained solely by particle size or crystallinity. Instead, the differences may be more closely related to variations in the phytochemical surface composition and elemental distribution resulting from the green synthesis process. The relatively higher nickel content detected in UDS-NiONPs may have contributed to the increased antibacterial activity of these nanoparticles by enhancing the availability of bioactive nickel species; however, further mechanistic studies are needed to confirm this relationship.

Overall, the current findings indicate that the phytochemical composition of plant extracts determines the surface chemistry of NiO nanoparticles and, consequently, governs their antimicrobial behavior. This study highlights the importance of selecting appropriate plant sources not only as reducing and stabilizing agents but also as key determinants of the biological functionality of nanomaterials produced via green synthesis. For this reason, the NiO nanoparticles developed here, as promising, environmentally friendly antimicrobial nanomaterials, provide a valuable framework for the rational design of plant-derived metal oxide nanoparticles in the future.

Declarations

Author contributions Conceptualization, ÖÖ methodology, ÖÖ, YA formal analysis, EMK, ÖÖ investigation ÖÖ, YA, resources, ÖÖ data curation, YA, ÖÖ; writing-original draft preparation, EMK; writing-review and editing, ÖÖ, YA; visualization, YA, EMK; supervision, ÖÖ; project administration, YA; funding acquisition, EMK. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest The authors declare no conflict of interest.

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Figures

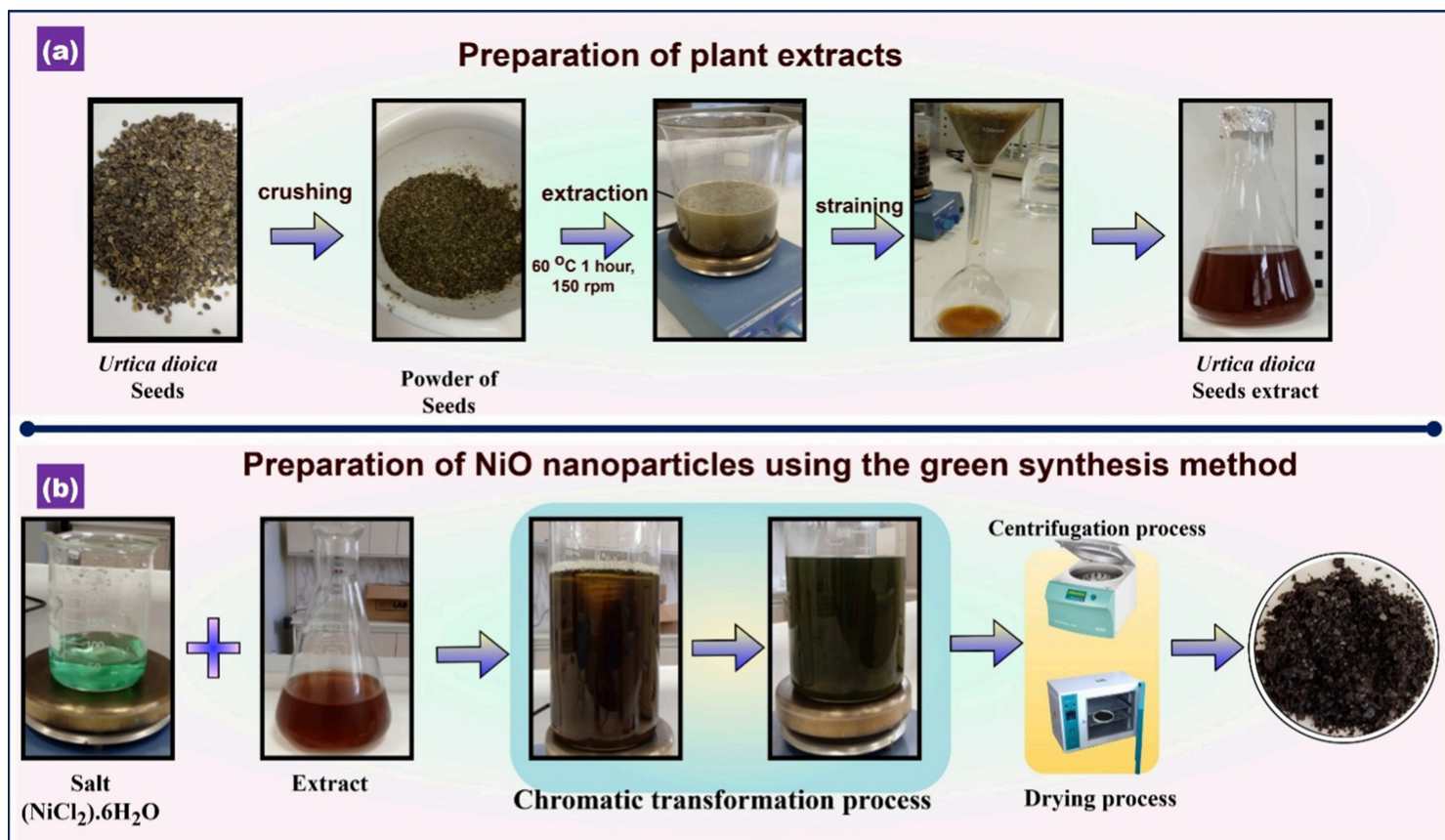


Figure 1

(a) shows the steps involved in the extraction process; (b) shows the stages involved in preparing the nanoparticles using the green synthesis approach

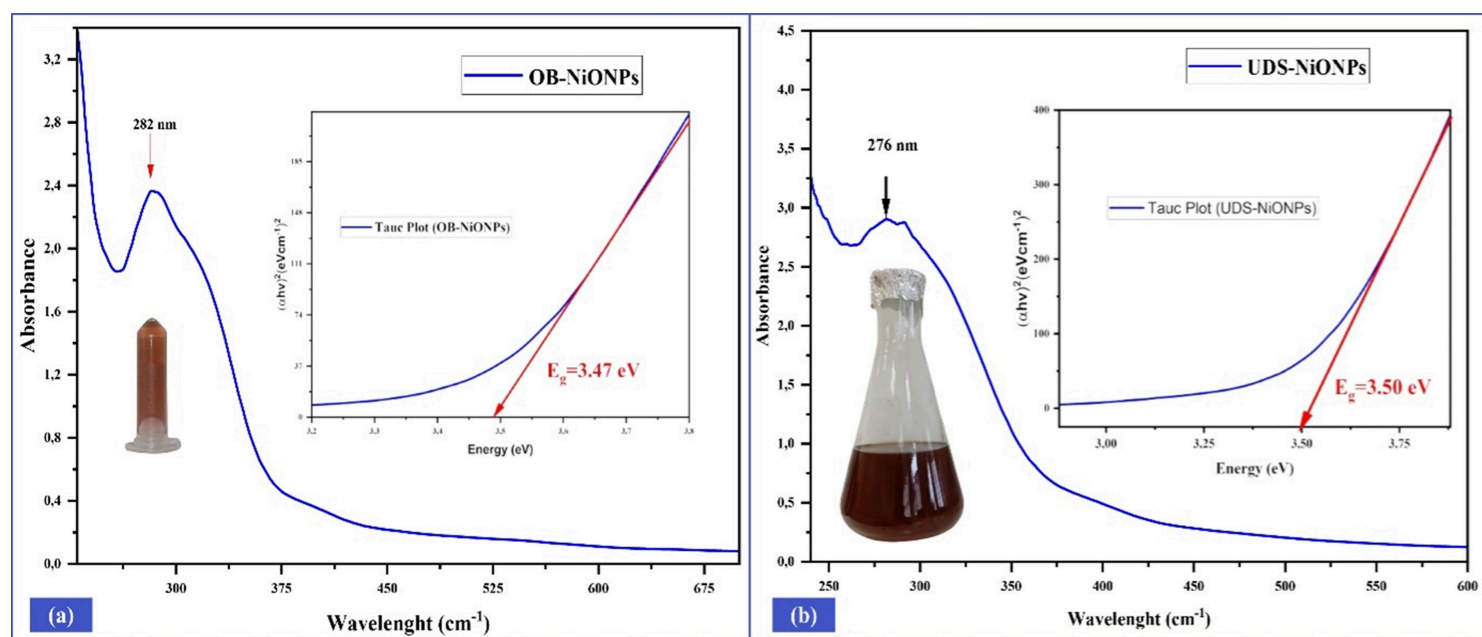


Figure 2

UV-Vis spectra and Tauc plots of (a) OB-NiONPs and (b) UDS-NiONPs

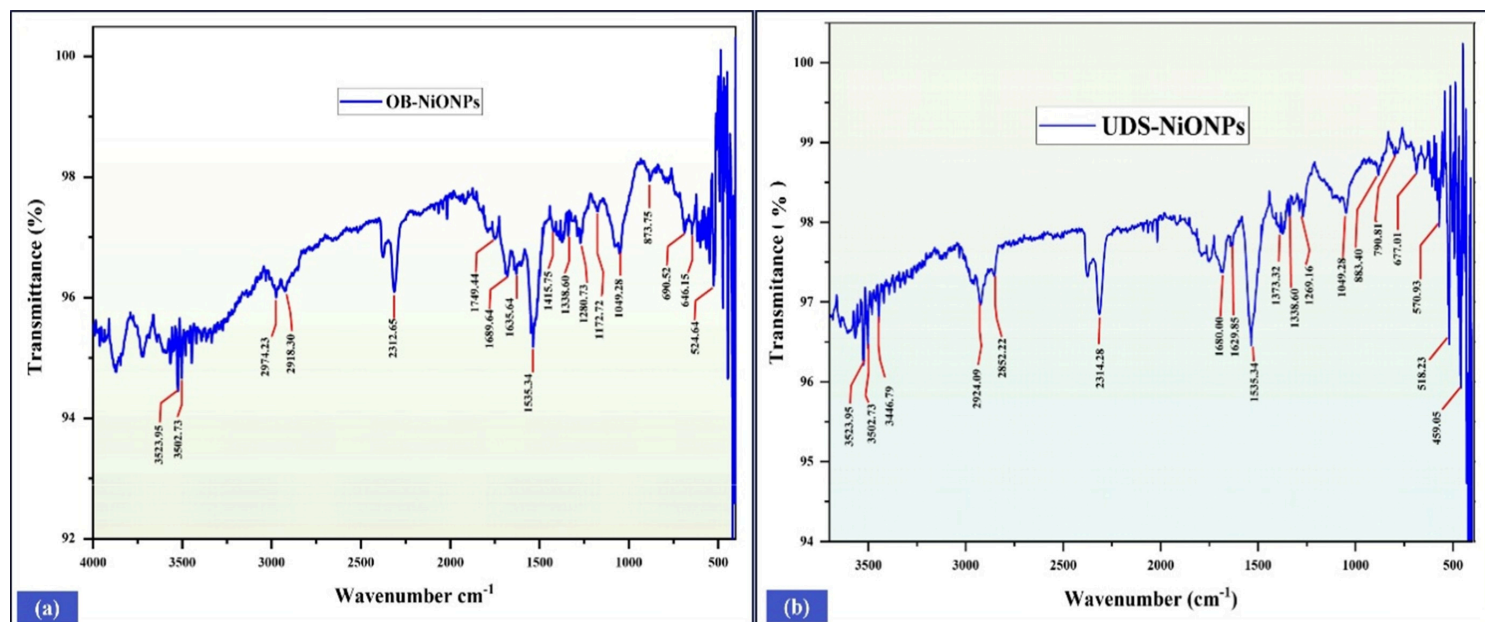


Figure 3

Fourier Transform Infrared (FT-IR) spectra of (a) OB-NiONPs and (b) UDS-NiONPs

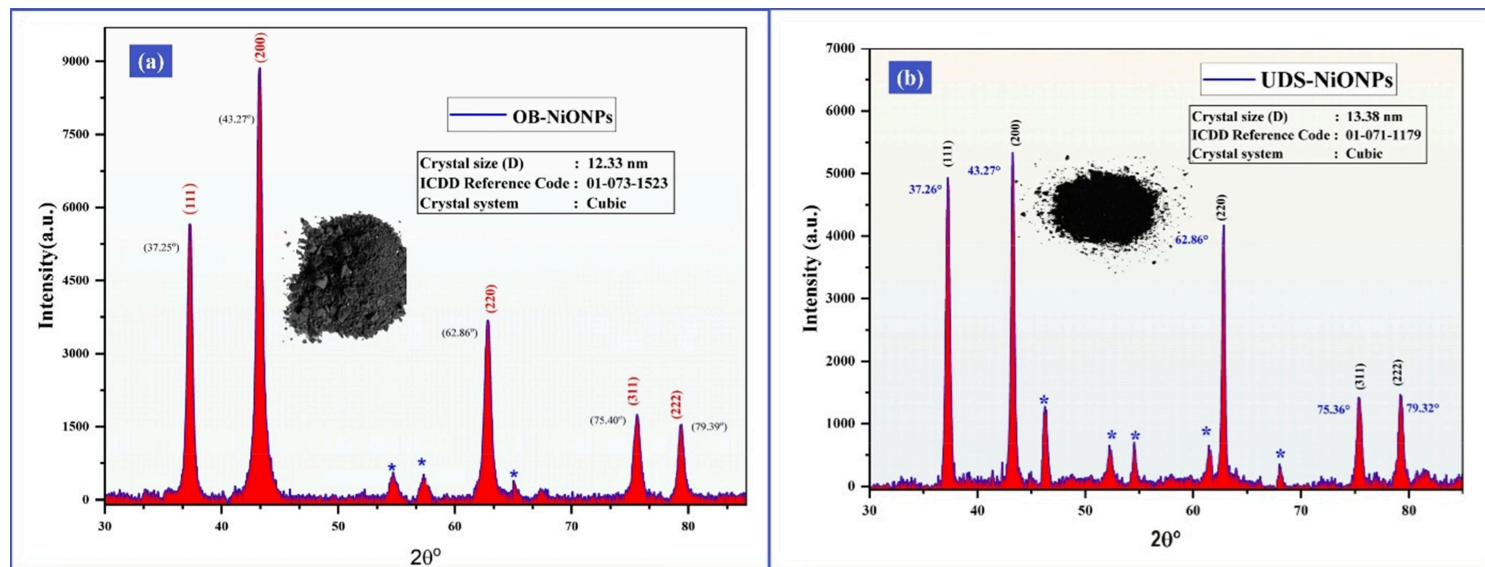


Figure 4

Graphs of (a) OB-NiONPs and (b) XRD UDS-NiONPs

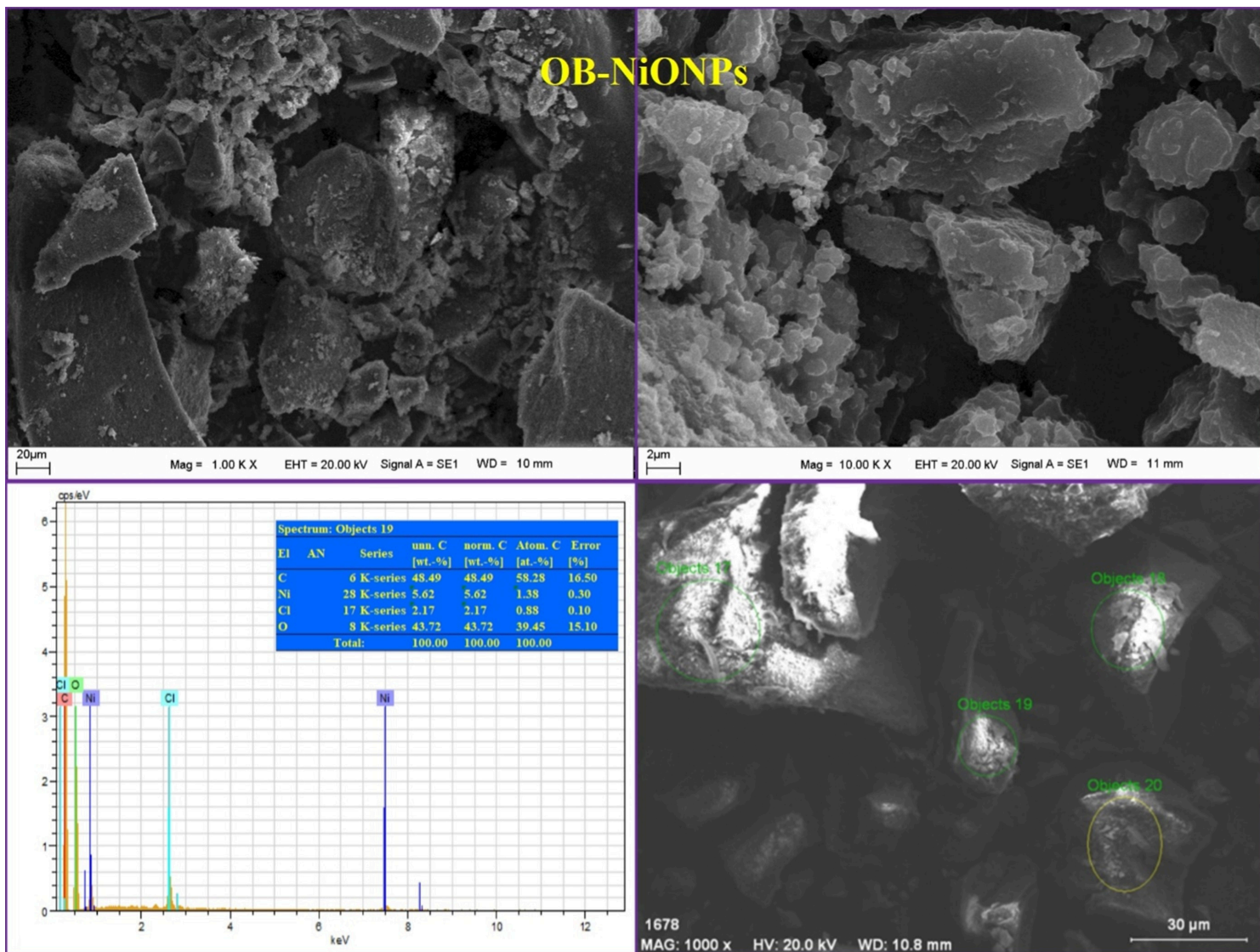


Figure 5

SEM images and EDX map of OB-NiONPs

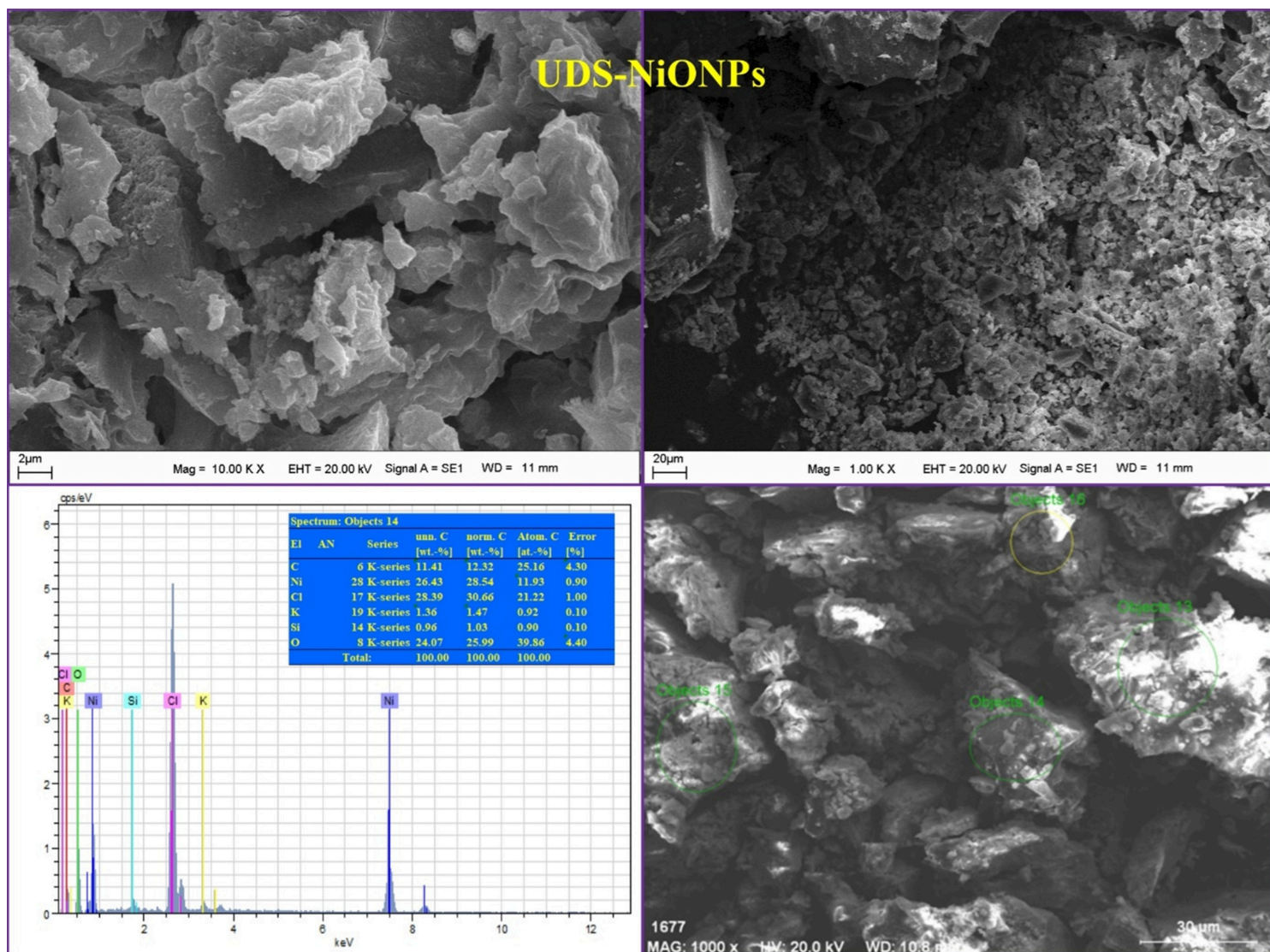


Figure 6

SEM images and EDX map of UDS-NiONPs

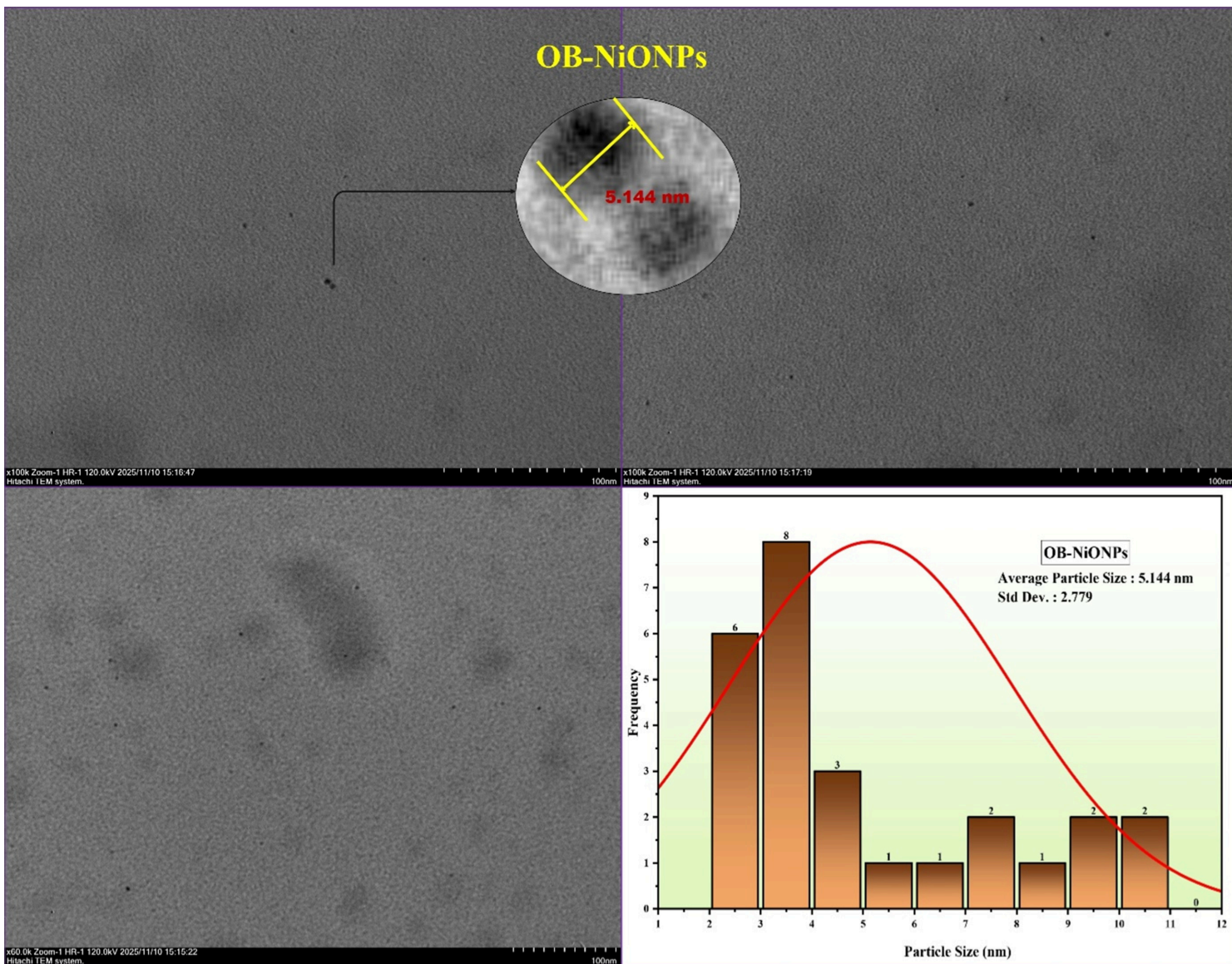


Figure 7

TEM images of OB-NiO NPs and a histogram of the nanoparticles

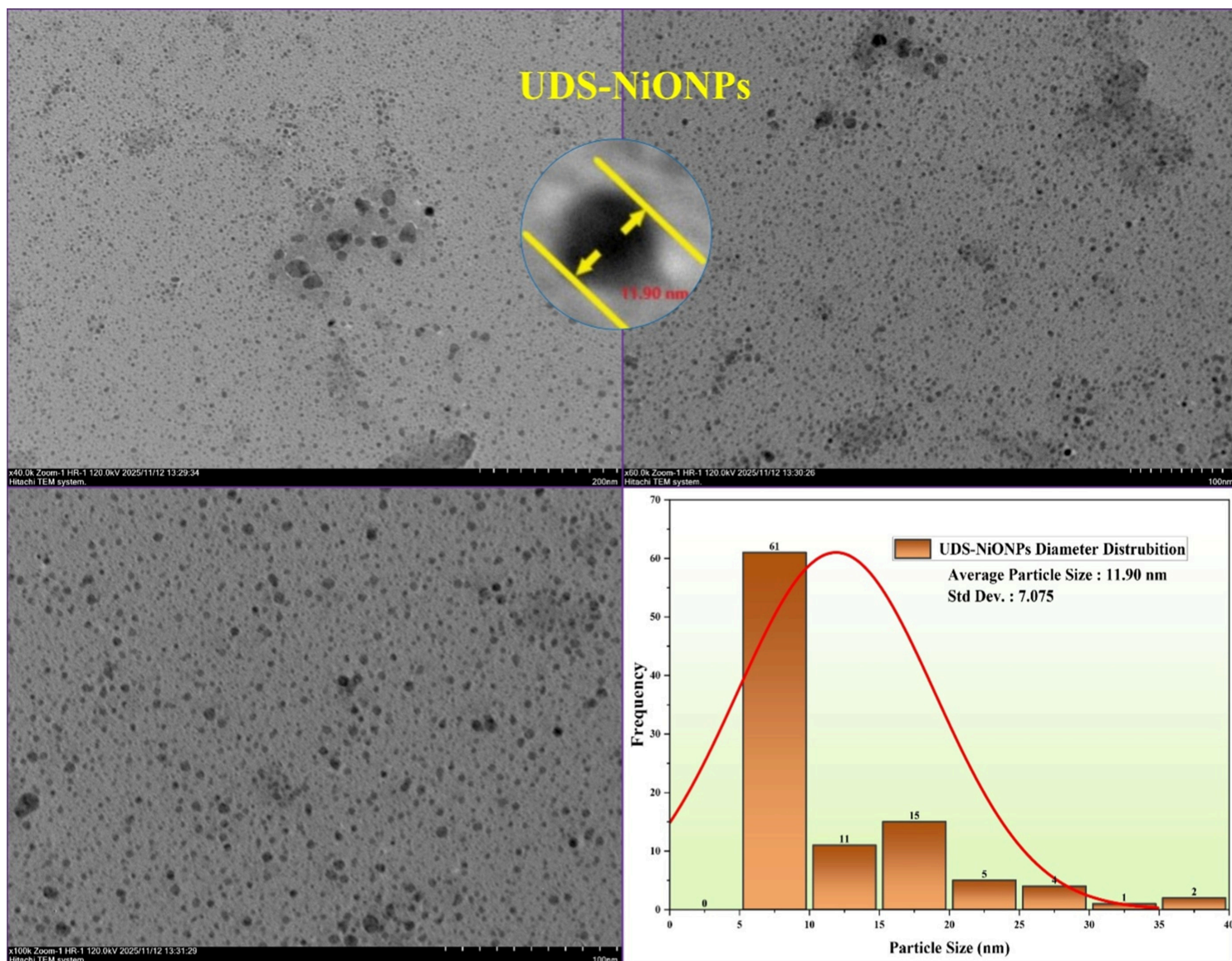


Figure 8

TEM images of UDS-NiO NPs and a histogram of the nanoparticles

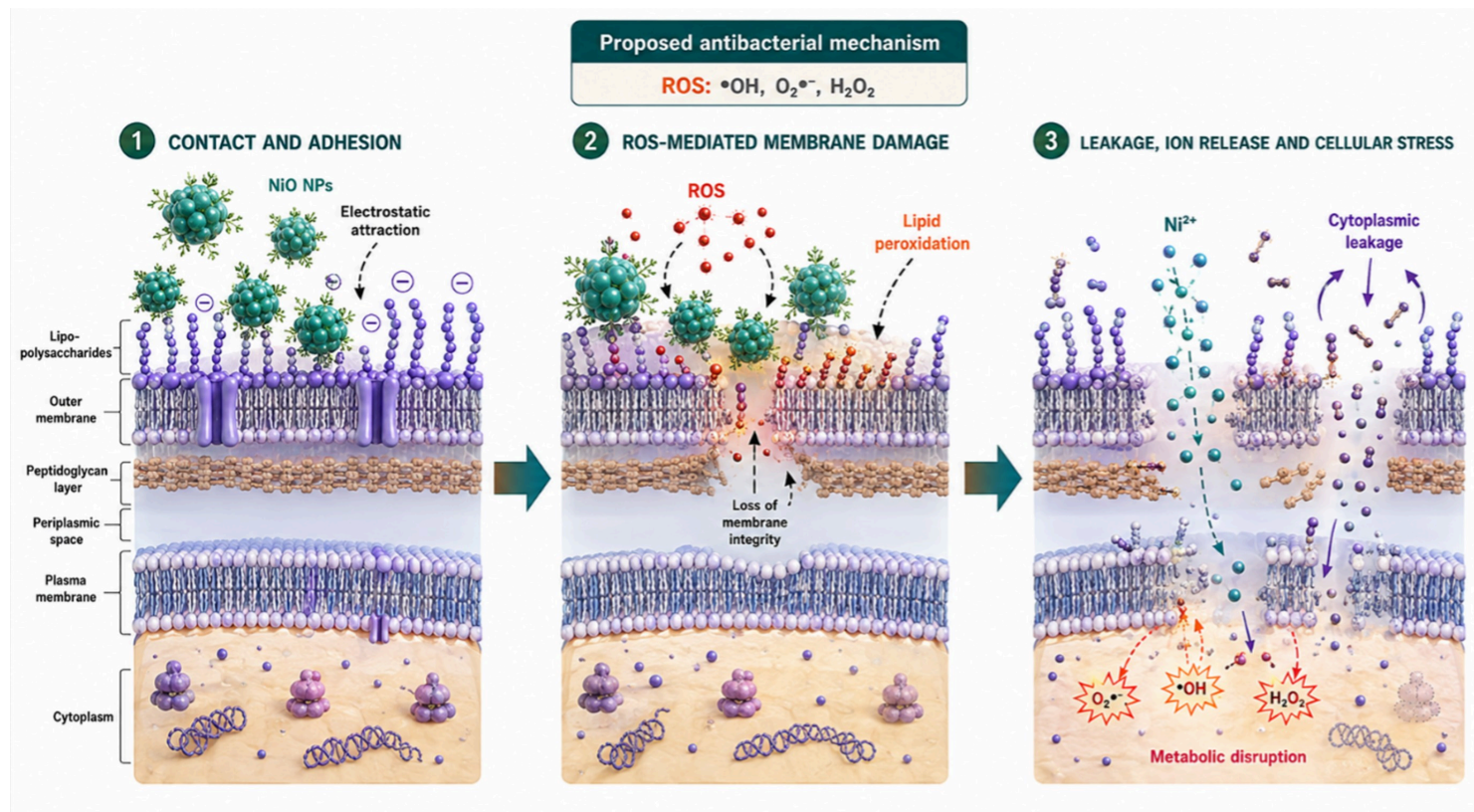


Figure 9

Adhesion of NiONPs to a bacterial cell membrane and their uptake into the cell