Research article

Extraction and characterization of nickel oxide nanoparticles from Hibiscus plant using green technology and study of its antibacterial activity

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ABSTRACT

Introduction and Aim: Nanomaterials are tiny particles varying in size from 1-100 nanometers with wide implications in the fields of medicine, biology, and pharmaceutical sciences. In this study, we aimed to extract Nickel oxide nanoparticles (NiO-NP) from the tropical hibiscus plant, and evaluate these nanoparticles for their structural features as well as study the antimicrobial activity of NiO-NPs against Gram-positive and Gram-negative bacteria.

Materials and Methods: NiO nanoparticles were synthesized by green nanotechnology using hibiscus extract and nickel nitrate as precursors. The NiO nanoparticles obtained were investigated for its surface properties using X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectroscopy and UV-Vis analysis. Transmission Electron Microscopy (TEM) was used in calculating the size of NiO nanoparticles. The antimicrobial activity of the NiO particles was tested against *E. coli* and *S. aureus* test strains.

Results: X-ray diffraction (XRD) and Fourier transform infrared (FT-IR) spectroscopy studies confirmed the formation of pure and well- crystallized NiO nanoparticles. The TEM analysis displayed that the size of nanoparticles to be within the limits of 71. The NiO-NP particles displayed antimicrobial properties and inhibited the growth of bacterial test strains used in this study.

Conclusion: The study demonstrated that nano Nickel oxide (NiO) particles could be successfully prepared using the hibiscus aqueous plant extract. The NiO-NP exhibited antimicrobial property against pathogenic *E. coli* and *S. aureus* bacteria, indicating that it could be used in formulations for treating infections by these organisms.

Keywords: NiO; nanoparticle; green nanotechnology; antibacterial.

INTRODUCTION

Nanomaterials are fine particles usually defined as a particle of matter with sizes between 1 and 100 nanometers in diameter (1). Nanoparticles, due to their ultrafine size and unique properties have been used in wide practical applications compared to their counterparts of larger dimensions (2), most notably among which are nano-metal oxides which have been widely utilized in the fields of medicine, biology, and pharmacy, biofuel production, wastewater treatments, etc., (3). As a result of the specific physical and chemical properties of nanoparticles that limit the growth of bacteria, there has been an increase in research on nanoparticles and their applications as antimicrobial agents. This is because nanoparticles inhibit the growth of bacteria, which is the cause of this phenomenon (4). In recent years, a wide variety of metal oxide nanoparticles have been created (5). These nanoparticles, such as copper, gold, and zinc, as well as NiO, are used in a variety of applications in the medical, biological, and pharmaceutical fields (6). For nanoparticles to be useful in biological applications, antibacterial activity against a diverse range of bacteria is required (7, 8) has demonstrated that alterations made to the structure, shape, and size of nanoparticles, as well as their functional group, can greatly improve the antibacterial capabilities of the particles. In contrast, Combining organic and inorganic nanoparticles is beneficial because inorganic nanoparticles are more stable and safer (9). Antibacterial activity of metal oxide nanoparticles has been demonstrated against both gram-negative and gram-positive bacteria (10), which means that bacteria, viruses, and fungi are rendered useless by the nanoparticles' binding to biological macromolecules and are subsequently destroyed (11). This antibacterial effect is one of the most important properties of metal oxide nanoparticles.

Noted among the metal oxide nanoparticles is NiO, which is a p-type semiconductor that possesses electrochemical, catalytic, photocatalytic, and antibacterial properties (9). Studies show that even at low doses, NiO exhibits antibacterial activity inhibiting the growth of a wide spectrum of microbes (12). Many physical and chemical methods were used to prepare NiO-NPs, which included the use of toxic and dangerous chemicals to humans and the environment (ecosystem), as well as requiring a long time, high costs and high energy depletion (13).

In addition, the resulting nano-materials are unstable and agglomerate quickly unless proven agents are added to them (14). In addition to releasing toxic and dangerous by-products to the environment, it was found necessary to go to simple methods in preparing nanomaterials (15), such as the green nanotechnology of preparation or the so-called (green synthesis) (16), which is characterized as being environmentally friendly, that is. Furthermore, it does less damage to the ecosystem and at simple costs and focuses on producing the required material without the presence of byproducts that are harmful to the environment and also enjoy their speed, purity and quality of the prepared oxide (17). The function of the plant extract in the preparation method is to reduce the salt and produce the corresponding nano-metal oxide (18). From all above, there are many pathogenic bacteria that have different virulence factors such as *Brucella melitensis* (19), *Pseudomonas aeruginosa* (20), *Clostridium perfringens* (21), *Staphylococcus aureus* (22), *Proteus vulgaris* (23), and Parasites such as Toxoplasma (24), were needed to find alternative anti-microbial and anti-parasitic agents.

MATERIALS AND METHODS

Synthesis of NiO-NPs

In this study, different sizes of NiO-NPs were synthesized from Hibiscus (Family: *Malvaceae*) plant material by using the sol-gel protocol described earlier (9). Flowers and leaves of the Hibiscus plant obtained locally and Ni (NO_3) $2.6H_2O$ (purchased from Central Drug House (P) Ltd – CDH, India) were utilized as the source of nickel element. Briefly, 3g of salt (Ni (NO_3) $2.6H_2O$) was dissolved in 100 ml of deionized distilled water and mixed using a magnetic stirrer at 50°C for 15 minutes. Simultaneously, 1 g each of the hibiscus leaves and flower were crushed to which 100 ml of distilled water was added and stirred by magnetic stirrer at 60°C for 20 min. until a clear red solution was obtained. This solution was further filtered using filter paper to get rid of the impurities. To the nickel-nitrate solution placed on the magnetic stirrer at a temperature of 50°C, 15 ml of the hibiscus plant extract was gradually added until the color of the solution changed and was concentrated. The resulting material contained the nanoscale nickel oxide (Fig. 1).

A B C D

Fig. 1: Experimental tubes containing A: distilled water; B: hibiscus flower extract; C: hibiscus leaf extract; D: resulting material containing nanoscale nickel oxide.

Methodology for examination of surface properties of NiO-Np

FT-IR analysis

Fourier transform infrared (FTIR) spectroscopy was performed to find out the presence of a functional group in NiO-NPs. The FT-IR analysis in this study revealed many absorption peaks within the wavelength limit from 4000 to 500 cm.

X-ray diffraction and TEM studies

To evaluate the crystal structure and particle size of nickel nanoparticles in this study, a X-ray diffraction study was undertaken. The size of the nanoparticles was evaluated by the Debye-Scherrier formula.

$$D = \frac{k\lambda}{\beta \cos\theta}$$

Where D is the particle size (nm), k = 0.94 is a constant, λ is the X-ray wavelength (0.154 nm), β is the line broadening at half the maximum intensity (FWHM), and θ is the Bragg angle.

Transmission electron microscope study was undertaken to know the shape and size of nickel oxide nanoparticles.

UV-Vis analysis

The value of band gap (Eg) for the nanoparticles was calculated based on the Tauc equation

$$(\alpha hn)^2 = A\,(hv - E_g)^n,$$

where, α refers to the absorption coefficient; h is the Planck constant, ν is the photon's frequency; hν stands for the photon energy, A would be an amount, and n is equal to 2 for a direct bandgap while being equated to 0.5 in the case of indirect bandgap. A Tauc curve was obtained by plotting $(\alpha hn)^2$ versus photon energy (hν).

Antibacterial activity studies of Nio-Np tested

To test the antibacterial effects of NiO-NPs two test strains *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive) were used. An antibacterial test was carried out by preparing a culture medium for bacteria. Two dishes were used, each dish for a type of bacteria. The prepared material was placed for the two samples in the same ratio.

RESULTS

Characterization

In general, a number of different techniques, such as FT-IR, UV-Vis, XRD, and TEM, have been utilized in order to examine the surface properties.

Fourier Transform Infrared Spectroscopy

The primary purpose of Fourier transform infrared (FTIR) spectroscopy is to provide evidence for the presence of a functional group in composite NiO-NPs. FT-IR analysis in this study revealed several absorption peaks within the wavelength limit of 4000 to 500 cm−1 (Fig.3). Among these, two major peaks were observed, one between wavelength 3650 and 2850 cm−1 (3325.28 cm−1) and another between 1700 and 1450 cm−1 (1631.78 cm−1) (Fig.3).

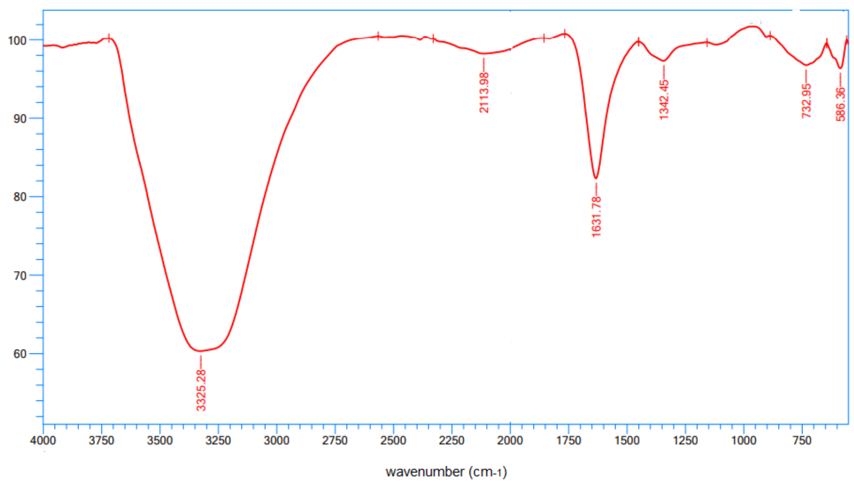

Fig. 3: The FT-IR spectrum of NiO-NPs

UV-Vis analysis

The UV-Vis plot for NiO-NPs at a wavelength range of 200 to 800 nm is presented in Fig. 4(a). As seen, the largest optical absorption occurred at a wavelength of 280 and 284 nm. The value of the energy gap was 3.43(eV).

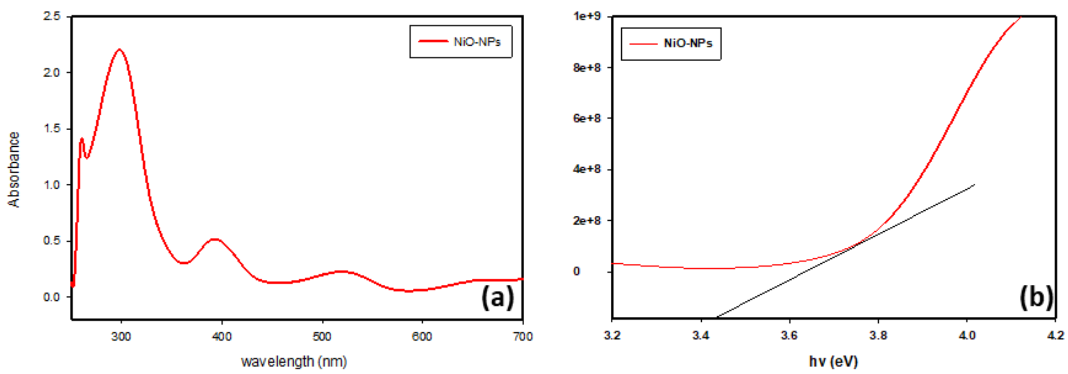

Fig. 4: The UV-Vis (a) and band gap (b) schematics of NiO-NPs

XRD study

Figure 5 shows the X-ray diffraction scheme for NiO-NPs. All XRD patterns clearly show the diffraction peaks of (102), (112), (103), (212), (301), (114), (303), (400), (111), (402) and (200) and (320) crystal planes, corresponding to a facet cubic (fcc) structure.

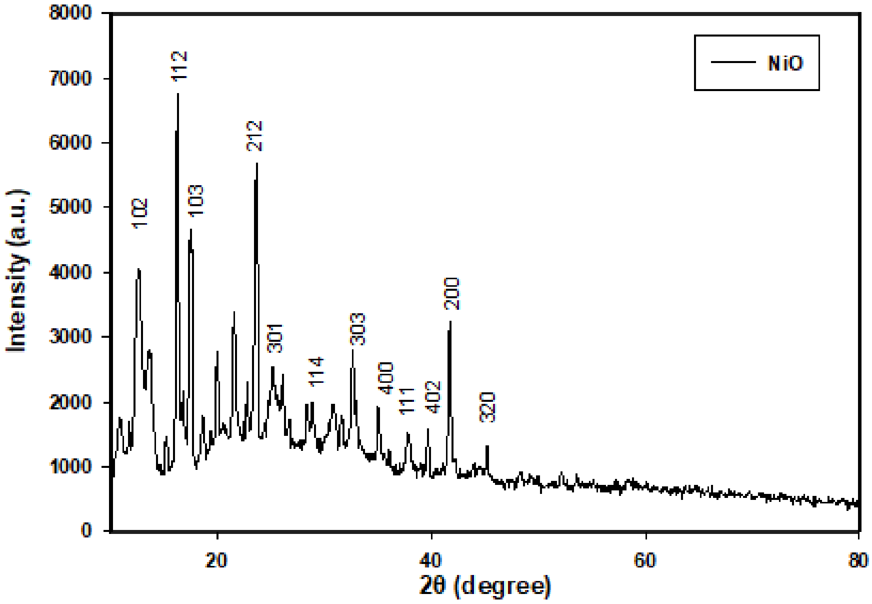

Fig. 5: The X-ray diffraction analysis of NiO-NPs

We were able to determine the diameters of the nanoparticles of nickel oxide. Consequently, with the help of TEM analysis images (D = 71 nm).

TEM image

Transmission electron microscopy revealed the NiO-NPs in this study to be spherical shaped with less amount of agglomeration (Fig 6a and b). The average size of the nanoparticles was determined to be around 71 nm which matched the X-ray diffraction assay (Fig 6c).

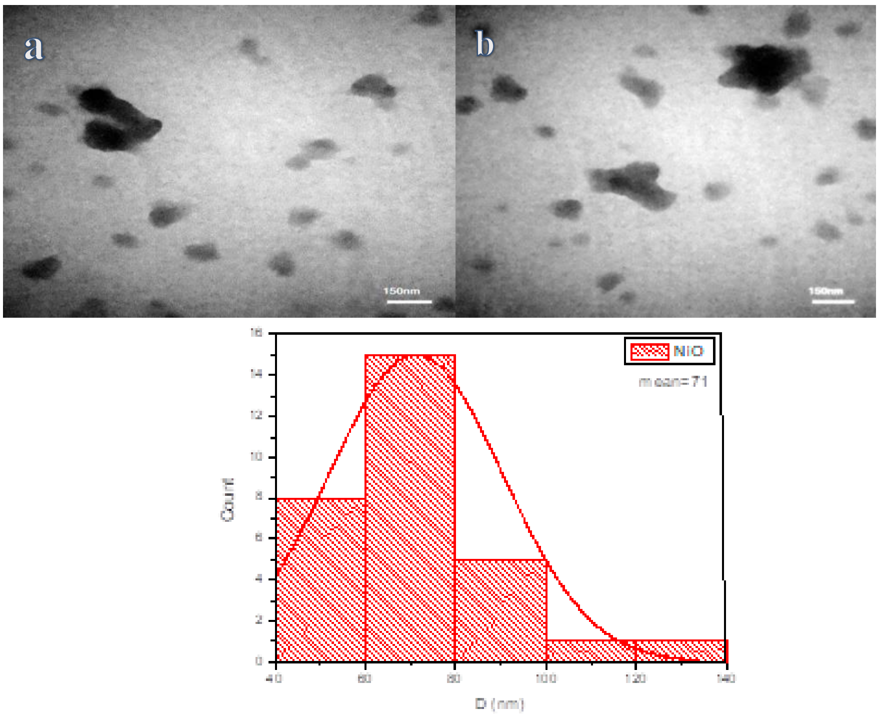

Fig.6: (a, b) TEM micrographs of NiO-NPs observed at 150 nm scales and **(c)** the size distribution of NiO-NPs

Assay of the antibacterial activity of NiO-NPs

The antibacterial activity of NiO-NPs was evaluated against two test bacteria *E. coli* and *Staphylococcus aureus* (Fig.3). The NiO-NP exhibited an average inhibition zone diameter of 26mm and 28mm for the test bacterium *E. coli* and *S. aureus* respectively.

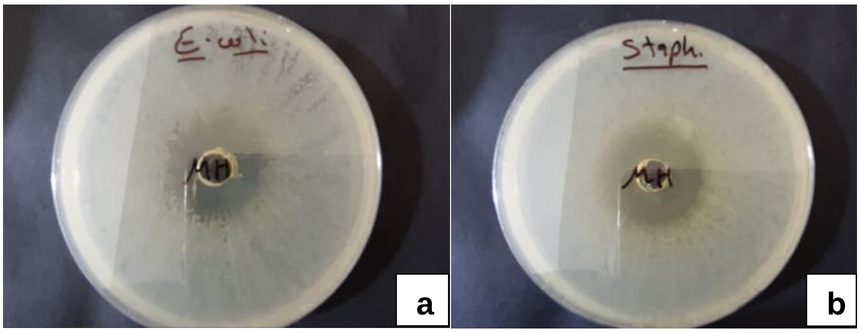

Fig. 3: The antibacterial assay for NiO nanoparticles tested on a. *Escherichia coli* and b. *Staphylococcus aureus*

DISCUSSION

Green method of NiO-NP synthesis in the current study was established through FTIR, UV-Vis Spectroscpy, XRD and TEM analysis. The FTIR spectrum of nickel oxide showed many ranges ranging from (4000-500). It shows the distinct peaks at 586.36 cm^{-1}, 732.95 cm^{-1}, 1342.45 cm^{-1}, 1631.78 cm^{-1}, 2113.98 cm^{-1}, 3325.28 cm^{-1}. Peaks indicate active groups in the compound. Absorption is observed between 500-750 cm^{-1} to the presence of NiO (fig. 4). The UV-Vis (a) and band gap (b) schematics of NiO-NPs it is possible to see that the energy gap for the synthesized nanoparticles is in the region of 3.45 eV. The size and shape of nanoparticles, dielectric constant of the medium and surface adsorbed species determine the spectral position of plasmon band absorption as well as its width. The synthesized NiO-NP showed a strong absorption peak at 280-284 nm which may be due to the functional groups present in the plant extract (25). Through the X-ray diffraction analysis, it is possible to demonstrate that nanoparticles that have been calcined are in the correct order. A rise in the height of the mountains indicates a rise in the crystallinity of the nanoparticles, while a decrease in the breadth of the mountains indicates an increase in the size of the crystalline particles that can occur as a result of the particles merging and growing. This can be explained by the fact that the size of the crystalline particles increases as the width of the mountains decreases. According to the TEM examination the particle morphology was spherical with little amount of agglomeration. The average size of the nanoparticles was determined to be around 71 nm which matches the X-ray diffraction assay. TEM and XRD results are in accordance with the findings of Lokapur *et al.,* (26) . The bacterial inhibition assay for the two types (positive and negative bacteria) that were examined indicates the effectiveness of the nanomaterial in inhibiting these bacteria. The indication of previous studies in the preparation of nanoparticles of nickel oxide varied in the size of the nanoparticle according to the method of work and the plant used. Some studies have recorded size 25nm (27) and other 21nm (28).

CONCLUSION

At room temperature, the Hibiscus extracts and nickel nitrate that were used as precursors in this study were able to be used to manufacture NiO-NPs utilizing a green nanotechnology technique. An attempt consisting of FT-IR, UV-vis, XRD, and TEM methods was made to characterize the nanoparticles that were produced as a result. The XRD and TEM studies that were done on them corroborated the shape and size of their crystals. It has been found that the energy of the band gap in NiO-NPs falls somewhere in the 3.45 eV range. Gram-negative bacteria have been tested and found to be susceptible to this product. According to the findings, the sizes of the inhibitory zones for bacteria (*Staphylococcus*, and *E. coli*, specifically) are, on average, 28 and 26 millimeters, respectively.

CONFLICT OF INTEREST

Authors declare that there is no conflicts of interest.

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